APPROPRIATE DORSO-VENTRAL GENETIC PATTERNING FOLLOWING REGULATIVE REGENERATION OF CHICKEN EMBRYO SPINAL CORD

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In the chicken embryo spinal cord neuronal subtypes are generated from spatially discrete progenitor domains, defined by the combinatorial expression of transcription factors, which are determined by gradients of sonic hedgehog (Shh) and bone morphogenetic proteins (BMPs), and by mutually repressive interactions.
The aim of our present work was to assess the regulative regeneration of the chicken embryo spinal neural tube with particular focus on the patterning of transcription factor expressions during and after regeneration.
Specific regions of the spinal cord neural tube were removed unilaterally by microsurgery sparing the floor plate. Of the embryos that survived, about 36% exhibited regulative regeneration, in which the missing tissue was reconstructed to normal size and morphology. During the regeneration (4-24 hours postoperative) the partially regenerated neural tissue showed appropriate expression patterns of several progenitor transcription factors (Pax 7, Pax 6, Nkx2.2 and Shh). Within the regenerated hemi-neural tube, neuron groups identified by postmitotic transcription factors (Brn3a, Lmx1b, Pax 2, Engrail 1, Islet 1, Islet 2, Lim 1/2, HB9 and Lim 3) appeared in their normal positions and virtually in the same number as on the contralateral unoperated side.
These results confirm regulative regeneration of the spinal neural tube in chicken embryos and demonstrate the capacity of appropriately patterned cellular distribution within the regenerated tissue.

NON-CELL-AUTONOMOUS ASPECTS OF THE DEGENERATION OF MOTOR NEURONS – NEW BASIS OF ATTEMPTS OF NEUROPROTECTION

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease leading to progressive loss of upper and lower motor neurons, resulting in paralysis and death. In vast majority of the patients (sporadic ALS), the trigger of the disease is not known, however, in the most common inherited form, point mutations of the (ubiquitously expressed) Cu/Zn superoxide dismutase (SOD1) have been identified as the cause of the degeneration. Transgenic animals, developed on the basis of some of these mutations serve, up to now, as the best models of the disease (mSOD1 mice).

Although, the etiology of the disease is basically unknown, several mechanisms have been identified which contribute to the degeneration of motor neurons. These include excitotoxicity, oxidative damage, abnormal neurofilament assembly and immune/inflammatory processes. Most, if not all of these mechanisms depend on the intracellular concentration of calcium, which may link and amplify the individual processes. To test, whether the cascade of pathological mechanisms could be broken by attenuating the intracellular calcium peaks, transgenic animals with elevated parvalbumin (PV) contents have been developed. This intervention, by crossing the mSOD1 and PV mice, resulted only in mild neuroprotection, suggesting that the restricted targeting the degenerating motor neurons is not sufficient for effective therapy, and treatment the neighboring glial cells might be necessary, as well.

Microglia are widely distributed cell population within the adult central nervous system, which rapidly activate in response to pathological changes. They produce various substances to coordinate the removal of potentially harmful cell debris, thus promote tissue repair. However, e.g. by releasing pro-inflammatory chemokines, they can act as double-edged sword and amplify the ongoing tissue damage. An understanding of molecular mechanisms of microglia proliferation and activation could serve a rational basis for targeted intervention on glial reactions to injuries in the CNS.

To determine whether expression of mSOD1 in motoneurons and astroglia, as well as in microglia, was required to produce motoneuron disease, PU.1-/- mice (that are unable to develop myeloid and lymphoid cells) were bred with mSOD1 mice. In mSOD1 x PU.1-/- mice, wild-type donor derived microglia significantly slowed motoneuron loss and prolonged disease duration and survival when compared with mice receiving mSOD1 expressing cells or mSOD1 mice.

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MODULATION OF DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION BY NITRIC OXIDE IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS

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Depolarization-induced suppression of inhibition (DSI) is a form of short-term modification of synaptic efficacy. During depolarization of hippocampal pyramidal cells the release of GABA is reduced from inhibitory terminals expressing CB1 cannabinoid receptors. This phenomenon is mediated by the action of a messenger. Nitric oxide (NO), a well-known intercellular signaling molecule, can also be released from neurons in an activity-dependent manner. In this study, we investigated how manipulation of NO levels may alter DSI.

Spontaneous action potential-dependent IPSCs were recorded from CA1 pyramidal cells of 15-18-day-old Wistar rats using a whole-cell patch-clamp technique. Depolarization from –60 mV to 0 mV for 1 s elicited DSI, which could be blocked by AM251, a CB_1 receptor antagonist. DSI was also prevented by inhibitors of NO synthase (L-NAME and 7-nitroindazole, 100 μM) and by extracellular or intracellular application of a membrane-impermeable NO scavenger (carboxy-PTIO, 0.5 and 1 mM). Exogenously applied L-arginine (0.2 - 1 mM), the precursor for NO, significantly prolonged DSI. One of the effective endogenous scavengers of NO levels in cells is glutathione (GSH). Reduction of the GSH levels by the GSH synthesis inhibitor BSO (30-100 μM) or BCNU (50 μM) increased the magnitude of DSI and prolonged its decaying phase. In contrast, elevation of the amount of GSH by blocking glutathione S-transferase with ethacrynic acid (1-20 μM) significantly reduced DSI.

Our results indicate that altering NO levels with L-arginine or with exogenous or endogenous NO scavengers significantly affects DSI. These data support the involvement of NO in retrograde signaling during DSI.

ROLE OF AMINERGIC SYSTEMS IN THE EMBRYONIC DEVELOPMENT OF THE POND SNAIL, LYMNAEA STAGNALIS

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Effect of monoamines (MA) on the duration of embryogenesis of Lymnaea was investigated in this study, coupled with the HPLC assay of the dopamine (DA) and serotonin (5-HT) levels from E12% of embryonic stage. In the DA content three distinct phases could be distinguished, including an early, gradual increase until metamorphosis (15.2-113.4 fmol/embryo), followed by a constant period (137-156.6 fmol/embryo), and then from E85% stage till hatching the DA content of the embryos showed a rapid and significant enhancement (184.5-415.3 fmol/embryo). The concentration of 5-HT was higher at E12% stage (47.2 fmol/embryo) than that of DA, then following a slow increase until E75% stage (47.2-64.1 fmol/embryo), it also showed a striking increase till hatching (160.1-491.5 fmol/embryo). Pharmacological interventions affected MA levels, synthesis and, consequently, the duration of the embryonic life as follows. 5-HT precursor, L-tryptophane (0.1 mM) enhanced the concentration of both DA (124±49.4%) and 5-HT (190± 40.6 %) and slowed down the embryonic development by 20% (2 days). DA precursor, tyrosine (0.05 mM) increased the DA level by 24% and prolonged the duration of intracapsular life by 55% (5.5 days). Tryptophan hydroxylase inhibitor, p-chlorphenylalanine (0.01 mM) decreased the concentration of both DA (43.5 ± 13.8%) and 5-HT (65.5±19.2%) and prolonged the embryonic development by 35% (3,5 days). Application of 0.01 mM m-hydroxybenzylhydrazine, a decarboxylase inhibitor, resulted in a 130□50.6% increase of DA level and a 4,5 days (40%) delay of snail embryos' hatching. From the DAergic neurotoxins, 0.1 mM 1-methyl-4-phenylpyridinium iodide (MPP+ iodide) decreased the DA content by 37 ± 6,1%, meanwhile 0,01 mM methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) did it by 46 ± 5%. Both neurotoxins retarded the embryonic life time: MPP+ iodide by 25% (2,5 days) and MPTP by 40% (4,5 days). Our results show that precursors, inhibitors and neurotoxins not only evoke significant changes of the MA levels in Lymnaea embryos, but whatever is the direction of the alterations, it is accompanied with a delayed embryonic development.

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UTILITY OF VISUAL EVOKED POTENTIAL (VEP) IN PEDIATRIC NEUROLOGY

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Background: Several authors have criticized the usage of VEP in nonverbal persons (e.g. infants), because the examination demands certain level of attention, fixation and cooperation from the participants. Using our data processing method including off line averaging, artifact rejection algorithm and applying proper statistics, it is possible to detect pathological waveforms and differentiate them from noise or artifacts.
Objectives: We demonstrate the use of pattern VEP in the diagnosis and follow up of hypoxic-ischemic cerebral white matter and oligodendrocyte lesions occurring in the critical period of visual development, during the perinatal stage and early childhood.
Subjects: Results are given for four infants, having endured damage in the visual pathways of different etiology (prematurity associated with ischemia, hydrocephalus and cranial trauma). The controls were healthy adults and infants.
Methods: VEPs to checkerboard pattern reversal (3.75 rev/s) in different size (120, 60, 30, 15 and 7.5 minutes of arch) were recorded during both monocular and binocular stimulation. Existence of stimulus synchronous signals was detected by T2 circ statistics.
Results and conclusions: With our methods the VEP results are correspondent in most cases to the clinical symptoms and can indicate the subclinical changes. The examination also proved to be useful in the estimation of the visual acuity of nonverbal persons.

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ELECTROPHYSIOLOGICAL MEASUREMENT OF STEREOPSIS: EFFECT OF INTEROCULAR DELAY ON DYNAMIC RANDOM DOT CORRELOGRAM (DRDC)

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Measuring visual evoked potential (VEP) response to DRDC is a reliable method to detect fusion and stereopsis in humans. DRDC-VEPs are sensitive to the interocular delay (IOD), which can be increased either due to a disorder (e.g. neuritis retrobulbaris, cataract). IOD can be artificially increased by neutral density filters placed in front of one eye creating an imbalance between the illuminations of the two retinas. The objective of our recent experiment was to study the correlation between the IOD and the DRDC-VEP. Five young adult individuals (between 18-30 years of age) were tested. DRDC, black-red and black-green color checkerboard pattern reversals (PR) were presented on red-green channels of a computer monitor and viewed through red-green goggles. Electrical scalp responses were recorded from O_z-F_z position. The Fourier component of the stimulus fundamental frequency in EEG was analysed by T^2_{circ} statistics. IOD was varied by placing different number of identical red or green filters in front of the eyes. IOD was calculated by subtracting the P100 latency obtained from PR-VEPs. An increase in IOD gradually reduced the amplitude of the DRDC-VEP, responses disappeared when IOD exceeded 20 ms. Since refresh rate of the DRDC pixel matrix was 60 Hz (i.e. 16.6 ms), theoretically, IOD greater than 16.6 ms renders it impossible to fuse correlated images. Our model precisely predicts this theoretical limit.

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EFFECT OF LUMINANCE ON VEP RESPONSE TO DYNAMIC RANDOM DOT CORRELOGRAM (DRDC-VEP)

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Measuring VEP response to cyclopean stimuli, e.g., DRDC is a generally accepted method to detect functional binocularity in human infants. Early assessment of disorders in the visual development could prevent amblyopia, which is the leading cause of vision loss in children. Therefore, DRDC-VEP could be a suitable screening method in the future. The objective of our recent study was optimization of DRDC stimulus parameters to achieve the largest and more reliable VEP response and to obtain further insight in the physiology of stereopsis. A total of 8 young adult individuals (between 18-25 years of age) were tested. DRDC on red-green channels and black-red and black-green color checkerboard pattern reversal were presented, while the subjects wore red-green goggles. Electrical scalp responses were recorded from O_z-F_z position. The Fourier component of the stimulus fundamental frequency in EEG was analysed by T^2_{circ} statistics. Decreasing luminance caused an increase in the P_{100} latency and a decrease in amplitude. The amplitude of DRDC-VEP showed a wide optimum as a function of luminance in the mesopic range. Large amplitude DRDC-VEP could be still recorded at the luminance when checkerboard response became hardly detectable. Our results suggest different processing mechanisms for the two stimuli: DRDC is probably processed by the magnocellular, while the checkerboard stimuli are processed by the parvocellular system. The luminance optimum may be explained with an interference between the two systems.

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CORRELATION BETWEEN ACTIVITY OF BASAL FOREBRAIN NEURONS AND THE CORTICAL UP- AND DOWNSTATES IN URETHANE ANESTHETIZED RATS

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The basal forebrain (BF) contains a diverse population of neurons, including cholinergic and non-cholinergic corticopetal cells that play a crucial role in the regulation of cortical activation. Under urethane anesthesia and in natural slow wave sleep cortical neurons display spontaneous rhythmic hyperpolarizing (downstate) and depolarizing (upstate) phases reflected in the electroencephalogram (EEG) as slow cortical rhythm. In this study we investigated in a systematic fashion the correlation between unit firing in the BF and cortical up- and down-states. Extracellular unit recordings of BF neurons were carried out with simultaneous EEG recording. After recording an attempt was made to juxtacellularly label recorded BF neurons with biocytin to identify their neurotransmitter. Based on the spike triggered average of the EEG, strong correlation was found between averaged unit firing and spontaneous EEG transitions. A group of BF neurons (43%) displayed significant positive or negative correlation with the cortical upstate. Changes in the BF neuronal firing always occurred with a delay in relation to the onset of the upstates. Based on these correlations we eventually described a group of neurons that significantly increased their firing rate at or shortly after the onset of the upstate (n=16), while another group of cells significantly decreased their firing rate after the onset of the upstate (n=6). Due to the small number of identified neurons (n=4) additional identification would be necessary to see if categorization based upon electrophysiological properties defines also a specific neurotransmitter class.

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DIFFERENTIAL DISTRIBUTION OF NCX1 CONTRIBUTES TO SPINE-DENDRITE COMPARTMENTALIZATION IN CA1 PYRAMIDAL CELLS

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Calcium spatiotemporal dynamics of spines of CA1 pyramidal cells is controlled by biophysical factors, influx, efflux, buffers, pumps, and stores (Koch C, 1998, Biophysics of computation; Oxford, UK: Oxford University Press; Majewska et al., J. Neuroscience, 2000, 20(5):1722-34). One of these factors the Na^+/Ca^{2+} exchanger is a high-capacity plasma membrane calcium pump which drives calcium extrusion in response to synaptic stimulation (Blaustein et al. 2002 Ann N Y Acad Sci. 2002, 976:356-66; Ranciat-McComb et al. Neurosci Lett. 2000 294(1):13-6). In a previous work Andrea Lőrincz and Gábor Tamás examined the subcellular distribution of NCX1 in pyramidal cells (IBRO International Workshop, 2004, Budapest). Quantitative electron microscopic analysis of preembedding immunogold reactions revealed uniform densities of NCX1 along the apical and basal dendritic shafts, and ~7- times higher densities in dendritic shafts than dendritic spines, indicating a preferential role in dendritic shafts. In order to investigate, whether this anatomical difference affects the calcium compartmentalization, we measured the spatiotemporal dynamics of calcium microdomains after blocking Na^+/Ca^{2+} exchange with 30 μM benzamil (Goldberg JH. et al., Neuron. 2003, 40(4):807-21). In recent publications (Yuste R. et al., 2000; Nat Neurosci. 2000 3(7):653-9. Review), as in our control experiments it has been shown that single synaptic input elicit well compartmentalized calcium signals restricted to spine without spreading to neighboring shaft. Benzamil did not change the calcium dynamics in these cases; however, with increasing stimulus number and intensity (activating dendritic 'computational subunits'), the calcium concentration of the dendritic shaft increased (Yasude R. et al., 2003 Nat Neurosci. 2003, 6(9):948-55). Since benzamil increased the amplitude and decay of the dendritic calcium transients, therefore is concluded that the high calcium concentration during repeated stimuli may be the substrate of the Na^+/Ca^{2+} exchanger located primarily on the membrane of dendrites. We have also found an increased probability of dendritic spiking during the blockade of Na^+/Ca^{2+} exchanger.

PRENATAL DEVELOPMENT OF GLUTAMATERGIC NEURONS IN THE HUMAN ENTERIC NERVOUS SYSTEM

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Vesicular glutamate transporter (VGLUT) mediates the packaging of the excitatory neurotransmitter glutamate into synaptic vesicles, therefore, it is considered to be a key component of glutamatergic neurotransmission and in consequence the specific marker for glutamatergic neurons. Three VGLUT subtypes have been identified so far, each with a characteristic expression pattern both in the adult and in the developing central nervous system. VGLUT2 was also found in the adult enteric nervous system (ENS), while VGLUT1 was not revealed there. While recent studies suggest an important role for glutamate in development, to date there has been no analysis of the developmental expression pattern of VGLUT in the ENS.

We have therefore aimed to investigate the expression of VGLUT in the ENS of human fetus between the 12^{th} and 23^{rd} weeks of gestation. We have first investigated the spatiotemporal distribution of VGLUT1 immunoreactive nerves and we have addressed the question of whether region and age-specific expression pattern of VGLUT1 are present in the myenteric plexus of the human fetal small intestine.

Myenteric neurons and varicose fibers with immunoreactivity for VGLUT1 were first detected at the 14^{th} week of gestation and they were present along the investigated fetal period. The expression pattern of VGLUT1 however varied in the different intestinal segments and the staining intensity decreased with age.

Our results demonstrate, for the first time, that VGLUT1 is expressed in the ENS of the human fetus between the 14^{th} and 23^{rd} weeks of gestation and the expression pattern of VGLUT1 appears to be regulated with age on a segmentspecific way.

COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT PEPTIDE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF RAT WITH DEFICIENT CCK-1 RECEPTOR

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In addition to the deletion of the CCK-1 receptor gene, the Otsuka Long Evans Tokushima Fatty (OLETF) rats express alterations to orexigenic hypothalamic peptides (e.g. NPY) and striatal dopamine. These deficits have been implied in development of hyperphagia, increased sweet preference and obesity in this strain. The aim of the present study was to investigate expression of the anorectic cocaine- and amphetamine regulated transcript (CART) peptide in various brain regions of adult (35-40 weeks), obese OLETF rats compared to age-matched lean controls using immunohistochemistry. Overall, the distribution of CART peptide-immunoreactive (CART-IR) neurons and axonal networks in OLETF rats and lean controls was identical, with no alteration in the appearance of CART-IR in areas related to feeding, as well as to stress and memory functions. In contrast, measuring the intensity of immunoreactivity, significantly lower level of intensity was found in the rostro-medial nucl. tractus solitarius ($p<0.05$) and in the rostral portion of the nucl. accumbens ($p<0.002$) in OLETF rats compared to lean controls. These findings suggest that in addition to impaired GI control of satiety and increased orexigenic signaling in the hypothalamus, the anorectic effect of CART peptide may also be impaired in OLETF rats. Although the affected areas appeared to be those that are also involved in taste functions and incentive learning, to reveal the exact nature of the effect and significance of these observations require further research.

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CORTICAL TOPOGRAPHY OF SINGLE LAYER IV SPINY NEURONS CORRELATE WITH THE REPRESENTATION OF VISUAL FEATURES IN AREA 18 OF THE CAT.

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We examined cat area 18 using optical imaging, unit recordings and single neuron reconstructions in order to analyze the relationship between the projection patterns of layer IV spiny neurons and orientation preference and visuotopic maps.
We show that orientation maps in cat area 18 are characterized by latero-medially (LM) elongated iso-orientation domains, which run nearly parallel to iso-elevation lines. The distal axonal fields of layer IV cells (N=18) varied from cell to cell; some showed less frequently axonal branching without obvious patchy distribution, while some showed more frequently axonal branching with several discrete patches. Distal axons of half of the cells showed bias to cross-orientations and the other half to iso-orientations. Furthermore, axonal elongation patterns on cortical and visual field dimensions correlated with orientation bias. Cells whose axonal field had latero-medial elongation, i.e. iso-elevation, had iso-orientation bias and cells whose axonal field had antero-posterior elongation, i.e. iso-azimuth, often had non-iso-orientation bias. Commonly, neighbouring cells projected to the same regions that resulted in strong overlap (i.e. convergence) of their bouton axon distributions. Bouton overlap was affected not only by distance between the parent somata, but also by similarity of their orientation preferences.
Taken together, the anisotropy of single cell projections with reference to the joint representation of preferred orientation and spatial position suggests that the processing of visual information along the horizontal versus vertical directions may require different cortical functional topography of the axons.

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EXPRESSION OF CALBINDIN AND COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT PEPTIDE IN GRANULE CELLS OF THE RAT DENTATE GYRUS DURING POSTNATAL DEVELOPMENT

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Granule cells of the dentate gyrus express cocaine- and amphetamine-regulated transcript (CART) peptide and calbindin (CB). CART peptide was suggested to act as a neuromodulator, and similarly to other neuropeptides, CART was supposed to exert neurotrophic effect. However, no information is available about the appearance of CART peptide in the hippocampal formation during development. Therefore, expression of CART peptide and CB was examined in the granule cells of the dentate gyrus using immunohistochemistry.
The fisrt CB-immunreactive granule cells appeared on the 7th postnatal day, 2 days earlier than CART peptide, which was first observed in granule cells on the 9th postnatal day. At this time, both markers were expressed by neurons of the dorsal blade of granule cell layer. CB-immunoreactive mossy fibers were detected on the 9th day in the stratum lucidum of the entire CA3 area. CART peptide first appeared on the 12th postnatal day in mossy fibers, in the stratum lucidum of the CA3 area, close to CA2. Number of the immunoreactive granule cells increased constantly with age, and the adult-like CB and CART peptide expression of granule cells and mossy fibers were observed around the 30th day.
Similarly to humans, where development of CB-immunoreactive granule cells and mossy fibers is completed during the first few months after birth, long-lasting postnatal maturation of the dentate gyrus was observed in the rat. Since functional maturation of the rat hippocampal formation has been described after the 14th postnatal day it can be assumed that both CB and CART peptide play a role in the adult-like synaptic function of mossy fibers.

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INHIBITORY SYNAPTIC TRANSMISSION IS CONTROLLED BY NITRIC OXIDE IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS.

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Several studies have shown that the efficacy of excitatory synapses could be controlled by nitric oxide (NO), a messenger molecule that is released from postsynaptic neurons in an activity-dependent manner, and is able to regulate glutamate release from presynaptic boutons. NO is produced by postsynaptically localized NO synthase (NOS) and can elevate cGMP levels by activating soluble guanylyl cyclase (sGC) in the axon terminals. In this study we examined whether NO signaling could control GABAergic synapses in the hippocampus.
Using whole-cell patch-clamp technique, we recorded pharmacologically isolated inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal cells. Measurements were obtained in hippocampal slices prepared from 14-17 day old male Wistar rats. We first investigated the effect of the endogenous NO precursor L-arginine on evoked IPSCs. L- arginine (0.5 mM) decreased the amplitude of evoked IPSCs, an effect that could be prevented by preincubation either in 0.1 mM L-NAME (an inhibitor of NOS) or in 0.01 mM ODQ (a blocker of sGC). Next we tested the action of an NO donor SNP and a cGMP analogue 8-Br-cGMP on IPSCs measured in the presence or absence of TTX. SNP (0.1 mM) and 8-Br-cGMP (0.1 mM) decreased both the amplitude and the frequency of TTX-sensitive IPSCs, whereas they did not change the properties of TTX-resistant miniature IPSCs.
These data suggest that NO produced by NOS can activate sGC at inhibitory synapses. Increase of cGMP levels regulates action potential-dependent, but not independent IPSCs, suggesting the presynaptic locus of action. Our results propose a novel mechanism controlling GABA release at hippocampal inhibitory synapses by a signaling cascade that involves nitric oxide.

EARLY EVOKED POTENTIALS DURING SIMULATED WEIGHTLESSNESS

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It is well known that cerebrovascular circulation is largely independent from the systemic blood pressure, but it is not known how acute hypoxia accompanied by blood loss influences cognitive functions. Adaptive changes of major body systems in astronauts during spaceflight can be simulated by bed rest combined with acute blood loss, a ground-based microgravity model that provides a meaningful opportunity to study mechanisms and possible countermeasures under controlled experimental conditions.
In our experiments 6 young, healthy, male volunteers were subjected to 60 degree head-up tilt combined with a blood donation of 500 ml. The changes in cognitive functions have been estimated with a follow-up of event-related potentials (ERPs). We measured the early ERPs: N1, P1, N2 and P2 in an "oddball" paradigm, when the subject detects an occasional "target" stimulus in a regular train of standard stimuli. In our experiment, "target" stimuli were pictures of animals of any species among a series of "non-animal" pictures. These ERPs are commonly used to characterize stages of processing at early cortical levels.
The amplitudes and latencies of the four waves did not change significantly after the blood donation. The amplitude differences between the "target" and "non-target" conditions, however, were considerably, but non-significantly reduced.

Our results show that 500 ml blood loss together with head-up tilt does not alter significantly early recognition processes, suggesting that autoregulation of cerebral circulation compensated these effects.

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CELLULAR DISTRIBUTION OF RAF PROTEIN KINASES IN THE BRAIN STEM OF THE ADULT RAT

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The Raf protooncogene product, a serine/threonine kinase, is a specific modulator of the MAPK/ERK signalling pathway. Three members of the Raf family are known, i.e. A-, B-, C-Raf. B- and C-Raf are expressed in neuronal tissue. The 95 kDa B-Raf is the main MAPK activator in the central nervous system.
The present study investigated the localization of B-Raf protein in the brain stem of the rat. Immunohistochemistry (IHC), electron microscopy (EM) and western blotting (WB) were used. Polyclonal anti-B-Raf serum was utilized for IHC and WB.
We found the widespread distribution of immunoreactivity in every segment of the brain stem. Strongest staining was detected in the motor cranial nerve nuclei, the giant neurons of the reticular formation, in the raphe nuclei and in the neurons of the locus coeruleus and the substantia nigra. The distribution of Raf-positive neurons was mapped, using cresyl-violet-stained cross sections and stereotaxic atlases. Some neuronal populations were identified by using monoclonal tyrosine-hydroxylase and dopamine-beta-hydroxylase antibodies. In EM, the Raf protein was localized in the perinuclear cytoplasm and in dendrites. We detected one highly immunoreactive band at 95 kDa with WB analysis from isolated tissues of the cerebral cortex and brain stem.
The neurons and their connections in the brain stem are functionally very different, which means that the chemical informations what they receive are different, too. However, it seems that neurons possess a similar intracellular molecular machinery. We think, that not the molecules themselves, rather their different intracellular localization and metabolism are responsible for the adaptation of neurons to a changing chemical environment. The postsynaptic localization points to the importance of Raf kinase in synaptic transmission and in postsynaptic information processing.

NUMBERS, DENSITIES AND CO-LOCALIZATION OF AMPA- AND NMDA-TYPE GLUTAMATE RECEPTORS AT INDIVIDUAL SYNAPTIC CONTACT AREAS IN THE SUPERFICIAL SPINAL DORSAL HORN OF RATS

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The well established role of AMPA- and NMDA-type ionotropic glutamate receptor mechanisms in the induction of spinal LTP and consecutive pain makes these receptors exceptionally important in spinal processing of nociceptive sensory signals. Here we applied the SDS-FRL method to laminae I-II of the spinal dorsal horn of rats and investigated the numbers, densities and co-localization of AMPA and NMDA receptors at individual postsynaptic active zones with a high molecular resolution. We demonstrated that the average surface area of glutamatergic postsynaptic active zones in laminae I-II was 0.0546 μm^2. We also showed that all glutamatergic postsynaptic membranes in laminae I-II expressed AMPA receptors, and most of them (96.2%) were immunostained also for the NR1 subunit of NMDA receptors. The numbers of gold particles labeling AMPA and NMDA type glutamate receptor ion channels at individual postsynaptic membranes varied in the range of 8-214 and 5-232 with mean values of 50.98 and 41.6, whereas their densities varied in the range of 325 – 3365 / μm^2 and 84 – 2263 / μm^2 with a mean of 1136.2 / μm^2 and 786.8 / μm^2, respectively. Concerning the subunits of AMPA receptors, it was revealed that virtually all (98.8%) investigated postsynaptic membranes expressed GluR2 subunits, and most of them (90.4%) were also immunoreactivity for GluR1. The size of postsynaptic surface areas showed a more or less linear correlation with the numbers of AMPA and NMDA receptors. However, GluR1 expression was exceptionally low in postsynaptic active zones larger than 0.1 μm^2.

MODULATION OF DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION BY NITRIC OXIDE IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS

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Depolarization-induced suppression of inhibition (DSI) is a form of short-term modification of synaptic efficacy. During depolarization of hippocampal pyramidal cells the release of GABA is reduced from inhibitory terminals expressing CB1 cannabinoid receptors. This phenomenon is mediated by the action of a messenger. Nitric oxide (NO), a well-known intercellular signaling molecule, can also be released from neurons in an activity-dependent manner. In this study, we investigated how manipulation of NO levels may alter DSI.

Spontaneous action potential-dependent IPSCs were recorded from CA1 pyramidal cells of 15-18-day-old Wistar rats using a whole-cell patch-clamp technique. Depolarization from –60 mV to 0 mV for 1 s elicited DSI, which could be blocked by AM251, a CB_1 receptor antagonist. DSI was also prevented by inhibitors of NO synthase (L-NAME and 7-nitroindazole, 100 μM) and by extracellular or intracellular application of a membrane-impermeable NO scavenger (carboxy-PTIO, 0.5 and 1 mM). Exogenously applied L-arginine (0.2 - 1 mM), the precursor for NO, significantly prolonged DSI. One of the effective endogenous scavengers of NO levels in cells is glutathione (GSH). Reduction of the GSH levels by the GSH synthesis inhibitor BSO (30-100 μM) or BCNU (50 μM) increased the magnitude of DSI and prolonged its decaying phase. In contrast, elevation of the amount of GSH by blocking glutathione S-transferase with ethacrynic acid (1-20 μM) significantly reduced DSI.

Our results indicate that altering NO levels with L-arginine or with exogenous or endogenous NO scavengers significantly affects DSI. These data support the involvement of NO in retrograde signaling during DSI.

METABOLIC CONSEQUENCES OF INTERLEUKIN 1beta MICROINJECTION INTO THE NUCLEUS ACCUMBENS OF THE RAT

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The nucleus accumbens (NAcc) is known to be intimately involved in the control of feeding and metabolism. In our previous investigations, interleukin mechanisms of the NAcc influencing feeding and body temperature were revealed. The present experiments were designed to elucidate the effect of direct administration of interleukin 1beta (IL-1b) into the NAcc on metabolic parameters such as plasma glucose (PG), total plasma cholesterol (TC), triglyceride (TG), uric acid (UA) and lactate dehydrogenase (LDH) concentrations. Blood samples were taken from trunk veins 10 minutes after bilateral intracerebral microinjection of the cytokine. In another experiment, intraperitoneal glucose tolerance test (IPGTT) was performed after bilateral IL-1b microinjection into the NAcc, and blood samples (from tail vein or trunk vein) were taken at certain times during as well as at the end of the test.
In the first study, PG, TC, TG, UA and LDH concentrations of the cytokine treated animals were found higher compared to those of control animals. In the second study, IL-1b microinjection into the NAcc resulted in pathological glucose tolerance of rats. Measurements from blood samples collected after the IPGTT revealed elevation of UA and LDH levels in the cytokine treated group.
Remarkable metabolic alterations seen in these experiments indicate that IL-1b mechanisms of the NAcc play important role in the central regulation of homeostasis.

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A COMPARATIVE STUDY OF THE FUNCTION OF GAP JUNCTIONS IN SEIZURES ON A CHRONIC AND A SEMICRHONIC EPILEPSY MODEL

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In our previous work, electrophysiological experiments combined with pharmacological manipulations of the gap junction channels (GJ), and measurements of neuronal and glial connexin mRNA levels were carried out on the semichronic 4-aminopyridine (4-AP) epilepsy model in rat neocortex. In the present study we compared the involvement of GJs in epileptogenicity, induction and maintenance of seizures on the chronic pilocarpine (Pc) and the simple 4-AP epilepsy models. The Pc rats gradually became epileptic in 2-3 months after a status epilepticus induced by pilocarpine. In order to analyse the effects of GJ manipulation, seizures were induced by 4-AP in acute experiments in both models. The GJs were opened by trimethylamine (TMA) and blocked either by carbenoxolone (a broad-spectrum GJ blocker) or quinine (a selective blocker of neuron-specific Cx36) before the induction (pretreatment) or at the active epileptic focus (treatment).
In the Pc animals, the 4-AP seizures were more pronounced indicated by elevated summated ictal activity and increased amplitudes of seizure discharges in comparison to the simple 4-AP ones. Pretreatment with TMA itself induced interictal-like discharges in the basic cortical activity (ECoG) only in Pc animals. As opposed to simple 4-AP rats, in Pc animals the blockade of Cx36 modified the epileptiform activity much efficiently both in the pretreatment and treatment experiments. Global blockade of the GJs after quinine treatment further decreased the summated ictal activity to a lower value in the Pc animals than in the simple 4-AP ones.
Based on these observations we suggest that the dramatic consequences of GJ manipulations both on the basic ECoG and on the expression of seizures could be explained by the higher level of GJal communication in the cortex of the Pc animals than in the simple 4-AP ones.

DISTRIBUTION OF TUBEROINFUNDIBULAR PEPTIDE OF 39 RESIDUES IN THE RAT BRAIN DURING EARLY DEVELOPMENT

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Tuberoinfundibular peptide of 39 residues (TIP39) was purified as an endogenous ligand of the parathyroid hormone 2 receptor. We have recently demonstrated that major TIP39 expression in the young adult rat brain is confined to neurons in the subparafascicular area at the thalamus-midbrain junction, and the medial paralemniscal area (MPL) in the lateral pontomesencephalic tegmentum. During postnatal development, TIP39 expression increases in both areas until postnatal day 14 (PND-14), is stable until PND-33, then decreases, and almost completely disappears by PND-125. In the present study, we examined the expression of TIP39 during embryonic development. TIP39-immunoreactive (TIP39-ir) neurons were detectable at embryonic day 14 (ED-14) in MPL, and the labeling was relatively intense thereafter. In contrast, TIP39-ir cells were not present in the subparafascicular area during embryonic life. However, we demonstrated TIP39-ir neurons in two additional brain areas - the posterior intralaminar complex of the thalamus (PIL) and the amygdalo-hippocampal transitional zone (AHi)- between ED-16 and PND-1. We confirmed the specificity of immunolabeling in these sites by demonstrating the presence of TIP39 mRNA using in situ hybridization histochemistry in them. Most TIP39-ir neurons in the PIL and all TIP39-ir neurons in the AHi disappear during early postnatal development. Most TIP39-ir fibers emerge only during postnatal development. However, fibers emanating from PIL can be followed in the supraoptic decussations at ED-18. Our data reveal a complex pattern of TIP39 expression during early development suggesting the involvement of TIP39 in transient functions during ontogeny.

LOCALIZATION OF LATENT TRANSFORMING GROWTH FACTOR BETA BINDING PROTEIN EXPRESSION IN THE RAT CENTRAL NERVOUS SYSTEM

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Transforming Growth Factor betas (TGFbeta-1, 2 and 3) regulate the survival and the differentiation of various cell types in different organs. A Latent TGFbeta Binding Protein (LTBP-1, 2, 3 or 4) is required for the secretion and matrix deposition of TGFbetas, and LTBPs may also play a role in the activation of TGFbetas. In the brain, the role and the location of TGFbetas and their binding proteins are largely unknown. In the present study, we used RT-PCR of dissected rat brain tissue to demonstrate that TGFbeta-1, 2 and 3, as well as LTBP-1, 3 and 4 are present in all major brain regions. In contrast, we found that LTBP-2 is not expressed in the thalamus, pons and medulla oblongata. In situ hybridization histochemistry for LTBP-1 and 2 confirmed their RT-PCR expressional data. In addition, a restricted expression of both LTBP-1 and 2 within major brain regions was revealed. For example, LTBP-2 mRNA expression in the hypothalamus was confined to neurons in the dorsolateral hypothalamus. We developed an antibody against LTBP-2, which confirmed the presence of LTBP-2 protein expression in the dorsolateral hypothalamus. Furthermore, double immunolabeling revealed that LTBP-2 is selectively expressed in orexin-immunoreactive neurons in the dorsolateral hypothalamus. Our data provide a morphological basis for the investigation of LTBPs functions in the central nervous system. The restricted expression of LTBP-1 and 2 suggests that they participate in region specific functions. In particular, LTBP-2 in the dorsolateral hypothalamus may play a part in the differentiation and survival of orexinergic neurons.

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REGIONAL-, AGE- AND GENDER-DEPENDENT DIFFERENCES IN CONCENTRATION OF NUCLEOSIDES IN THE HUMAN BRAIN

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Nucleosides as neuromodulators participate in the neuronal functions of the brain. For better understanding function of nucleosides in the central nervous system, to reveal distribution of nucleoside levels and changes of nucleoside concentrations by age and sex are actual requirements.
Using post mortem samples (n=975) from 61 human brain and 4 spinal cord areas we established *in vivo* tissue levels of four nucleosides (uridine, inosine, guanosine, adenosine) and three of their metabolites (uracil, hypoxanthine, xanthine). Average *in vivo* concentrations of the brain samples investigated (regardless to their anatomical locations, gender or age; mean±S.E.M.) were the following (pmol/mg wet tissue weight): adenosine 8.5±0.58, inosine 74.8±2.84, guanosine 13.2±0.62, uridine 35.1±1.26, uracil 7.1±0.36, hypoxanthine 58.3±1.58 and xanthine 35.1±1.17. Concentration of nucleosides were uneven in the human central nervous system and highest in the temporal cortex, amygdala, caudate nucleus, vestibular and cochlear nuclei while lowest in the habenula, zona incerta, substantia nigra, inferior colliculus and locus coeruleus. We demonstrated that concentrations of uridine, inosine, guanosine and adenosine in the frontal cortex and the cerebral white matter are age- and gender-dependent. Our findings support the hypothesis that the nucleoside microenvironment in the brain could be an important factor in aging process.

IDENTIFICATION AND CHARACTERIZATION OF THE MEDIAL PARALEMNISCAL NUCLEUS IN THE LATERAL PONTOMESENCEPHALIC TEGMENTUM OF THE RAT

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While mapping the topographical distribution of the newly purified neuropeptide, tuberoinfundibular peptide of 39 residues (TIP39), we have described a TIP39 cell group in the paralemniscal zone of the lateral pontomesencephalic tegmentum and termed the area as medial paralemniscal nucleus (MPL). To further identify the MPL, in the present study, we characterized it cytoarchitectonically, myeloarchitectonically, neurochemically, and also examined its neuronal inputs in the rat. By analyzing coronal, horizontal and sagittal sections stained with cresyl-violet and the Luxol Fast Blue myelin dye we demonstrated that neurons in the area corresponding to the MPL have a laminar distribution distinct from adjacent areas. Double labeling of TIP39 and a fluorescent Nissl dye demonstrated that TIP39-immunoreactive (TIP39-ir) neurons are distributed in the cytoarchitectonically defined area indicating that it corresponds to the MPL, and that TIP39-ir neurons amount to about 50% of the neurons in the MPL. The borders of MPL are the intermediate nucleus of the lateral lemniscus laterally, the Kölliker-Fuse nucleus laterocaudally, the pontine reticular formation medially, and the rubrospinal tract ventrally while double immunolabeling of TIP39 and tyrosine-hydroxylase demonstrated that the A7 noradrenergic cells are located immediately caudomedial to the MPL. We also determined (using the retrograde tracer cholera toxin B subunit) that the MPL has afferent neuronal connections distinct from adjacent brain regions including major inputs e.g. from the auditory cortex, and from the hypothalamus, especially from the ventromedial nucleus. The presented cytoarchitectonic, myeloarchitectonic, neurochemical and connectional characterizations form the foundation of further functional studies on the MPL.

INVESTIGATION OF A NEW DERIVATIVE OF KYNURENIC ACID *IN VIVO* AND *IN VITRO*

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Overactivation of the excitatory amino acid receptors plays a definitive role in neuronal death in neurodegenerative disorders. It seems to be plausible to use excitatory amino acid receptor antagonists in neuroprotection. The metabolism of trytophan along the kynurenine pathway yields several neuroactive intermediates, including kynurenic acid (KYNA), which is one of the few known endogenous N-methyl-D-aspartate (NMDA) receptor inhibitors; in parallel with this, it is an α7 nicotinic acetylcholinergic receptor (nAChR) antagonist. As kynurenic acid has a very limited ability to cross the blood-brain barrier, the use of this compound as a neuroprotective agent is practically excluded. Accordingly it is very important to develop such analogues and derivatives of KYNA, which are able to pass into the central nervous system (CNS) with good efficiency, and have retained property of NMDA receptor antagonist at the same time.
In the course of our experiments we examined our recently synthetized KYNA derivative (KSz-27) *in vivo* and *in vitro*. Applying KSz-27 *in vitro* -equimolar to KYNA (16 µM)- resulted in decrease of field EPSP (fEPSP) amplitudes recorded from hippocampal CA1 region. The attenuating effect of KSz-27 was similar to that experienced when applying KYNA. Also, we examined the action of KSz-27 in a dose of 4,9 mg/kg -equimolar to KYNA (3 mg/kg, i.p.)- on the CA3 stimulation-evoked activity of the CA1 pyramidal cells in the hippocampus *in vivo*. We found that KSz-27 was effective in reducing the amplitudes of population spikes in contrast with KYNA, which has only a limited ability to penetrate into the CNS. Above all, animals were treated with systemically administered KSz-27 in doses of 4.9 and 49 mg/kg and examined whether these pharmacological manipulations could lead to increased brain concentrations of KSz-27. Samples of liquor were taken 1,5 h after the drug application, and a reversed phased *HPLC/MS* *(ESI)* technique was used to analyse them. The measured data shows that KSz-27 in a dose of 4,9 mg/kg was able to cross the blood-brain barrier, because we could detect its presence at the samples of liquor in nanomolar range (~ 172,2 nM). Over against this concentration, the applied KSz-27 in a dose of tenfold (49 mg/kg) resulted in only a double concentration of KSz-27 (~ 327,5 nM) in the cerebrospinal fluid.
In vitro and *in vivo* electrophysiological results just as analytical measurements suggest that we could develop a hopeful NMDA receptor antagonist, which is able to pass the blood-brain barrier.

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AXONAL AND DENDRITIC EFFECTS OF NEUROGLIAFORM CELLS IN RAT AND HUMAN NEOCORTEX

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Neurogliaform cells have a unique position among cortical interneurons because they can elicit combined $GABA_A$ and $GABA_B$ receptor-mediated inhibition on pyramidal cells. Moreover, they establish electrical synapses with each other and with other interneuron types.
We measured the pre- and postsynaptic effects of neurogliaform cells applying simultaneous whole-cell recordings in layers I-IV of rat somatosensory cortex and in human association cortex *in vitro*.
Apart from the $GABA_A$ receptor mediated component in postsynaptic responses, single action potentials in neurogliaform cells elicited $GABA_B$ receptor mediated responses in neurogliaform, regular spiking and fast spiking interneurons in rat cerebral cortex.
Neurogliaform cells recorded in human cortical brain slices evoked $GABA_A$ and $GABA_B$ receptor mediated slow inhibition in various types of inteneurons and one of them established heterologous electrical coupling. These are the first multiple patch clamp recordings which analyse the functions of neuorgliaform cells in human cortex.
These cells can effectively recruit $GABA_B$ receptors not only on classical postsynaptic compartments like dendritic spines and shafts but on presynaptic axon terminals as well. This presynaptic inhibitory effect can reduce synaptic transmission and this is reflected in the altered paired pulse ratios and reduced amplitudes of the evoked postsynaptic potentials. In one case we show phamacological dissection of this presynaptic modulation by applying $GABA_B$ receptor antagonist.
Our results highlight the peculiar role of neurogliaform cells in cortical circuits and extend their contributions to slow inhibition in cortex

ELECTRONIC DEVICES MAKING MULTIPLE RECORDINGS EASYER

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Here we describe the implementation of some utilities for that laboratories which have a commercially available 8- or 16-channel data equisition system for electrophysiology recording.
Microdrive: Utilizing several years of experience with our original PCB microdrive we redesigned it to improve its stability and usability. The improved design led to a low cost and light-weight multi-channel microdrive with outstanding modularity for extracellular field, single unit or multiunit tetrode recording up to 64/128 channels.
Preamps: *Low noise, low power, low input bias opamps have been applied on the unity gain 32-channel headstage amplifiers.*
Tetrode selectors I-II: The tetrode selectors can be used to select 2 or 4 tetrodes out of 8 ones for recording multiple unit discharges. In these experiments it is advisable to implant 8 tetrodes, adjust their position by a PCB microdrive, use a 32-channel unity gain headstage preamp, 8 to 16-channel amplifier and 16-channel ADC cards and select the 2 or 4 tetrodes that seem to provide high probability of successful spike sorting.
ISO-PS: We have developed a galvanically isolated low-noise power supply board. Applying this board there is no need for using rechargeable batteries as power supplies of the preamps and Faraday shielding.

THE UNKNOWN THALAMUS

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According to the classical view the main role of the thalamus is the faithful transmission of peripheral sensory signals to the neocortex. This, however, applies only to the primary sensory relay nuclei which constitute only the smaller part of the thalamus. The role of the rest of the thalamus is hardly explored. Not only the function of these thalamic nuclei are unknown but the type of information relayed, the synaptic organization of their major afferent systems and the basic mechanisms of synaptic integration are all poorly understood. Since all cortical areas require intact thalamic connections to perform their task understanding the computation in the "rest of the thalamus" is indispensable.
By analyzing giant thalamic excitatory and inhibitory terminals and their impact on thalamic relay cells in rodents and primates here I present data indicating that far from being a simple relay station non-primary (or higher order) relay nuclei are characterized by complex interaction of three major afferent systems: excitatory ("driver") afferents from subcortical areas and from layer V pyramidal cells of the neocortex and inhibitory terminals arising outside the reticular thalamic nucleus (extrareticular GABAergic system).
Large thalamic territories are entirely devoid of subcortical inputs and are basically driven by neocortex, in other regions extrareticular inhibition dominates and exerts powerful control on relay cell activity. Convergence of cortical and subcortical drivers on the same relay cell allows performing "integration" rather than "relay" (sensu stricto) of the incoming signals.
The data suggest that non-primary relay nuclei transmit rich, contextual information to wide cortical territories. The transmitted message is probably contingent on the final cortical (motor) output and may participate in updating neocortex about its motor instructions and/or linking the activity of distant functionally related cortical areas.

EFFECT OF NEUROMEDIN U ON PASSIVE AVOIDANCE IN RATS. INVOLVEMENT OF NEUROTRANSMITTERS

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Neuromedin U (NMU) the member of 'neuromedins ' family of peptides was isolated from porcine spinal cord, two of which are known to posses similar biological activity (NmU-23 and NmU-8). Two NMU receptors, NMU-R1 and NMU-R2 were isolated. The NMU-R1 is expressed in the periphery, especially the gastrointestinal tract, whereas NMU-R2 is predominantly expressed in the CNS (hypothalamus, hippocampus and spinal cord) Current evidence suggests roles of NMU in pain sensation, in the immune system, in the regulation of smooth-muscle contraction of the gastrointestinal and genitourinary tracts, in the control of blood flow and blood pressure, and in the regulation of feeding and energy homeostasis and stress responses. NMU also increases gross locomotor activity. Furthermore, NMU, a potent endogenous anorectic peptide, serves as a catabolic signaling molecule in the brain.

In the present paper the action of neuromedin U was studied on learning and memory in a passive avoidance task. The neuromedin U was administered into the lateral brain ventricle in male rats. The possible involvement of neurotransmitters in the action of neuromedin U was followed by pretreating the animals with receptor blockers. The receptor blocker was injected ip. or icv.The neuromedin U administered into the lateral brain ventricle showed an amnesic action. The following receptor antagonists, haloperidol, phenoxybenzamine, and atropine prevented the amnesic action of neuromedin U.However propranolol, naloxone, biculline were ineffective. Pretreatment with nitricoxyde synthase inhibitor, nitro-L-arginine, was also ineffective.

The result suggest that the amnesic action of neuromedin U is mediated by dopamine 2, alpha adrenergic and muscarinic cholinergic receptors.

FUNCTIONAL RELEVANCE OF THE IMMUNOREACTIVITY OF BASAL LAMINA COMPONENTS AND LAMININ RECEPTORS- A STUDY IN RAT BRAIN. III. EPENDYMA

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Lamina basalis and its receptors play an important role maintaining the connection between extracellular matrix and cells. Components of basal lamina (e.g. laminin and agrin) as well, as its receptor-complex (such as dystroglycan, dystrophin, utrophin) can be found, however in the ependyma too. Present study concerns with the distribution of these molecules specifically along the rat ventricular system. Ependyma and astrocytes were visualized by the immunostaining of S100 and GFAP. Immunostaining of dystroglycan and utrophin revealed „dots" arranged at the basis of ependymal cells. These „dots" were superficial to the paraventricular GFAP-immunopositive layer throughout the ventricular system except the ventral part of the 3rd ventricle. In that line, where the general ependyma is replaced by tanycytes, the dots ceased, as did the underlying GFAP-immunopositive zone. This area seems to extend caudally from the arcuate nucleus along the hypothalamic part of the 3rd ventricle comprising the median eminence, the floor of the 3rd ventricle.

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ALPHA2-ADRENERGIC RECEPTORS MODULATE DENDRITIC ACTION POTENTIAL GENERATION IN LAYER 5 PYRAMIDAL NEURONS

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Recent research highlighted the integrative role of the backpropagating action potentials in neurons. These dendritic signals carry information to the active synapses about the recent activities of the cell in order to detect coincidence with current inputs. In our experiments the role of noradrenergic transmission through alpha2-adrenergic receptors was investigated on the backpropagating action potentials in layer 5 pyramidal neurons of the prefrontal cortex. Two-foton laser scanning microscopy was used to measure the calcium transients and dendritic spikes in the apical dendrite evoked by backpropagating action potential trains with different frequencies. At a critical frequency, the spike train could evoke an all-or-none type response identical to dendritic spikes. The alpha2-agonist clonidine, which was added to the bath, caused hyperpolarization. The effect of clonidine on the calcium transients showed frequency and distance dependence: (i) at distal apical dendrites trains were able to produce dendritic spikes (ii) at frequencies that normally fail to induce spikes. Our data revealed an unknown mechanism of noradrenaline acting on alpha2-adrenergic receptors in the prefrontal cortex that gives a weight to information arriving at the distal dendrite and increase the likelihood of generating dendritic spikes.

THE CENTRAL EFFECTS OF ALPHA-MELANOCYTE-STIMULATING HORMONE ON FOOD INTAKE IN RATS OF VARIOUS NUTRITIONAL STATES

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Alpha-melanocyte-stimulating hormone (alpha-MSH) is an endogenous agonist of the pro-opiomelanocortin/melanocortin system and provides a good opportunity to investigate the catabolic effects of this system. Earlier we found that acute central administration of alpha-MSH caused anorexia in rats fasted for 24-h. In the present study the effects of centrally injected alpha-MSH were analyzed in rats of various body weight and composition. The importance of food deprivation was also analyzed in the different groups. Chronic intracerebroventricular cannula was implanted into the right lateral cerebral ventricle of male Wistar rats through which alpha-MSH or saline was administered. According to the mean body weights three groups (359 + 8,81, 493 + 10,98 or 520 + 15,57 g) were separated. The effects of alpha-MSH on 1-h consumption of powdered rat chow (food intake-FI) were measured without fasting or after a 24-h food deprivation (Feed-Scale Monitoring System). After the experiments the perirenal fat tissue and the musculus tibialis anterior were isolated, removed and weighted from each animal. One-way ANOVA with Scheffe's *post hoc* test was used for statistical analysis. We have found that alpha-MSH injection does not influence the spontaneus FI, but it reduces the hyperphagia induced by previous fasting. Furthermore, the anorexigenic effect of central alpha-MSH appears earlier if the relative rate of the perirenal fat tissue is low, suggesting that a higher fat ratio decreases the efficacy of alpha-MSH. Accordingly, body composition seems to influence the effects of alpha-MSH. It is suggested that low amount of fat tissue enhances the FI-reducing effect of the peptide.

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EFFECTS OF CENTRAL ALPHA-MELANOCYTE-STIMULATING HORMONE ON THERMOREGULATION IN RATS

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In the regulation of energy balance neuropeptides of the hypothalamus play an important role. One of these is the pro-opimelanocortin/melanocortin system. Its endogenous agonist is the alpha-melanocyte-stimulating hormone (alpha-MSH), which is supposed to act as one of the main catabolic agents. According to our earlier studies alpha-MSH reduces food deprivation-induced hyperphagia and elevates metabolic rate (MR) and core temperature (Tc), others described antipyretic effects. In the recent study we investigated in details the acute thermoregulatory effects of centrally applied alpha-MSH at different initial core temperatures. Male Wistar rats had chronic cannula implanted into the right cerebral ventricle. Through this, alpha-MSH or saline was injected at different ambient and core temperatures. MR was measured by indirect calorimetry, together with thermocouple measurements Tc and skin temperature (Ts). At near thermoneutrality, and relatively low initial Tc alpha-MSH injection induced a rapid rise of MR and Tc and a delayed skin vasodilatation. At relatively higher initial Tc elevation of MR and Ts developed together, the hyperthermic effect was prevented. In warm environment at high initial Tc skin vasodilatation appeared without a change in MR, consequently Tc decreased. At low ambient and initial core temperature MR and Tc rose, Ts elevation was inhibited. It is suggested that the thermoregulatory effects of alpha-MSH correlate with the initial Tc. It tends to elevate MR, but it can be also antipyretic because of its heat loss inducing effect.

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EFFECT OF CHRONIC FLUOROKINOLONE TREATMENT ON THE STRUCTURES INNERVATING THE SALIVARY GLANDS OF THE RAT

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Fluorokinolones (i.e. Peflacine, PEF) are used in the dental and medical therapy. It was demonstrated that chronic treatment on the rats resulted in disturbance in the secretory function of the salivary glands accompanied by the morphological sign of atrophy in the secretory units. The mechanism of this impairment is unknown. Because the peripheral neuropathy was previously described as the toxic side effect of the chronic PEF treatment we proposed that the morphological and functional disorder of salivary glands developed on the basis of a neuronal disorder. Earlier studies described, that the mast cells could release nerve growth factor (NGF), which is important in the surviving of neurons. The lack of NGF results degenerative processes in the peripheral neurons which can change the expression of neuropeptides (i.e. serotonine, SER).
The aim of this study was to determine the number of mast cells and the qualitative and quantitative changes of SER immunoreactive (IR) nerve terminals in the salivary glands after PEF treatment. Adult rats were treated with PEF for 3 and 7 days. For the visualization of mast cell we have used Toluidine blue staining. Immunohistochemical methods were used to detect the SER containing fibers on the salivary glands. After the chronic treatment we could detect the increased number of mast cells which supports the protective role of the NGF. The number of SER IR fibers decreased compared to the control. The changes in the number of IR fibers support our previous results, that local denervation of salivary gland can cause the atrophy of the acini.

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VESTIBULORTIGEMINAL PATHWAYS IN THE FROG

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Previous physiological studies on mammalian species revealed that the activity of vestibular system is involved in the control of jaw muscle tone during head movements. Transneuronal tracing studies showed that the activity of vestibular receptors is mediated indirectly to the masticatory motoneurons by way of neurons of the vestibular nuclei. We have previously demonstrated that the boutons of primary afferent vestibular fibers are found within the dendritic arborization area of the trigeminal motor nucleus. In this study we have examined whether the primary vestibular afferent fibers establish direct connections with the trigeminal motoneurons. The experiments were carried out on common water frog. The trigeminal and vestibular nerves were cut, and their proximal stumps were labeled with retrograde fluorescent tracers (nV: Rhodamine binding dextran-amine; nVIII: Fluorescein binding dextran-amine) simultaneously. By using of confocal laser scanning microscope we could detect connections between the vestibular fibers and the branches of dorsolateral dendritic array of the trigeminal motor nucleus that belongs to the jaw closing motoneurons. Our results suggest that the vestibulo-trigeminal pathway of the frog has monosynaptic connection between the vestibular apparatus and trigeminal motoneurons giving the possibility of a very quick response.
In agreement with the results obtained on mammalian species our findings suggest that the vestibular-trigeminal relationship is quite complex and uses multiple systems to connect the vestibular apparatus with the trigeminal motor nuclei.

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THE EFFECTS OF CORTICOTROPIN-RELEASING FACTOR PEPTIDE FAMILY ON [3H]GABA RELEASE FROM ELECTRICALLY STIMULATED RAT AMYGDALA

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Corticotropin-releasing factor (CRF) is the major neuromodulator of hypothalamic-pituitary-adrenal axis. It also has neurotransmitter actions along with the lately discovered members of CRF peptide family, urocortin I (UCN I), urocortin II (UCN II) and urocortin III (UCN III). It has been demonstrated that stress induces CRF release in the paraventricular nucleus of hypothalamus and both CRF and GABA release in the amygdala.
We used an *in vitro* superfusion system to study the effect of CRF on [3H]GABA release from the rat amygdala stimulated by electric impulse. As the role of urocortins in the stress response has not been elucidated yet, they have been also tested. CRF and UCN I increased significantly the release of [3H]GABA from the rat amygdala slices following electric stimulation. UCN II and UCN III were ineffective. The effects of CRF and UCN I were blocked by selective CRF_1 receptor antagonist antalarmin, but were not inhibited by selective CRF_2 receptor antagonist astressin 2B, administered in equimolar doses.
Our results demonstrate that the release of GABA from amygdala during stress is mediated by CRF through the activation of CRF_1 receptors. The action of UCN I in acute stress response seem to wear the same patterns as that of CRF, while UCN II and UCN III, acting preferentially on CRF_2 receptors, could have role in coping with chronic stress.

FUNCTIONAL RELEVANCE OF THE IMMUNOREACTIVITY OF BASAL LAMINA COMPONENTS AND LAMININ RECEPTORS – A STUDY IN RAT BRAIN. I. VESSELS AND MENINGES OF INTACT ADULT BRAIN.

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Former studies suggested that immunoreactivities of laminin and its receptor dystroglycan-β, or their absence may characterise developmental or functional stages of brain vessels, actually, those of the basal lamina. In the mature, aldehyde-perfused brain tissue the vascular laminin immunreactivity is confined to the entering segments, i.e. just below the brain surface, and to the circumventricular ogans. In the present study In the present study utrophin (another component of the dystrophin-dystroglycan complex), fibronectin (another adhesive proteoglycan), and agrin (also basal lamina component) were investigated in the intact, mature rat brain, following paraformaldehyde perfusion. None of them was detectable by immunohistochemical reaction throughout in the brain vessels. The meningeal surface, however, was immunoreactive in every case, as well as the entering segments of the brain vessels. The vessels surrounded with brain tissues by developmental fusions of meningeal surfaces (e.g. in the hippocampal fissure, in the midline of the septum) proved to be immunoreactive, as well as the choroid plexus, and the vessels of some circumventricular organs (subfornical organ, organon vasculosum laminae terminalis, and area postrema), but not in the vessels of the subcommissural organ, where the blood-brain barrrier of perfect. Considering these phenomena together with those found formerly in the case of laminin and dystroglycan, as well as those presented in our other poster (Pócsai et al.), the immunoreactivity of fibronectin, agrin, and utrophin may also depend on the functional stage of brain vessels, especially on that of their basal lamina.

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EFFERENT CONNECTIONS OF THE VENTROBASAL TELENCEPHALON IN THE DOMESTIC CHICK

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The connectivity of the core (AcC) and shell (AcS) areas of the nucleus accumbens (Ac), a part of the avian basal ganglia, was studied using the anterograde tracer biotinylated dextrane amine (BDA, 10 kDa). Pressure injections were placed into the AcC, partially covering the mediodorsal region of the medial striatum, while further injections have targeted AcS. The projection patterns of the two subregions were largely similar with some minor alterations. Most of the efferents remained ipsilateral, however, few contralateral fibres were apparent primarily in the brain stem.

Within the telencephalon, terminating axons were found in the dorsomedial cortex, hyperpallial areas, mesopallium, entopallium, medial and caudolateral nidopallial regions, arcopallium, nucleus taeniae, lateral septal nucleus, ventral and dorsal striatal and pallidal areas.

Further fibres were seen in the limbic dorsal thalamus, as well as in the nucleus paramedianus internus, the habenular complex, the mammillary nuclei and in the lateral and paraventricular hypothalamic areas.

A massive projection from Ac was detected in the midbrain central gray and the cathecolaminergic areas including the substantia nigra, ventral tegmental area, the locus coeruleus, the subcoeruleus nuclei. Less numerous fibres were seen in the deep tectal nuclei and the brain stem reticular formation, including the raphe nuclei.

In the lower brain stem, AcC, but not AcS, efferents were apparent in the hypoglossal nucleus, the nucleus intercalatus and the nucl. tractus solitarii reflecting a minor difference between the projection patterns.

Retrogradely labelled somata were seen in the bilateral nucleus tegmentalis ventralis and ipsilaterally in the substantia nigra and the habenular complex.

ALTERED GENE EXPRESSION AND FUNCTIONAL ACTIVITY OF μ OPIOID RECEPTORS AFTER ACUTE NOLADIN ETHER TREATMENT IN THE CEREBELLUM OF CB_1 CANNABIS RECEPTOR KNOCKOUT MICE

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Growing body of evidence suggest the existence of a functional interaction between opioid and cannabinoid systems. The purpose of this study was to evaluate whether acute treatment by endogenous cannabinoid agonist noladin ether (2-arachidonyl glyceryl ether) modulate the expression of μ-(MOR) opioid receptor mRNA level and functional activity in the cerebellum of transgenic mice deficient in the CB_1 type of cannabis receptors. We examined the effect of noladin ether pretreatment on MOR mRNA expression by using reverse transcription and real-time polymerase chain reaction (PCR) and on DAMGO (MOR agonist) stimulated G-protein activation by using [^{35}S]GTPγS binding assay in CB_1 wild-type ($CB_1^{+/+}$) and CB_1 cannabinoid receptor deficient ('knockout', $CB_1^{-/-}$) mice. The acute administration of noladin ether markedly reduced MOR-mediated G-protein activation and caused a significant increase in the level of MOR mRNAs in the cerebella of $CB_1^{+/+}$ mice. No significant differences were observed in MOR mRNA expression and functional activity in $CB_1^{-/-}$ animals as compared to control. Taken together, these results indicate that acute treatment with noladin ether causes alterations in MOR mRNA expression and functional activity in the cerebella of $CB_1^{+/+}$ mice but not in $CB_1^{-/-}$ indicating that CB_1 agonist noladin ether exert modulatory effect on μ-opioid receptors.

DISCRIMINATION OF SINGLE NEURONS - A NEW SPIKE SEPARATOR SYSTEM

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Visual information processing seems to be a critical role of the SN. The SN neurons may respond with excitation or inhibition to visual stimulation. The visual receptive field organization of the SN is, however, have not yet been clarified.
In our presentation, we describe our developed system with automatized spike separation that allows the simultaneous recording of excitatory and inhibitory neuronal responses.
Extracellular single-cell recordings were performed with tungsten microelectrodes in the SN pars reticulata of anaesthetized, immobilized cats to moving visual stimulation. Depending on the physical properties of the electrode, the activity of 2-3 neurons could be separated, recorded and evaluated simultaneously. Our system provides a spike shape discriminating method to separate neuronal activity. Activity of a single-cell was highly dependent on the size and the velocity as well as the direction of the visual stimulus. Each neuron was able to exhibit both excitatory and inhibitory responses. The neuronal activation or inhibition strongly depended on the visual stimulus parameters.
Our results did not meet the previous notions on separate excitatory and inhibitory neurons in the SN. We suggest the existence of broad and narrow direction and velocity tuned units in the SN.

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TASTE REACTIVITY AFTER ACQUISITION OF CONDITIONED TASTE AVERSION IN MEDIAL PREFRONTAL CORTEX LESIONED RATS

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Our previous results showed that the medial prefrontal cortex (mPFC) is involved in taste aversion learning and mPFC neurons respond to taste stimuli. The aim of this study was to examine the role of mPFC in taste related learning mechanisms, taste perception and hedonic evaluation of tastants.
Kainic acid (KA) was applied microiontophoretically into the mPFC of rats to damage intrinsic neurons, whereas 6-hydroxidopamine (6-OHDA) was injected into the same brain region to destroy catecholaminergic projection fiber terminals. Intraoral tubes were chronically implanted for application of 0.18 % saccharine solutions to detect taste reactivity responses.
Conditioned taste aversion (CTA) was developed by i.p. injection of 0.15 M LiCl 5 min after infusion of saccharine in lesioned animals and vehicle treated controls (paired groups: p-KA, p-6-OHDA, p-CO). In other lesioned animals saline was applied i.p., instead of LiCl (unpaired groups: un-KA, un-6-OHDA). Ingestive and rejective responses were tested 4 days after LiCl or saline injections. Neither the ingestive nor rejective reactions of the lesioned groups differed from the controls before CTA. Due to the development of CTA in the p-CO group the number of ingestive responses decreased and the rejective responses increased on the test day, while in the unpaired lesioned animals no change were observed. The number of rejective responses did not increase in the paired lesioned groups and during the first minute of the test less rejective responses were recorded similarly to those observed in the unpaired lesioned groups. Our results show that lesions of mPFC modulate the hedonic evaluation of tastants.

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A RECENTLY REVEALED INTRANEURONAL STRUCTURE CAN COUNTERACT THE OSMOTIC PRESSURE THROUGH A NON-ENZIMATIC PROCESS

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According to the present state of knowledge, under conditions unfavorable for enzyme-mediated processes, the volume of a cell is determined solely by the osmotic gradient between the intra- and extracellular spaces, since the plasma membrane is thought to be semi-permeable. Surprisingly, the results presented here cannot be explained in this way: Physiological saline containing SDS in a sub-critical micelle concentration was perfused transcardially in rats for 5 min. prior to perfusion fixation. As a result, a low proportion of neurons in the central nervous system, together with their dendrites but never with their axons, have lost more then half of the original volume, while the neighboring neurons remained either unchanged or became swollen. The ultrastructural elements in the swollen neurons displayed partial or complete disintegration, which corresponds to the known effects of SDS. In contrast, the ultrastructural elements in the shrunken neurons appeared to be intact, but the distance between them were dramatically decreased (compaction). Furthermore, the extracellular spaces around the compacted neurons remained unchanged, while adjacent astrocytic processes became extremely swollen. All these allow the conclusions that (i) enzyme-independent direct channels exist between neurons and astrocytes, and (ii) some non-enzymatic and non-osmotic process is capable of pressing fluid out of neurons through these channels. The present one of us (Gallyas et al., Biol. Cell. 96:313-324, 2004) is able to execute this „work", while the ultrastructural elements visible in the conventional transmission electron microscope are not.

NON-SPECIFIC EFFECTS OF ELECTRO-MAGNETIC FIELDS: ANIMAL MODELS?

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Although electromagnetic fields (EMF) represent a potential threat to health, very few consequent studies have been published about their general effect. However, public complaints suggest that some of the – frequently unnoticed – effects have a non-specific character. Since human accounts are polluted by expectations, and media influence, only animal models can offer a firm ground to accept or refuse these claims. The aim of our work has been to work out such models.Non-specific health problems are difficult to examine since a multitude of diffuse and overlapping symptoms exist. Animal models have an advantage to be free of cognitive distortions and verbal pollutions, but, on the other hand, animals cannot be interviewed thus the advantage turns into a disadvantage at this point. There seem to be one way to get around this problem: to use a multiple-method approach in which different behavioural tests are run and evaluated in a complex, multivariate way.
In a long and still ongoing series of experiments, rats had been exposed to a low-frequency electromagnetic field (500 μT, 50 Hz) for short and long intervals as embryos, pups or adults, and were tested during, immediately, or long after the exposure, respectively. Our experiences accumulated so far have shown that tests for general activity and preference changes as well as situation-anxiety tests (e.g. plus-maze, or the novel-object test) both have a relatively good predictive power; on the other hand , traditional social-anxiety tests (e.g. repeated social-interaction, territorial behaviour) seem to be non-sensitive to EMF exposition. The newly developed 'resource-competition' test may prove better for this purpose.

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TYR-TIC-(2S,3R)-b-MEPHE-PHE-OH IS A NEW d1-OPIOID RECEPTOR ANTAGONIST

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Our goal was to investigate the ligand binding and signaling of a new, proteolytically resistant analog of TIPP, Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH in mouse brain membranes. Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH *per se* had no effect on the basal activity of [^{35}S]GTPγS binding at any GDP concentration tested showing the antagonistic character of the ligand. DPDPE, the putative δ_1 agonist caused a 137 % stimulation of the basal [^{35}S]GTPγS activity with an EC_{50} of 834 nM. Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH was very potent in inhibiting the DPDPE-induced stimulation of [^{35}S]GTPγS binding, 100 nM of the antagonist completely wiped out the effect of DPDPE. Conversely, it had no effect on DAGO-stimulated [^{35}S]GTPγS binding even at 1 μM. While DPDPE stimulation was greatly attenuated, DAGO stimulation of [^{35}S]GTPγS binding in δ-opioid receptor knock-out mouse brain membranes was similar to that in wild type. *Intrathecal* analgesia induced by DPDPE (8 μg) was greatly reduced by a similar dose of Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH. However, the antinociceptive effect of the hypothetical δ_2 agonist Ile^{5,6}-deltorphin was not antagonized by Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH. Specific δ ligands competed with [^{3}H]Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH (specific activity 53.7 Ci/mmol) with a rank order of potency: naltrindole>Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH>Ile^{5,6}-deltorphin. Specific μ and κ ligands showed poor affinity in competition binding assays. These results show that Tyr-Tic-(2S,3R)-b-MePhe-Phe-OH is a highly potent antagonist with preference for a distinct population that may represent the proposed δ_1-subtype of the δ-opioid receptor.

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MORPHOFUNCTIONAL ANALYSIS OF GABAERGIC TERMINALS WITH SINGLE AND MULTIPLE RELEASE SITES IN THE THALAMUS

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In this study, we compared the 3D ultrastructure and the electrophysiological characteristics of two major GABAergic inputs in posterior thalamic nucleus (Po).
GABAergic terminals in the Po originating in the anterior pretectal nucleus (APT) were large, innervated the proximal dendrites of relay cells and always contacted a single postsynaptic target via multiple release sites (up to 16). In contrast, boutons of the reticular thalamic nucleus (nRT) were small, randomly selected postsynaptic dendrites, frequently had multiple targets and the vast majority of them contacted their targets with single release sites.
In brain slices with preserved connectivity from both nRt and APT whole-cell patch-clamp recordings were performed in Po neurons. GABAergic spontaneous, miniature and evoked (e) inhibitory postsynaptic currents (IPSCs) were characterized, and the quantal parameters of the synaptic transmission were determined by variance-mean analysis. Surprisingly, the amplitude of eIPSCs, the quantal amplitude, number of release sites, and the probability of release did not show major difference between the nRT-Po and APT-Po connections. These data are compatible with the anatomy only if we assume multiple nRT terminals converging on the same Po neuron. APT-mediated eIPSCs, however, showed much smaller paired-pulse depression rate (PPR = 0.9 vs. 0.6 for APT and nRt, respectively) and smaller steady state depression rate compared to nRt-mediated events.
Our conclusion is that high frequency GABAergic events are transmitted more faithfully if similar number of release site is concentrated on a single terminal rather than distributed on several terminals.

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CONVERGENCE OF CORTICAL AND PERIPHERAL DRIVERS IN HIGHER ORDER THALAMIC RELAYS.

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Higher order thalamic nuclei receive large excitatory afferents from cortical layer V pyramidal cells and are innervated by subcortical drivers as well. This raises the question whether or not the two systems interact at the level of individual neurons. We addressed this question using *in vivo* intracellular recordings and anatomical methods.
Intracellular recording of relay cells was performed in the higher order somatosensory posterior (Po) and in the first order ventral posteromedial (VPM) nuclei in anaesthetized rats with S1 cortex EEG recording. *In vivo* intracellular recordings in Po demonstrated fast rising, large amplitude EPSPs coherent with slow cortical oscillation. These EPSPs disappeared following cortical inactivation, demonstrating their cortical origin. Cortical EPSPs showed similar characteristics to peripheral, so called driver type EPSP, evoked by whisker stimulation. In VMP cells, which lack cortical driver input, cortical upstates were associated with inhibitory potentials, presumably reflecting activity of GABAergic reticular thalamic neurons.
The anterograde labeling of large cortical terminals originating in layer V and immunostaining of peripheral terminals with vGLUT2 antibodies revealed that both populations of terminals overlap in Po. Peripheral and cortical drivers were found in close proximity (less than 10 microns). Electron microscopic analysis of vGLUT2 immunostaining in Po showed that vGLUT2-positive and negative large excitatory terminals innervate the same neurons, demonstrating the convergence of excitatory driver afferents with different origins on the same relay cell.
The convergence of peripheral and cortical drivers on the same cell suggests that higher order relays may serve as AND gates with output determined by the temporal coincidence of cortical and peripheral inputs.

UNREVEALED MITOCHONDRIAL EFFECT OF VINPOCETINE IN PRIMARY CORTICAL CULTURES

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Vinpocetine (Cavinton®) has been introduced to the market for 28 years in the treatment of cerebrovascular syndromes. The experimentally proved mechanism of neuroprotective effect of vinpocetine includes the inhibition of voltage-dependent sodium channels, stabilization of intracellular calcium homeostasis, and the inhibition of the adenosine uptake. The observed respiratory-enhancing effect on isolated mitochondria as well as the relatively high binding affinity for t Molecular Probes peripheral benzodiazepine receptor (PBR) drew our attention to the possible subcellular effect of vinpoetine. It was reinforced also by the utilization of labeled vinpocetine as an effective PET tracer for the distribution of PBR in the brain.

The uncoupling-like effect and its binding to the PBR at the outer membrane of the mitochondria raise the possibility of the mitochondrial membrane potential ($\Delta\Psi$m)-modifying effect. The experiments have been started on rat primary cortical cultures with JC-1 (Molecular Probes) staining followed by flow cytometry measurements (FACScan, Becton Dickinson). The known uncouplers and the classic PBR ligand FGIN-1-27 strongly reduced the $\Delta\Psi$m. The effect appeared in the micromolar range and was time-dependent with the maximal effect at 24 h. Vinpocetine proved to be more potent in the reduction of $\Delta\Psi$m and its effect was concentration-dependent and appeared from 20 min to 24 h. The mitochondrial volume change (proportional with the monomeric dye fluorescence intensity) was concomitant with the disruption of $\Delta\Psi$m. The remarkable swelling of the vinpocetine-treated mitochondria was appeared for 20 min, but normalized for 12 h. It points to the partially different effect of vinpocetine form the classical PBR ligands, an the reversibility of the effect of vinpocetine on the mitochondria. The primary cortical cultures are heterogeneous containing neural and astrocyte cells. According to our results, the uncoupling effect appeared on both populations.

As PBR plays role in the assembly and function of the mitochondrial permeability transition pore (mPTP) which functions as a fast and high capacity Ca^{2+} channel. We measured $[Ca^{2+}]c$ with plate reader-based fluorimeter (Fluoroskan Ascent). Vinpocetine evoked a significant, time– and concentration-dependent rise in $[Ca^{2+}]c$. 15 % of this response was extra mitochondrial and in higher concentration 45% extracellular Ca^{2+} component could be measured. These results also reinforcing the vinpocetine's effect on the PBR and the mPTP, respectively. There was obvious discrepancy between the binding affinity and the in vitro effect on the mitochondrial function; however the same is true for the classical PBR ligands. The described uncoupling and mPTP activating effect of vinpocetine appeared only in micromolar concentrations in vitro. While full and extended uncoupling would deteriorate cells, transient and/or moderated uncoupling might be neuroprotective in vitro and in vivo. The mechanism based on the dissipated $\Delta\Psi$m-related reduction of free radical production, the inhibition of the mitochondrial membrane hiperpolarization and the *de novo* mitochondrial production which in turn leads to improved energetization of the neurons.

CHANGES OF CELL ADHESIVE PROPERTIES IN THE COURSE OF IN VITRO NEURON FORMATION: STUDIES ON CLONED NEUROECTODERMAL (NE-4C) STEM CELLS

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Selective cell adhesivity is a basic biological principle, which provides mechanisms for a number of inevitable processes from phagocytosis to building multicellular organisms. Differential adhesion plays fundamental roles in the genesis and regeneration of the neural tissue as well, and seems to be responsible for the restriction of migration of neural stem cells in the adult brain parenchyma. Despite of its fundamental importance, the molecular interactions and cell-biological mechanisms behind the adhesive behavior of neural stem cells are not understood. For understanding some characteristics of neural stem cell attachment, the adhesive behavior of primary neural cells and NE-4C neuroectodermal stem cells was investigated by novel approaches combining video-microscopic[1] recordings and biological adhesion-assays with optical waveguide spectroscopic(OWLS)[2] and molecular biological methods. The interaction of stem cells with different types of cells was investigated by chimera-aggregation techniques, and the kinetics of cell attachment on different matrix components was studied by OWLS. The composition of the adhesion-receptor set on the surface of stem cells was analyzed by using synthetic peptide ligands3, and by investigating the pattern of adhesion molecule expression in the course of *in vitro* induced neurogenesis.

The studies had been carried out in collaboration with researchers of [1]Dept. of Physical Biology, ELTE, [2]MicroVacuum Ltd. and [3]Peptide Chemistry Research Laboratory, ELTE-HAS.

INTERACTION BETWEEN CANNABINOID AND GABAB RECEPTORS IN RAT BRAIN HIPPOCAMPUS

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The $GABA_B$ receptors are the only known G-protein coupled receptors that require hetero-dimerization between two subunits, R1 and R2 for functional expression. It has been discovered that hetero-oligomerization between distinct receptors may also take a place and this profoundly alters the binding and signaling properties of the receptors. $GABA_B$ and CB_1 receptors share many physiological functions and have overlapping anatomical localization in the brain. We have performed a detailed characterization of the $GABA_B$ and CB_1 cannabinoid receptors by using ligand-stimulated [^{35}S]GTPγS binding assays in rat hippocampal membranes. The CB_1 agonist R-Win55,212-2 displayed high potency (ED_{50}=33 ± 6 nM) and efficacy (48 ± 2.5 %). This effect was completely blocked by the CB_1 antagonist, AM251. The $GABA_B$ agonist baclofen and SKF 97541 also elevated [^{35}S]GTPγS binding by 71 and 66 %, respectively. The $GABA_B$ antagonist phaclofen was a weak inhibitor against baclofen. However, nanomolar concentrations of phaclofen slightly but significantly lowered the maximal stimulation of [^{35}S]GTPγS binding of R-Win55,212-2. The pharmacologically inactive stereoisomer S-Win55,212-3 had no effect either alone or in combination with phaclofen proving that the interaction is stereospecific. Combination of the CB_1 antagonist AM251 with the $GABA_B$ receptor agonist SKF 97541 also displayed a ligand binding profile distinct from that of the individual receptors. These results suggest that the CB_1 and $GABA_B$ receptors may interact with each other in rat hippocampus.

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NEURONAL AND NON-NEURONAL EXPRESSION OF THE TRPV1 RECEPTOR IN RODENTS

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Vanilloid receptor type 1 (TRPV1) is a molecular integrator of various painful stimuli, such as capsaicin, endogenous lipids, noxious heat, and low pH. TRPV1 receptor expression is well characterized in a capsaicin-sensitive and peptide-containing sub-population of primary sensory nerves. Recent reports document that TRPV1 is also expressed in the CNS and in non-neuronal cells at the periphery, but the functional significance of the presence of TRPV1 receptor at these sites is unknown. In this preliminary study, we used immunohistochemistry to investigate the distribution of TRPV1 in neuronal and non-neuronal structures. TRPV1 immunoreactive axons were found in the subepithelial regions of the trachea and in nerve terminals innervating hair follicles of the ear skin. In the CNS, we observed TRPV1-positive neurons in the hippocampus and neocortex and fibers in the cerebellum and spinal cord. Strong TRPV1 expression was visible in the eminentia mediana of the hypothalamus. TRPV1-positive glial cells, with astroglial appearance, were seen in the corpus callosum. Pronounced TRPV1 expression was detected around numerous small vessels in a variety of brain structures. The non-neuronal expression of TRPV1 was confirmed by positive labeling in the parietal cells of the gastric mucosa. As an attempt to examine the functional relevance of this non-neuronal expression, we examined whether resiniferatoxin (RTX) pre-treatment could modulate the expression of TRPV1. RTX pre-treatment suppressed TRPV1 expression in the gastric mucosa providing a model for analyzing the function of the non-neuronal TRPV1 receptor.

CHRONIC SOCIAL STRESS INHIBITS CELL PROLIFERATION IN THE ADULT MEDIAL PREFRONTAL CORTEX: HEMISPHERIC ASYMMETRY AND REVERSAL BY FLUOXETINE TREATMENT

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Profound neuroplastic changes have been demonstrated in various limbic structures after chronic stress exposure and antidepressant treatment in animal models of mood disorders. Here we examined in rats the effect of chronic social stress and concomitant antidepressant treatment on cell proliferation in the medial prefrontal cortex (mPFC). Animals were subjected to 5 weeks of daily social defeat by an aggressive conspecific and received concomitant, daily, oral fluoxetine (10 mg/kg) during the last four weeks. Bromodeoxyuridine (BrdU) labeling and quantitative stereological techniques were used to evaluate the treatment effects on proliferation and survival of newborn cells in limbic structures such as the mPFC and the hippocampal dentate gyrus, in comparison with nonlimbic structures such as the primary motor cortex and the subventricular zone. Phenotypic analysis showed that neurogenesis dominated the dentate gyrus, whereas in the mPFC most newborn cells were glia, with smaller numbers of endothelial cells. Chronic stress significantly suppressed cytogenesis in the mPFC and neurogenesis in the dentate gyrus, but had minor effect in nonlimbic structures. Fluoxetine treatment counteracted the inhibitory effect of stress. Hemispheric comparison revealed that the rate of cytogenesis was significantly higher in the left mPFC of control animals, whereas stress inverted this asymmetry, yielding a significantly higher incidence of newborn cells in the right mPFC. These pronounced changes in gliogenesis after chronic stress exposure may relate to the abnormalities of glial cell numbers reported in the frontolimbic areas of depressed patients.

THE ROLE OF PKD IN NEURONAL TRANSPORT PROCESSES: VESICULAR TRANSPORT AND ALTERED MORPHOLOGY OF TRANSFECTED HIPPOCAMPAL NEURONS

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Protein kinase D (PKD) participates in diverse cellular processes, including membrane remodelling, Golgi organization and basolateral membrane protein targeting. Neurons are extremely polarized cells assuming a complex and strictly regulated protein transport machinery. Some data have already indicated the involvement of PKD in neuronal protein transport, however, several key questions have so far been unanswered.
Mouse primary hippocampal neuronal cultures were transfected with GFP labeled constructs including wild type (PKDwt) and kinase dead (dominant negative) forms of PKD (PKDkd) and with empty EGFP vector as control. The effects of the overexpression or the deactivation of PKD was examined 24 hours after transfection in fixed and living cultures.
PKDwt-GFP was distributed evenly in the cytoplasm, while PKDkd-GFP formed larger patches and was located almost exclusively in the dendrites and the soma. PKDkd expression led to distinct morphological changes in the neurons: primary dendrites seemed to be thickened upon transfection and Golgi structure became fragmented. Our initial experiments on vesicular transport in hippocampal neurons investigated by fast live cell imaging revealed differences in the direction and motility of the GFP labeled vesicle movements between PKDwt and PKDkd transfected neurons.
These initial findings already emphasize the role of PKD in neuronal transport processes.

P2-RECEPTOR MEDIATED MODULATION OF NORADRENALINE RELEASE IN RESPONSE TO ELECTRICAL FIELD STIMULATION AND ISCHEMIC CONDITIONS IN SUPERFUSED RAT HIPPOCAMPUS SLICES

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1 In this study, the regulation of [^{3}H]noradrenaline release was investigated in rat hippocampus slices subjected to electrical field stimulation (EFS) and *in vitro* ischemic-like conditions (combined oxygen and glucose deprivation).
2 EFS enhanced the [^{3}H]noradrenaline release in a $[Ca]_o$ –dependent manner, in contrast, the excess release in response to ischemic-like conditions was $[Ca]_o$-independent. The Na+ channel blocker, tetrodotoxin (1-3 µM) abolished both EFS-and ischemia-evoked release of tritium.
3 The P2 receptor agonist ATP, ADP and 2-methylthioadenosine 5'-diphosphate (2-MeSADP) decreased concentration dependently the tritium overflow with the potency order of ADP>2-MeSADP > ATP.
4 The inhibition by ATP (300 µM) was prevented by the P2 receptor antagonist PPADS (30 µM), by the $P2Y_1$ receptor antagonist MRS2179 (10 µM) and by the $P2Y_{12/13}$ receptor antagonist 2-MeSAMP (10 µM). Under ischemic-like conditions the $P2X_1$ receptor antagonist PPNDS (1 µM) inhibited the outflow of [^{3}H]NA, whereas MRS2179 significantly increased the tritium outflow (10 µM). PPADS and 2-MeSAMP did not affect ischemia-evoked [^{3}H]NA efflux.
5 RT-PCR analysis revealed that mRNA encoding $P2Y_{12}$ and $P2Y_{13}$ receptor subunits were expressed in the brainstem containing catecholaminergic nuclei projecting to the hippocampus.
6 These data indicate that the pharmacological profile of the receptor mediating the inhibition of EFS-evoked [^{3}H]noradrenaline outflow resembles the $P2Y_1$ and $P2Y_{13}$ receptor phenotype, and the endogenous activation of $P2X_1$ and $P2Y_1$ receptors contribute to the modulation of noradrenaline efflux upon combined oxygen and glucose-deprivation.

VESTIBULAR LESION-INDUCED CHANGES IN THE EXPRESSION OF HYALURONAN IN THE RAT BRAINSTEM

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Unilateral labyrinthectomy results in postural deficits and disturbances in the eye movements in mammalian species. The disturbances are spontaneously restored after a shorter or longer period of time during the vestibular compensation, which is a favorite model of neuronal plasticity in the adult CNS. Recent studies have shown that the extracellular matrix (ECM) molecules have an important role in the neural plasticity however, their permissive or non-permissive roles have not yet completely evaluated. In the present study the expression of hyaluronan (HA) was examined in the vestibular nuclei of the rat brainstem.
After unilateral labyrinthectomy the animals were sacrificed on different survival days and the brainstem was immersed into Sainte-Marie's fixative. Sections of brainstem were cut in transverse plane and a specific biotinylated hyaluronan binding probe was applied to detect the HA.
During the 1st, 3rd postoperative days the intensity of HA reaction decreased in the vestibular nuclei on the operated side. The perineuronal net (PN), the ECM containing cell coat of neurons, could not be distinguished from the surrounding neuropil that may explain the drop in HA reaction intensity. By the time of 7th day the staining pattern of PN began to restore indicating the stabilization of newly established synaptic contacts.
Our results may provide new insights into fundamental mechanisms of vestibular plasticity, and into the role of ECM in the CNS. The results may also assist in developing new therapeutic strategies for the treatment of symptoms of vestibular lesion.

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MOLECULAR BASIS OF RETROGRADE ENDOCANNABINOID SIGNALING AT NEOCORTICAL GLUTAMATERGIC SYNAPSES

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Endocannabinoids are important retrograde signaling molecules in the neocortex, where they contribute to homosynaptic and heterosynaptic depression and involved in synaptic remodelling during activity-dependent plasticity. The endocannabinoid system comprises of several lipid molecules as well as their synthetic and degrading enzymes. To unravel the molecular basis of retrograde endocannabinoid signaling at neocortical glutamatergic synapses, we investigated the precise regional, cellular and subcellular localization of diacylglycerol lipase alpha (DGL-α), the synthesizing enzyme of 2-arachidonoyl-glycerol (2-AG), the most numerous endocannabinoid in the brain. In situ hybridization revealed that DGL-α mRNA is abundantly expressed by principal cells throughout the neocortex of the mouse. DGL-α immunostaining resulted in a punctate pattern in the neuropil across all layers. At the electron microscopic level, these puncta corresponded to selective labeling of dendritic spines receiving asymmetric synapses from terminals bearing CB_1 receptors. The density of staining in the barrel field was higher in the termination zone of thalamocortical afferents, which are thought to be devoid of CB_1 receptors. Double immunostaining for DGL-α and either VGluT1, a marker of intracortical glutamatergic axon terminals, or VGluT2, a marker of thalamocortical terminals, revealed that DGL-α is present in spines targeted by both types of glutamatergic fibers. Since spines postsynaptic to thalamocortical afferents are also innervated by CB_1-positive GABAergic terminals, we hypothesize that 2-AG produced at this site by DGL-α may be involved in heterosynaptic depression between thalamocortical glutamatergic and local GABAergic synapses, whereas 2-AG may mediate homosynaptic depression at intracortical excitatory synapses.

EFFECTS OF STEROID TREATMENT ON THE RETURN OF CIRCADIAN TEMPERATURE AND ACTIVITY RHYTHMS AFTER SURGICAL STRESS IN MICE.

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Circadian rhythm of body temperature is disturbed by stress situations. Both amplitude and duration of the rhythm take 6-8 days to return to normal after transmitter implantation. After repeated stimuli the time required for the development of normal pattern becomes increasingly shorter. Our aim was to determine whether the steroid methylprednisolone affected the onset of normal rhythmicity in a series of experiments inducing stress by surgical incision. A radiotransmitter was implanted in the peritoneal cavity of 16 C57BL/6 mice under general anaesthesia. Post implantation 10 mice received 50 mg/kg methylprednisolone IP at 08:30 daily (MP group), while 6 mice were injected with 0,06 ml 0.9 % NaCl solution (control group [CT]) throughout the experiment.Following the return of normal pattern three consecutive laparotomies were carried out in each group 6 days apart. Core temperatures of the mice were detected by a radiotelemetry method and data were analyzed.After the first laparotomy post transmitter implantation, 4-5 days were required to develop a normal pattern of oscillation , while after the second and third laparotomy this period decreased to 3-4 and 2-3 days, respectively, both in the MP and the CT group. There was no significant difference in the length of the time required to reach the normal oscillation in the MP and CT groups. These data suggest that a factor other than the disturbance of the HPA axis is responsible for the shortening of the time needed to recover normal patterns of daily temperature rhythmicity. Our aim is to further evaluate this problem by using endogenous neurotransmitters, such as melatonine to find the explanation for this phenomenon.

STRUCTURAL AND FUNCTIONAL DIVERSITY OF DEEP SHORT AXON CELLS OF THE RAT OLFACTORY BULB

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In the main olfactory bulb (MOB), the majority of interneurons (INs) exert their inhibitory effects on principal cells by releasing GABA at dendro-dendritic synapses. Although primarily composed of small, axon-less granule cells, the deep inframitral layers of the MOB also host a variety of larger INs, termed deep short axon cells (dSACs). We investigated the physiological properties of dSACs using *in vitro* patch-clamp recordings in acute slices, followed by subsequent immunofluorescent, light- and electron-microscopic analysis.
In sharp contrast to their name, dSACs were found to have extensive and characteristic axonal ramifications in various layers of the MOB, based on which they were divided into three subgroups. Approximately 40% of the dSACs had extensive axonal arbors in the glomerular layer, 46% in the external plexiform layer and the remaining of the cells mainly innervated the granule cell layer. The input resistance, membrane time constant, spontaneous firing activity, action potential threshold of the dSACs significantly differed among these subgroups. Among the subgroups, some morphological (e.g. number of axonal varicosities, mean axonal segment length, axonal Sholl maximum) and molecular (expression of the $GABA_A$ receptor $\alpha 1$ subunit) properties of the cells were also significantly different. The axon terminals of all dSACs subtypes were immunopositive for GABA and formed symmetrical synapses on other interneuron somata or dendrites.
Our results reveal the presence of distinct dSAC subtypes, which are specialized to influence the activity of the MOB by selectively innervating distinct INs.

SERTRALINE POTENTIATES THE SPINAL REFLEX INHIBITORY ACTION OF MEMANTINE IN RATS, IN VITRO

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Although NMDA receptor antagonism is regarded as the main mechanism of action of the antispastic agent, memantine, the drug has significant affinity also for many other receptors and ion channels, including voltage gated sodium channels (VGSCs). Co-administration of selective serotonin reuptake inhibitors (SSRIs), such as sertraline, increases the efficacy of VGSC blockers via a PKC mediated phosphorylation of the channel. On the other hand, phosphorylation of NMDA receptors by PKC results in an increased NMDA antagonistic effect of memantine, since Mg^{2+} ions, which compete with memantine, are released from the channel.
The aim of this study was to analyze the mechanism of action of memantine, co-applied with sertraline, in the rat hemisected spinal cord model. Monosynaptic reflex potentials (MSR) were recorded, and like other NMDA antagonists, memantine had no effect on this response. In the presence of sertraline (0.5 μM), however, the drug dose-dependently inhibited MSR (IC50: 35 μM). A weaker blocking effect (15% inhibition at 20 μM) by the memantine-sertraline combination was seen when NMDA receptors had been fully antagonized by high concentration of APV.
To explain these findings we hypothesize, that phosphorylation by PKC released NMDA receptors from Mg^{2+} blockade, allowing the involvement of NMDA receptors in the mediation of the reflex response. Thus, synaptic responses became sensitive to memantine. Alternatively, an enhanced VGSC blocking action of memantine can explain why it inhibited the responses in the presence of sertraline. Since a complete blockade of NMDA receptors only partially prevented the ability of memantine to block the reflex, both VGSCs and NMDA receptors seem to be involved in the inhibition.

SOCIAL DEFEAT STRESS INCREASES C-FOS EXPRESSION IN RAT BRAIN: PARTIAL CO-LOCALIZATION WITH CRF2 RECEPTOR mRNA IN THE MEDIAL AMYGDALA

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Social defeat in rodents is an experimental model of social stress when an intruder animal is subordinated and then threatened by a territorial resident. The neural substrates mediating responses to social defeat are not completely understood. In the present study, the expression of the immediate early gene product, c-Fos, was used as a marker for neuronal activation to identify neural populations activated by social defeat. A resident-intruder paradigm was used in which experimental rats (n=6) were intruders in the cage of an aggressive, veteran resident Long-Evans rat. After defeat, intruders were put in a small wire-mesh chamber, which was later returned to the resident's cage to continue non-physical threat. Handled rats served as control (n=12). Rats were perfused transcardially with fixative 75 min after onset. Serial coronal sections (25 μm) of the forebrain were cut and stained for c-Fos-immunoreactivity (IR). Defeated rats had increased c-Fos-IR in the medial amygdala (MeA) and ventromedial and arcuate nuclei of the hypothalamus (VMH, ArcN), brain regions dense with CRF2 receptor expression. To examine whether the corticotropin-releasing factor (CRF)/urocortins system is involved in social defeat, a combined in situ hybridization/immunohistochemistry study was performed to visualize c-Fos and CRF2 mRNA simutaneously. Substantial co-localization of CRF2 mRNA and c-Fos-IR was observed in the MeA, but not in VMH and ArcN, suggesting that identified neural populations activated by social defeat and MeA CRF2 neurons may play a role in responses to social conflict.

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AN ALLOPREGNANOLONE DERIVATIVE IS A NEUROSTEROID ANTAGONIST OF CEREBELLAR α6ßδ GABAA RECEPTORS

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High-affinity, subtype-selective antagonists of the neurosteroid binding sites of ionotropic γ–aminobutyric acidA ($GABA_A$) receptors are not yet available. This is an attempt to develop and characterize such an antagonist.
Allosteric modulation of cerebellar $GABA_A$ receptors by an allopregnanolone derivative, (20R)-17ß-(1-hydroxy-2,3-butadienyl)-5α-androstane-3α-ol (HBAO), was studied via receptor binding and electrophysiological methods. $GABA_A$ receptors of rat cerebellar synaptic membranes were labelled with [^{3}H]ethynylbicycloorthobenzoate (EBOB). The ionophore function of $GABA_A$ receptors was studied by whole-cell patch clamp electrophysiology in cultured rat cerebellar granule cells.
Nanomolar, partial displacement of [^{3}H]EBOB binding by HBAO was attenuated by 0.1 mM furosemide, a selective antagonist of $GABA_A$ receptors containing α_6 subunits. Displacement curves by HBAO were shifted to the right by 30 nM GABA. The nanomolar, but not the micromolar phase of displacement of [^{3}H]EBOB binding by GABA was attenuated by 100 nM HBAO. Submicromolar HBAO did not affect [^{3}H]EBOB binding to cortical and hippocampal $GABA_A$ receptors. HBAO up to 1 μM did not affect GABA-elicited chloride currents, while it completely abolished potentiation by 1 μM allopregnanolone with nanomolar potency. Furosemide attenuated the inhibition by 100 nM HBAO.
In conclusion, HBAO is a high-affinity, selective antagonist of allopregnanolone, a major endogenous positive modulator via neurosteroid sites on cerebellar α_6ßδ $GABA_A$ receptors.

HETEROGENEITY OF THE EFFERENT PATHWAYS IN THE ANTERIOIR PRETECTAL NUCLEUS OF RAT

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The anterior pretectal nucleus (APT) and the zona incerta (ZI) are diencephalic nuclei, which exert strong inhibitory influence selectively in higher order (HO) thalamic relays. Beside thalamus, APT is also known to project to ZI, but the anatomical details of the APT-ZI projection has not been described.
We investigated the morphological heterogeneity of the APT efferent pathways within the APT-ZI-thalamus network, using anterograde and retrograde tracing in combination with pre- and postembedding immunocytochemical stainings and in situ hybridization.
The vast majority of APT fibers selectively innervated the parvalbumin-positive, ventral part of ZI, which is known to contain neurons projecting HO thalamic nuclei. The APT-ZI pathway consisted of both GABA-negative and GABA-positive components demonstrated both at the level of terminals in ZI, using electron microscopic analysis, and at the level of somata in APT, by visualizing GAD 67 mRNS together with retrograde labeling from ZI. The combination of parvalbumin immunostaining with retrograde tracing showed that in the APT strongly and weakly parvalbumin-positive and parvalbumin-negative neurons were all among the ZI projecting cells. Similar heterogeneity was found among the thalamus projecting APT cells, but double retrograde tracing from topographically matched HO thalamic and ZI region disclosed hardly any APT neuron with dual projections.
Our data suggest that both ZI and the HO thalamic relays are innervated by distinct, physiologically heterogeneous APT neurons. These various efferent pathways probably interact via the rich recurrent collaterals of the projecting APT cells. The powerful, GABAergic APT and ZI outputs to the thalamus are apparently synchronized in a synergistic manner via dual excitatory and inhibitory APT-ZI connections.

THE EFFECTIVENESS AND METABOLISM OF SOME OF THE NEUROLEPTICUMS ON THE GST PATHWAY.

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Neuroleptics (antipsychotics) is a group of compounds with a strong blocking effect on the central nervous system. As a result of their effectiveness and wide-range of applicability with intensive research an increasing number of medicine became available. These compounds have a significant number of – sometimes dangerous - side effects. The reason for the appearance of these is still not entirely clear. Neuroleptics are divided into two large groups, the traditional and new-generation drugs, the latter having significantly less side-effects. In this paper I examine the Glutathione-S-Transferase (GST) induction of five neuroleptics from the two groups in various organs. The GST enzyme binds xenobiotics to Glutathione (GST) and forms a conjugate. As a result of this detoxification mechanism, glutathione conjugated metabolites are created that may take part in the development of side-effects and probably in the development of efficiency.
Five selected drugs were given to SPF Wistar rats intraperitoneally during *in vivo* treatment through 10 days, in a dose used in human therapy (in body mass ratio). In all treated groups the GST activity of the liver increased. The same tendency was recognized in the case of the brain stem homogenate as well, although the GST activity in that case was 20-30 times smaller than in the liver. The new generation neuroleptics usually result in less GST induction than the traditional –with more side effects– drugs. The findings show that the examined psychoactive compounds have GST enzyme inductive effect as well. At the same time, as a result of the brain tissue GST activity the glutathione conjugates of the compounds can develop in the brain tissue as well. The glutathione conjugates, similarly to other γ-glutamine peptides and glutathione analogues, can influence the glutamatergic neurotransmission of the central nervous system, this way contributing to the development of various side effects.
The GST induction experienced makes the additional research of GST-Neuroleptic conjugates necessary.

EFFECT OF mu-OPIATE RECEPTOR AGONIST ON FAST NETWORK OSCILLATIONS IN THE CA3 REGION OF HIPPOCAMPAL SLICES.

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In vitro models are used to elucidate the cellular mechanisms underlying fast oscillations in the hippocampus. One of the most studied models is the cholinergically-induced rhythmic activity. In the hippocampus, at least two functionally distinct types of inhibitory cells innervate the perisomatic region of pyramidal neurons. Immuncytochemical data suggest that mu-opiate receptors (MOR) are selectively expressed on the axon terminals of parvalbumin-immunoreactive GABAergic neurons, but not on cholecystokinin-immunopositive basket cells providing a tool to clarify which type of these GABAergic cells plays role in the oscillations.
We studied the effect of 10 microM DAMGO, a MOR agonist, on oscillations induced by bath application of a cholinergic receptor agonist carbachol (CCh) in the CA3 region of hippocampal slices. Local field potentials were recorded in the stratum pyramidale at room temperature. DAMGO reversibly abolished or decreased the power of the oscillations depending on the initial power of the rhythmic activity. This inhibitory effect of DAMGO were blocked by CTAP (a MOR antagonist, 1 microM). In MOR knockout mice DAMGO did not changed the power or frequency of oscillations. To reveal the possible mechanisms the effect of DAMGO on synchronous activity, miniature inhibitory or excitatory postsynaptic currents (mIPSCs or mEPSCs) were measured in the CA3 pyramidal cells. DAMGO did not altered mEPSCs, but it decreased the frequency of mIPSC without changing their amplitude.
Our results suggest that parvalbumin-containing perisomatic inhibitory neurons could play dominant role in the generation of CCh-induced fast network oscillations.

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MICROGLIA ACTIVATION FOLLOWING NERVE INJURY IN THE OCULOMOTOR NUCLEUS: EFFECT OF HORMONAL ENVIRONMENT.

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Microglia are a relatively under-recognized, widely distributed cell population within the adult central nervous system and their most characteristic feature is the rapid activation in response to even minor pathological changes in the central nervous system. In response to brain injury microglia respond in a very dynamic way by secreting trophic factors that are essential for sustaining neuronal function and mounting evidence indicates microglia as neuroprotective, immunocompetent cells of the CNS. It produces various pro- or anti-inflammatory chemokines, cytokines and growth factors thought to participate in the orchestration of cellular responses aimed at rapid re-establishment of tissue integrity and subsequent repair. Activated microglia can remove potentially harmful cell debris, promote tissue repair and thus facilitate the return to tissue homeostasis. An understanding of molecular mechanisms of microglia proliferation and activation could serve a rational basis for targeted intervention on glial reactions to injuries in the CNS.
In the present experiments we used target deprivation model of nerve injury and studied the time course of expression of microglia markers. We could demonstrate the first sign of microglia activation after 24 hours, the maximal effect was found at four days. In ovariectomized females 17ß-estradiol treatment decreased significantly the microglia reaction. Our results show that microglia response to nerve injury is influenced by the estrogenic status, which may have direct consequences for structural and functional recovery.

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CELLULAR MECHANISMS OF HIPPOCAMPAL GAMMA OSCILLATIONS

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Gamma frequency (30-70 Hz) oscillations are thought to be important in cognitive processing, including hippocampal memory operations. The function of these oscillations remains poorly understood despite extensive research *in vivo*, and the cellular mechanisms underlying their generation are largely unknown. Our investigation of an *in vitro* model of gamma oscillations induced by an acetylcholine receptor agonist carbachol in the CA3 region of hippocampal slices revealed that during oscillations the firing of all phase-coupled inhibitory interneurons followed the discharge of pyramidal cells with a delay of monosynaptic neurotransmission. Using combinations of current source density analyses with imaging of voltage-sensitive fluorescent dyes, we demonstrated that active current sinks (excitation) and subsequent sources (inhibition) were restricted to the perisomatic region of the pyramidal cells. Thus, the activity of inhibitory cells innervating the somata and the proximal dendrites of CA3 pyramidal cells is crucial in the synchronization. In addition, the analyses of postsynaptic currents during oscillations uncovered that the dominant synaptic input received by phase-coupled perisomatic inhibitory interneurons was excitatory, while the dominant input to pyramidal cells was inhibitory. Differences in synaptic input could account for the differences in firing properties both between individual cells and between cell types.
In summary, our results support a synaptic feedback model of gamma oscillations generated by pyramidal cells and inhibitory interneurons targeting their perisomatic region in CA3 neuronal networks, and suggest that the firing of phase-coupled neurons during rhythmic activity is largely under the control of chemical synaptic transmission.

MODULATION OF VIOLENT AGGRESSIVE BEHAVIOUR VIA NK1 RECEPTORS

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Non-peptide antagonists of the Neurokinin1 (NK1) receptor are well known for their potential modulating emotional behaviour, but their possible role in the modulation of aggression is unclear. The aim of our study was to describe the effects of peripheral NK1 receptor antagonism in Wistar males during aggressive behaviour. Moreover, the activation of NK1 receptor positive neurons was assessed in limbic areas during aggressive behaviour.
The NK1 antagonist L-703,606 (0.1 and 1 mg/kg ip) applied 30 minutes before the resident intruder test dose-dependently decreased the number of hard bites delivered by intact residents towards smaller male intruders. Interestingly, the number of soft bites was not affected. In glucocorticoid deficient residents, L-703,606 was able to abolish dose dependently the attacks on vulnerable targets. In the medial amygdala and hypothalamic attack area, the number of double labelled cells for c-Fos and NK1 receptor was significantly increased during aggressive behaviour. The highest number of activated NK1 positive cells was found in the hypothalamic attack area. Preliminary results suggest that selective lesion of NK1 positive neurons in this area accompanies with a decrease in violent components of aggressive behaviour.
These results indicate that NK1 receptor plays a crucial role in the formation of violent aggressive behaviour, and non-peptide NK1 antagonists might be useful in the treatment of pathological aggression.

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EFFECT OF LOCAL (INTRACEREBRAL AND INTRACEREBROVENTRICULAR) ADMINISTRATION OF TYROSINE HYDROXYLASE INHIBITOR ON THE NEUROENDOCRINE DOPAMINERGIC NEURONS AND PROLACTIN RELEASE

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Dopamine (DA), the physiological regulator of pituitary prolactin (PRL) secretion is synthesized in the neuroendocrine DAergic (NEDA) neurons of the mediobasal hypothalamus that projects to the median eminence (ME) and the neurointermediate lobe (NIL) of the pituitary gland. The rate-limiting step of DA biosynthesis is catalyzed by the enzyme, tyrosine hydroxylase (TH) that produces L-3,4-dihydroxy-phenylalanine (L-DOPA) from tyrosine. The aim of our present study was to investigate the effect of inhibition of the DA biosynthesis locally in the hypothalamic arcuate nucleus (ARC). Therefore, the well known TH inhibitor, α-methyl-p-tyrosine (αMpT) was injected either intracerbro-ventricularly (ICV) or directly into the ARC of freely moving rats and plasma PRL concentration was measured by RIA. ICV administration of αMpT has no effect on basal PRL concentrations, unlike the intra-ARC injection of enzyme inhibitor, which resulted a slight but significant elevation of plasma PRL. On the other hand, systematic application of the αMpT that inhibits mainly the TH activity of the DAergic terminals located in the ME and the NIL, resulted a lot more pronounced elevation of plasma PRL. These results suggest that αMpT administered close to the NEDA neurons was able to inhibit the neural TH activity. However, this comparative data suggest that the majority of DA released into the pituitary portal circulation is synthesized or activated in the axon terminals of the hypothalamic NEDA neurons.

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SUBTYPE-SPECIFIC MODULATION OF [^{3}H]GLUTAMATE RELEASE BY P2X AND P2Y RECEPTORS IN THE RAT SPINAL CORD

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The superficial dorsal horn of the spinal cord plays a crucial role in pain transmission. Glutamate release from the primary afferent terminals and intrinsic neurons of the spinal cord are important target sites to influence sensory transmission, which might be modulated by the purinergic system. ATP is stored and released together with glutamate from glutamatergic nerve terminals and in this study the P2 receptor mediated modulation of [^{3}H]glutamate release was examined in rat spinal cord slices. ATP, ADP and 2-methylthioadenosine 5'-disphosphate (2-MeSADP) decreased the electrically evoked [^{3}H]glutamate overflow, the rank order of potency was ADP>2-MeSADP>ATP. The inhibitory effect of ATP was antagonized by suramin (300 μM) and by the P2Y12/13 receptor antagonist 2-methylthioadenosine 5'-monophosphate (2-MeSAMP, 10 μM), and partly by PPADS (30 μM) and by the P2Y1 receptor antagonist MRS 2179 (10 μM). On the other hand, 2-methylthioadenosine-5'-triphosphate (2-MeSATP, 100-300 μM) increased electrically evoked [^{3}H]glutamate overflow and this effect was prevented by the P2X1 receptor selective antagonist NF449 (100 nM). RT - PCR analysis showed that mRNAs encoding P2Y1, and P2Y13 receptors are present in rat dorsal root ganglion and spinal cord, and the expression of these receptors have also been confirmed at the protein level.
Our results show that nucleotides exert dual and opposite modulation of glutamate release in the spinal cord of the rat. The inhibitory effect is mediated by P2Y13 and probably also by P2Y1 receptors, whereas the stimulatory effect is mediated by P2X1 receptors.

THE NEUROANATOMICAL BACKGROUND OF FEEDING MODULATION IN GASTROPODS (Helix) WITH SPECIAL ATTENTION TO THE SEROTONIN AND DOPAMINE CONTAINING ELEMENTS

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In gastropods the cyclic feeding movements as the protraction and retraction of the radula as well as swallowing are generated by feeding central pattern generators (CPG). The activity of the CPG is modulated by serotonin (5HT) and is commanded by dopamine (DA). Our preliminary results showed that during food intake both 5HT and DA levels increased in the ganglia containing feeding CPG. The firing activity of the cerebral giant serotonergic neuron MGC (the main 5HTergic modulator neuron of the feeding CPG) is elevated during food intake, suggesting that the activity of the MGC is modulated by 5HTergic and DAergic inputs.
In isolated CNS preparations externally applied 5HT (1-100 µM) increases the firing rate of the MGC, while DA (1-100 µM) decreases or inhibits the firing activity. However, when the firing frequency was first elevated by 5HT, the consecutive DA application is not able to inhibit the firing activity. Intracellular neurobiotin labeling of the MGC combined with 5HT and tyrosine hydroxylase (TH) immunostaining showed that the MGC receives numerous 5HT- and TH- immuno stained fibers. Retrograde neurobiotin labeling of peripheral nerves of the cerebral ganglia combined with 5HT and TH-immunostaining showed no 5HTergic cells, but numerous TH- immunostained receptor cells in different segments of the foregut which may reach the MGC.
The present results suggest that the firing activity of MGC is modulated both by 5HT and DA containg elements. The putative 5HTergic inputs (5HT-immunostained) to the MGC originate from central neurons whereas the putative DAergic inputs (TH-immunostained) partly originate from receptor cells located in the different segments of the foregut.

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SOURCES AND ULTRASTRUCTURE OF GLUTAMATERGIC FIBERS IN THE HYPOTHALAMIC MEDIAN EMINENCE AND POSTERIOR PITUITARY OF THE RAT.

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Several peptidergic neurosecretory systems regulating hypophysial functions have been shown to contain the glutamatergic cell marker type-2 vesicular glutamate transporter (VGLUT2). In the present studies we attempted to identify the neuronal sources of glutamate in the median eminence and posterior pituitary, the main terminal fields of hypothalamic neurosecretory cells. Neurons projecting to these regions were visualized by the uptake of the retrograde tracer Fluoro-Gold (FG) from the systemic circulation, whereas glutamatergic cell bodies of the neuroendocrine hypothalamus were identified via the radioisotopic *in situ* hybridization (ISH) detection of VGLUT2 mRNA. The majority of neurons accumulating FG and also expressing VGLUT2 mRNA were observed around the organum vasculosum of the lamina terminalis (OVLT) and within the paraventricular, periventricular and supraoptic nuclei. Dual-immunofluorescent studies of the median eminence and posterior pituitary confirmed the presence of VGLUT2 protein in peptidergic terminals. Electron microscopic studies using preembedding colloidal gold labeling revealed the association of VGLUT2 with synaptic vesicles which often formed segregated groups from the peptide-containing large dense-core vesicles.
These data together suggest that classical neurosecretory neurons around the OVLT and within the periventricular, paraventricular and supraoptic nuclei co-secrete glutamate into the pericapillary space of fenestrated vessels in the median eminence and the posterior pituitary. The functional aspects of the putative glutamate co-release from neuroendocrine terminals remain to be investigated.

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GLUTAMATERGIC PRESYNAPTIC VESICULES ARE POSSIBLE INTRACELLULAR SOURCES OF THE GLUTAMATE INDUCED INCREASE OF THE CYTOPLASMIC ZINC CONCENTRACION

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Hippocampal glutamatergic mossy fiber terminals in CA3 area contain high levels of vesicular free zinc ions (Zn^{2+}). Zn^{2+} may serve as a neuromodulator affecting basic synaptic function, and influencing plasticity and memory. Electrical stimulation or chemical treatment (e.g. KCl) of the mossy fibres evokes release of presynaptic vesicular zinc into the synapse, together with glutamate. Glutamate activates, while Zn^{2+} inhibits NMDA receptors simultaneously. Following the depletion of Zn^{2+} and glutamate containing vesicles, a persistent Zn^{2+} deficit will emerge. This could lead to overactivation of NMDA receptors. However, both high and low levels of Zn^{2+} concentration are also toxic to the neurons. In this study, we report that synaptic vesicles also contribute to intracellular Zn^{2+} release. We report the development of a simple and rapid method to assess the vesicular zinc release and the effects of zinc binding chelators in rat acute hippocampal slices. By combining the usage of membrane permeable and impermeable Zn-specific fluorescence dyes, TFLZn and FluoZin-3 and chelators, TPEN and CaEDTA with the evident efficiency of a fluorescent plate reader, we show that a relatively high amount of intracellular Zn^{2+} release occurs from the presynaptic vesicles in the hippocampus after glutamate treatment.

THE DYSTROPHIN-ASSOCIATED PROTEIN COMPLEX IN THE CENTRAL NERVOUS SYSTEM: ITS ROLE IN CELL- EXTRACELLULAR MATRIX AND CELL-CELL INTERACTIONS.

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The dystrophin-associated protein complex (DAPC) is a membrane-spanning multi-protein adhesion complex with tissue- and developmental state- specific expression pattern. Abnormalities in the expression or posttranscriptional processing of the protein components lead to defective cellular functioning in divers tissues. Despite the widespread expression of several DAPC members in neurons and glial cells, the function of the complex in the central nervous system is still elusive.

In glial cells the localization of DAPC members in the perivascular endfeet of astrocytes is particularly remarkable. Two cytoplasmic members of the complex, dystrophin and dystrobrevin, has been found to localize predominantly on the endfeet membrane contacting the basal lamina. Furthermore, a splice variant of a third cytoplasmic component, Dp71, occured preferentially in the laminin-contacting subpopulation of the astrocytes. The adhesion and the motility pattern of cultured Müller glial cells is characteristically affected by the presence of laminin. These results substantiate the notion that DAPC is a key player in the interactions of glial cells with the extracellular matrix.

Within neurons, DAPC components are identified in the postsynaptic density. Neurexins, a family of presynaptic cell surface proteins, are able to bind to the extracellular part of the DAPC, raising the possibility that DAPC takes part in the interactions of the presynaptic and postsynaptic membranes. In line with this, our results indicate that in excitatory synapses the presynaptic element is likely to influence the protein composition of the postynaptic DAPC complex.

PEPTIDERGIC INNERVATION OF GABAERGIC NEURONS IN THE LATERAL SEPTUM

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The lateral septum plays an important role in the regulation of several vegetative functions. Its reciprok connections with the hypothalamic nuclei has already been proven, moreover similar neuropeptides (eg. CART, galanin, neuropeptide Y, opioid peptides, etc.) occur in both brain parts. The main inhibitory neurotransmitter, GABA is also detectable in many cells which may be among the postsynaptic targets of peptidergic axons. The aim of our studies was to reveal the distribution of some neuropeptides, to describe the fine structure of the peptidergic elements, and their relationship with the GABAergic system of LS. We used 50-60 μm serial vibratome sections from perfusion-fixed rat- and transgenic (GAD-65 - GFP) mouse brains for light- and electron microscopic pre-embedding immunocytochemistry and immunofluorescence. Both in the rat and mouse the above neuropeptides were present in varicose nerve fibers. Only NPY occurred in perikarya. The fine structure of peptidergic synaptic terminals revealed the presence of both small agranular vesicles and dense-core vesicles. Their targets were mainly dendrites or dendritic spines. Colocalization studies proved that each peptidergic fiber-type made contacts with inhibitory cells. Gal and Leu-enk formed multiple contacts (pericellular baskets) around the GFP-labelled LS neurons, CART and NPY gave 1-2 synaptic contacts. Our results serve as basis for further experiments aiming at studying the effect of food deprivation on

EEG EFFECTS OF OLFACTORY AND GUSTATORY STIMULATIONS ANALYZED BY POINT CORRELATION DIMENSION (PD2i) METHOD

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Background: The effects of the smell and taste stimulations are known from functional magnetic resonance imaging (fMRI) and electroencephalographic (EEG) studies. They have shown that the caudal orbitofrontal cortex, amygdala, insular cortex and anterior cingulate are activated by both modalities. Linear EEG investigations in time and frequency domain showed power and coherency changes in the alpha as well as theta bands depending on the quality of the stimulation. Only some analyses of non-linear nature were found.
Aim/objective: The study was set up to investigate the short term effects of an acute smell and taste stimulation in healthy adult volunteers by the PD2i analysis of Skinner and Molnár.

Methods: Nine persons (seven female, two males) with an average age of 49±19 years participated in the examination. Sixty seconds of 16 channel EEGs – according to 10/20 system – were recorded by an EEG 16X equipment and digitalized by a LI-01/A interface (Mikromed) at a rate of 256 sample/s. After the first 30 sec which was taken as a basal period for the comparison a fresh perfume cap or a piece of milk chocolate was presented. The period after the stimulation was divided in two 15 sec long parts and their PD2i values were compared to the basal condition by non-parametric signed rank test of Wilcoxon.

Results: It was found, that there was a significant decrease in the average of the PD2i after the olfactory stimulation on both sides. A short lasting about 15 sec long decrease was found in the F3, O1 and C4 leads. Longer decreases have appeared in the F7, T4, P4 leads and the SD values decreased around the above mentioned positions. The effects after gustatory stimulation were less pronounced and late. Significant decrease of the average PD2i was found only in the right side, on the C4 and O2 leads.

Conclusion: The PD2i analysis of the EEGs looks suitable for finding differences and similarities in the data processing after olfactory and gustatory stimulations.

BILATERAL OLFACTORY BULBECTOMY (OBX) AS A MODEL OF DEPRESSION – BEHAVIOURAL AND ENDOCRINE CHANGES

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Objective: OBX is considered to be an animal model of depression. The aim of the present study was to examine whether the observed endocrine and behavioral changes following bulbectomy, were in accordance with the symptoms of depression.
Methods and Results: Bilateral bulbectomy was performed in anesthetized male Wistar rats, the tests were performed 3 weeks later. Sham-operated animals served as controls.
1.) Observations corresponding to the symptoms of depression were as follows:
Elevated corticosterone level measured by RIA.
Hyperlocomotion in novel surroundings.
Impaired memory in the passive avoidance test.
Decreased frequency of swimming and stuggling in the forced swimming test.
2.) Observations not corresponding to the characteristic symptoms of depression were as follows:
Lack of increased anxiety in the elevated plus maze test.
No difference compared to controls in sucrose preference and in the conditioned place preference induced by morphine, suggesting lack of anhedonia.
No response to the inescapable shock in learned helplessness test (number of escape failures did not increase, as it did in control animals in this animal model of depression).
Conclusion: The data indicate that some, but not all characteristic symptoms of depression appear after bilateral bulbectomy, indicating that OBX can be considered as a special animal model of depression.

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FUNCTIONAL RELEVANCE OF THE IMMUNOREACTIVITY OF ADHESIVE FACTORS IN BRAIN VESSELS

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This presentation is a summary of our investigations and the literatural data. The pial surface and the intracerebral vessels are surrounded by a basal lamina produced by astrocytes. The adhesive factor laminin, and its receptors are essential in the formation of basal laminae. In mature, aldehyde-perfused brain the laminin immunoreactivity is confined, whereas in immature or lesioned brains the vessels are immunoreactive. A probable explanation is that there is a common gliovascular basal lamina, which has been formed by the fusion of the basal laminae of the endothel and the perivascular glia. This fusion may 'cover' the laminin epitops from immunoreagents. Immunoreactivity of cerebral vessels to adhesive factors laminin, fibronectin, and laminin-receptor component beta-dystroglycan were investigated during brain development, and in the post-lesion period. The following assemblies were found, suggesting subsequent developmental stages between E12-P10: a) fibronectin +, laminin +, dystroglycan –; b) fibronectin +-, laminin +, dystroglycan +, c) fibronectin -, laminin +, dystroglycan +; d) fibronectin -, laminin +-, dystroglycan +; e) fibronectin -, laminin -, dystroglycan +. These may correspond to stages of the organization of gliovascular connections and common basal lamina. During the post-lesion glial reaction, beside the transitory appearance of laminn immunoreactivity, a transitory disappearance of dystroglycan also occurs. These phenomena may indicate the desorganisation and reorganisation of gliovascular connections, and characterise stages of these processes. Presently, our studies are extended over other components, as agrin, fibronectin, and utrophin. These latter results are presented in posters (Bagyura et al., Pócsai et al., Adorján et al.).

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LONG-TERM CHANGES OF POTASSIUM CHLORIDE COTRANSPORTER TYPE 2 EXPRESSION IN PILOCARPINE-INDUCED EPILEPSY

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Potassium-chloride cotransporter KCC2 is a membrane protein responsible for the regulation of cell volume and intracellular Cl- and K^+ concentrations, and its reduced activity leads to a depolarizing effect of GABA. We studied the expression pattern of KCC2 in pilocarpine-induced epilepsy using CD1 mice. Based on behavioral signs of status epilepticus, mice were classified as “strongly” and “weakly” epileptic using the Racine-scale. In the strong group the seizures were frequent with intense motor symptoms (Racine 4-5), whereas in the weak group there were only a few mild seizures (Racine 1-3).We found that, in the strong group, the hippocampal CA1 region became sclerotic in most cases. At light microscopic level, the membranes of surviving pyramidal cells became strongly immunopositive. Dentate granule cells displayed considerably stronger immunopositivity in sclerotic animals. The number of KCC2-positive interneurons increased both in sclerotic and non-sclerotic animals. Somewhat increased density of KCC2 immunstaining was found also in the weak animals without sclerosis in the hippocampi. In the electron microscope, we found more numerous immunopositive profiles in the epileptic hippocampi than in the controls. Interneuronal cell bodies and dendrites showed stronger immunopositivity than the surviving principal cells. The enhancement of KCC2-expression in this model may represent a neuroprotective reaction of the tissue, since it may prevent excitotoxic damage (swelling) via increased efficacy of volume regulation. However, if the chloride (and potassium) transport is reversed due to the high extracellular potassium concentration during seizures, GABA effects may become depolarizing and will aggravate seizures.

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ANATOMY, PATTERN AND DEVELOPMENT OF THE GABAergic SENSORY SYSTEM IN THE MODEL ANIMAL EISENIA FETIDA (ANNELIDA, OLIGOCHAETA)

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Presence of GABA in sensory system of earthworms has been reported by some laboratories, but systematic observation of GABAergic sensory system has not carried out yet. This study focuses on the GABA-immunoreactive (GABA-IR) sensory system of earthworms applying light and electron microscopic immunocytochemistry.

Based on investigations of whole mount preparations and serial sections distribution pattern of GABAergic primary sensory cells was determined on the whole body surface. Labelled cells belong to two distinct groups: (i) solitary sensory cells that are thought to be mechanoreceptors and (ii) grouped sensory cells characterised by various morphology. Peripheral processes of sensory cells join the subepidermal plexus while ventral processes enter the central nervous system via segmental nerves and concentrated in the ventrolateral and ventromedial longitudinal sensory axon bundles.

Ultrastructural observations revealed that GABA-IR sensory processes form synapses with other sensory processes of the subepidermal plexus. Further, ventral giant axons receive several GABAergic inputes from central sensory fibres. These results suggest that GABA modulates the activity of sensory fibres at periphery and it has direct influence on the activity of ventral giant axons. During the embryonic development, the first appearing GABA-IR sensory cells are characterised by seemingly random distribution. Later, migrations of sensory cells were observed, so at hatching similarly to adult animals, most of them concentrated in sense organs showing characteristic distribution pattern.

Present results support earlier findings, namely GABA acts as transmitter and neuromodulator in the earthworm nervous system. Our data also suggest that GABA-positive sensory cells have direct effect on the motor activity of earthworms via ventral giant axons.

TRANSIENT CHANGES IN THE LOCALIZATION PATTERN AND JOINT ACTIVITY OF NTPDASES AFTER LPS TREATMENT IN RAT HIPPOCAMPUS

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Pathological conditions, including inflammation and ischemia, increase the extracellular release of ATP. The concentrations of extracellularly released nucleotides is controlled by ecto-nucleotidases but the precise physiological roles of the NTPDases (ectonucleoside triphosphate diphosphohydrolases) in the modulation of P_2 receptor signaling is still unclear. Our current experiments demonstrated altered IL-1 beta protein expression 2 hours after *in vivo* LPS (2 mg/kg i.p) treatment; however, increased expression of NTPDase1 or 2 was not observed, although changes in the localization pattern of ecto-ATPase activity and changes in the morphology of endothelial cells were evident. The enzyme activity of NTPDase1, present on the luminal side of the endothelial cells, was transiently lost, while other nucleotidase activity, presumably due to NTPDase2, increased, and thus the overall ecto-ATPase activity of the cell remained unchanged. After 2-3 days these transient changes were no longer evident. Transient loss of NTPDase1 activity (but not protein) can be explained by transient alterations in the membrane structure due to the LPS-evoked inflammation, since NTPDase1 activity is sensitive to changes in its transmembrane domains. Quantitative immunoblotting and HPLC analyses did not demonstrate any significant differences in the overall expression levels of NTPDase1 or 2, and in the ecto-nucleotidase activities of the control and treated hippocampi only the faster rate of ATP hydrolysis at low initial ATP concentrations 1 day after LPS treatment. In summary, we conclude that LPS treatment can cause cell-specific responses in purinergic signaling, mediated, at least in part, by changes in the activities and expression of NTPDases.

PATHOGENESIS OF HUNTINGTON'S DISEASE

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Huntington's disease is a neurodegenerative disorder characterized by the progressive development of involuntary choreiform movements, cognitive impairment, neuropsychiatric symptoms, and premature death. Huntington's disease is a member of a group of diseases caused by CAG repeat expansions. The protein called huntingtin. The mutant huntingtin disrupts a number of vital cellular processes, including energy metabolism, gene transcription, intraneuronal trafficking, and postsynaptic signaling, but the crucial mechanism is still unclear. A large body of evidence, however, supports involvement of mitochondrial dysfunction and energy deficit in the disease mechanism. Thus, excitotoxicity, apoptosis, and oxidative damage, have been implicated in the mechanism of selective neuronal damage in HD.

COULD IT BE DONE BETTER? MEDICINE PACKAGE LEAFLETS AS POSSIBLE SOURCES OF POSITIVE SUGGESTIONS

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From an ethical point of view, the therapeutic usage of placebos is prohibited, mainly because of deception. On the other hand, the placebo effect itself – although usually inadvertedly - seems to be a part of every therapeutic procedure. A possible way of improving this placebo effect is issuing positive suggestions. A proper suggestion has beneficial subjective (hope, optimism) and objective (bodily changes caused by expectancy and better compliance) effects, and is able to accelerate recovery. A possible way for communicating positive suggestions is package leaflets of medicinal products. Representatives of complementary and alternative medicine usually harness the advantages of the placebo effect much better than conventional practitioners. We, therefore, hypothesized, that the leaflets of alternative (e.g. natural healers') products also deliver more positive suggestions to patients about effectivity and recovery than those of the conventional pharmaceutical products. To test this hypothesis, altogether 92 package leaflets of conventional and alternative health care products were inspected by content analysis. The positive suggestive power of the leaflets was evaluated by two independent raters by a simple scoring system. The results support our hypothesis: information sheets of alternative products proved to be more powerful as of positive suggestions than of the conventional products.
The mandatory existence of these leaflets provides a convenient and cheap way for using health-improving suggestions in both ethical and effective manner. The authors of leaflets of alternative products seem to know this fact very well. On the other hand, this possibility is mainly unutilised by the producers of conventional medicines.

ASTROGLIA-DERIVED RETINOIC ACID INDUCES NEURONAL DIFFERENTIATION OF STEM CELLS

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Recently we reported that in astroglia/stem cell co-cultures astrocytes instruct non-committed neuroectodermal stem cells to adopt a neuronal fate. The large-scale neuronal induction was due to some soluble, easily degradable, short-distance acting factors released by astrocytes. Neuronal fate commitment could be induced by *all-trans* retinoic acid (ATRA) as well, in all of the stem cell-populations used in our experiments (mouse-derived ES, EC [P19] and neuroectodermal stem [NE-4C] cells). Based on the apparent similarities between ATRA and glia-induced neuron formation, we presumed, that astroglia-derived all-trans retinoic acid is one of the key factors directing glia-induced neuron formation. Astrocytes were isolated from various brain regions of neonatal (P0-P7) mice derived from non-transgenic CD1 and FVB/N strains or from transgenic mice expressing eGFP under the control of human GFAP promoter. The ATRA-production by cultivated astrocytes was investigated by HPLC, by bioassay using a retinoic acid-responsive cell-line and by RT-PCR demonstration of transcriptional activity of retinaldehyde dehydrogenase (RALDH) enzyme coding genes. The data provide strong evidence on the key role of astroglia-derived retinoic acid in glia-induced neuronal differentiation of stem cells.

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NTPDase 3 IN THE CNS OF THE RAT: MAPPING AND FUNCTIONAL CONSIDERATIONS.

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Antisera were raised against three distinct amino acid sequences of ecto-nucleoside triphosphate diphosphohydrolase 3 (NTPDase3), characterized by Western blot analyses, and used to determine the distribution of NTPDase3 protein in adult rat brain. NTPDase3-immunoractivity (IR) was detected exclusively in neurons. IR was localized primarily to neuronal processes with prominent staining of synaptic elements. Specific perikaryal staining was detected in scattered neurons near the lateral hypothalamus and the perifornical area. High densities of IR axon- and dendrite-like fibers were present in midline regions of the brain. Scattered NTPDase3 positive fiber-like profiles were observed in the cerebral cortex, the hippocampus and the basal ganglia. Very high densities of IR punctate structures were detected in the hypothalamus and the molecular layer of the cerebellum. High densities of NTPDase3/IR terminals were also associated with noradrenergic neurons. Co-localization studies revealed that NTPDase3 IR was not localized within the noradrenaline cells/terminals, nor in GABAergic neurons. In contrast, nearly all of the NTPDase3-IR hypothalamic cells, and most fibers in the mid- and hindbrain also expressed orexin-A. The pattern of expression and co-localization with hypocretin-1/orexin-A suggests that NTPDase3, by regulating the extracellular turnover of ATP, may modulate feeding, sleep–wake and other behaviors through diverse homeostatic systems.

MULTISENSORY MECHANISMS UNDERLYING OBJECT MOTION AS SHOWN BY FMRI

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Real-life moving objects are often detected by multisensory cues. We investigated the cortical activity associated with coherent visual motion perception in the presence of a stationary or moving auditory noise source using functional magnetic resonance imaging. Twelve subjects judged episodes of 5-s random-dot motion containing either no (0%) or abundant (16%) coherent direction information. Auditory noise was presented with the displayed visual motion that was moving in phase, was moving out-of-phase, or was stationary. Subjects judged whether visual coherent motion was present, and if so, whether the auditory noise source was moving in phase, was moving out-of-phase, or was not moving. Performance was greatest for a moving sound source that was in phase with the visual coherent dot motion compared with when it was in antiphase. A random-effects analysis revealed that auditory motion activated extended regions in both cerebral hemispheres in the superior temporal gyrus (STG), with a right-hemispheric preponderance. Combined audiovisual motion led to activation clusters in the STG, the supramarginal gyrus, the superior parietal lobule, and the cerebellum. The size of the activated regions was substantially larger than that evoked by either visual or auditory motion alone. The congruent audiovisual motion evoked the most extensive activation pattern, exhibiting several exclusively activated subregions.

IS THE MOTION PERCEPTION ABNORMAL IF THE CORTICAL EXCITABILITY IS TOO HIGH?

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Migraine is a very common disorder in which the central nervous system dysfunction has a pivotal role. Abnormal cortical excitability has been suggested to play an important part as a possible factor in predisposing sufferers to the sponteous, cortical spreading depression that has been suggested to represent the pathological basis of the aura experienced during migraine. A number of previous studies identified differences in visual perception and related psychophysical performance in migraineurs between attacks. The aim of this study was to determine whether cortical motion processing abnormalities are present in individuals with migraine with aura (MA) and without aura (MO), due to higher cortical excitability. Functional magnetic resonance imaging (fMRI) was performed on 18 migraineurs (9 MA, 9 MO) and 12 age- and gender-matched healthy subjects at 3 Tesla, whereby different moving dot stimuli (vertical, horizontal, rotational, radial, and random) were contrasted against a static dot pattern. All motion stimuli activated a distributed cortical network, including previously described motion-sensitive striate and extrastriate visual areas. There was no significant difference in BOLD repsonse between migraineurs and controls in MT, which is considered necessary for optimal human motion perception. However, the adjacent MST that is activated by optic flow and during pursuit eye movements was more active in migraineurs (both MA and MO subjects) than in controls. These results imply that the abnormal excitability of MST may be repsonsible for the altered motion perception and disturbance of eye-movements in migraine.

INHIBITORY MECHANISMS OF VISUAL ATTENTIONAL SELECTION

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Visual attentional selection enhances the neural response to relevant objects and suppresses the response to competing irrelevant ones. Traditionally, research has been focused on how attention facilitates processing of the selected visual information. As a result, we know surprisingly little about the neural mechanisms of attentional suppression of the task-irrelevant visual information.
Our goal was to isolate and characterize the perceptual and neural mechanisms of attentional suppression using psychophysics and fMRI in human observers. We designed a visual learning experiment, which enabled us to investigate the learning-induced neural plasticity of the inhibitory mechanisms of attentional selection. Observers were trained on a motion speed discrimination task using a bidirectional transparent motion display, consisting a task-relevant and a task-irrelevant motion component. We tested the effect of learning on observer's perceptual sensitivity for as well as the BOLD responses to the two motion directions that were present during training and a third, control direction.
It was found that perceptual sensitivity for the task-irrelevant motion direction is decreased as a result of training. Learning also affected the BOLD responses to the motion direction that was ignored during training: significantly stronger BOLD responses were measured in human MT+ complex to the task-irrelevant motion direction as compared to the task-relevant direction after but not before training.
These results provide evidence for the mechanisms of direct attentional suppression of task-irrelevant visual information, the efficacy of which can be improved with learning.

NEURAL CORRELATES OF VISUALLY INDUCED SELF-MOTION IN DEPTH

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Optic flow fields are able to generate the conscious illusion of self-motion in a stationary observer. Here we used functional magnetic resonance imaging to reveal the differential processing of self-motion and object-motion in the human brain. Subjects were presented a constantly expanding optic-flow stimulus, composed of disparate red-blue dots, viewed through red-blue glasses to generate a vivid percept of 3-D motion. We compared the activity obtained during periods of illusory self-motion with periods of object-motion percept. We found, that the left MT+ and precuneus, as well as areas bilaterally along the dorsal part of the intraparetal sulcus, and along the left posterior intraparietal sulcus were more active during self-motion perception than during object-motion. Additional signal increases were located at the depth of left the superior frontal sulcus, over the ventral part of the left anterior cingulate, in the depth of the right central sulcus and in the caudate nucleus/putamen. We found no significant deactivations, associated with self-motion perception. Our results suggest that the illusory percept of self-motion is correlated with the activation of a network of areas, ranging from motion specific areas to regions, involved in visuo-vestibular integration, visual imagery, decision making and introspection.

THE POSSIBLE ROLE OF CART(55-102) PEPTIDE IN EXCITATORY NEUROTRANSMISSION IN THE SPINAL DORSAL HORN OF RATS

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Cocaine- and amphetamine- regulated transcript peptides (CART) have been implicated in regulation of several physiological functions including pain. Dense plexus of CART immunoreactive fibers have been described in the superficial laminae of the spinal cord, which are key areas in sensory information processing and pain transmission. We have shown that the majority of CART positive fibers in lamina I originate from nociceptive primary afferents and terminate on projection neurons. In this study the neurochemical features of CART-erg fibers have been further investigated.
We found that 70% and 35% of CART-erg axons are primary afferents in lamina I and II, respectively. Vesicular glutamate transporter 2, a marker of glutamatergic excitatory interneurons, was found in 50% of CART-erg boutons in lamina I and II, suggesting that the remaining CART labelled fibers belong to local interneurons. Many of these terminals also contained SP or somatostatin/neurotensin without CGRP. Colocalization of CART with vesicular GABA transporter was very sparse. Coexpression of CART with serotonin, a marker for one of the biggest descending systems targeting the dorsal horn was never found. At electronmicroscopic level, most of the CART terminals contained round vesicles and formed asymmetrical synapses mainly with dendrites and, in some cases, axo-axonic synapses were observed.
Although the exact role of the two excitatory interneuron populations containing substance P or somatostatine/neurotensin has not been determined, their subpopulations expressing CART could be involved in the glutamate mediated physiological effects including nociception.

TABLETS AND EFFECTS - STUDYING EXPECTATIONS TOWARD COLOURS AND SIZES OF PILLS

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A considerable part of the total effect of a drug treatment can be due to the so-called placebo or non-specific effects. Important sources of such non-specific effects are the expectations evoked by the perceptual characteristics (e.g. colour, size) of the curatives. The aim of the present work was to study how colour/size of tablets influence expectations toward the effects/side effects.
100 undergraduate university students were asked to fill out a test containing 12 common drug effects and 9 side effects. In each case, participants had to choose 3 out of 15 pictures of differently coloured and shaped tablets which they thought were the mostly associated with the given effect/side effect. As a result, significant differences from the theoretical equal distribution were found in 19 out of 21 (colours) and in 16 out of 21 (sizes) cases. After grouping the effects according to 6 body functions, all distributions differed significantly from equality regarding both pill colour and size. The same significant difference remained if only two groups of effects had been composed (stimulant/sedative). The colour preferences we have found are mainly in accordance with the previous results but are surprisingly strong as compared to other data. Size preferences are also significant but their interpretation can be dubious.
The expectations evoked by perceptual characteristics of drugs can interact with their biological effects, and can affect recovery in many ways: via direct psychophysiological influence and via improving patients' compliance. From this point of view, studying and understanding non-specific drug effects/side effects and their sources (hereditary, cultural or learned origin) can help to design curatives which are more effective and have less side effects.

BEHAVIORAL MONITORING AFTER DIFFERENT SEVERITY OF TRAUMATIC BRAIN INJURY USING A RAT MODEL OF IMPACT ACCELERATION

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Traumatic brain injury (TBI) is the leading cause of death and disability under the age of 40. While the significance of diffuse axonal injury (DAI) evoked by/associated with TBI is well known in terms of its neuropathological aspects, neurobehavioral deficit has been examined less extensively. In light of above the aim of this study was to examine neurobehavioral alterations in an impact acceleration TBI model primarily characterized with DAI in rat.
Adult male rats were injured in three groups with a brass weight of 450g falling from the height of 100, 150 and 200cms, respectively. Next we have examined alterations of the motor and cognitive functions with beam-balance, open-field (OF) and elevated plus-maze (EPM).
In the beam-balance test, we observed significant difference between groups representing mild and severe TBI. The OF- test revealed reduced activity of the severely injured rats in all parameters compared to any other experimental group. Results of the EPM revealed that TBI did not cause any difference in the anxiety scores between the experimental and control groups. These results indicate that DAI in this model –and in the scope of the functional test applied- primarily affects the motor system a finding well explained by our previous morphological observations concerning the magnitude of DAI in the corticospinal tract in the brainstem.

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COMPARATIVE ANALYSIS OF S100B PROTEIN IN THE CEREBROSPINAL FLUID IN SEVERE TRAUMATIC BRAIN INJURY PATIENTS –CASE REPORT

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The S100B protein belongs to the Ca^{2+}-bindig protein family and primarily localized to neurons. After traumatic brain injury (TBI) it gets to cerebrospinal fluid (CSF) and according to some observations its concentrations in the CSF are associated with the magnitude of head injury. The aim of this study was to determine the quantity and the time course of S100B protein in the ventricular CSF in six severe TBI-patients using the BioVendor S100B sandwich ELISA test.
Our results indicate that in those patients, who had survived, both the value as well as the time course of S-100 accumulation differed markedly from the one of those patients who failed to survive. While S100B protein concentration of the survivors remained under 2500 pg/ml few days after the head injury, the values of those patients who died has never decreased under 15000 pg/ml and the latter group displayed a secondary rise in CSF values.
Based on our examinations we suggest, that the S100B protein, as its diagnostic role was described in another diseases, could have a role as biomarker at the diagnosis of severe traumatic brain injury. The results of our present examinations can be considered as a pilot-study, witch could be corroborated by further processing the samples of the "Pécs Severe Head Injury Databank".

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QUEST FOR A SPECIFIC PARATHYROID HORMONE RECEPTOR 2 (PTH2R) ANTAGONIST

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Chronic (neurogenic or inflammatory) pains are caused by a primary lesion, dysfunction or transitory perturbation in the peripheral or central nervous system (CNS) or by any persistent local inflammatory response. Various clinical strategies have been designed to deal with the different pathophysiological mechanisms underlying chronic pain, but all the efforts deployed have been, at best, partially effective The recently characterized neuropeptide ligand–receptor pair, the tuberoinfundibular peptide (TIP39)–prathyroid hormone receptor-2 (PTH2R) distribution in the CNS is characteristic to the hypothalamus, the dorsal horn of the spinal cord and to the dorsal root ganglia. Most of these structures are involved in the ascending pain pathway, thus inhibiting the modulatory (enhancing) function of this receptor-ligand interaction a well-targeted and adequate result could be obtained. This G-protein-coupled receptor might play unique and central role in the nociceptive input processing at the spinal level because of its dual signaling nature. The cAMP response and the simultaneous rise in Ca^{2+} level elicited by TIP39 endows this receptor with the ability to initiate the maladaptive spinal learning process, central sensitization. The lack of a selective small molecule inhibitor of PTH2R limits the exploration of it's role in the chronic pain.

In quest for a specific ligand, first, a functional HTS assay on HEK293-hPTH2R-CNG cells utilizing the ACT:One system has been completed. For LTS characterization of HTS hits, the Atto Bioscience's Act:One system HEK293-CNG (cyclic nucleotide-gated)-PTH2R cell line has been also optimized for FlexStation (Molecular Devices) measurement. This dynamic $[Ca^{2+}]_i$ measurement was utilized to characterize the inhibitory efficacy of the selected compounds against the EC_{80} concentration (150 pM) of TIP39, the peptide agonist. We applied Flex mode with end-point measurement. To exclude those compounds that directly inhibit adenylate cyclase or CNG channel (the biosensor), forskolin-induced $[Ca^{2+}]_i$ measurement on HEK293-hPTH2-CNG- was set up and optimized as well. Here we describe the characteristics of the HEK293-hPTH2R-CNG cell line as a suitable tool for drug discovery at both HTS and LTS level, investigating specific, G-protein coupled receptor ligands.

REACTIVE OXYGEN SPECIES PRODUCTION IN ISOLATED NERVE TERMINALS

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Increased production of Reactive Oxygen Species (ROS) is a major factor in the pathomechanism of ischemia-reperfusion injury. Mitochondria play an important role in the intracellular ROS production. In isolated mitochondria (energized from complex I. or from complex II.), ROS production is highly dependent on the mitochondrial membrane potential ($\Delta\Psi_m$). In the synaptosomes (isolated nerve terminals), mitochondria are in physiologic „in situ" conditions. In the present work we studied the synaptosomal ROS production and the relationship between $\Delta\Psi_m$ and ROS generation. The membrane potential of "in situ" mitochondria was modulated by changing the demand and production of ATP of the synaptosomes. Mitochondrial functions were followed measuring $\Delta\Psi_m$ with JC-1 fluorescent dye and oxygen consumption with oxygen electrode. ROS production was followed with Amplex Red fluorescence and with chemiluminescence.
Inhibition of catalase by aminotriazol and glutathion-peroxidase by merkaptosuccinate increased release of H2O2 from synaptosomes. These results indicate the presence of efficient ROS scavenging mechanisms in synaptosomes Decrease of mitochondrial membrane potential by uncoupler FCCP or by increased Na^+ load (veratridin) did not decrease ROS production in synaptosomes. We concluded that mitochondrial ROS production under "in situ" conditions can not be decreased by decreasing mitochondrial membrane potential.

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OVEREXPRESSION OF GAD67 IN THE MOUSE LENS RESULTS IN MULTIPLE OCULAR DEFECTS

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γ-aminobutyric acid (GABA), the principal inhibitory transmitter in the CNS, during both neuronal and non-neuronal development acts as a trophic factor to control processes including cell proliferation, migration and differentiation. As we reported before GABA and other components of GABAergic signaling are expressed in the mouse lens from early embryonic stages. Because of its simplicity and its predictable pattern of development and differentiation, the lens has attracted the attention as a model system.

To uncover the possible role of GABA signaling in the lens, we have created transgenic mice overexpressing glutamic acid decarboxylase (GAD67) -the enzyme catalyzing the rate limiting step in the production of GABA- under the regulation of the □A-crystallin promoter, which drives lens fiber-specific expression. We have found that elevated GABA levels, established by HPLC and immunohistochemistry, induced strong ocular defects: multilayer lens epithelium, cataract, lens-retina fusion, retinal foldings and ectopic retina. To evaluate the role of GABA during lens development, we analyzed mice overexpressing and mice lacking GAD67 in the lens by Ki67 (marker for actively dividing cells) immunohistochemistry. Statistical analysis revealed increased epithelial cell proliferation in mice overexpressing and decreased proliferation in mice lacking GAD67.

Ocular defects of transgenic mice with genetically altered GABA levels are consistent with a role for GABA in the differentiation of lens epithelium into fiber cells, which correlates with previous findings of GABA being a modulator of proliferation, differentiation and migration during neuronal development. Further detailed phenotypic and molecular analysis will help to understand the exact molecular mechanism of GABA action in the development.

EFFECT OF NEUROTENSIN IN AMYGDALOID LEARNING MECHANISMS

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In the central nervous system tridecapeptide Neurotensin (NT) has been demonstrated to modulate neurotransmission in a number of brain regions. It has been shown that NT in the ventral tegmental area is capable of inducing positive reinforcing effects. It was indicated that microinfusions of NT antagonist into the nucleus accumbens core impair spatial learning. Via its receptors in the ventral mesencephalon NT stimulates dopamine cell firing and axonal dopamine release in limbic terminal fields. Experimental results on NT - dopamine interactions suggest that endogenous NT is involved in the control of behavioural actions, motivation and learning. By means of immunohistochemical and radioimmune methods it was shown that the human and rodent amygdaloid body is rich in NT immunoreactive elements and NT receptors. Our previous results indicated that in the rat central nucleus of amygdala (ACE) NT has positive reinforcing effects.
The aim of our study was to examine in the ACE the possible effects of NT on spatial learning in Morris water maze paradigm. Male wistar rats were microinjected bilaterally with 100 ng NT or 250 ng NT (Sigma: N 3010, dissolved in sterile saline, injected in volume of 0.4 µl) or vehicle solution into the ACE. Application of 100 ng NT and 250 ng NT significantly reduced latency to find the safe platform located in one of the quadrants of the maze. After removal of the platform, 250 ng NT treated rats spent significantly more time at the previously learned place of the platform. Our results show that in the rat ACE NT facilitates place learning and memory.

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AMPA RECEPTOR DESENSITISATION CONTROLS SEIZURE-LIKE EVENT LENGTH IN JUVENILE RAT HIPPOCAMPAL SLICES

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Cyclothiazide (CTZ; 100 µM) an allosteric positive modulator of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) type Glu receptors increased the length of low-[Mg^{2+}]-induced seizure-like events (SLE), recorded from the CA3 *stratum pyramidale* of juvenile rat hippocampal slices. CTZ increased the length of tonic discharge phase of SLEs in all slices tested and made the SLEs endless in 2 of 9 slices by stabilizing clonic-like bursting. 30 µM CTZ had no effect, while 300 µM CTZ made the SLEs endless in 4 of 8 slices. Effects of CTZ were abolished when co-applied with 100 µM GYKI-52466, an allosteric inhibitor of AMPA receptors. CTZ is also known to increase Glu release by a mechanism independent of the inhibition of AMPA receptor desensitisation. To differentiate between these possible mechanisms of CTZ action, we compared the effects of 4-aminopyridine (4-AP), a compound that increases Glu release without affecting AMPA receptor desensitisation. 4-AP (50 µM) transformed recurrent SLEs into an incessant epileptiform pattern, distinguishable from that observed under CTZ application. Co-application of CTZ (100 µM) with 4-AP (100 µM) resulted in an intermittent tonic-like pattern. The self-similar slow down of SLEs by CTZ suggests that AMPA receptor desensitisation shapes internal SLE dynamics, while increased Glu release disrupts the recurrent pattern.
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THALAMOTECTAL PRJECTIONS IN THE FROG

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The locations and morphology of thalamotectal projection neurons are poorly understood. To extend our knowledge on this system transport techniques were used to label thalamic neurons that send their axons to the optic tectum. Texas Red-labelled dextran amine (BDA-TR, 10000 MW) and biotinylated dextran amine (BDA, 3000 MW) were iontophoretically deposited in various places of the optic tectum, and the animals were left to survive for 5 to 12 days. The retrogradely transported tracers labelled piriform neurons in the pretectal region, in the posterodorsal and posteroventral division of the lateral thalamic nucleus (Lpd, Lpv), and the posterior and central thalamic nuclei (P, C) on the ipsilateral side. A few cells were also labelled in the contralateral Lpd and P. Small, round cells with a few, short dendrites were filled ipsilaterally in the dorsal hypothalamic nucleus. More rostrally, cells were labelled only ipsilaterally in the C, ventromedial and ventrolateral thalamic nuclei, some in the posterior entopeduncular nucleus, lateral and the anterior thalamic nucleus. Most neurons were piriform. The dendrites of neurons located in the dorsal thalamus were directed laterally. The axon originated from the main dendrite and joined the medial aspect of the marginal optic tract. The dendrites of the neurons labelled in the ventral thalamus pointed to the ventrolatereal direction and their axons arched over the lateral forebrain bundle to rich the marginal optic tract.

In an earlier HRP study, the tectal projection of the pretectal cell groups was already described. Also in mammals, some ventral thalamic cell groups and the dorsal hypothalamic nucleus project to the superior colliculus (tectum).

N-CADHERIN FUNCTION DURING NEURAL CELL DIFFERENTIATION IN THE MAMMALIAN CNS

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N-cadherin expression is downregulated in GABAergic cells of the adult nervous system and this has been tentatively linked to switch of GABAergic cell function from excitatory to inhibitory (Benson and Tanaka, 1998). We demonstrate here using two.color in situ hybridization however that N-cadherin mRNA is cleared from future GABAergic cells much earlier, during their fate determination, therefore we decided to examine the role N-cadherin might play in this process *in vivo*. Since N-cadherin also plays vital roles during early events of development, examination of their later functions by simple knockout experiments is impossible. To overcome this, we are building up a tissue-specific, tetracycline-inducible transgenic mouse model system in which we could study N-cadherin function in a temporally and spatially controllable manner. The application of bicistronic constructs makes the following of the introduced N-cadherin expression readily available. Moreover, by using the Tet-inducible system it will be possible to cross as many expression lines as desirable to study the redundancy among multiple cadherins at the same time. In the tissue-specific driver constructs we are including promoters that drive early neuroepithelial expression as well as ones that provide neuronal cell-type specific expression at later stages. The expression constructs contain in combination N-cadherin or its deleted dominant negative mutant version in combination with *in vivo* and *in vitro* detectable reporter genes.

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HIPPOCAMPAL CONTRIBUTIONS TO CONTROL - A NORMATIVE THEORY

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The mammalian brain possesses multiple memory systems, including the striatum, the locus of procedural memories underlying habits, the neocortex, the main site for long-term general perceptual and semantic knowledge, and the hippocampus, the area largely responsible for storing and processing specific autobiographical memories. These three systems and their interactions have been extensively studied, both empirically and theoretically, but their collective roles in guiding optimal behavior have only rarely been addressed. The contribution of episodic memory is particularly mysterious. Here, we develop a normative framework of control in the face of uncertainty, in which each plays a precisely delineated part.

Uncertainty arises in control from inherent stochasticity of the underlying tasks, and ignorance of the controller about the tasks, even if they are otherwise deterministic. Following others, we see the neocortex as a learning system making efficient use of available information in such conditions. A key aspect of such a system is that it represents not only single values of relevant variables, but also the uncertainty surrounding those values, thereby at least approximately performing optimal statistical inference. However, we also observe that while it may be ideal – if at all possible – to keep account of uncertainty for learning, ironically, it is precisely the same careful bookkeeping of uncertainty that renders impractical planning sequences of actions using such a model. The more uncertainty there is to represent, the less feasible it is to model all the consequences of taking an action by enumerating recursively all possibilities that it entails in the future. This problem becomes exponentially harder as the time horizon of the task broadens, making direct approximations, such as pruning or sampling, suffer from serious biases. The striatal habit-based system has been suggested to offer a solution to this problem, but is only effective in the limit of substantial samples.

We suggest that a different class of approximations, namely recalling episodic memories of specific behavioral sequences that proved successful in the past, can be a powerful alternative to the other two systems. The eventual reduction of ignorance-related subjective uncertainty and the steady accumulation of sufficient information to license reliable habits, imply that the hippocampus should be particularly important in the early stages of training on a task or exploring an environment.

Our results suggest normative accounts of the widely observed time-limited role of the hippocampus in processing memories, and the apparently more semantic characteristics of distant memories. This offers a different perspective from the popular, but computationally challenging hypothesis that memories are consolidated out of the hippocampus and into the neocortex, or elsewhere.

IDEAL BAYESIAN LEARNING IN HUMAN SCENE PERCEPTION

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Humans are confronted with enormous amounts of visual information that must be collapsed into meaningful chunks in memory to form the features, objects, and their parts for later recognition. Chunk formation requires a classic model-selection trade-off: choosing the right inventory of chunks that accurately captures the structure of attested scenes, but is not so specific that it fails to generalize to new scenes. Here we address this model-selection problem by asking two questions: what are the optimal learning strategies for the formation of visual chunks, and do humans employ these strategies during unsupervised learning? We implement an ideal learner that uses Bayesian model comparison, a principled statistical solution for the model-selection problem, to extract and store only those chunks that are minimally sufficient to encode the scenes. We show that, in contrast to earlier accounts of human learning, our ideal Bayesian learner can reproduce the results of a large set of previous empirical findings. We also contrast the Bayesian model directly with previous pair-wise associative accounts of learning in a human visual learning experiment. We show that, in accordance with the Bayesian model but contrary to the associative model, human performance is well above chance when pair-wise statistics in the scenes contain no relevant information. Our results suggest that human learners extract complex information from visual scenes by generating optimally economical representations and not by encoding the full correlational structure of the input.

FIRING RATES AND PHASES IN THE HIPPOCAMPUS: A COMBINED MODELING, IN VITRO, IN VIVO APPROACH

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In the hippocampus, there is evidence that information processing involves both the the instantaneous spiking rate of pyramidal cells and the phases of those spikes relative to the ongoing theta oscillation. However, it is as yet unclear how a dual rate-and-phase scheme encodes critical information or how its dynamics support memory processing, a key function accorded to the hippocampus.

We have developed a normative theory as to how hippocampal neural dynamics implement high quality memory retrieval. In the theory, the content of memory traces is specified by the firing phases of pyramidal cells relative to theta cycles, being stored by spike timing-dependent plasticity, whereas the degree of certainty in the current memory being retrieved is conveyed by the number and concentration of spikes per cycle (burst strength). The theory specifies, and therefore predicts, how such spiking neurons should interact according to their relative rates and phases. We have shown that such interactions lead to competent memory retrieval performance in simulated networks.

To test the theory's predictions about the effects of the timing and burst strength of presynaptic stimulation on the timing of postsynaptic firing, we recorded phase response curves from rat hippocampal CA3 pyramidal cells *in vitro*. We also analysed the cycle-by-cycle dynamics of putative excitatory cells in ensemble recordings from the hippocampi of behaving rats. Experimental results are in good qualitative agreement with the theory.

These results suggest that neural interactions in the hippocampus may be optimal for retrieving memory traces encoded by firing phases augmented with an important uncertainty signal conveyed by burst strengths.

CHARACTERIZATION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP)-INDUCED PIAL ARTERIOLAR DILATION IN PIGLETS.

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Pituitary adenylate cyclase activating polypeptide (PACAP) has been found neuroprotective in numerous *in vivo* and *in vitro* experimental models of cerebral ischemia. PACAP-induced neuroprotection *in vivo* - at least partially - is based on its vasorelaxant effect. Little is known, however, on the mechanism of PACAP-induced cerebrovascular changes in newborns often affected by asphyxia and hypoxia.

In this study, we sought to characterize the vascular reactivity to PACAP-27 and PACAP-38 (the two naturally existing molecular forms), as well as shorter (artificial) PACAP sequences in a piglet model that shows good correlation with the vascular physiology of newborn babies. Using the non-selective cyclooxygenase (COX) inhibitor indomethacin (5 mg/kg, iv), the selective COX 1 inhibitor SC 560 (1 mg/kg, iv) and the selective COX 2 inhibitor NS 398 (1 mg/kg, iv), we investigated if COX-derived metabolites play a role in the cerebrovascular effects of PACAP. Further, we determined the effect of the nitric oxide synthase (NOS) inhibition (by N-nitro-l-arginine methyl ester [L-NAME, 15 mg/kg, iv]) on tis process. Finally we tested the influence of short PACAPs (10^{-5} M topically) on PACAP 27 and -38 induced vasodilation.

Anesthetized (Na-thiopenthal 40 mg/kg, ip, followed by α-chloralose 40 mg/kg, iv), ventilated piglets (1 day old, 1-2 kg, n=54) were equipped with closed cranial windows. Pial arteriolar diameters (baseline ~100 μm) were determined via intravital microscopy.

Topical PACAP-27 and PACAP-38 elicited similar, repeatable, dose-dependent pial arteriolar dilations. Percent changes in diameters to 10^{-8}, 10^{-7}, and 10^{-6} M PACAP-38 and to PACAP-27 were 6±1, 16±2, and 40±4, and 9±2, 19±3, 36±4 (mean±SEM), respectively. In contrast, the shorter segments of the peptides (6-27, 6-38, 6-15, 20-31, 1-15) did not display any vasoactivity. Arteriolar dilations to PACAP-38 were abolished 20 min after indomethacin and SC-560 (to 10^{-6} M from 35±4% to -1±1%*, and from 36±6% to 1±1*, *p<0.05), but the response was not significantly affected by NS-398 (to 10-6 M 39±4% and 45±8%, before and after the treatment, respectively). L NAME proved to be ineffective too (to 10-6 M 34±5% and 26±5%, before and after the treatment).

Co-application with PACAP 6 27 and 6 38 (PACAP receptor antagonists) reduced the PACAP induced caliber changes efficiently (for instance the changes were 46±10% and 7±3%* before and after PACAP 6 27 to 10-6 M PACAP38), however other short segments had no impact on this response.

In summary, PACAP is a potent vasodilator in the neonatal cerebral circulation, and this vascular reaction appears to depend critically on COX 1 derived prostanoids, whereas the role of NO seems to be ancillary.

LOW OVERLAP RECALL: EFFECTS OF STOCHASTIC RELEASE ON ASSOCIATIVE MEMORY

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To build a cortical memory storage model some kind of repeatability of the evoked spatial temporal activity patterns is indispensable. Stochastic, low probability synaptic release, low average activity and the relatively sparse cortical network connectivity results in that exact or small error recall of the stored patterns are not achievable at the physiological spike time dependent plasticity (STDP) levels. Stochastic release of the cortical synapses indicates that the organization of the cortical memory is quite different than the basic assumptions of the so far used, even detailed and sophisticated neural network models. As the recall is to be deciphered by other neural networks therefore there is even no need of perfect recall. The only requirements are that the recalled pattern overlap to the stored one should be significantly different then its overlap to other patterns and a similar network should be able to make stable recalls using these noisy patterns as input. A simple neural network model is introduced to model the storage of spatial-temporal activity patterns. Stochastic release caused large input noise in the neurons makes the application of the detailed multi compartment, Hodgkin-Huxley type models futile. However, the model incorporates and based on the known STDP rules. This model show, that pattern with low but significant overlap to the stored ones can be recalled using input biases. Random evoked, recalled patterns are in god accordance with experimental findings. Basin of attraction of the stored patterns can be much higher than that of the earlier models and their inherent randomness minimize the stored patterns overlap which considerably limits the storage capacity of the classical associative memories.

TELEMETRIC INVESTIGATION OF FEBRILE ANOREXIA IN CAPSAICIN TREATED RATS

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After the administration of endotoxins (LPS) fever and sickness behavior develop. The latter consists of sleepiness, altered social behavior, anorexia, etc. Our former studies suggested that the local stretch-induced component of the postprandial hyperthermia can be prevented by perivagal or small-dose intraperitoneal (IP) capsaicin (CAPS) treatment. We also found that the IP, but not the perivagal CAPS treatment abolished the first phase of LPS fever. These data suggested that IP CAPS may influence fever through non-vagal mechanisms, e.g. through an action on the liver. In the present study we analyzed by telemetry whether IP or subcutaneous (SC) CAPS treatment can influence the LPS-induced anorexia. Wistar rats were implanted with biotelemetry transmitters for core temperature, heart rate and general activity measurements. 10-14 days after implantation IP CAPS (2+3 mg/kg) or saline was administered. SC CAPS was given in 5 increasing doses, totaling 400 mg/kg. After recovery a jugular vein cannula was implanted, through which LPS or saline was administered following a 24-h food deprivation. Feeding behavior was recorded by infrared feeding monitor assembly. Without CAPS treatment intravenous LPS administration induced the usual biphasic fever coupled with elevation of heart rate. The general activity was also suppressed in this group. Without LPS the rats ate significant amounts in the first 30-60 min. The LPS-anorexia caused a 30 min delay and strong attenuation of the re-feeding hyperphagia. IP CAPS treated rats showed a similar pattern of LPS-anorexia. After SC CAPS treatment the immediate re-feeding hyperphagia was not abolished by LPS. It is suggested that LPS-fever and LPS-anorexia develop by different ways.

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IMMEDIATE AND ACUTE NEUROBEHAVIOURAL EFFECTS OF A GLUTAMATE AGONIST AND AN ANTAGONIST IN RATS

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The mitochondrial toxin 3-nitropropionic acid (3-NP) is used to model human neurodegenerative diseases in animals. It also has indirect glutamatergic effect and induces behavioral alterations. MK-801, a highly selective non-competitive NMDA antagonist produces hyperlocomotion and impaired sensorimotor gating in rats, and was shown to be neuroprotective in 3-NP treated rats.
In this study, the interaction of 3-NP and the NMDA-antagonist MK-801 was examined in immediately and acutely treated rats on behavioral outcomes.
Male Wistar rats (10 weeks old, 150-160 g body weight, 10 in a group) were treated with a single dose of 20 mg/kg 3-NP i.p. (group 1), 0.8 mg/kg MK-801 (group 2), MK-801 followed by 3-NP (group 3), or the same drugs in reverse order (group 4). Controls were injected with saline. Spontaneous locomotor activity, rota-rod performance, acoustic startle response, and pre-pulse inhibition of acoustic startle were tested before administration, and 30 minutes (immediate scheme) or 24 hours (acute scheme) after it.

3-NP caused horizontal hypo- and local hyperactivity both immediately and acutely. Vertical activity decreased significantly 30 minutes after 3-NP administration. MK-801 had a significant effect on vertical activity in both treatments. The effect of the two substances on the number of noise-positive acoustic startle responses was opposite after 30 minutes but similar after 24 hours. Sensorimotor gating was reduced both by 3-NP and MK-801 in the immediate, and by MK-801 in the acute, scheme.
The effects of the two substances proved to be opposite in several endpoints. Therefore, glutamate antagonists like MK-801 may offer another opportunity in the therapy of chronic neurodegenerative diseases.

SPATIO-TEMPORAL FILTER PROPERTIES OF VISUALLY ACTIVE NEURONS IN THE CAUDATE NUCLEUS

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The motor functions of the basal ganglia have been widely investigated, however, much less is known about the sensory background of their visuomotor coordinating function. Our experiments deal with the analysis of visual information processing in the feline caudate nucleus (CN) in anaesthetized, paralyzed, artificially ventilated, adult cats. Extracellular single-cell recordings were performed via tungsten microelectrodes in 103 neurons in the dorsolateral part of the CN. The neurons were stimulated by drifting visual gratings at various spatial and temporal frequencies. The CN units responded optimally to grating patterns of very low spatial and very high temporal frequencies. Accordingly, the CN units exhibited extremely low spatial and very high temporal resolution. The CN neurons produced fine spatial and temporal tuning. Thus, they can act as accurate spatio-temporal filters. The spatial and temporal visual properties of the CN neurons are very similar to those of other feline extrageniculate visual structures, but they markedly differ from those of the primary visual cortex and the lateral geniculate nucleus. This suggests a functional relationship between the CN and the extrageniculate tecto-thalamo-cortical visual system of the feline brain. The above detailed spatio-temporal visual properties of the CN suggest the role of the CN in motion detection and velocity analysis and the connected behavioral actions.

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ELECTROPHYSIOLOGICAL STUDY OF PEPTIDES EFFECTIVE AGAINST THE SYNAPTOTOXICITY INDUCED BY BETA-AMYLOID PEPTIDE

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Amyloid peptide (Aβ) plays a critical role in the pathogenesis of Alzheimer's disease. Aβ is neurotoxic, a character which correlates well with the degree of its aggregation. In pathological conditions, fibrils and senile plaques develop in consequence of aggregation.
From our previous results it is known that 10^{-5} M Aβ(1-42) is able to produce a long-lasting decrease in cortical fEPSPs elicited through L2/3 stimulation of horizontal connections. In the present experiments we examined the following compounds against the fEPSP amplitude-attenuating effects of Aβ(1-42) at 5-fold over-excess cocktails: MAT1, P29, P59 and MAT5. The P29 showed a 40% decreasing effect in cortical fEPSPs. The MAT1 alone were unable to modulate cortical fEPSPs, while P59 and MAT5 alone caused only a slight decrease (~10%).
Examined in cocktails, the most effective compounds were MAT1 and MAT5 to protect neurons against the attenuating effect of Aβ(1-42) through a mechanism, which needs further examination and could they therefore be promising candidates for pharmaceutical research on Alzheimer's disease.

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INHIBITION OF THE PSEUDORABIES VIRUS SPREADING IN THE NERVOUS SYSTEM: EFFECTS OF FLUORESCENT TRACERS

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The Bartha strain of pseudorabies virus (PrV) retrogradely spreads through chains of neurons in the nervous structure and therefore is used for mapping neuronal networks.
The intriguing question raised, whether PrV and fluorescent tracers (e.g. Fast Blue, FB) can be applied together.
We examined the effect of FB on the PrV infection in rat spinal cord at different survival times after the virus injection and model these processes *in vitro*.
Hindlimb muscles were inoculated with the PrV and after 24 or 48 hours FB crystals were applied to the sciatic nerve. In control rats the muscles were injected with the virus only.
Differentiated neuronblastoma cells were treated with FB and virus was added after one hour incubation time. In controls the cells were treated with the virus only.
In control rats the virus infected the motoneurons after 50 hours and spread into the linked interneurons by 80-96 hours. In animals where the sciatic nerve was labelled with FB 24 hours after the PrV injection interneurons did not become infected at 80h survival time, while after 96 hours survival many interneurons were infected. In rats where FB was applied 48 hours after the PrV injection at 96 hours survival time the virus appeared in motoneurones and interneurones.
In control neuron culture the virus appeared in the cells after 5-6 hours incubation, but in neurons pretreated with FB the appearance of the virus was delayed by about 1 hour.
Our results show that the FB delivered to the primarily infected neurons within 24 hours after PrV injection prevents the PrV infection of the lumbar motoneurons whereas the virus is able to spread to the linked neurones producing productive infection.

ROLE OF EXTRACELLULAR MATRIX IN THE NEURAL REGENERATION AND PLASTICITY

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The macromolecules of extracellular matrix (ECM) play an important role in the regeneration and plasticity of the nervous system; however their specific function is still poorly understood. One of the characteristic places of occurrence of ECM is the perineuronal net (PN), which is a condensation of the ECM around the perikaryon, proximal dendrites and axon hillock. It has been shown that PN plays an important protective role in the maintenance of the normal function of neurons and in the stabilization of synaptic connections. Another distinguished site of occurrence of ECM molecules is the transitional zone (TZ) of motor and sensory radices, where they leave or enter the medullospinal neuroaxis. Growing axons can penetrate TZ in the embryo, but it turns into a barrier against regenerating fibers in the postnatal life. The possible excitatory or inhibitory function of the ECM macromolecules during regeneration can be best studied in lower vertebrates because contrary to the mammalian species the regenerating fibers of these animals grow into the CNS and it is followed by partial or complete restoration of function. We have studied the qualitative and quantitative changes in the distribution of hyaluronan, tenascin C and laminin following trans-section of the optic and vestibulocochlear nerves of the frog in the diencephalic and mesencephalic visual centers and in the vestibular nuclei of the brainstem. Our results demonstrated that the lesion of these nerves is accompanied by the modification of ECM expression pattern, which runs parallel to the functional restoration in the visual and vestibular system.

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CHANGES IN ADAPTABILITY FOLLOWING PERINATAL DRUG EXPOSURE, IN RATS

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Objective: Perinatal exposure to psychoactive drugs induces higher risk of morbidity or mortality. The aim of the present study was to examine the consequences of morphine (MO) exposure during the whole gestation and lactation periods in offspring at different postnatal ages.
Methods: Pregnant Wistar female rats, from the day of mating, were treated daily with MO (10 mg/kg sc.) until the 21st postpartum day (PD), when the offspring was separated. Offspring of female rats treated with physiological saline served as control. Locomotor activity in novel surroundings, behaviour in elevated plus maze test (EPM), resting and forced swimming stress induced corticosterone and ACTH levels were measured at PD 23-25. Intensity of conditioned place preference (CPP) evoked by MO treatment was tested at adult age.
Results: 1. The extinction of the initial investigatory locomotor activity was slower in MO-exposed animals, and their total movement in the EPM apparatus was also higher, however, they did not show either anxiogenic or anxiolytic-like behaviour. 2. Both the resting and the stress-induced corticosterone and ACTH levels were significantly lower in the MO-exposed offspring. 3. MO treatment in CPP test resulted in significantly more intensive place preference in MO-exposed offspring than in the control peers. This effect was stronger in females.
Conclusion: On the basis of these data we may conclude that perinatal MO exposure decreases adaptability and enhances the sensitivity to reinforcing effect of MO, indicating a higher risk of abuse liability.

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THE MYSTERIOUS HIGH AFFINITY DESENSITIZATION OF THE P2X3 RECEPTOR

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It has been proposed that P2X3 receptors possess a unique mechanism of agonist-induced conformational transitions (Pratt et al., J Neurosci 25:7359, 2005). Recovery from ATP-induced desensitization was found to be very slow (taking several minutes) during which period a special agonist binding site was supposed to be formed, which should bind the agonist with high (nanomolar) affinity, and promote desensitization without activation. The authors supposed that this high affinity binding site is absent from "naïve" (not activated) receptors.
In contrast with this hypothesis, Sokolova et al. (Mol Pharmacol 70:373, 2006) argued, that even resting receptors expressed the high affinity agonist binding site, and high affinity desensitization developed regardless of previous agonist-induced conformational changes. They proposed a simple kinetic model of the receptor. Simulations reproduced all peculiar characteristics observed experimentally, without a need to introduce any extraordinary mechanism. However, their kinetic model was incorrect, lacking microscopic reversibility, and proposing a highly unlikely mechanism for coupling between agonist binding and gating.
We attempted to find a more realistic kinetic scheme that could reproduce experimentally observed behavior, and hopefully give a hint on the mechanism of the coupling of agonist binding and receptor gating. We found no difference in the affinity of the binding site depending on previous activation. We studied the kinetics of low agonist concentration-evoked currents in order to test possible activation mechanisms. Based on the concentration-dependence of amplitude and kinetics, we propose a more likely mechanism for the activation and desensitization of the P2X3 receptor.

CHROMATOGRAPHIC ANALYSIS FOR THE MEASUREMENT OF NEUROTRANSMITTER AMINO ACIDS IN TISSUE OVER-FLOW FLUID

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For the separation and determination of neuroactive amino acids and the distribution of labeled glutamate (Glu) or γ-aminobutiric acid simultaneously, a method is presented. The assay is based on a direct injection of 0.5 ml volume of in situ precolumn derivatized isoindol amino acids onto the column. A simple guard column, in an extra loop position achieved the sample enrichment. Method precisions for the different amino acids were between 3.8 and 12.3 % RSD; detection limits were between 0.3-0.9 nM. The method was demonstrated by its application to determination of amino acid neurotransmitters released into superfusion fluid from *in vitro* brain slices.
In vitro ischemic-like conditions evoked a significant ($p<0.05$) increase of Glu release from rat hippocampus slices 18-21 min following the onset of ischemia. At the end of ischemia the rise of metabolism were typical indicated by an increase in the amount of Asp and Ala derivatives; the recovery of [^{3}H] was 78.3±5.5 % (n=12).
Potassium depolarization increased the release of Glu in synaptosomal preparations of both rats and mice (from 137±11.7 and 65.5±12.3 pmol/min to 271.6±56.3 and 81.7±11.1 pmol/min, respectively). Interestingly the Asp concentrations were similarly high either in rat and mouse samples. During routine application of separation the chromatographic behavior of [^{3}H]Glu or [^{3}H]Gaba deviated from fluorescence chromatograms, and the presence of more [^{3}H]atoms led to an increase in the retention time of both compounds and their metabolites. The longer retention time of tritiated isoindol amino acid derivatives then would point to a decreased polarity of these counterparts in comparison with their unlabeled compounds.

CONTRASTING PROPERTIES OF THETA OSCILLATIONS GENERATED THROUGH DIFFERENT MECHANISMS IN A NETWORK MODEL OF HIPPOCAMPAL AREA CA1

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Field oscillations in the theta frequency range are prominent in the hippocampus during specific behavioral states including exploration and REM sleep, and are thought to make an essential contribution to hippocampal computations. The firing patterns of various classes of hippocampal neurons in relation to the theta oscillation have also been determined. However, the nature of the mechanisms which give rise to the observed activity patterns, and to theta rhythmicity itself, have been controversial. The goal of the present study was to analyze, using our existing computer model of the CA1 network, the possible contributions of external and internal factors to theta generation in this area.

Our large-scale network simulations contained simplified models of CA1 pyramidal neurons, basket cells, and O-LM interneurons, and included external inputs from area CA3, entorhinal cortex, and the medial septum. Rhythmic septal inhibition of basket cells in itself resulted in oscillatory activity in the pyramidal cell population. However, even in the absence of phasic external input, networks comprising reciprocally connected pyramidal and O-LM cell populations were also capable of generating theta frequency oscillations. These oscillations required that both afferent input and feedback inhibition target the distal apical dendrites of CA1 pyramidal cells, and depended on the generation of dendritic spikes, but were insensitive to many details of the model cells and network. In the full network, the two combined mechanisms gave rise to a single coherent oscillation phase-locked to the septal input, and phase preferences for all cell types were consistent with experimental data.

THE INTERACTION OF ANANDAMIDE AND ADENOSINE AT SPINAL LEVEL

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Anandamide and adenosine, endogenous cannabinoid and adenosine receptor agonists, respectively, have significant role in pain mechanisms. The goal of this study was to determine how adenosine and/or caffeine influence the antinociceptive potency of anandamide after intrathecal administration.
After obtaining institutional ethical approval, intrathecal catheters were implanted into male Wistar rats. Unilateral inflammation of a hindpaw was produced by carrageenan, and the pain threshold was assessed by paw withdrawal test. caffeine was injected after the determination of post-carrageenan baseline value (-20 min), and the 2nd injection (adenosine) at -10 min, and the 3rd one (anandamide) was applied at 0 min. The paw withdrawal latencies were registered in every 10 minutes until 70 min. Groups were compared by ANOVA with $P<0.05$ considered significant.

Anandamide by itself (1, 30, 100 µg) dose-dependently decreased the thermal hyperalgesia, however, the highest dose caused temporary painful behaviour. Neither adenosine (100 µg) nor caffeine (400 µg) by themselves changed markedly the pain sensitivity; however, their combination caused a short-lasting antihyperalgesia. Both adenosine and caffeine decreased the antihyperalgesic potential of the largest dose of anandamide, while adenosine-caffeine pretreatment temporarily increased its antihyperalgesic effect.

Our results show that adenosine and caffeine has small influence on the antinociceptive potential of anandamide. Since all of these drugs have effects on several receptors and/or systems, the net effect after their coadministration is be due to the complex changes.

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THE ROLE OF CONE-SPECIFIC INPUTS IN CHROMATIC OPPONENCY OF PARVOCELLULAR RECEPTIVE FIELDS

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A typical property of primate parvocellular (PC) neurons is their opponent response to stimulation of long- and medium-wavelength-sensitive (L and M) cones, which underlies red-green color vision. Here, we tested by physiological methods several predictions of the "random wiring" hypothesis, which claims that the two types of cone contribute to the input of PC cells in a random fashion. We measured in the lateral geniculate nucleus of anesthetized paralyzed marmoset monkeys the ratio of inputs from the two types of cone to the excitatory and inhibitory mechanisms of PC receptive fields (RFs). We could essentially isolate these mechanisms by using suitably sized spot and annulus stimuli, respectively. The measured RFs (n=34) ranged from the fovea up to 30 deg eccentricity. According to our results, the entire range of M-L mixtures can be found in both mechanisms. The strength of chromatic opponent response depended on the segregation of cone types to the excitatory and inhibitory mechanisms but it did not require pure input from one cone type to either mechanism. These data are compatible with random wiring. Contrary to its predictions however, chromatic opponent responses did not break down at high eccentricities. Moreover, the inhibitory mechanism was often dominated by one cone type, although its area was on average about 36 times larger. Finally, the contribution of cone types showed a negative correlation between the two mechanisms. These effects may increase the proportion of chromatic opponent neurons in the PC population.

HYPOTHALAMIC, LIMBIC AND MEDULLARY INNERVATION OF THE STOMACH AND THE DUODENUM IN VAGOTOMIZED RATS

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In our previous study, gastric- and duodenum-projecting forebrain and brainstem neurons have been localized by imunohistochemical verification of two types of pseudorabies virus (modified versions of the Bartha strain) injected into the wall of the antrum and the upper part of the duodenum, in rats. The viral labeling is retrograde and trans-synaptic: the virus infects all of the participants of the neuronal pathway from the peripheral terminals up to central neurons at the various levels in the brain, 3-5 days after inoculation. To obtain information about the possible routes of these axons through the vagal nerve *versus via* a multiple transfer through spinal sympathetic neurons, BDA virus with red and BDL virus with green fluorescence were injected in the same rats, 10-14 days after subdiaphragmatic vagotomy. Like in intact rats, 3.5 days after inoculation, sympathetic preganglionic neurons in the spinal cord and vagal neurons both in the dorsal motor vagal nucleus and the nucleus of the solitary tract were virus-labeled in vagotomized rats. The density of labeled cells in vagotomized rats was markedly lower in the vagal nuclei than that in intact rats, at the exactly same post-inoculation period of time. This observation indicates the significance of the spinosolitary tract through vagal neurons may influence the sympathetic outflow to the upper gastrointestinal tract. In both intact and vagotomized rats, a various percentage of neurons in brain areas that project to either vagal or spinal preganglionic neurons (A5, A6 and C1 catecholaminergic neurons, paraventricular nucleus, lateral hypothalamic area) showed single or double virus-labeling, at 4.0-4.5 days after inoculation. Some of these cells established double labeling.

STRUCTURAL BASIS OF ENDOCANNABINOID SIGNALING AT GLUTAMATERGIC SYNAPSES IN THE NUCLEUS ACCUMBENS

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The addictive potential of several abused drugs are based on the alteration of long-term synaptic plasticity in neurons of the brain's reward circuitry. A form of long-term depression is induced by electrical stimulus at prefrontal cortical afferents to the nucleus accumbens and is entirely dependent on an intact endocannabinoid system. In this paradigm, anterograde presynaptic activity measured by postsynaptic type 5 metabotropic glutamate receptors (mGluR5) is translated into a retrograde signal measured by presynaptically located CB_1 cannabinoid receptors. In the present study, we aimed to uncover the precise molecular and anatomical organization of retrograde cannabinoid signaling underlying this form of synaptic plasticity. Modest, but widespread expression of diacylglycerol lipase alpha (DGL-□), the synthetic enzyme of the endocannabinoid, 2-arachidonoyl-glycerol (2-AG) was found in neurons of the nucleus accumbens. Immunocytochemistry revealed a granular localization pattern of the enzyme protein at the light microscopic level. Further electron microscopic analysis demonstrated that this pattern is due to DGL-□ located on the head of dendritic spines at the perisynaptic domain of glutamatergic synapses. Notably, presynaptic axon terminals forming excitatory synapses on these spineheads were immunostained for CB_1 receptors. Double immunostaining showed that these spines can colocalize either D1 or D2 dopamine receptors with DGL-□, thus both types of medium spiny neurons are equipped with the synthetic machinery for retrograde endocannabinoid signaling. Finally, both types of spines were also decorated with mGluR5 receptors. Taken together, our results suggest that molecular interaction between the glutamatergic and cannabinoid systems in triggering endocannabinoid synthesis may contribute to addiction-related synaptic plasticity in the brain region.

ROLE OF ASCENDING ACTIVATING SYSTEMS IN THE REGULATION OF SLEEP-WAKE CYCLE IN RATS

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The alteration of sleep and wakefulness is the most obvious example of biological rhythms. Sleep, however, has homeostatic functions as well, indicated by its rebound following extended periods of wakefulness. After sleep deprivation paradoxical sleep length is restored more or less completely while deep sleep loss is compensated by an increase in its length and intensity. Intensity seems to be related to the power of the delta frequency band of the EEG which depends on the diminished activity of the ascending activating systems. Therefore, we examined the effects of cholinergic, adrenergic, dopaminergic, and serotoninergic drugs on sleep and wakefulness in rats.

The results show that activating systems differently influence the delta power. Activation of the noradrenergic system did not produce any increased need for recovery. In contrast, shorter lasting enhanced activity of the other activating systems caused rebound, which was most prominent after treatment with apomorfine.

Paradoxical sleep was eliminated by yohimbine treatment, but it was not followed by any rebound during the registration time. Similarly, fluoxetine inhibited paradoxical sleep for a long time without any recovery. The rebound of paradoxical sleep could be only seen following the administration of apomorfine. Eserine was ineffective at this dose.

The lack of rebound following enhanced effect of some of the activating systems on the thalamocortical circuitry means that activation of these systems probably do not produce any increased need for restoration. On the other hand, profound and long lasting rebound in other cases indicates some role of that system in sleep homeostasis indicated by enhanced delta activity or lengthened duration of paradoxical sleep.

COMPARATIVE ANALYSIS OF THE EFFICACY OF NMDA ANTAGONIST MEMANTINE IN DEVELOPING RAT NERVOUS SYSTEM

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Several pharmacons acting on different ion channels might be used as antiepileptics. In recent series of experiments we tested the dose-dependent effect of the non-competitive NMDA receptor antagonist memantine in immature nervous system.
In our electrophysiological investigations the changes of evoked responses and paired-pulse stimulations of rat brain were analyzed on 12th, 18th and 25th postnatal days. In *in vivo* experiments memantine was administered intraperitoneally (10 or 20 mg/kg), while in brain slice experiments memantine was applied into the perfusion solution (0.75 and 1.5 μM).
Memantine affected the early component of evoked field potentials in *in vivo* measurements, while *in vitro* it reduced the amplitude of late component. The maximal inhibition was about 31.58 % percent in the *in vivo* and 34.78 % percent in the *in vitro* experiment. The inhibitory effect was most pronounced in 12d and 25d old rats *in vivo* and 18d old rats *in vitro*, however only lower concentration of memantine was effective. In paired-pulse stimulation experiments a relatively strong facilitation of the late component of evoked response was observed in 25d old rats at 50 and 100 ms interstimulus interval.
Our data indicate that memantine as an NMDA channel blocker has considerable inhibitory effect on evoked responses, and weak facilitating influence on short-term plasticity. As memantine posses different inhibitory potency in different age groups, an important postnatal rearrangement of NMDA receptors composition and change in the sensitivity might be supposed in the rats round 18th postnatal day.

These investigations were supported by grants from Hungarian-Czech Intergovernmental S & T Cooperation Programme (CZ-7/04).

COMPLEMENT C5a RECEPTOR IS EXPRESSED IN THE NEURONS OF HYPOTHALAMIC BRAIN SLICE AND MODULATED BY ESTROGEN

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The brain has its own immune system including the complement cascade. The neuroendocrine hormones are known modulators of the peripheral immune response, however, little is known about this function in the brain. Our earlier results showed that neurons of the gonadotropin hormone-releasing hormone (GnRH)-producing GT1-7 cell line of hypothalamic origin express complement C5a receptor (C5aR) and that 17beta-estradiol (E2) increased expression of the C5aR in these cells. In addition, E2 pretreatment elevated the C5a-agonist evoked calcium influx in the GT1-7 neurons. In the present study we examined presence of the C5aR in hypothalamic neurons of the brain slices of the rat and mouse and effect of estrogen on the C5a-agonist (PL37-MAP) evoked calcium influx using whole cell clamp electrophysiology. Magnocellular neurons of the supraoptic and paraventricular nuclei responded to the PL37-MAP peptide with inward ion current pulses. In addition, GnRH-producing neurons of the brain slice of the GnRH-GFP transgenic mice also reacted to the peptide treatment with calcium influx. The amplitude of the inward ion current elicited by PL37-MAP in GnRH neurons of the brain slice of ovariectomized animals, however, was significantly lower than in slices obtained from E2-substituted animals. The hypothalamic neuroendocrine neurons could therefore be putative sites in the brain where immune and neuroendocrine systems can interact. Our results thus provide better understanding of the inflammation-induced defects of the hormone regulation and role of hormones in the immune responses.

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NEUROPROTECTIVE EFFECT OF OXALOACETATE IN CORTICAL PHOTOTROMBOTIC LESION MODELL

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It is well established that abnormally high glutamate levels in brain tissue are the hallmark of several neurodegenerative conditions that result from acute events, such as cerebral ischemia, traumatic brain injury or epilepsy. The removal of excess glutamate could prevent the glutamate excitotoxicity that causes long-lasting neurological deficits. Nowadays attention has been given to the Glu transporters present on brain blood vessels. Extracellular Glu is transported via Na^+-dependent transporters located on the antiluminal membrane and accumulates into endothelial cells. When its concentration exceeds that in plasma, Glu is facilitatively transported across the luminal membrane into blood. The larger Glu concentration gradient between ISF/CSF and blood plasma could provide an increased driving force the brain-to-blood Glu efflux. This driving force was achived by exploiting the Glu scavenging properties of the blood resident enzyme glutamate-oxaloacetate transaminase (GOT) in the presence of the respective Glu co-substrate oxaloacetate (OxAc).
In the present work we tested the prediction that oxaloacetate-mediated blood glutamate scavenging causes neuroprotection in a pathological situation such as cortical photothrombotic lesion. The cortical photothrombotic lesion was carried out by i.v. injection of Rose Bengal (3 mg/100 g) and cold light exposure (20 min). Animals in treated group were administered with i.v. oxaloacetate (50 μmole/min 30 min) immediately after cold light exposure. Four hours later, the animals were transcardially perfused, the brain were cut into 36 μm thick slices and the section stained with Fluoro-Jade B (FJB). The volume of the hemispheric lesion and the number of FJB pozitive cells were calculated for each animal. The results demonstrated that the lesion extension and the number of FJB lebelled cells were significantly smaller in OxAc treated group. It is concluded that even a single posttraumatic administration of OxAc may be of substantial therapeutic benefit in the treatment of focal brain injury.

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STUDY ON THE CHANGES OF GLUTAMATERGIC RECEPTOR SYSTEM IN 4-AMINOPYRIDINE INDUCED EPILEPSY MODEL

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4-aminopyridine (4-AP) application provokes relatively short convulse episodes. In the present investigation the effect of repeated onset of seizure was studied on neocortical excitability. The changes of underlying glutamatergic transmission system were analyzed using electrophysiological and histoblot methods.
Male Wistar rats received daily intraperitoneal dose of 4-AP during 12 days. Horizontal brain slices were prepared one day after finishing treatments. Extracellular field potentials, evoked by corpus callosum stimulation, were analyzed. Changes in the excitability and the effect of different glutamate receptor antagonists (GYKI 52 466 and APV) was tested. Alteration in glutamate receptor subunit-composition was determined in parallel investigations.
Amplitude of early component of evoked potentials was slightly increased in slices obtained from treated animals, while late components were usually smaller. AMPA receptor antagonist GYKI 52 466 decreased the amplitude of early components, NMDA receptor antagonist APV had a stronger inhibitory effect on late components, respectively. The alterations, however, were not significant in every case. In parallel histoblot measurements slight alterations in number of AMPA and NMDA subunits were detected.
4-AP applications were usually followed by one, exceptionally two ictal episodes. Between treatments and during the subsequent period no phenotypic interictal discharges were detected. So we can conclude that remainder effect of 4-AP differs from the late effects of other convulsants (e.g. kainite or pilocarpine), which suggests that there might be different underlying processes taking place in receptor structure and network activity.

ELECTROPHYSIOLOGICAL INVESTIGATION OF CALCIUM-INDEPENDENT CALPAIN ACTIVATION ON RAT BRAIN SLICES

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Calpains, the cytoplasmic Ca^{2+}-activated proteases, have been suggested to play an important role in several physiological and pathological neuronal processes. Calpastatin protein is specific endogenous inhibitor of ubiquitous calpains. Previous biochemical investigations shoved that calpains might be activated on a Ca^{2+}-independent way by synthetic peptides, which contain equivalent amino acid sequences as A & C domains of calpastatin.
The aim of our present experiments was to check the hypothesis that the calpastatin-derived peptides can activate calpains also in living systems. If these activation proved to be successful, an opportunity can be developed to investigate calpain-effects without activation of any other Ca^{2+}-dependent cytoplasmic processes.
Experiments were performed on neocortical and hippocampal rat brain slices, extracellular field responses were compared. Changes in excitability and short- and long-term plasticity were examined in paired-pulse tests and following LTP induction. Activators were applied during a pre-incubation period.
A slight but significant dose-dependent increment in the excitability was detected in both types of slices. Synaptic responses shoved both short and long term facilitation. At higher peptide concentration, however, over-activation and synaptic suppression developed.
We can state that the activator assembly is functioning also in *ex vivo* conditions, the measure of exposition is critical, too high activator concentration or prolonged exposure may produce exaggerated responses, the sensitivities of different brain areas are also different.

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EFFECTS OF LOW-FREQUENCY ELECTROMAGNETIC FIELD IN RAT BRAIN SLICES

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Exposure to low-frequency electromagnetic field is supposed to cause non-specific health problems. Our aim was to examine if there could be any changes in neuronal excitability underlying these phenomena.
Long-term (60 hours, 500 μT) *in vivo* and short-term (1 hour, 350 μT) *in vitro* EM field (50 Hz) exposure was applied. Treated rats were sacrificed 3-10 days after treatment (long-term effect), while treated slices were used within a few hours (short-term effect).
Experiments were performed on neocortical and hippocampal slices by means of measuring extracellular field potentials. Effects on the excitability and short- and long-term plasticity were examined, paired-pulse tests were performed and long-term potentiation (LTP) was induced. Spontaneous seizure activity was studied using Mg^{2+}-free Ringer-solution (MFR).
Amplitude of the evoked potentials in slices obtained from chronic animals was larger than those from the controls, while in *in vitro* treated slices, the responses were smaller. This tendency could be observed in both cortical and hippocampal slices.
Paired-pulse inhibition (neocortex) and facilitation (hippocampus) weren't influenced by EM exposure. The efficacy of LTP-induction was also nearly the same in the control and in both treated groups.
After treatment with MFR, spontaneous seizure-like activity was more likely to develop in control and *in vivo* treated slices than in *in vitro* treated slices. *In vivo* treatment promoted the development of a second component of the evoked response, while *in vitro* treatment seemed to inhibit it.
Our results demonstrate that exposure to low-frequency electromagnetic field can cause slight short- and long-term changes in basic neuronal activity. It appears that an inhibitory effect occurs shortly after treatment but after several days the effect turns into excitation.

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PARVALBUMIN KNOCK-OUT ANIMALS: DISAPPEARENCE OF PARVALBUMIN BUT PERSISTENCE OF GABA IN LARGE CALYCIFORM PRESYNAPTIC TERMINALS OF THE RETICULAR THALAMIC NUCLEUS AND SIMULTANEOUS APPEARENCE OF ONCOMODULIN IMMUNOREACTIVE AXONS IN LAMINA MEDULLARIS EXTERNA OF THE THALAMUS

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Coexistence of GABA and the calcium binding proteins parvalbumin, calbindin, calretinin and calcineurin in giantic calyciform presynaptic terminals of the reticular thalamic nucleus [1, 2] raises the question whether or not, GABA and calcium binding proteins are not only structurally but also genetically related to eachother. Young adult male homozygous parvalbumin-knockout (PV -/-) mice [3] were subjected to immunohistochemical analysis after transcardial fixation with 4 % formaldehyde, 0.5 % glutaraldehyde added. Brains have been removed and processed in an ascending series of sucrose, containing 4% formaldehyde. Serial cryostat sections, 20 um thick, were obtained in the paramedial plane, comprizing the thickness of 1-3 mm as measured from the midsagittal plane. The reticular nucleus of the thalamus has been localized using the parameters of the Sidman-Angevine-Pierce stereotactic atlas [4] It was found that GABA immunoreaction of giantic calyciform presynaptic terminals persisted in PV knockouts, while PV disappeared completely, not only from neuronal parikarya but also from calyciform presynaptic terminals. At the same time, numerous varicous PV immunoreactive axons appeared in the lamina medullaris externa, surrounding the thalamus; these axons were found to contain oncomodulin or beta-PV [5, 6], a protein which was known until now to be present in normal mammals only in the Corti organ of the inner ear [7, 8]. . At the same time, numerous varicous PV immunoreactive axons appeared in the lamina medullaris externa, surrounding the thalamus; these axons were found to contain oncomodulin or beta-PV [5, 6], a protein which was known until now to be present in normal mammals only in the Corti organ of the inner ear [7, 8]. Oncomodulin-immunopositive fibre bundles originate from small oncomodulin-immunoreactive neurons and are closely related to ill-defined oncomodulin-immunoreactive cellular structures, resembling macrophages. Accordingly, our studies seem to support a recent theory [9] about the origin of oncomodulin from macrophages, and its role in the outgrowth of axons. The possibility to induce axonal regeneration in the central nervous system by application of oncomodulin presents an unsurpassed challenge for clinical neurologists and neurosurgeons. The question of the genetical background of calcium-binding proteins in GABA-ergic synapses, however, remains enigmatic.

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PERCEPTUAL AND NEURAL MECHANISMS OF DECISION MAKING ABOUT MOTION DIRECTION

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Decision making in a visual discrimination task depends on how strong and unambiguous is the task-relevant sensory signal. Our goal was to investigate the neural correlates and dynamics of decision making using electroencephalography (EEG).
Observers performed a motion direction discrimination task. Discrimination difficulty was regulated by changing the percentage of coherently moving dots in the random dot motion display used as stimulus (there were six coherence levels). EEG (64 channels) data were analyzed using the traditional average-based method as well as using a newly developed single trial analysis, involving a linear discriminator.
Average event related response (ERP) amplitudes on the occipito-temporal electrodes were modulated by the motion coherence level within two intervals: 250-380 ms after stimulus onset amplitudes were more negative whereas between 400-600 ms they were more positive with increasing motion coherence. On the parietal and frontal electrodes, however, motion coherence-dependent modulation started later: amplitudes become more positive starting from 350 ms as coherence was increased. The onset delay of the later component on the occipito-temporal electrodes and the onset of the modulation on the parietal and frontal electrodes was inversely correlated with motion coherence.
These results suggest that decision difficulty is reflected in ERP responses and that in the motion discrimination task used in our study decision difficulty component arises ~ 350 ms after stimulus presentation.

fMRI-PERIMETRY: SIMULTANEOUS NEURAL AND BEHAVIORAL MAPPING OF THE VISUAL FIELD

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Visual field loss is characteristic symptom of various ophthalmologic disorders, e.g. glaucoma, optic neuritis, etc. A critical unresolved question concerns the plastic brain processes that are triggered by these disorders.
Our goal was to develop an fMRI-perimetry method, which would allow (1) simultaneous mapping of perceptual and neural visual field deficits as well as (2) assessment of neural plasticity processes underlying spontaneous and medication-induced visual field recovery.
Neural and behavioral function of a patient with unilateral anterior ischemic optic neuritis was evaluated on both eyes. The subject reported – in 2-AFC manner – on the color of a 1.66° diameter circle patch pseudo-randomly overlaid on standard fMRI retinopic mapping stimuli. fMRI acquisition was done at 3T (Philips Achieva 3T, 18 functional slices perpendicular to the calcarine, TR: 1.2 s, resolution: 3.4×3.4×3.4 mm), data was preprocessed, analyzed and visualized in Brainvoyger QX using standard methods.

fMRI-perimetry provided a reliable voxel-based quantification of the extent of neural visual field deficits. The pattern of BOLD responses in retinotopic visual cortical areas – in particular in the primary visual cortex – showed close correlation with the performance in the perimetry tests obtained during scanning.
In conclusion, fMRI-perimetry is suitable for the simultaneous assessment of the functional and behavioral aspects of visual field loss, thus providing an opportunity to investigate neural plasticity in affected patient populations.

BRIEF, REPEATED SEIZURES INDUCE PLASTIC CHANGES IN DISTRIBUTION OF IONOTROPIC GLUTAMATE RECEPTOR SUBUNITS IN THE HIPPOCAMPUS OF RATS

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Repeated, abnormal, synchronized discharges of cortical neurons are considered to be epileptogenic. The neurochemical background of epileptogenesis was investigated in rats subjected to daily epileptiform seizures.
Male Wistar rats were injected daily with 4-aminopyridine (4-AP) for 12 days. One group of animals was sacrificed one day after the series of 4-AP injections, and the other group was killed 5 weeks later. Optical density of ionotropic glutamate receptor subunits were measured in layers of the hippocampal formation from animals of both groups by histoblot technique. The brains of the longer-surviving rats were used to evaluate the density of the mossy fibers using Timm's staining. For spatial memory test, we applied the Morris water maze procedure.
Daily treatment with 4-AP resulted in an increased NR2A level, while NR2B remained unchanged. The GluR2 subunit, which regulates Ca^{++} via AMPA receptors, decreased significantly in every layer. The density of mossy fibers in the str. lucidum increased three times of the control level. During the memory tests, the animals with induced convulsions scored significantly weaker.
Our results indicate that short, repeated convulsions evoke changes in the glutamatergic transmission, in the background of which the decrease of GluR2 subunit may be determinant, since the increased level of intracellular Ca^{++} ions may inhibit the NMDA receptors. This explains the weaker performance of the animals in the memory tests.

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GUSTATORY STIMULATION ELICITED CHANGES IN THE HUMAN BRAIN: AN fMRI STUDY

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Functional magnetic resonance imaging has been gaining increasing significance in elucidating physiological and pathological processes of the human brain. Although the technique has been widely used for studying several functions, to date it was rarely employed in the investigation of brain mechanisms associated with taste perception. In the present series of experiments, therefore, our purpose was to detect cerebral fMRI activity changes following gustatory stimulations in healthy subjects.
Six healthy adult subjects (1 woman and 5 men) participated in this study. The investigations, in two sessions, took place in a 1T Siemens MR scanner at the Pécs Diagnostic Center. The solutions (in 5 ml volume) were delivered via polyvinyl tubes positioned intraorally. In the first session, sucrose (0.1 M), a pleasant taste, in the second session, quinine HCl (0.5 mM), an aversive taste were used as stimulus during the active phase. In both sessions, 5 ml of distilled water was delivered in the passive phase. A standard headcoil and a standard EPI sequence (TR/TR: 2500/80 ms, flip angle: 90º, receiver bandwith: 752 Hz, filed of view: 192 mm, matrix: 64x64, slice thickness: 6 mm) were employed. Group analysis was made by using SPM5 software package.
The two hedonically differential taste solutions elicited characteristic activity changes. Activation was found in the insula and the anterior cingulum after the pleasant taste sucrose. The aversive taste quinine led to activation in the insula, the cingulum, and the amygdala as well.
After having demonstrated gustatory stimulation elicited changes in the brain of healthy subjects, our purpose is to extend these studies to the field of taste perception alterations in pathological conditions.

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EXPRESSION OF HCN2 ION CHANNELS IN THE SUPERFICIAL SPINAL DORSAL HORN OF RATS IN FREUND'S ADJUVANT-INDUCED INFLAMMATORY PAIN CONDITION

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In one of our previous papers we have demonstrated that hyperpolarization-activated and cyclic nucleotide-gated cation channel subunit 2 (HCN2) is strongly expressed by terminals of peptidergic nociceptive primary afferents in laminae I-IIo of the rat spinal dorsal horn. The majority of HCN2-immunoreactive primary afferent terminals also contain substance-P indicating that HCN2 ion channel mechanisms may play some role in spinal pain processing. In the present study, we investigated how Freund's adjuvant-induced inflammation of the hindpaw influences expression of HCN2 ion channels in the lumbar spinal dorsal horn of rats. The distribution of HCN2 protein was investigated in laminae I-II of the dorsal horn by immunocytochemical methods at the level of the spinal cord where the inflammation of the hindpaw evoked elevated c-Fos expression. We found that Freund's adjuvant-evoked inflammation of the hindpaw did not cause any substantial change in the density of HCN2 immunoreactive axon terminals in laminae I-II of the spinal dorsal horn. On the other hand, however, the co-localization between HCN2 and substance-P was found to be altered. HCN2 immunoreactivity was detected in more axon terminals that was negative for substance-P in the experimental than in control animals.

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GFAP AND EZRIN IMMUNOREACTIVITIES ARE DIFFERENTIAL MARKERS FOR REACTIVE CHANGES IN ASTROCYTIC PROCESSES FOLLOWING TRANSIENT ISCHEMIA

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Protective functions of astrocytes are compromised during ischemia and reperfusion resulting in glutamate excitotoxicity, oxidative stress, acidosis and ultimately neuronal death. The glial-neuronal communication through the extracellular space is mediated by the peripheral astrocytic processes (PAPs), the short term plastic changes of which are thought to be regulated by the ERM protein, ezrin.
The goal of the present study was to reveal the effects of ischemia and reperfusion (0h, 0.5h, 1h, 2h, 4h, 24h or 48h) on ezrin immunoreactivity (IR) in the rat forebrain and compared to GFAP-IR after one hour occlusion of the middle cerebral artery (MCAO). GFAP is present in the perikaryon and primary processes of astrocytes, but is absent from the PAPs.
In control animals, strong ezrin-IR was detected in the cortex, striatum, hippocampus and lateral septum. Moderate IR was observed in the thalamus and globus pallidus. The IR in the hypothalamus was rather low. Astrocytic territories were marked by the immunoreactive PAPs, but individual cells could not be distinguished, as the astrocytic stem processes and the perinuclear region contained little ezrin.
In contrast to the GFAP-IR, 1h MCAO resulted in a significant decrease of ezrin-IR in the cortex and striatum and a circumscribed region of the preoptic/hypothalamic area on the ipsilateral side. In these regions the ezrin-IR exhibited a further reduction with increasing reperfusion time. In addition, a high number of astrocytes showed increased perikaryal ezrin-IR on the ipsilateral side, less were observable on the contralateral side. In 24h samples ezrin-IR was greatly reduced in the ipsilateral striatum and cortex, but an intensively stained penumbra appeared next to the area of infarct. By 48 hours, striatum and cortex became immunonegative, but ezrin positive astrocytic perikarya were still present in reduced number. These suggest that changes in ezrin-IR detect early effects of ischemia on the PAPs, as well as reveals secondary effects, induced possibly by edema on the contralateral side. Changes in blood supply of the brain are accompanied by changes in ezrin-IR and possibly in patterns of PAPs. The latter clearly needs to be confirmed at the ultra structural level.

GONADOTROPIN RELEASING HORMONE PRODUCING CELLS EXHIBIT MITOCHONDRIAL IMMUNOREACTIVITY FOR ESTROGEN RECEPTORS

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Estrogens have been implicated in multiple physiological and pathological processes in mammalian cells. Many of these processes are mediated via estrogen receptors α and β (ERα; ERβ). The possible association between these ERs and mitochondria has been examined in rat and mouse preoptic neurons, including gonadotropin releasing hormone (GnRH)-producing cells. Immunocytochemistry with ER antibodies raised against the F domain of the receptor proteins revealed both types of nuclear immunoreactivity (IR) in the preoptic area after ovariectomy. In addition, a punctate ERα signal was apparent extranuclearly; this signal was attenuated by estrogen replacement. At the ultrastructural level, the extranuclear IR appeared to be associated mainly with mitochondria. Immunocytochemical double-labeling identified ERα-IR in mitochondria in GnRH neurons and in other unidentified neurons. Subcellular distribution of ERs was also investigated in GnRH-secreting immortalized GT1-7 cells. Fixation and permeabilization conditions greatly influenced the detectibility of ERs within GT1-7 cells. After mild fixation and permeabilization with Triton X-100, cells showed immunoreactivity primarily within the nucleus. In contrast, a stronger fixation and avoidance of Triton X-100 resulted in ERα- and ERβ-IR in the cytoplasm. Simultaneous detection of ERs and mitochondrial markers showed an association between the extranuclear ERα and ERβ signals and mitochondria in GT1-7 cells. The presence of the ER-IR in GT1-7 mitochondria was confirmed by electron microscopy. Given the evidence for a protective role for oestrogen in mitochondria (Nilsen & Brinton, 2004), we speculate that the mitochondrial ERs detected in this study may provide supportive functions for GnRH neurons.

FEED-FORWARD NETWORKS ACTIVATED BY SINGLE PYRAMIDAL CELLS IN THE HUMAN CEREBRAL CORTEX

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Functional characterization of microcircuits of the cerebral cortex of rodents, carnivores and to some extent, monkeys has been propelled by simultaneous multiple recordings of neural connections combined with anatomical and molecular analysis of the recorded cells. In the human cortical microcircuit, however, only single cells were characterized to date, interactions between identified neurons were not studied. We recorded from pairs, triplets and quadruplets of neurons and analysed their interconnections in slices of human association cortices. Single action potentials in identified human pyramidal cells frequently triggered reliable and stereotyped series of multiple postsynaptic potentials in simultaneously recorded pyramidal cells and interneurons. Such temporally correlated series of postsynaptic events were composed of both excitatory and inhibitory responses and lasted up to ~35 ms. A limited number of synaptic junctions was sufficient for triggering postsynaptic action potentials in interneurons by single spikes in pyramidal cells. The amplitude of EPSPs evoked by pyramidal cells in human interneurones was significantly higher than those recorded in rat. Distinct type of interneurons contributed to polysynaptic IPSPs triggered by single pyramidal action potentials and feed-forward inhibition could be up- and downregulated pharmacologically.
Our results show that groups of human neurons are functionally coupled by spike-to-spike transmission suggesting that powerful and abundant feed-forward connections increase the saliency of single neurons and support effective propagation and retrieval of complex information packages in the human neocortex.

THE ROLE OF ZINC ION IN NEURODEGENERATION AND NEUROPROTECTION

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Zinc (Zn^{2+}) is a very important trace element in biological systems. The main role of the vesicular Zn^{2+} is the neuromodulation. It is redox inert and has structural, catalytic, and regulatory roles in cellular biology. The role of zinc is versatile: it is a co-factor of over 200 proteins and enzymes. The sources of chelatable zinc pool are vesicular compartments, loosely zinc-binding proteins, and Zn^{2+}. Among these, the vesicular zinc pool is quantitatively the most significant. Chelatable Zn^{2+} is easily detectable by using various histological techniques e.g. fluorescent methods.

Zn^{2+} is released together with glutamate from the hippocampal mossy fiber terminals during spontaneous activity after stimulation. It is evoked electrically or by administration of potassium ions. The amount of Zn^{2+} that may be released from synaptic vesicles of the mossy fiber terminals is approximately 8% of the total hippocampal Zn^{2+}. Zinc transporter 3 (ZnT3), which is a membrane bound transporter protein, is responsible for the uptake of Zn2+ into the synaptic vesicules.

The abnormal Zn^{2+} level in the brain causes high risk of the development of neurodegenerative diseases. Zn^{2+} is a double-faced ion: both the decreased and the increased level of this element are toxic. By low Zn^{2+} concentrations, essential biochemical processes are impaired; at high concentration of Zn^{2+} Aβ aggregation and plaque formation are enhanced. Metallothioneins (MTs) prevent Zn^{2+} deficiency and toxicity *in vivo*. MTs are able to bind up to a total of 7 g Zn^{2+} per mol thionein protein. Old MTs are unable to compensate the Zn^{2+} equilibrium shifts, therefore Zn^{2+} may be accumulated at high and toxic concentration in the brain.

Alzheimer's disease (AD) is characterized by amyloid deposits within the brain. The main component of these predominantly extracellular collections is the amyloid beta protein (Aβ). Considerable evidence has indicated that the aggregation and toxicity of Aβ in AD are mediated by impaired interactions with metal ions, especially with Zn^{2+}, Cu^{2+} and Fe^{3+}.

USE OF TAIL FLICK METHOD IN STREPTOZOTOCIN-INDUCED DIABETES FOR MEASUREMENT OF ANALGESIC EFFECT OF DRUGS IN MICE

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Abstract:
Neuropathy is one of the most common complications of diabetes accompanied by complex disorders. In the past decades, a number of pharmacological approaches have been developed to alleviate the diabetic neuropathy. However, clinical potential of present therapeutics is not satisfactory due to limited efficacy or significant adverse effects.
The aim of the present study was to establish an experimental animal model to assess the effects of test compounds with different mechanisms of action. Administration of a single dose (250 mg/kg, i.p.) of streptozotocin (STZ) in NMRI mice induces type 1 diabetes, and subsequently, diabetic neuropathy (DNP). Following STZ-treatment the hyperglycemia and hyperalgesia are developed within a two week period. The DNP was measured using the tail flick assay. The latency (L) of the tail flick response at pre- and post-treatment with test drugs was measured using a tail flick analgesia meter (IITC). The analgesic effect of the selected drugs was characterized by calculating the percentage reversal of hyperalgesia: Reversal $\%=((L_{postDrug}-L_{preDrug})/(L_{preSTZ}-L_{preDrug}))*100$; Compounds were tested on days 10-20 post-STZ. Following acute administration of Morphine (5 mg/kg, p.o), Gabapentin (300 mg/kg, p.o), Duloxetin (50 mg/kg i.p) and SSR-240612 (bradykinin B1 receptor antagonist, 30 mg/kg, p.o.), the thermal hyperalgesia was alleviated by increasing the tail flick reaction time by 113%, 93%, 38% and 76% respectively.
This experimental animal model provides a sensitive and reproducible tool for the evaluation of new analgesic compounds for treating DNP.

PREDICTION OF OUTCOME IN SEVERE TRAUMATIC BRAIN INJURY BASED ON COMPUTED TOMOGRAPHIC EXAMINATIONS

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The Marshall computed tomographic (CT) classification identifies six groups of patients with traumatic brain injury (TBI), based on morphological abnormalities on the CT scan. This classification is increasingly used as a predictor of outcome. While nowadays this is the one and only widely used scoring system it is not considered predictive in terms of outcome.
In our study we have analyzed 99 patients initial CT scan based on our severe head injury databank. In 46 cases we could analyze only conventional printed CT images, while in the remaining 53 cases we carried out an accurate computed analysis of digital images with DicomWorks 1.3.5 software. Beside Marshall's classification we examined several other abnormalities of CT scans frequently related to elevated intracranial pressure in clinical observations. For statistical processing we used logistic regression analysis.
Our results indicate that the Marshall CT classification based on only printed CT images has not provided a significant relationship with the outcome (p=0,106). On the other hand when we utilized computer based algorithm to measure the volume of intracranial haemorrhages, according to the advice of the latest protocols, we got an unambiguous connection with mortality (p=0,009). Further our results show that the addition of some of other investigated parameters (traumatic subarachnoid haemorrhage; skull fracture with impression etc.) could significantly improve the predictive power of Marshall CT classification.

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COMPARATIVE DISTRIBUTION OF ANDROGEN-RECEPTOR- AND VESICULAR GLUTAMATE TRANSPORTER 2-CONTAINING NEURONS IN THE SEPTUM, HYPOTHALAMUS AND AMYGDALA OF THE RAT

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We examined the presence of androgen receptors in glutamatergic neurons. As first step we tried to get information about the distribution of glutamatergic neurons (using vesicular glutamate transporter 2, VGluT2 as marker of glutamatergic elements) and androgen receptor-containing nerve cells in the lateral septum, hypothalamus and amygdala. The cell density of the androgen receptor ir. and VGluT2 ir. neurons was the following: lateral septum: 4+/2+; median preoptic nucleus: 3+/2+; medial preoptic area: 3+/3+; anterior periventricular nucleus: 3+/2+; paraventricular nucleus: 2+/2+; arcuate nucleus: 3+/2+; ventromedial nucleus: 4+/3+; ventral premamillary nucleus: 4+/2+; lateral mamillary nucleus: 3+/3+; amygdala: 4+/2+ and the internal capsule: 3+/3+. So far we detected colocalization of androgen receptor and VGluT2 in the hypothalamic ventromedial and arcuate nuclei, the mamillary complex and the internal capsule. Besides cell nuclear labeling we observed extranuclear androgen receptor labeling in the cell bodies and dendrites of the bed nucleus, the amygdala and the internal capsule. Our observations are consistent with the view that glutamatergic neurons may be involved in the mediation of the action of androgens on the central nervous system.

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VESICULAR GLUTAMATE TRANSPORTER 2 FIBERS TERMINATE ON VASOACTIVE INTESTINAL POLYPEPTIDE-, ARGININE-VASOPRESSIN- AND GABA-CONTAINING NEURONS OF THE RAT SUPRACHIASMATIC NUCLEUS

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The hypothalamic suprachiasmatic nucleus is the key-structure of the control of circadian rhythms. We studied the relationship between glutamatergic axon terminals and vasoactive intestinal polypeptide (VIP), GABA and arginine-vasopressin (AVP) neurons of the cell group. In addition, we examined the presence of glutamatergic neurons in the nucleus. Vesicular glutamate transporter 2 (VGluT2) was applied as marker of the glutamatergic elements. Single- and double-label immunocytochemistry was used. VGluT2 ir. fibers and terminals were distributed in all parts of the nucleus. The most intense network was observed in the ventrolateral part of the cell group. There were only very few VGluT2 positive elements in the dorsomedial part. Double label immuno-electron microscopy revealed that most of the VGluT2 boutons terminated on GAD-67 ir. neurons distributed in the whole cell group. Less frequently, VGluT2 axon terminals formed synaptic contact with VIP positive neurons concentrated in the ventrolateral part of the cell group. Synapses between AVP positive neurons located in the dorsomedial part of the nucleus and VGluT2 terminals were also seen, however, only very rarely. The ultrastructure of the synaptic contacts showed in each case the asymmetric type. In addition, we detected VGluT2 ir. perikarya which did not contain VIP, AVP or GAD-67. The VGluT2 containing neurons were found to be in synaptic contact with GAD-67 and/or VGluT2 positive axon terminals.

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GLUTAMATERGIC AFFERENT INPUTS TO CHOLINERGIC AND GABAERGIC NEURONS LOCATED IN VARIOUS SUBREGIONS OF THE RAT BASAL FOREBRAIN

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The aim of the present study was to investigate glutamatergic terminals synapsing on cholinergic and GABAergic neuronal elements in subdivisions of the basal forebrain (BF). The combinations of vesicular glutamate transporter type 2 (VGluT2)-immunocytochemical silver-gold labelling with DAB staining of cholin acetyltransferase (ChAT) or vesicular acetylcholin transporter (VAChT) as cholinergic and parvalbumin (PV) or GAD-67 as GABAergic markers were used. The frequency of occurrence of synaptic contacts between VGluT2-labelled terminals and DAB-stained cholinergic or GABAergic target elements was estimated in the horizontal diagonal band (hDB), the ventral pallidum (VP) and the substantia innominata (SI). - EM findings: VGluT2-gold labelled axon terminals occurred regularly in hDB and the VP-SI areas. On sections double-labelled either with VAChT or GAD-67/PV gold-labelled terminals made synaptic contacts on unidentified and on DAB-immunostained target sites immunopositive for VAChT or GAD-67/PV, forming in all cases the asymmetrical type of synapse. Synaptic contacts on GABAergic targets were seen more frequently compared to cholinergic neuronal elements. On sections double labelled but DAB-negative perikarya were also observed in VP and SI subdivisions. - The data suggest: a) glutamatergic innervation in various subdivisions plays an intense role in excitation of neurons participating in BF functions; b) GABAergic neurons in these subdivisions are mainly the direct targets for glutamatergic inputs; c) intrinsic glutamatergic neurons are also present in the investigated BF areas.

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CHEMICAL-NEUROANATOMY OF THE 5-HTERGIC SYSTEM IN THE BUCCAL COMPLEX OF THE SNAIL (HELIX AND LYMNAEA)

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Serotonin (5-HT) is a general modulator in the snail nervous system, including its role in the regulation of feeding behavior. Although the distribution of 5-HT immunoreactive (IR) neurons was described in details in the CNS of the pulmonate snails *Lymnaea stagnalis* and *Helix pomatia*, little attention has been paid to the organization of the 5-HTergic system in the buccal ganglia and the buccal mass, responsible for the execution of active feeding. In this study, therefore, we analyzed two aspects of the 5-HT-IR innervations: the organization and possible form(s) of axo-somatic and neuromuscular connections. The *nerve cell body layer of the buccal ganglia* was richly supplied with 5-HT-immunoreactive (IR) processes. Most of the neural perikarya, including the identified giant B1-4 efferent neurons, were surrounded by immunolabeled varicose processes. These fibers originated from the buccal ganglion neuropil containing a dense arrangement of 5-HT-IR axon processes branching from the primary axon of the identified giant 5-HTergic neuron (CGC-*Lymnaea*/MGC-*Helix*). In the *buccal mass*, 5-HT-IR bundles entering arborized first into smaller axon branches or individual axon processes running between the muscle bundles, which thereafter sent thin varicose elements to the muscle cells. End-plate or spider-like forms of terminal branchings were also observed. Both the longitudinal and circular muscle bundles displayed a similar 5-HT-IR innervation pattern. A gradual maturation of 5-HT-IR axo-somatic and buccal mass innervation could be observed during late embryonic and early postembryonic development of *Lymnaea*, corresponding to the appearance of adult feeding behavior at this time. It is suggested, that the 5-HT-IR axo-somatic innervations of neural perikarya in the buccal ganglia represents an additional, yet unknown, level of 5-HTergic regulation of feeding, whereas at the neuromuscular contacts, 5-HT may act as an extrinsic general modulator subtance.

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A SCULL MOUNTED MICRODRIVE-POSITIONER ASSEMBLY (MPA) FOR EXTRACELLULAR NEURONAL RECORDING IN THE DEEP BRAIN STRUCTURES OF ANESTHETIZED CATS

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We developed a new MPA for recording extracellular neuronal activity in anesthetized cats. In order to provide stable neuronal recordings the microdrive is mounted onto the scull to minimize the appearance of mechanical movements. The MPA consists of a microdrive, a driver-holder and a platform. The microdrive is made of Plexiglas and it has a 25 mm-long advancing range with 0.5 mm/rev. The duralumin platform is attached to the scull with dental acrylic cement. The first part of the removable driver holder is fixed on the duralumin platform by the M2 screw, while the second part has a conical shaped hole for the microdrive and the cylinder core that can be rotated inside in the first part of the driver-holder (in order to change the aiming angle of the electrode penetration). The advantages of our construction are the following: precise electrode positioning, stable recordings of single unit activity up to four hours, possibility to use metal electrodes with different diameter, possibility to modify the penetration angle in two dimensions, performing penetration tracks in the same structures up to 10-12 times and wide range of precise electrode positioning up to 23 mm in the brain. The weight of the whole MPA is less than 4 g. The small size provides the possibility to install three microdrives for targeting the same brain structure.
The MPA was successfully tested in four anesthetized cats. During experiments we recorded neuronal activity in several brain structures, i.e. cerebral cortex, hippocampus, substantia nigra, superior colliculus and lateral geniculate nucleus. Our MPA suggests an alternative solution to the heavy and expensive electrode microdrives that can be mounted on a stereotaxic frame.

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OSCILLATIONS AND SYNCHRONIZATION IN HYBRID CIRCUITS OF BIOLOGICAL AND ELECTRONIC NEURONS

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Our earlier theoretical work on the bursting-spiking neurons of the lobster pyloric central pattern generator showed that low dimensional polynomial models of the Hindmarsh-Rose type reproduced several aspects of their voltage dynamics. These H-R models express voltage oscillations very similar to those observed in the biological neurons, which have been shown to display low-dimensional behavior under synaptic isolation. Subsequently, we have developed analog implementations of 3 and 4 dimensional versions of modified H-R models and connected these "electronic neurons" (ENs) to the biological pyloric circuit as both extrinsic inputs and as replacements for specific neurons that had been removed from the circuit by photoablation. Electronic neurons were connected via artificial synapses using the dynamic clamp method. We studied the cooperative behavior of such assemblies using spike density functions, Fourier analysis and tools of nonlinear dynamics. The major disruption of the pyloric rhythm caused by removal of the AB pacemaker neuron could be corrected by substituting an electronic, intrinsically bursting AB-type neuron into the circuit. More surprisingly, when both electronic and biological neurons displayed chaotic dynamics in isolation, coupling them with artificial electrotonic or chemical synapses initiated regularized and synchronous bursting in them. This phenomena suggests a novel way of burst generation in networks containing intrinsically irregular components. Here we report the dependence of the observed regularization on the synaptic parameters of the hybrid network.

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COMPUTATIONAL ANALYSIS OF ACTIVE PROCESSES DETERMINING THE FIRING PATTERN OF HIPPOCAMPAL NEURONS

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Hippocampal neurons are able to produce multiple distinct firing patterns according to their inputs and internal state. We investigated the roles of different active processes and the morphology of the cell in the development of characteristic firing patterns using computational models, implemented in the GENESIS simulation environment. One such phenomenon is the ability of CA1 pyramidal cells to fire at arbitrarily low frequencies in response to sustained current pulses. We used models of different complexity in our investigation. In a simplified model neuron, which consisted of only one compartment, we were able to completely describe this aspect of the cell's behavior using an analytical formula and predict the firing mode. In particular, we showed that it is possible to get a slow firing cell without large time constants for the channels, in agreement with *in vitro* measurements. The other model we used was a detailed model of a CA1 pyramidal cell (consisting of 455 compartments). In this case it was not sufficient to analyze the active conductances, because the morphology of the cells was shown to influence whether the cell can spike at slow frequencies or not. Therefore, we investigated the influence of the morphology of the firing rate. We found that a large dendritic tree can modify the cell's behavior, and change the set of channel parameters required for the generation of slow action potential trains.

IMMUNOHISTOCHEMICAL STUDIES ON THE PROTECTIVE EFFECT OF PRECONDITIONING ON ISCHEMIA-INDUCED RESPONSES IN NEURONS OF THE HIPPOCAMPUS AND OTHER BRAIN AREAS IN RATS

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The AP-1 transcription factor family has been widely studied in the response to ischemic brain injury. Previously, we have reported that bilateral ischemic preconditioning differently reduced the global ischemia induced c-fos activation in rat hippocampal areas. In the present study we found that unilateral ischemic preconditioning (PC) can also reduce c-fos activation following bilateral ischemic insult. Fos-positive neurons decline by 20-90% in hippocampal CA1, CA2, CA3 areas and the dentate gyrus bilaterally, with ipsilateral dominance. The Fos-related antigen (Fra-2) has demonstrated a potential for controlling neuroprotective gene expression. We investigated the FRA-2 expression after 4 min unilateral or bilateral ischemic PC. Three days after bilateral PC, FRA-2 expression was markedly elevated in the dental gyrus and all pyramidal layers and this elevated state persisted, still on the 7th day but it was weaker. In the unilateral PC group FRA-2 expression showed the same pattern as in the bilateral PC group, but the staining was less intense. We found no significant difference from the ipsilateral and the contralateral hemisphere. Three days after PC, Fra-2-positive neurons were seen in the nucleus tractus diagonalis and the nucleus suprachiasmatis in contrast to the sham-operated animals. These two areas are morphologically and functionally connected to the hippocampus, therefore they may serve as good candidates to be targets for further studies – parallel with the hippocampus – to elucidate the possible mechanism of PC on brain ischemia.

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NEURONAL PROJECTIONS OF THE INTERSTITIAL NUCLEUS OF CAJAL

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Vasoactive intestinal polypetide/PHI (VIP/PHI)-expressing neurons are localized in the rostral part of the periaqueductal central gray and in the nucleus interstitialis of Cajal in rats using *in situ* hybridization histochemistry. Diencephalic coronal hemisection that cut ascending brainstem fibers both in the periventricular area and the medial forebrain bundle reduced or blocked completely VIP/PHI mRNA expression in the central gray neurons but failed to affect on Cajal neurons. Surgical hemisection through the pons reduced VIP/PHI mRNA expression in the Cajal nucleus unilaterally, on the side of the lesion while this hemisection did not influence the expression of VIP/PHI neurons inside the central gray. Short (1.5 mm wide) coronal paramedian cut in the pons was as effective on Cajal neurons as the complete hemisection indicating that VIP/PHI axons descend to the medulla in a location close to the midline. To localize the topography of VIP/PHI axons, anterograde tracer biotynilated dextran amine (BDA) was stereotaxically injected into the Cajal nucleus and various areas of the rostral throughout the central gray. Labeled fibers were seen throughout the forebrain, especially in limbic and hypothalamic areas after injections into the subependymal rostral central gray but not to the Cajal nucleus. In contrast, labeled descending fibers could be followed from the Cajal nucleus to the inferior olive in the medulla oblongata and the spinal cord. This observation, combined with lesion-induced alterations in the Cajal nucleus indicates that axons from VIP/PHI- expressing Cajal neurons participate in the descending pathway, among the fibers of the medial longitudinal fasciculus.

GLUTAMATE UPTAKE-COUPLED GABA RELEASE FROM BOTH NEURONS AND ASTROCYTES

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The balance between inhibition and excitation is dominated by GABA and Glu, the major inhibitory and excitatory neurotransmitters in the brain. Interplaying inhibitory and excitatory neurotransmissions has been demonstrated in the past decade, however, direct coupling between these functionally antagonistic neurotransmitters has remained undisclosed.
We discovered that activation of Glu transporters induces inhibitory feedback by increasing extracellular GABA level. A Ca^{2+}-independent GABA efflux into the extracellular space has been identified, which can be triggered by Glu transporter substrates both *in vitro* and *in vivo*. This GABA release was eliminated by the blockade of Glu transporters with the non-transportable inhibitors DHK and TBOA, but only partially affected by the inhibition of Glu decarboxylase with semicarbazide. We propose a neuronal and a glial mechanism responsible for Glu-induced GABA release, both of them are operating through the reversal of GABA transporters. This transporter-mediated interplay represents a direct link between inhibitory and excitatory signalling, which may function as a negative feedback mechanism to avoid hyperexcitability. Under physiological conditions, Glu-induced GABA release may contribute to tonic inhibition, whereas it can provide a new therapeutic strategy to combat intense excitation in diseases such as epilepsy and ischemia.

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ROLE OF ENTORHINAL CORTICAL GRID CELLS IN DISTANCE REPRESENTATION: A MODEL STUDY

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Medial entorhinal cortex of the rat was recently shown to contain so called 'grid cells' that show a highly regular spatial firing pattern: each grid cell is activated whenever the animal's position coincides with any vertex of a triangular grid spanning the surface of the environment (Hafting et al., 2005). The goal of our study was to investigate whether this neural map may support a distance estimating function in the rat navigation system. A layer of 'distance cells' (DC) were defined where different cells received input from grid cells of different spacings (distance between the vertices of the grid) and synaptic weights between grid cells and DCs were set proportional to the grid cell firing frequency at the goal site (implicitely assuming that synaptic plasticity takes place when the animal occupies the significant location). Firing frequencies of DCs were calculated in the case of various rat and goal locations and we concluded that distance (up to ~ 200 cm) of the virtual rat from the goal site can be determined based on the identities of the most and least active DCs. An appropriate candidate for the area containing DCs is the hippocampus, since it receives a significant projection from the entorhinal cortex. Interestingly, a lesion study demonstrated that dentate gyrus region of the hippocampus plays a crucial role in the correct performance of a spatial separation task (Gilbert et al., 2001). We suggest that this can be explained by the supposed distance measuring function of the area containing DCs.

REGIONAL-, AGE- AND GENDER-DEPENDENT DIFFERENCES IN CONCENTRATIONS OF NUCLEOSIDES IN THE HUMAN BRAIN

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Nucleosides as neuromodulators control neuronal functions in the brain. For better understanding the functions of nucleosides in the central nervous system, distribution of nucleoside levels and changes of nucleoside concentrations by age and sex are actual requirements has to be known. Using *post mortem* samples (n = 975) from 61 human brain and 4 spinal cord areas we established tissue levels of four nucleosides (uridine, inosine, guanosine, adenosine) and three metabolites (uracil, hypoxanthine, xanthine). Average concentrations of the brain samples investigated (mean ± S.E.M.) were the following (pmol/mg wet tissue weight): adenosine 8.5 ± 0.58, inosine 74.8 ± 2.84, guanosine 13.2 ± 0.62, uridine 35.1 ± 1.26, uracil 7.1 ± 0.36, hypoxanthine 58.3 ± 1.58 and xanthine 35.1 ± 1.17. Concentration of nucleosides were uneven in the human central nervous system and highest in the temporal cortex, amygdala, caudate nucleus, vestibular and cochlear nuclei, while lowest in the habenula, zona incerta, substantia nigra, inferior colliculus and locus coeruleus. We demonstrated that concentrations of uridine, inosine, guanosine and adenosine in the frontal cortex and the cerebral white matter are age- and gender-dependent. Our findings support the hypothesis that the nucleoside microenvironment in the brain could be an important factor in aging process.

HYPOTHALAMIC AND MEDULLARY INNERVATION OF THE STOMACH AND THE DUODENUM IN VAGOTOMIZED RATS

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In our previous study, gastric- and duodenum-projecting forebrain and brainstem neurons have been localized by imunohistochemical verification of two types of pseudorabies virus (modified versions of the Bartha strain) injected into the wall of the antrum and the upper part of the duodenum, in rats. The viral labeling is retrograde and trans-synaptic: the virus infects the participating neurons of the pathway from peripheral terminals up to the forebrain, at the various levels in the brain depending on the post-infection periods of time. To obtain information about the possible routes of axons through the vagal nerve *versus via* a multiple transfer through spinal sympathetic neurons, BDA virus with red and BDL virus with green fluorescence were injected in the same rats, 10-14 days after subdiaphragmatic vagotomy. Like in intact rats, 3.5 days after inoculation, sympathetic preganglionic neurons in the spinal cord and vagal neurons both in the dorsal motor vagal nucleus and the nucleus of the solitary tract were virus-labeled in vagotomized rats. The density of labeled cells in vagotomized rats was markedly lower in the vagal nuclei than that in intact rats comparing them at the exactly same post-inoculation period of time. This observation indicates the significance of the spino-solitary tract through vagal neurons may influence the sympathetic outflow to the upper gastrointestinal tract. In both intact and vagotomized rats, a various percentage of neurons in brain areas that project to either vagal or spinal preganglionic neurons (A5, A6 and C1 catecholaminergic neurons, paraventricular nucleus, lateral hypothalamic area) showed single or double virus-labeling, at 4.0-4.5 days after inoculation. Some of these cells established double labeling.

FEEDING- AND REFEEDING-INDUCED NEURONAL ACTIVITY IN THE HYPOTHALAMUS OF GOLD THIOGLUCOSE-TREATED MICE

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Gold thioglucose (GTG) represents one of the most effective chemical agents that generates extreme obesity. A single injection of GTG to young mice induces lesions in the hypothalamic ventromedial nucleus (VMN) and the animals begin to get fat. During the first 2 days, the entire VMN becomes ischemic and stains negative for NeuN, a specific marker for neuronal cells. After 2-3 days, a glial scar develops at the ventromedial portion of the VMN that borders and almost covers the arcuate nucleus (AN) disconnecting it to other hypothalamic nuclei. The medial part of the AN constitutes a gate for food intake-related humoral signals (leptin, ghrelin, insulin) to the hypothalamus. We used 2- and 4-days GTG-injected mice, and the effect of 2 days food restriction (fasting) and the consecutive 2 hours refeeding on the activity of hypothalamic neurons was demonstrated by immunostainings for Fos and *Fra-2*, markers for short- and long-term neuronal activity, respectively. Fos- and *Fra-2*-labeled neurons were numerous in the AN and in the VMN around the ischemic area (2 days) or the glial scar (4 days). In addition, few cells were labeled in the dorsomedial nucleus (DMN) and the dorsolateral hypothalamic area (DLH). Important to note, that tanycytes in the ventral half of the third ventricle´s ependyma establish very strong Fra-2 expression in GTG mice. GTG did not influence the fasting and refeeding induced very strong Fos or *Fra-2* expressions in the NA, DMN, and the DLH 2 days after injection, but these effects were only moderate in 4 days GTG mice. To understand the mechanism through GTG can influence food intake, this study should be extended over investigations on longer post-injection periods and combine it with immunohistochemistry for orexigenic and anorexigenic neuropeptides in the hypothalamus of GTG-mice.

CHARACTERIZATION OF SUCCINATE BINDING SITE IN BRAIN SYNAPTIC MEMBRANES

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Previously, a central intermediate in the TCA cycle succinate, was postulated to mimic some of the actions of γ-hydroxybutyric acid (GHB). Here we report on the presence of specific, pH_o-dependent succinate binding sites in both human nucleus accumbens (NA) and rat forebrain synaptic membranes. Binding of succinate ($IC_{50,SUCC}$= 2,9 ± 0,6 μM), GHB ($IC_{50,GHB}$= 2,1 ± 1,3 μM), the GHB receptor antagonist NCS-382 ($IC_{50,NCS}$= 0,8 ± 0,2 μM) and gap junction blockers (carbenoxolone $IC_{50,CBX}$= 7,1±5,8 μM and β-glycyrrhetinic acid $IC_{50,GRA}$= 13,6 ± 10,6 μM) has been shown in measurements of [^{3}H]succinate binding in human NA synaptic membrane fraction. There was no interaction between specific $GABA_B$ receptor ligands and this site. A similar binding profile was found in rat forebrain synaptic membrane fractions. To compare synaptic binding with high-affinity uptake process, we tested succinate and GHB as inhibitors of [^{3}H]succinate uptake. The effective concentrations for inhibition of uptake were IC50,GHB > 1 mM and IC50,SUCC = 6,7 ± 0,2 μM. In addition, [^{14}C]succinate release from rat NA punches has also been measured. Efflux of the radioligand was greatly accelerated by adding 2 mM succinate to the external medium, whereas 2 mM GHB did not affect [^{14}C]succinate release.
We conclude the existence of a pH_o-dependent synaptic membrane site for the intermediary metabolite succinate. The pharmacological and functional properties of this recognition site may possibly suggest the existence of a hemichannel-like target for succinate.

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MODELING HUMAN GAT-1 TRANSPORTER*

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Gamma-aminobutyric acid (GABA) transporter subtype GAT-1 performs termination of synaptic transmission and replenishes neuronal GABA pools. Certain pathological conditions, including epilepsy can be treated by inhibition of GAT-1 functions.
Based on the experimental structure of a bacterial homologue, *in silico* model of the human GAT-1 (hGAT-1) was built. Our hGAT-1 model showed that conserved amino acids controlling transporter activity were either parts of the active site or they have a role in stabilizing it. Two sodium ions are co-transported with GABA. Moreover, the model showed that the active site would not be complete without them, the interactions between ligands and protein were weaker if they were formed.
To screen compounds for hGAT-1 inhibitory activity, several known substrates with widely different inhibitory activity were docked in the hGAT-1 protein-model, and "score values", - indicating ligand-protein interaction energies - were compared with the experimental binding constants. The model, the docking methods and the approximations of the interactions were refined by serial docking experiments. Our approach to predict hGAT-1 inhibitory activity of compounds opens up a new avenue in the field of more specific therapeutic intervention.

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DORSOLATERAL HYPOTHALAMIC PROJECTIONS TO MEDULLARY AND SPINAL AUTONOMIC NEURONS

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The major projecting orexigenic neurons (orexins, melanin-concentrating hormone [MCH]) distribute almost exclusively in the dorsolateral hypothalamus (DLH), where they are arranged in different topographical patterns. In neuroanatomical respect, the DLH represents a poorly outlined area composed by cytologically and biochemically different cell types and cell groups. On the basis of cell types and density, as well as the neurotransmitter/neuropeptide characters of the cells, the DLH can be divided (mainly for didactical reasons) into 7 subdivisions in rats: dorsal, posterior, juxtafornical, perifornical, subfornical lateral hypothalamic, and "far lateral" (Forel's field I) areas. Orexin and MCH neurons are present, in various number and density, in each of the 7 areas and, in addition, in the dorsomedial hypothalamic nucleus. To localize the projections of neurons located in these individual DLH areas to medullary and spinal autonomic neurons, an anterograde tracer, biotynilated dextran amine (BDA) was injected stereotaxically. Labeled fibers were localized in both parasympathetic (dorsal motor vagal nucleus) and sympathetic (intermediolateral cell column in the thoracic spinal cord) preganglionic neurons. In parallel, using immunostainings, the distribution patterns of BDA-labeled, orexin- and MCH-immunopositive fibers were mapped and compared. The fine localization of BDA-labeled nerve terminals on medullary preganglionic neurons have been verified at electron microscopic level.

ALTERATIONS IN THE NUMBER OF SOMATOSTATIN-IMMUNOREACTIVE NEURONS IN THE HIPPOCAMPUS, HYPOTHALAMUS AND AMYGDALA IN RESPONSE TO ACUTE EXPERIMENTAL BRAIN ISCHAEMIA AND PRECONDITIONING IN RATS

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Neurons of distinctive parts of the brain react differently to ischemic damage. Hypoxic preconditioning makes temporary resistance to ischemia in some regions, especially in the hippocampus. Ischemia has been carried out with two groups: 12 rats with and 6 rats without preconditioning (3 min of carotid occlusion, one day after the coagulation of the vertebral arteries, „four vessels model"). Three days after preconditioning, the carotids were occluded for 10 min, uni- or bilaterally in preconditioned or only in vertebral coagulated rats. Control group was formed with the rats without carotid occlusions. The number of somatostatin immunostained (SOM-ir) cells (3-fold immunoreactivity over the background) was counted in 9 selected brain areas. Statistical significance was determined by the Mann-Whitney test. Acute brain ischemia depleted SOM immunoreactivity, and consequently, the „visible" number of SOM-ir interneurons in the hippocampus. Preconditioning, however, did not alter the number of intensively stained SOM-ir interneurons in hippocampal CA1, CA2, CA3 areas and the dentate gyrus. The number and the immunoreactivity of SOM-ir cells in the hypothalamus (peri- and paraventricular nuclei) have been influenced neither by ischemia nor preconditioning. In the amygdala, the ischemia-induced reduction in SOM-ir was highly significaant in the medial, moderate in the central and basolateral nuclei, which was not influenced by preconditioning. It is more likely, that the preserving effect of preconditioning on brain neurons (especially in the hippocampus) may not be mediated by somatostatin.

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EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY ON FASTING- AND REFEEDING-ELICITED NEURONAL ACTIVITY IN RATS WITH AND WITHOUT MONOSODIUM GLUTAMATE PRETREATMENT

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Immunostainings for Fos and *Fra-2* oncogenes were applied to demonstrate short- and long-term neuronal activities in the nucleus of the solitary tract (NTS), the dorsal motor vagal nucleus (DVN) and the area postrema (AP) in response to 2-days food restriction (fasting), and a consecutive 2 hours refeeding. The experiment was repeated in rats with subdiaphragmatic vagotomy. No Fos-positive neurons were seen in the investigated nuclei in intact and vagotomized control (feeded) rats. Fasting elicited minor Fos or *Fra-2* activities in the AP and the NTS both in intact and vagotomized rats. In vagotomized rats, neurons in the DVN displayed no *Fra-2*, and very limited Fos immunostaining. In intact rats, strong Fos immunoreactivity was seen in neurons of the medial and commissural NTS and the AP in response to refeeding. This effect was even stronger in vagotomized rats. In a second experiment, monosodium glutamate (MSG)-treated animals were used. MSG was injected to newborn rats during the first postnatal week. By the 4 months of the treatment, MSG destroyed blood-brain barrier-free brain areas, like the medial portion of the arcuate nucleus or the border area between the AP and the NTS, and it strongly influenced food intake and body weight. In MSG-treated rats, with or without vagotomy, fasting elicited minor if any Fos or *Fra-2* expressions in the NTS and the AP. MSG-treatment did not influence the fasting-elicited scattered Fos expression in the DVN. The refeeding-induced strong Fos expression in the NTS and the AP was weaker in MSG-treated than in control or in MSG-treated vagotomized rats. Data presented here indicate that neurons in the vagal nuclei and the AP react diversely to food intake-related neuronal and humoral inputs.

THE ROLE OF TUBEROMAMILLARY HISTAMINERG NEURONS IN FOOD INTAKE

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Recent data indicate the participation of brain histamine in the central regulation of the food intake. In the brain, histamine is expressed almost exclusively by neurons in the tuberomamillary nucleus (TMN). Immunostainings for *Fos* and *Fra-2* oncogenes were applied to demonstrate short- and long-term neuronal activities in the TMN in response to 2-days food restriction (fasting), and a consecutive 2 hours refeeding. Fasting exerted a minor influence on the activity of TMN neurons, but refeeding elicited strong *Fos* and *Fra-2* expression there. The experiment was repeated in rats with subdiaphragmatic vagotomy. Vagotomy, without any treatment established strong neuronal activity in the TMT that was even stronger in fasted and refeeded rats. In a third experiment, monosodium glutamate (MSG) was injected to newborn rats during the first postnatal week. MSG lesions blood-brain barrier-free brain areas in the arcuate nucleus where food intake-related humoral signals (leptin, ghrelin, insulin) may rich the hypothalamus. In MSG-treated rats, fasting elicited minor if any effect in the TMN, and MSG did not influence the refeeding-induced strong *Fos* and *Fra-2* expression. In contrast, vagotomy was less effective in MSG rats. In an attempt to identify the neuronal inputs that cause *Fos* and *Fra-2* activation in the TMN, orexigenic neuropeptides (neuropeptide Y, orexin-A, melanin-concentrating hormone, galanin) were immunolabeled. TMN neurons are innervated by axons originating from these neuropeptides expressing neurons in the arcuate nucleus or the dorsolateral hyypothalamus. The intensity of imunolabeling was increased in galanin-containing fibers in response to refeeding. The present data provide neuroanatomical support to functional studies on the role of histamine in food intake.

EFFECT OF REPEATED RESTRAINT STRESS ON PROLACTIN-PELEASING PEPTIDE MRNA EXPRESSION IN THE RAT BRAIN

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We investigated the effect of chronic restraint stress on prolactin-releasing peptide (PrRP) mRNA expression in the CNS of male and female rats. In the brainstem, we also examined tyrosine-hydroxylase (TH) mRNA expression. Bilateral lesions of the hypothalamic paraventricular nucleus (PVN) were performed on two groups of male rats and one week later, one group of the rats was exposed to the first restraint. Under basal conditions in the brainstem the PrRP, but not the TH mRNA expression was higher in the females than in males. In the dorsomedial hypothalamic nucleus (DM), there was no gender related difference in the PrRP mRNA expression. Chronic restraint resulted in an elevation of both the PrRP and the TH mRNA expression in the A1 cell group, in both genders. In the A2 cell group, the PrRP and mRNA expression was higher only in male rats, accompanied with slight increase in the TH mRNA expression. In the DM, the PrRP mRNA expression also increased in both genders. Bilateral PVN lesions alone reduced the PrRP and the TH mRNA expression the A2 cell group, but did not prevent the response for stress. Percents of the PrRP and TH colocalizing cells among the only TH expressing cells in the brainstem were determined for each experimental group. The ratio of PrRP and TH expressing cells increased for the chronic stress, in both genders in the A1, but only in males in the A2 cell group. The ratio of colocalizing cells decreased in the A2 cell group after the bilateral PVN lesions. Our data show, that there is a gender difference under basal conditions in PrRP mRNA expression in the rat brain, the PrRP positive neurons respond to the chronic restraint stress, and the PVN exerts a tonic positive effect on the A2 PrRP/TH mRNA expressing cells, without influencing the response for the chronic restraint stress.

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CHANGES IN ENDOGENOUS PACAP LEVELS IN THE BRAIN FOLLOWING FOOD AND WATER DEPRIVATION

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic neuropeptide exerting diverse actions in the central and peripheral nervous systems. A few studies indicate that PACAP is involved in the regulation of feeding and water homeostasis. The aim of the present study was to investigate changes in PACAP38 concentrations in different brain areas following food or water deprivation in male and female rats. Rats were sacrificed 12, 36 and 84 hrs after water or food removal. PACAP levels were determined by radioimmunoassay. Our results show that levels of PACAP decreased in the hypothalamus in both sexes after water deprivation, with a more marked, significant decrease in females at 12 hrs. A decrease was observed also in the telencephalon, with a similar pattern in both genders: levels were lowest after 12 hrs, and showed a gradual increase at the other two time-points. PACAP levels increased in the brainstem of male rats, while females had a decrease 12 hrs after water deprivation. The pattern of changes in PACAP levels was very different after food deprivation. In male rats, PACAP levels showed a significant increase in the hypothalamus, telencephalon and brainstem 12 hrs after the beginning of starvation. In females, a less marked increase was observed only in the hypothalamus while no changes were found in the other brain areas. Our results show a sensitive reaction in changes of endogenous PACAP levels to water and food deprivation in most brain areas, but they are differentially regulated in male and female rats.

RELATIONSHIP OF NEURONAL VULNERABILITY AND TYPE 2 POTASSIUM-CHLORIDE COTRANSPORTER (KCC2) IMMUNOREACTIVITY IN HIPPOCAMPUS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA

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Cation chloride cotransporters have been reported to be expressed in neurons in the hippocampus and to regulate intracellular Cl^- concentration. The neuron-specific K^+-Cl^- cotransporter 2 (KCC2) is necessary for fast synaptic inhibition via maintaining the low intracellular chloride concentration required for the hyperpolarizing actions of GABA.
In this study we examined the vulnerability of KCC2-containing neurons in the rat hippocampus following 15 min ischemia induced by four-vessel occlusion.
72 kDa heat shock protein (HSP-72)-like immunoreactivity was used to investigate the extent of damage in neuronal populations previously shown to be vulnerable to ischemia. At 6-24h after ischemia, when the pyramidal cells in the CA1 region showed no morphological signs of damage, a minor rise of KCC2 immunoreactivity was already observed. At a survival time of two days, when the CA1 pyramidal cells started to degenerate, a progressive downregulation of the KCC2 protein was visible. Interestingly, in same areas, the parvalbumin containing interneurons showed no signs of ischemic damage and an elevated level of KCC2 immunoreactivity was maintained on their membrane surface.
In CA1 pyramidal cells, the reduction in KCC2 expression may lead to an elevation of intracellular Cl^- concentration, which causes a shift in E_{Cl} toward more positive levels, converting GABAergic transmission from inhibitory to excitatory. The reduced inhibitory action of GABA through a Ca-dependent downregulation of KCC2 function may be causal to delayed neuronal death in the CA1 pyramidal cells.

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EFFECTS OF THE NEUROPEPTIDE PACAP ON THE SURVIVAL OF ENDOTHELIAL CELLS

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a widely distributed neuropeptide that has various different functions in the nervous system and in non-neural tissues. Little is known about the effects of PACAP in endothelial cells. The aim of the present study was to investigate the effects of PACAP on endothelial cell survival and apoptotic signaling pathways under oxidative stress. Mouse hemangioendothelioma (EOMA) cells were exposed to 0.5 mM H_2O_2 which resulted in a marked reduction of cell viability and a parallel increase of apoptotic cells assessed by MTT test and flow cytometry. Co-incubation with 20 nM PACAP1-38 increased cell viability and reduced the percentage of apoptotic cells. Flow cytometry analysis showed that oxidative stress reduced the phosphorylation of the anti-apoptotic ERK and increased the phosphorylation of the pro-apoptotic JNK and p38 MAP kinases. PACAP1-38 treatment ameliorated these changes: levels of phospho-ERK were elevated and those of phospho-JNK and p38 were decreased. All these effects were abolished by simultaneous treatment with the PACAP antagonist PACAP6-38. In summary, our results show that PACAP effectively protects endothelial cells against the apoptosis-inducing effects of oxidative stress.

CHARACTERIZATION OF THE NUCLEAR FACTOR-KB RESPONSIVENESS OF THE HUMAN *dio2* GENE

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Type 2 deiodinase (D2) activates T4 by deiodination to T3, a process being the source of most T3 present in the brain. In the mediobasal hypothalamus, expression of the *dio2* gene is potently activated by administration of bacterial lipopolysaccharide (LPS), which in turn mediates the modifications in thyroid homeostasis typically observed in patients with nonthyroidal illness syndrome (NTS). Here we show that LPS-induced D2 expression is also observed in human MSTO-211H cells that endogenously express D2. LPS rapidly doubled D2 activity by a mechanism that was partially blocked by the nuclear factor-kB (NF-kB) inhibitor sulfasalazine. Next, the human *dio2* 5'-flanking region promoter assay was used in HC11 cells and the p65/NF-kB responsiveness mapped to the 3' ~600-bp region with an approximately 15-fold induction. Semiquantitative EMSA identified the strongest NF-kB binding sites at the positions -683 bp (called no. 2) and -198 bp (no. 5) 5' to the transcriptional starting site. Only site no.5 possessed transactivation potency in the presence of the p65 subunit of NF-kB. Our results indicate that inflammatory signals regulate D2 expression predominantly via the NF-kB pathway in a direct transcriptional manner and could contribute to the changes in thyroid economy observed in nonthyroidal illness syndrome during infection.

ADRENERGIC REGULATION OF ARGININE VASOPRESSIN SECRETION IN RAT NEUROHYPOPHYSEAL TISSUE CULTURES

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The effects of adrenaline (A) and noradrenaline (NA) on vasopressin (VP) secretion were studied in 13-14-day cultures of isolated rat neurohypophyseal (NH) tissue. The VP contents of the supernatant media were determined by radioimmunoassay after a 1-h incubation. Significantly increased VP levels were detected in the tissue culture media following A (an α receptor agonist) administration, depending on the A dose. The VP secretion elevation was totally blocked by previous administration of the A antagonist phentolamine (an α_1+α_2 receptor antagonist) or corynanthine (an α_1 receptor antagonist). Yohimbine (an α_2 receptor antagonist) did not influence the VP secretion increase induced by A. After NA (a β receptor agonist) administration, a VP secretion elevation was again detected, but the degree of enhancement proved smaller than that of the VP secretion increase induced by A. However, propranolol (a β_1+β_2 antagonist) prevented the VP secretion increase before NA administration. Atenolol (a β1 antagonist) did not block the VP secretion elevation induced by NA. Surprisingly, pindolol (PDL, a β_2 antagonist) administration enhanced VP secretion. This contradictory effect can be explained in that PDL not only acts as a blocker, but also exerts intrinsic sympathomimetic action and a strong agonist effect.
Conclusions: The α_1A and β_2NA receptors are mainly involved in the A-, or NA-induced increase of VP secretion in isolated NH tissue cultures. The results indicate that VP release is influenced directly by the adrenergic system, and the adrenergic control of VP secretion from the NH tissue in rats can occur at the level of the posterior pituitary.

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DISTRIBUTION OF POTASSIUM-CHLORIDE COTRANSPORTER 2 (KCC2) IN THE SUPERFICIAL SPINAL DORSAL HORN

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GABA-mediated fast-hyperpolarizing inhibition depends on extrusion of chloride from the cytoplasm into the extracellular space by KCC2. Disruption of KCC2 effectively renders the $GABA_A$ receptor mediated postsynaptic effect of GABA excitatory rather than inhibitory. In the present experiments we investigated the distribution of KCC2 in the superficial spinal dorsal horn of rodents. Using fluorescence immunohitochemistry and confocal microscopy we found a strong immunostaining for KCC2 in laminae I-II, the nociceptive receptive layers of the spinal dorsal horn. Careful examination of sections double stained for KCC2 and markers of axon terminals of various origin like substance P, CGRP, IB4 (markers of nociceptive primary afferents), VGLUT1-3 (markers for glutamatergic axon terminals), GAD65/67 (markers for GABAergic axon terminals) showed that all types of axon terminals were negative for KCC2 in laminae I-II. In contrast to axon terminals, KCC2 was strongly expressed in cell membranes of neural somata and dendrites. However, investigating KCC2 immunoreactivity of perykaria of calbindin D28k (CaB) and μ-opioid receptor (MOR) positive excitatory neurons in the rat and GAD65 containing inhibitory neurons in GAD65-eGFP transgenic mice we found that the density of KCC2 immunoreactive puncta varied from neuron to neuron and 7,2% of CaB-IR, 0,6% of MOR-IR and 1,1% of GAD65 expressing neurons did not show any detectable immunoreactivity for KCC2. Our result suggests that $GABA_A$ receptor mechanisms may play a more complex role in pain processing neural circuits in the superficial spinal dorsal horn as it was believed before.

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EFFECTS OF *IN VITRO* PACAP EXPOSURE ON THE PINEAL EXPRESSION OF CLOCK GENES IN BIRDS

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Data indicate that PACAP is involved in the transmission of light information to the SCN, the master circadian clock in vertebrates. PACAP is known to phase-shift the circadian rhythm of clock gene expression in the mammalian SCN *in vitro*. The molecular oscillator of the chicken pineal gland shows functional homology with that of the mammalian SCN, however, the effects of PACAP on the pineal expression of clock genes in birds were not studied earlier.
In this study, changes in Cry1 and Clock mRNA contents of chicken pineal glands were analyzed after exposed to PACAP *in vitro*. To reduce the effect of environmental synchronizing factors, chicken embryos were grown under continuous darkness and random handling. On the 17th embryonic day (ED 17), pineal glands were removed and placed to a dynamic *in vitro* (perifusion) system. On ED18 the glands were exposed to PACAP-38 for 1 hour. Explanted glands were collected for RT-PCR analysis in 4 hour intervals beginning 2 hours before the exposure.
In control experiments, both Cry1 and Clock mRNA contents showed episodic changes under continuous darkness. PACAP exposure reduced mRNA contents of Cry1 and inhibited the induction of Clock within 2 hours. Ten hours after the exposure, both Cry1 and Clock mRNA contents exceeded that of the controls. Our results support that (1) clock gene expression in the pineal gland may become rhythmic even in the absence of periodic environmental stimuli during development. Also, (2) the molecular clock machinery is sensitive to PACAP in the chicken pineal gland similar to that observed in the mammalian SCN. Based on our previous and present data, PACAP affects not only the MT synthesizing apparatus, but also it may be involved in mechanisms which regulate the circadian oscillators of the chicken pineal gland.

SOMATOSENSORY-MOTOR NEURONAL ACTIVITY IN THE SUPERIOR COLLICULUS OF THE PRIMATE BRAIN

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The superior colliculus (SC) plays an important role in visually guided reaching. In the present study we investigated whether the SC is similarly involved in the interaction with a target. Extracellular single-cell recordings were performed in the intermediate and deep layers of the SC of two awake, behaving macaque monkeys in visually guided reach paradigms. Interestingly, we found a large number of SC units that were not active during reaching but instead of that, they were strongly active during contacting and pressing the reached target. These somatosensory-motor neurons had very low or no spontaneous activity. The large majority of them was not responsive to cutaneous stimulation, but about half of these SC units responded to passive arm movements. The majority of the somatosensory-motor SC units was selective for target location. The target location that elicited maximal response varied among the recorded cells. The somatosensory-motor SC units were bimanual. The magnitude of the somatosensory-motor responses of SC neurons depended on the strength of the pushing, stronger pushing elicited higher discharge rates than lighter pushing. The somatosensory-motor activity of the SC units may monitor the force of the active muscles as well as the spatial position of the target and serve for the stabilization of the arm to maintain an interaction with a target with a particular force at a certain spatial position.

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PLACEBO IN THE EFFECT OF ALCOHOL: TO KNOW OR NOT TO KNOW?

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Our experiment was designed to study a phenomenon which is widely known through anecdotes: people become tipsy by consuming alcohol-free beer. Therefore, we hypothesised that the effects of alcohol are partially mediated by placebo effect, which works the best if the person expects consuming alcohol.
University students recruited by advertisements were tested in groups of 10 to 15. They had consumed a mixture of 4cl rum in 20cl coke every ten minutes, four times altogether; however, half of the subjects drank alcohol-free rum aroma instead of rum. Short term memory, standing balance, and self-evaluation of subjective physical, emotional and social state were recorded regularly before and after each round. In the first experiment, all subjects believed that they were consuming alcohol, whereas in the second round they were told that half of them had received placebo. In addition, 28 more subjects had only to imagine that they were drinking rum with coke (cognitive group). The same tests were done with all subjects.
Results showed that in the first experiment, placebo and alcohol consumption groups produced highly similar results in all tests with a slight difference only after the fourth round in a few measures. On the contrary, if subjects had been informed about the possibility that they might have consumed only aroma, the two groups differed in most of the tests. Finally, the cognitive group differed significantly from any of the other groups showing that expectations had not been built on a pure cognitive basis. Thus, it seems that the effects of alcohol consumption in smaller portions are in part mediated by a placebo effect at least in subjects who believed that they consumed alcohol. These findings support our general hypothesis that placebo effect in humans is frequently mediated by expectations.

THE NEUROENDOCRINE ACTIONS OF NEUROMEDIN S

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Neuromedins are representative members of a smooth-muscle-stimulating peptide family with prominent and versatile impact on several neuroendocrine processes. In the present experiments we focused on the effects of the recently discovered neuromedin S on open-field behaviour and hypothalamic-pituitary-adrenal (HPA) activation. The peptide was administered intracerebroventricularly to freely moving rats and 30 minutes later certain neuronedocrine parameters were investigated. Our results disclosed that neuromedin S has a profound and dose-dependent action on the HPA system, evoking a threefold increase in plasma corticosterone level in a dose of 1 μg. It also activated grooming in a dose of 0.25 μg. The latter action displayed a bell-shaped dose-response curve. However, the neuropeptide did not influence square crossing, rearing and defecation in open field behaviour studies. Our results suggest that, indeed, neuromedins are important regulators of neuroendocrine processes and shed light on the possible functions of the newly described neuromedin S in the central nervous system. It appears, that centrally administered neuromedin S can stimulate such CRF-dependent processes as corticosterone release and grooming.

TRANSPLANTATION OF EMBRYONIC MOTONEURONES INTO THE INJURED CERVICAL SPINAL CORD: FUNCTIONAL REINNERVATION

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Spinal cord injury usually results in irreversible loss of function and morphological structures of the cord. Injury affecting the cervical segments of the cord induces tetraplegia and denervation of vital respiratory muscles. Therefore in this study we intended to find therapy to improve lost spinal cord function following a spinal hemisection injury.

The right cervical 6th spinal segment was hemisected, the 6th ventral root avulsed and an embryonic spinal cord graft enriched in motoneurones was placed into the hemisection cavity. Then the avulsed spinal root was reimplanted into the graft. Control animals underwent the same operation but received no graft. After 3 months survival the corticospinal and rubrospinal tracts were labelled anterogradely with BDA, the 6th spinal nerve was labelled retrogradely with Fast Blue and various immunhistochemical markers were detected.

The C6 ventral root was reinnervated by axons of host motoneurones and neurones of the lateral spinal nucleus. Grafted cells did not contribute in considerable number to the reinnervation of the ventral root. However, grafts promoted the regeneration of host neurones as significantly more neurones were retrogradely labelled in grafted animals than in control ones. The grafted animals showed improved functional reinnervation in their affected forelimbs as compared to that of controls.

The grafts received various supraspinal inputs: serotonergic fibres and rubrospinal fibres readily entered the graft and established functional synapses on the grafted neurones, while corticospinal fibres avoided the territory of the graft.

These results suggest that embryonic spinal cord grafts act as bridges between the injured spinal cord stumps and promote the regeneration of the host neurones rather than contributing directly to the reinnervation of denervated forelimbs.

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PHENOTYPIC SWITCH OF NOCICEPTIVE PRIMARY SENSORY NEURONS INDUCED BY PERINEURAL TREATMENT WITH CAPSAICIN OR RESINIFERATOXIN

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Perineural treatment with the potent TRPV1 agonists, capsaicin or resiniferatoxin (vanilloids) producing long-lasting selective regional analgesia has been suggested to induce sprouting of Abeta myelinated spinal primary afferent fibres labelled with the B subunit of choleratoxin (CTB), which specifically binds to GM1 ganglioside. The present study was initiated in an attempt to clarify the role of capsaicin-sensitive C-fibre afferents in this process. In control rats, injection of CTB-horseradish peroxidase (HRP) conjugate into the sciatic nerve resulted in an intense labelling of the spinal dorsal horn except laminae I-II. Vanilloid treatment of the sciatic nerve 2 weeks prior to the injection of CTB-HRP resulted in an intense labelling also of these superficial laminae. Examination of transganglionic labelling 6 weeks after perineural treatment with vanilloids revealed labelling of the deep dorsal horn but not that of laminae I and II. In relating spinal ganglia, a significant increase in the proportion of labelled small cells was seen 2 but not 6 weeks after vanilloid treatment. Taken together with previous findings showing a substantial reduction in the number of C-fibres 6 weeks after perineural vanilloid treatment, the present results indicate that vanilloid-induced increased labelling of the substantia gelatinosa may be attributed to a phenotypic switch of C-fibre sensory ganglion neurons rather than a sprouting response. The findings also suggest an important role of GM1 ganglioside metabolism in the regulation of nociceptor function and may provide a novel approach to interfere with pain mechanisms.

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CHANGES IN TRPV1 RECEPTOR EXPRESSION FOLLOWING PERINEURAL TREATMENT WITH CAPSAICIN AND RESINIFERATOXIN: IMPLICATIONS FOR THE ANALGESIC EFFECT OF VANILLOIDS.

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Changes in the expression of the capsaicin receptor TRPV1 were studied after epineural application of vanilloids or section of the sciatic nerve using immunohistochemistry and colour in situ hybridization. In control rats, quantitative morphometric and statistical analyses of TRPV1 receptor mRNA expression in L4-5 dorsal root ganglion cells revealed distinct populations of large TRPV1-negative (type A) and small (type C) and small to medium (type B) TRPV1-positive neurons with very high and moderate optical density of the hybridization signal, respectively. Immunohistochemistry revealed populations of large TRPV1-negative and small to medium TRPV1-positive neurons. In immunohistochemical experiments significant decreases in the number of strongly fluorescent small neurons were observed after sciatic nerve transection, capsaicin or resiniferatoxin treatment. In situ hybridization revealed dramatic decreases (up to 85%) in the proportion of type C neurons 3, 14 and 30 days after sciatic nerve section. In contrast, perineural treatment with capsaicin or resiniferatoxin resulted in a similar substantial decrease in the proportion of type C neurons only after 3 days while after 14 and 30 days the reduction in the number of these neurons was less profound and amounted to 60% and 40%, respectively. The findings indicate that the antinociceptive and antiinflammatory effects of perineural capsaicin/resiniferatoxin treatment involve distinct changes in neuronal TRPV1 mRNA expression and alterations in (post-) translational modification.

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INSULIN AND INSULIN-LIKE GROWTH FACTOR-I INDUCE THERMO-SENSITIVE IONIC CURRENTS IN CAPSAICIN-SENSITIVE PRIMARY SENSORY NEURONS

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Primary sensory neurons (PSNs) express different thermo-sensitive transient receptor potential (TRP) channels including vanilloid-type 1 (TRPV1), which are regulated by the activation of tyrosin kinase neurotrphin receptors. Here we studied whether stimulation of the insulin receptor (IR), which is co-expressed with the TRPV1 in a sub-population of PSNs, has a similar effect on TRPV1 activity.

Neuronal cultures were prepared from dorsal root ganglia of rats and wild type or TRPV1-/- mice. Capsaicin ($5x10^{-7}$M)-, insulin (10^{-5}M-10^{-7}M)-, insulin-like growth factor I (IGF-I; 10^{-7}M-10^{-9}M)-, menthol (10^{-4}M)- or heat-induced whole cell currents were recorded.

In rat, insulin evoked inward currents in 30.9% of capsaicin-sensitive, but none in capsaicin-insensitive, PSNs. This current was blocked by ruthenium red but not by the competitive TRPV1 antagonist capsazepine. Similarly, IGF-I also induced inward currents in insulin-sensitive neurons.

In the TRPV1+/+ mice, insulin evoked responses in 9.3% of the neurons (all capsaicin-sensitive); however in TRPV1-/- only 5% of the cells were activated. While in TRPV1-/- mice none of the cells were sensitive to capsaicin, three insulin-responsive neurons we tested exhibited heat-evoked currents.

In rat PSNs both moderate cooling (≥18ºC), and warming (≤41°C) influenced the insulin-induced current: while warming mainly enhanced it, in menthol-sensitive neurons cooling also produced potentiation.

These findings indicate that insulin and IGF-I acutely influence the function of a subpopulation of capsaicin-sensitive PSNs possibly by modulating the thermo-sensitive TRP channels, including TRPV1.

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REGENERATION OF INJURED SPINAL MOTONEURONS AFTER STEM CELL TRANSPLANTATION

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Motoneurons of spinal cord are severely injured in case of the spinal cord injury or central nervous system diseases. The stem cell transplantation is a possible way to replace injured motoneuron populations, or possibly to enhance their regeneration. In this study we examined whether it is possible to replace the damaged motoneurons with neuroectodermal stem cells transplantation and regeneration of injured spinal motoneurons is influenced by grafted stem cells. In our experimental model the lumbar 4 (L4) segment of spinal cord of SD rats was exposed in deep chloral-hydrate anaesthesia and the L4 ventral root was avulsed. Stem cells were grafted into L4 segment of the spinal cord and the avulsed ventral root was reimplanted. In control animals only the L4 ventral root was avulsed and reimplanted without stem cell transplantation.
The grafted cells survived and started to differentiate. The transplanted cells settled down in the dorsal horn and in the intermedier gray matter and to a least extent in the ventral horn. If low number of stem cells (n< 50000) were implanted the transplanted cells differentiated to neurons or astrocytes. However, the grafted cells did not contribute to the innervation of the reimplanted root but promoted the survival and regeneration of injured host motoneurons. When higher number of grafted cells (n=100000) was used the ventral root was reinnervated by axons of grafted and host cells but the grafted cells did not differentiate to motoneurons.
Our results have presented evidence that a major effect of grafted stem cells is to support the regeneration of host neurons. We suggest, that this effect may be more effective in case of degenerative diseases than the replacement therapy.

MORPHOLOGICAL AND PEPTIDE-EXPRESSION CHANGES IN DORSAL ROOT GANGLIA IN NEUROPATHY FOLLOWING LIMB LENGTHENING IN RABBITS

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Limb-lengthening is a frequently used surgical procedure to lengthen and/or straighten deformed bone segments. Nerve injury is one of the most serious complications of this type of operation resulting neuropathy with pain, sensory loss or motor weakness. Despite of the fact that limb-lengthening is a common practice, the precise aetiology of the symptoms is still unknown. In this study, we examined the morphological and neurochemical changes associated with the limb lengthening in the rabbit dorsal root ganglia.
Three groups of rabbits (5 rabbits / group) were used. Both in group A and B tibial osteotomy, external fixateur application and bone compression for 7 days were carried out. After the callus formation in group A, 1 mm distraction was applied once a day for 20 days to achieve 120% of the original length, while in group B, 1mm lengthening was performed three times a day for 10 days to reach 30% increment. Untreated animals formed the group C.
Using immunohistochemistry and confocal image analysis, the total number of neurons and their substance P (SP) immunreactive portion were determined in dorsal root ganglia (DRG) in the three groups. Data were analysed with the help of logistical regression analysis.
In groups A and B, huge vacuoles appeared in large ganglion cells on the ipsilateral side of the operation. The morphological appearance of the vacuoles in rabbits was very similar to those existing after nerve transsection in rats. However, the number of the SP immunoreactive cells in the DRGs decreased (by 10%) while the total number of DRG neurons was not changed.
Our results suggest that vacuolisation and changes in substance P expression in DRGs could contribute to different pain symptoms in patients during and after limb-lengthening.

SPATIO-TEMPORAL FILTER PROPERTIES ALONG THE TECTO-THALAMO-CORTICAL PATHWAY

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Although the morphological connections and the physiological properties of the multisensory pathways including the superior colliculus (SC), the suprageniculate nucleus (Sg) of the posterior thalamus and the cortex along the anterior ectosylvian sulcus (AES) of the feline brain were widely investigated we have scare knowledge on the function of these structures. To contribute to the description of the behavioral role of the tectal visual system we aimed to investigate the still missing spatial and temporal filter properties of visual neurons in the SC, the Sg and the AES cortex.
Extracellular single-unit recordings were performed in halothane-anesthetized, immobilized, artifically ventilated cats to drifting sinusoidal grating stimulation of various spatial and temporal frequencies. The spatio-temporal response properties of the SC, the Sg and AES cortex neurons were surprisingly similar. Hence, we can summarize the spatial and temporal visual properties irrespective of the region in question. Most of the cells were strongly sensitive to the direction of drifting gratings. The large majority of the neurons displayed vigorous responses to extremely low spatial and very high temporal frequencies and possessed narrow spatial and temporal tuning.
The spatio-temporal filter properties of the tectal visual structures aiding the velocity detection and the analysis of the object in motion. The SC-Sg-AES cortex axis can be involved in the optic flow processing and could be good candidate for tasks involved in the perception of self-motion. It may play a role in recording movements of the visual environment relative to the body and thus it may participate in the adjustment of motor behavior in response to environmental challenges.

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TRANSSYNAPTIC GENE DELIVERY BY PSEUDORABIES VIRUS VECTORS

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In this study we have constructed recombinant pseudorabies virus (PRV) vectors for delivery of fluorescent activity marker genes to specific neurons across synapses. Troponeon is a calcium sensor, which indicates the activity of the targeted neurons. An ideal virus vector selectively spreads across synapses in only one direction and exerts a negligible effect on cell physiology in a relatively long period. Strain Bartha is a commercially available retrograde spreading strain of PRV, which is a widely used tract-tracing tool in neurobiology. However, according to our experience, transgenes, especially those of with complex structures become unstable (mutated or eliminated) in the Bartha background. Therefore, we have constructed wild type-based PRV vectors with an exclusively retrograde spreading characteristic by eliminating the gE and gI genes, which are essential for the anterograde spread of PRV. The PRV virion host shut-off (*vhs*) gene is a ribonuclease degrading the mRNAs of the host cell, and is a major virulence factor of the virus. We obtained a highly nonvirulent virus by deleting this gene. Mature virion particles derived from an infected cell can be passed to neurons across synapses (specific spread) or they can get to the intracellular space and infect synaptically non-linked neurons (non-specific spread). Potential non-specific spread can be eliminated by reducing viral virulence via eliminating various genes or DNA segments, including vhs, early protein 0 gene, antisense promoter (ASP) region, etc. Furthermore, in order to visualize the stage of viral infection, we have also inserted the mRFP gene to the virus genome. The mRFP has a much longer maturation time than that of GFP and other GFP-derived fluorescence proteins, therefore, neurons with GFP but without mRFP signal indicate a very early stage of infection and hence, an intact physiology. Recombinant viruses are being tested in the visual pathway of mouse.

GLUTAMATE-EVOKED GABA RELEASE THROUGH TRANSPORTER INTERACTION IN THE RAT HIPPOCAMPAL SLICE

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Growing amount of experimental data suggests an intricate interplay between inhibitory and excitatory neurotransmission. Glutamate (Glu) and gamma-amino butyrate (GABA) are two major neurotransmitters of the central nervous system, whose extracellular levels depend not only on the balance between synaptic release and uptake but also on extrasynaptic release and the metabolic state of the tissue.
We examined the effect of Glu (0.1 mM) on the radiolabelled GABA release from rat brain hippocampal slices. Inhibition of the dominant GABA transporter subtype by the non-transportable GAT1 inhibitor NNC-711 (100 μM) disclosed a Glu-induced GABA release (169 ± 12 % of the control), which was Ca^{2+}-independent. The effect could not be blocked by inhibiting Glu receptors with 10 μM CNQX, 50 μM AP5 and 500 μM MCPG. As the effect in nominally Ca^{2+}-free, high Mg^{2+} (20 mM) solution was the same (169 ± 24 %), the observed GABA release is only partially attributable to a direct, receptor-mediated effect of Glu on the synaptic release of GABA. In contrast, 0.1 mM and 1 mM D,L-TBOA decreased the effect of Glu on the GABA release to 135 ± 13 % and 122 ± 5 %, respectively. Because Glu-induced GABA release is markedly decreased by TBOA, a specific inhibitor of Glu transport, a plausible mechanism is the reversal of GABA transporters upon activation of Glu transporters. This transporter-mediated interplay represents a direct link between inhibitory and excitatory neurotransmission.

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FUNCTIONAL RELEVANCE OF THE IMMUNOREACTIVITY OF BASAL LAMINA COMPONENTS AND LAMININ RECEPTORS – A STUDY IN RAT BRAIN. II. PHENOMENA DURING MATURATION AND FOLLOWING LESION.

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Former studies suggested that immunoreactivities of laminin and its receptor dystroglycan-□, or their absence may characterise developmental, or functional stages of brain vessels, actually, those of the basal lamina. In the present study utrophin (another component of the dystrophin-dystroglycan complex), fibronectin (another adhesive proteoglycan), and agrin (also basal lamina component) were investigated following paraformaldehyde perfusion, in developing brains (E14-P15 days), and in lesioned brains. None of them was detectable by immunohistochemistry in the vessels of the intact mature brain (see the poster of Bagyura et al.) During development, however, immunoreactivities of utrophin and fibronectin transiently visualized the brain vessels already at E14, and gradually disappeared postnatally. Agrin also was found at E14, in the ganglionic eminence, but only at E16 in the pallium, and disappeared by E18. On the meningeal surface all the immunreactivities persisted. Following lesions (stab wounds in ketamine-xylazine anaesthesia), perilesional vessels were immunoreactive to utrophin in postoperative days 14 to 28. Fibronectin and agrin immunoreactivities were inconsequent. In the post-lesional, so-called 'secondary' basal lamina the utrophin immunoreactivity was rather similar to that of the original one, whereas that of agrin, and even more that of fibronectin became rough and uneven. These phenomena seem to correlate with former observations on the immunoreactivities of laminin and dystroglycan, and may complete those data in following the developmental and functional changes of the basal lamina.

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ORGANIZATION OF THE ARP2/3COMPLEX IN HIPPOCAMPAL DENDRITIC SPINES

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Synaptic plasticity is associated with morphological changes in dendritic spines. The actin-based cytoskeleton plays a key role in regulating spine structure, and actin reorganization in spines is critical for the maintenance of long term potentiation.
Among the actin binding proteins (ABPs) known to contribute to actin reorganization in model systems is the Arp2/3 complex, a seven subunit protein complex, which occurs in a wide range of organisms from yeast to mammals. Arp2/3 initiates actin polymerization and branching and is essential for actin-reorganization. However, despite its crucial role in actin remodeling, its localization and organization in brain is not known.
We performed immunoelectron microscopy on rat hippocampus to elucidate the subcellular distribution of the Arp2/3 complex. We here show that this ABP concentrated away from the non-synaptic membrane, but also away from the geometric centre of the spine. Since the Arp2/3 plays a pivotal role in reorganizing actin filaments, filament branching and polymerization is likely co-localizes to this “wedge-like” area of the spine.
These data add further weight to the idea of functionally-distinct core and shell regions of the dendritic spine: the Arp2/3 complex and cortactin concentrate in the spine core, where they polymerize actin filaments and establish branch-points, whereas dynamic depolymerization of this actin-spinoskeleton takes place in the spine shell, which is mediated by cofilin. Our present data imply a remarkable spatial segregation of actin regulatory processes within dendritic spines.

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COMPOSITION OF THE PERINEURONAL NET OF MOTONEURONS IN THE BRAIN STEM

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Lecticans, hyaluronan (HA) binding proteoglycans (PG), are major components of specialized extracellular matrix (ECM) coat surrounding the neurons called perineuronal net (PN). The PN is usually strong around fast spiking neurons expressing Ca-binding proteins especially parvalbumin. The absence of Ca-binding proteins in the motoneurons in pathologic conditions causes a neurodegenerative disorder called amyotrophic lateral sclerosis (ALS). Recently described, that the progress of ALS was associated with the Ca-buffering capacity of motoneurons of the oculomotor and the hypoglossal nuclei. The organization of PN around these motoneurons is not described to date but its polyanionic character may be associated with Ca buffering capacity of the ECM.
Since the ALS is never described in frog we studied the organization of PN of the oculomotor and hypoglossal nuclei compared with the mouse using monoclonal antibody against chondroitin-sulphate (ChS) and WFA histochemistry.
Strong WFA stained PN was found around the oculomotor neurons and the motoneurons of the hypoglossal nerve in mouse however no obvious WFA signal was detected in frog. The distribution pattern of the ChS differed from the WFA labeling. More ChS positive cells were found in mouse oculomotor and hypoglossal nuclei then WFA staining.
The absence of the WFA labeled neurons in frog may be associated with its regeneration potential and with the fact that the ALS is not described in frog.

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EFFECTS OF HYPOXIC/ISCHEMIC INJURIES ON NEUROBEHAVIORAL DEVELOPMENT OF NEWBORN RATS

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Severe perinatal hypoxia/ischemia is an important cause of brain injury in both full-term and premature newborns, with a high risk of future behavioral and neurological deficits. The most commonly used animal models are the unilateral ligation of the common carotid artery followed by exposure to hypoxia in 7-day-old rats and perinatal asphyxia, where the pups are delivered by cesarian section 15 min after decapitation of the mother. The aim of the present study was to describe the neurobehavioral development of the newborn rats following these injuries. Somatic growth, appearance and performance of neurological reflexes (sensory, grasping, gait, righting and negative geotaxis), and motor coordination were tested. Somatic development was more severely retarded in animals with carotid occlusion. Rats of both models displayed retardation in the appearance of several reflexes. Carotid occluded pups also displayed retarded performance in righting, geotaxis and gait reflexes. Motor coordination was affected in both models: performance of animals in rota-rod and foot-fault tests was significantly worse than in normal pups. Brain areas were reduced in hypoxic animals. However, there was no correlation between the severity of reduction in brain volumes and the early neurological deficits. Retinas of the injured animals also showed a significant reduction in thickness. Our results provide a basic set of neurological tests that can serve for further studies with neuroprotective substances.

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MENINGEAL NOCICEPTOR FUNCTION IN DIABETIC RATS: IMPAIRED CAPSAICIN-INDUCED NEUROGENIC SENSORY VASODILATATION

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Neuropathic alterations associated with diabetes affect cephalic pain mechanisms and vascular responses mediated by sensory nerves. Capsaicin-sensitive nociceptive afferents innervate the rat dura mater and may elicit vasodilatation through the release of calcitonin gene-related peptide (CGRP). This study was initiated in an attempt to reveal diabetes-induced alterations in the function of nociceptors of the dura mater by studying capsaicin-induced vasodilatation and CGRP release under *in vitro* conditions using laser Doppler flowmetry and measurement of peptide levels. Diabetes was induced with streptozotocin in adult male Wistar rats. Epidural application of capsaicin (10^{-7}-10^{-6} M) in a cranial window preparation produced distinct vasodilatory responses in control animals. In diabetic rats, capsaicin-induced vasodilatation was significantly reduced or even abolished 6 but not 2 or 4 weeks after the induction of diabetes. However, vasoconstriction, a non-neurogenic response to capsaicin at higher concentrations (10^{-5} M) was not altered in diabetic rats. The vasodilatory effects of histamine (10^{-5} M), acetylcholine (10^{-4} M) and CGRP (10^{-5} M) were similar in control and diabetic animals. In diabetic rats, *in vitro* experiments revealed a significant inhibition of capsaicin-induced release of CGRP. The results indicate an impairment of meningeal vasodilatory mechanisms involving capsaicin-sensitive nociceptors in diabetic rats whereas endothelium dependent vascular responses seem to be preserved. Hence, the findings suggest that limited removal of inflammatory mediators and/or tissue metabolites may contribute to the enhanced incidence of headaches in diabetics.

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THE EFFECT OF PLASMA MEMBRANE CHOLESTEROL DEPLETION ON THE ACTIVATION OF THE TRPV1 RECEPTOR

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Vanilloid receptor 1 (TRPV1) is a ligand-gated non-selective cation channel expressed by a subset of primary sensory neurons, notably the polymodal nociceptors. The plasma membranes of many cell types contain microdomains, called lipid rafts which are rich in cholesterol and sphingolipids and can be associated with various membrane receptors. Some members of the TRP receptor family has been shown to form signal complexes in lipid rafts, therefore we wanted to investigate how lipid raft disruption effects the TRPV1 receptor.

Cholesterol, the essential component of the rafts was depleted from the membrane of the cells by methyl-β-cyclodextrin (MCD) treatment. First, mammalian cells expressing the cloned TRPV1 receptor were incubated with 1 to 10 mM MCD and receptor activation induced by different treatments were measured by an assay using radioactive ^{45}Ca. In these experiments, MCD decreased the effect of 100 nM capsaicin activation, while had no effect on resiniferatoxin or low pH treatment. Next, the effects of MCD on TRPV1 receptor activation in isolated rat primary trigeminal ganglia neurons were studied by FURA-2 microfluorimetry. 10 nM MCD greatly reduced the effect of 333 nM capsaicin treatment on these TRG neurons as well. Finally, the effect of MCD on TRPV1 receptors situated in the nerve endings of polymodal nociceptors were investigated by measuring capsaicin induced neuropeptide release from nerve terminals in isolated rat trachea. In these experiments, 100 μM MCD treatment reduced the effect of 1 μM capsaicin, while had no effect on electric stimulation induced CGRP peptide release. These results clearly indicate that MCD treatment inhibits the effects of capsaicin on the TRPV1 receptor.

HISTOCHEMICAL DEMONSTRATION OF EXTRACELLULAR MATRIX COMPONENTS IN THE ADULT AND DEVELOPING CENTRAL NERVOUS SYSTEM OF SNAILS (*LYMNAEA STAGNALIS, HELIX POMATIA*)

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In the present study the distribution of extracellular matrix (ECM) molecules was investigated in the adult and developing CNS of two pulmonate snail species, *Lymnaea stagnalis* and *Helix pomatia*, applying different histochemical methods. At pH 4.0, the basic thiazin dye, toluidine blue, resulted in a selective, fluoromethachromatic staining of glial cells and ganglion neuropil in both species. Staining with alcian blue at acidic pH and with acridine orange at neutral pH indicated that acidic proteoglycans, among them presumably mainly hyaluronic acid, were present more abundantly in *Helix* ganglia. On the other hand, non-acidic proteoglycans, detected by acriflavine-Schiff staining, seemed to frequently occur in the ganglion neuropil of both the adult *Helix* and *Lymnaea*, and they appeared from E80% embryonic stage in developing *Lymnaea*. Applying 14 different fluorescence lectin probes, those labeling N-acetyl-glucosamine oligomers (jimson weed, tomato, potato and wheat germ lectins) or recognizing N-acetyl-galactosamine oligomers (Jack been, castor been and peanut lectins) visualized the presence of ECM in different regions of the CNS of both species, such as the layer of neuronal and glial perykarya, the ganglion neuropil and the periganglionic connective tissue sheath. In the ganglion neuropil, some proteoglycans, demonstrated by jimson weed, tomato, and potato lectins, displayed a transient occurrence from E40% embryonic to early postembryonic stages. On the other hand, proteoglycans, labeled with wheat germ lectin, could be observed in the ganglion neuropil throughout the whole gangliogenesis, but the intensity of their labeling was increasing until adulthood. Our results are the first demonstrating ECM molecules in the gastropod CNS, and they indicate significant differences in the composition of the extraneuronal environment of the aquatic *Lymnaea* and terrestrial *Helix*. It is also suggested that the molecular composition of ECM is different in the developing and adult *Lymnaea* CNS.

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LOCALIZATION OF SINGLE CELL MEMBRANE CURRENTS BASED ON EXTRACELLULAR POTENTIALS PATTERNS.

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Traditional current source calculation method (CSD) method allows calculation of current source distribution from the exracellular potential patterns, thus provides important information for neurophysiology. The traditional CSD method is based on strong physical foundations, but uses some assumptions, which can not hold for single cell activity. By this reason, traditional CSD method gives false results for single cell activity.
A new, single cell CSD method have been developed, directly designed for revealing current source density distribution of a single cell, during firing. This new method were applied on extracellular spatial potential patterns of spikes. The spikes were measured *in vivo* cat primary auditory cortex with a 16 channel chronically implanted linear probe. Using our new method, many fine details of the spatio-temporal dynamics uncovered. Dendritic back propagation was proven to be much more frequent than it was known before, it was observable in almost every cell. The speed of back propagation was typically different in the appical and basal directions. In contrast to the literature, forward propagation preceding the spikes was also observable. In perspective, this new method raises the possibility of identifying synaptic inputs, which causes a cell fire.

EFFECTS OF HIPPOCAMPAL CANNABINOID IMPLANTS ON MEMORY IN RATS

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Cannabinoids have strong effects on memory as well as emotional responses. These effects are mediated at least partly by the hippocampus, as this brain structure is densely packed with cannabinoid CB1 receptors. The present study aimed at investigating the effects of dorsal hippocampal cannabinoid implants on memory. Besides placing capillaries filled with cannabinoid agonist WIN-55,212-2 alone or in combination with cannabinoid antagonist SR-141716A into the hippocampus, we made a check-up test which helped to clarify whether the given compounds are really traceable in the brain tissue. In this latter investigation the implantations contained a combination of WIN-55,212-2 and radioactive ^{3}H-WIN-55,212-2.
Our results showed that dorsal hippocampal cannabinoid implants affect memory: the cannabinoid agonist WIN-55,212-2 inhibited the recognition of a novel object, but this effect disappeared when the agonist was implanted together with the cannabinoid antagonist SR-141716A. In the check-up test we could find significant difference in the level of radioactivity measured in the hippocampal extracts of control vs implanted animals.
On the basis of these results dorsal hippocampal cannabinoid signaling appears to be involved in memory. Additionally it seems that our implantation methods are appropriate means for independent stimulation of the cannabinoid system of a limited, small brain area.

ROLE OF STAGE SPECIFIC EMBRYONIC ANTIGEN 1 (SSEA-1) IN THE MAINTENANCE AND NEURAL DIFFERENTIATION OF THE EMBRYONIC NE-4C NEURAL STEM CELLS

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SSEA-1/Lex/CD15 is a cell-surface carbohydrate antigen carried by ES cells, bone marrow stem cells, adult and embryonic neural stem- and progenitor cells. It is supposed to play roles in cell adhesion, migration, growth factor responses of stem cells, but its function in neural differentiation is not clear.
In non-induced cultures, 40-60% of the one cell derived, neuroectodermal NE-4C stem cells show high SSEA-1 immunoreactivity. The amount of SSEA-1 carried by individual NE-4C cells seems to reflect the physiological and developmental state of the cells. FACS selection of non-differentiated NE-4C cells shows that SSEA-1 positivity of sorted populations correlates positively with the rate of growth. During retinoic acid (RA) induced neural differentiation, the differentiated progenies of NE-4C cells lose SSEA-1 immunoreactivity. The mRNA of α1,3-fucosyltransferase IX, the enzyme catalyzing the final step of the biosynthesis of SSEA-1 epitope, remains detectable in the cultures, in the entire period of *in vitro* neural differentiation. In differentiated cultures which contain neurons and astrocytes as well, a sustaining population of stem cells bears the SSEA-1 antigen.
While the expression of SSEA-1 seems to be bound to some non-differentiated cell-state, the function of SSEA-1 molecules in the proliferation or survival of stem cells needs further elucidation.

AN ANALYSIS OF GEOMETRICAL AND FUNCTIONAL DISTRIBUTIONS OF RETICULOSPINAL AND PROPRIOSPINAL SYNAPSES RECEIVED BY MOTONEURONS IN FROGS (*RANA ESCULENTA*)

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Investigation of identified single fibre monosynaptic connections provide us with the opportunity to study the effectivity of synapses; how they can affect the membrane potential of the soma in the postsynaptic neuron. By comparing the size of unitary synaptic potentials (uPSP), the dendritic morphology of the postsynaptic neuron, the number and location of boutons between the axon and the dendrites we can study factors that may play a role in synaptic efficiencies.
We studied two sets of connections received by motoneurons (MN) in adult frogs: propriospinal axon – MN pairs (n=3) and reticulospinal – MN pairs (n=3) in the lumbar and brachial spinal cord respectively. Unitary PSPs evoked by intraaxonal stimulations were recorded in the MN soma, axons and MNs were filled by neurobiotin, MNs spatially reconstructed and close appositions between boutons and dendrites were marked. Statistical analysis showed that the mean uPSPs were the same despite the significant difference in the mean path distances between the boutons and soma with similar number of boutons /axon-MN pair in the two sets of connections.
We used computer models of the MNs and their connections to look for possible factors that may compensate for the double mean distance of putative synapses in the propriospinal connections. Size of synaptic conductances and the morphoelectrotonic distributions of synapses over the dendrites were studied by cluster analysis of morphoelectrotonic matrices.

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NITRIC OXIDE SIGNALING IN HIPPOCAMPAL GABAERGIC SYNAPSES

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Nitric oxide (NO) plays an important role in synaptic plasticity as a retrograde messenger. Here we propose a new mechanism whereby it can modify transmission at GABAergic synapses, which terminate on hippocampal pyramidal cells. We describe for the first time that in hippocampal pyramidal cells nNOS is associated to the postsynaptic active zones of symmetrical synapses in somatic, dendritic and axon initial segment synapses established by GABAergic cells, in mice and rats. The NO receptor, nitric oxide sensitive guanylyl cyclase (NOsGC) is present in the brain in two functional subunit composition: α1β1 and α2β1. The β1 subunit is expressed both in pyramidal cells and in interneurons in the rat hippocampus, however the exact localization of the α subunits was unknown. Here we describe the expression pattern of NOsGC α1 and α2 subunits in the CA1 region and show that their cell-type specific expression. We also found that α1 subunit positive interneurons were always positive for β1 and vice versa, however pyramidal cells stained only for β1, but not for α1 subunit. With double immunofluorescent staining, we found that two thirds of cholecystokinin positive, three fourth of parvalbumin positive, one third of somatostatin positive and one fifth of neuronal nitric oxide synthase (nNOS) positive neurons are positive for the α1 subunit.

Using preembedding immunogold and immonoperoxidase double labeling technique, we also investigated whether NOsGC is transported to the axon terminals. We found that the α1 subunit is present in parvalbumin and cholecystokinin positive basket cell terminals and in cholecystokinin positive terminals on pyramidal cell dendrites. Our results suggest that NOsGC composed of α1β1 subunits is selectively expressed in different types of interneurons, and is transported to the terminals as well, where it potentially detects NO released from pyramidal cells or interneurons.

THE PROTECTIVE EFFECT OF THE WLD^S GENE ON THE RAT LUMBAR MOTONEURONES FOLLOWING AVULSION INJURY

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The slow Wallerian degeneration gene, *Wld^S*, delays Wallerian degeneration and axon pathology for several weeks in mice and rats. Interestingly, neuronal cell death is also delayed in some *in vivo* models, most strikingly in the progressive motoneuronopathy mouse. Here, we tested the hypothesis that *Wld^S* has a direct protective effect on motoneuron cell bodies *in vivo*. By intravertebral avulsion of L4 ventral root in rats, cell death of the corresponding motoneurons was induced and simultaneously most of the motor axon length was removed, thus minimizing the possibility that the protective effect toward axons could rescue cell bodies secondarily.
There was no significant difference between the survival of motoneurons in control and *Wld^S* rats suggesting that *Wld^S* gene has no direct protective effect on cell bodies.
We also tested for any delay in apoptotic motoneuron death following neonatal nerve injury in *Wld^S* rats and found that, unlike *Wld^S* mice, *Wld^S* rats show no delay in cell death. However, the corresponding distal axons were preserved, confirming that motoneuron cell bodies and motor axons die by different mechanisms.
Thus, *Wld^S* does not directly prevent death of motoneuron cell bodies. It follows that the protection of neuronal cell bodies observed in several disease and injury models where axons or significant axonal stumps remain is most probably secondary to axonal protection.

LOCALIZATION AND DENDRO-DENDRITIC CONNECTIONS OF THE COCHLEAR EFFERENT NEURONS IN GUINEA PIG

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The hair cells in the organ of Corti are innervated by the efferent neurons of the superior olive (medial olivocochlear efferents, MOC) in the brainstem. Most of the neurons are excited by only one ear with a binaural facilitation. The central parts of the MOC pathways are not yet completely characterized with morphological methods, therefore we have labeled the cochlear nerve with neuronal tracers in guinea pigs. In the anesthetized animals the cochlear nerve was exposed and labelled in the basal part of the modiolus. In one group of animals biotinilated dextrane amine (BDA) was applied to the injured nerve. In the other group of animals the bilateral cochlear nerves were explored and fluorescein binding dextran amine (FBDA) was applied onto the injured cochlear nerve at one side and tetramethylrhodamine dextran amine (RBDA) was the tracer on the other side. After three-four days the animals were perfused transcardially with 4% paraformaldehyd and serial sections of the brainstem were cut in transverse plane. The BDA was visualized with ABC-DAB chromogen reaction. The positions of labeled efferent neurons were reconstructed with Neurolucida. We have detected the labelled cells bilaterally in the superior olivary complex and in the trapezoid body. By using of confocal laser microscope we could detect dendro-dendritic connections suggesting synaptic contacts between the bilateral olivary neurons. These connections might serve as one of the underlying mechanisms of the binaural facilitation mediated by the olivocochlear reflex.

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EARLY OLFACTORY DEPRIVATION INDUCED MORPHOLOGICAL CHANGES IN THE RAT RMS.

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The subventricular zone (SVZ) persists as a germinal zone into adulthood and thus functions as the largest neurogenic region in the adult brain. SVZ cells migrate along the rostral migratory stream (RMS) to the olfactory bulb where they differentiate into interneurons. Although neurogenesis occurs continuously throughout adulthood, the rate of proliferation and the fate of new cells may be affected by exogenous factors. The aim of our study was to investigate the effect of early olfactory deprivation (OD) on neurogenesis in the rat RMS.
Maternal separation, a well-characterized model of early life stress in rodents, has been used as a model of OD. Rat pups from postnatal day 1 (P1) to P21 were separated from their mother for 3 h daily, and compared to pups that remained with their mother. At the end of OD, resp. one week later (P28) the rats were injected with BrdU (50 mg/kg) and perfused after 2 h. Dying cells were visualized by Fluoro-Jade B histochemistry.
The RMS of OD rats in the sagittal sections had typical L-shape and its three regular parts, the vertical arm, elbow and horizontal arm, were preserved after both times of survival. However, the results of BrdU immunohistochemistry showed that OD specifically affects cell proliferation in the migratory stream. At P21 dividing cells in the RMS vertical arm almost disappeared. At same time, the number of dying cells noticeably increased in this part of the RMS. In P28 animals the amount of dividing cells in the RMS vertical arm lightly increased but was still reduced in comparison with control rats. Our results show that adverse experience early in life may induce acute site-specific changes in the RMS morphology.

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DOES WHOLE-BODY MICROWAVE EXPOSURE INDUCE CHANGES OF POSTNATAL NEUROGENESIS IN RATS?

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In comparison to the wide range of literary data about the health effect of electromagnetic field (EMF), the data studying morphological changes in nervous system of experimental animals exposed to the microwave radiation are rare. Our study deals with immunohistochemical analysis of rostral migratory stream (RMS) after whole body exposure of newborn and aging rats.

Newborn (P7) and aging rats (2 years) were irradiated with a pulsed-wave EMF of 2.45 GHz at average power density 6.7 mW/cm^2 and 2 mW/cm^2 in a purpose-designed exposure chamber for 3 and/or 2 days (5h/day). Acute changes were evaluated 1 day after irradiation and chronic changes after 1, 2 and 3 weeks of post-irradiation survival. 4 h before perfusion fixation the animals were injected by a single BrdU i.p. injection. BrdU immunohistochemistry was provided on 24 μm cryocut sections. Statistical analyses were performed by ANOVA and Tukey-Kramer tests.

EMF at 6.7 mW/cm^2 induced in P7 significant decrease of BrdU-positive cells and in addition, enlargement and protrusion of lateral ventricles into the RMS vertical arm evident in each post-irradiation survival period. The P7 rats irradiated at 2 mW/cm^2 showed increased number of the BrdU-positive cells in acute post-irradiation survival. The RMS of aging rats was characteristic by decreasing of BrdU positive cells.

The present study put evidence that EMF of 2 and 6.7 mW/cm^2 induces age dependent and dose dependent changes of postnatal neurogenesis manifested as increase/decrease of proliferating cells number within the rat RMS.

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COMPARATIVE IMMUNOHISTOLOGICAL AND ELECTROCHEMICAL EXAMINATIONS ON SEROTONINERGIC INNERVATION OF MUSCLES IN *EISENIA FETIDA*

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Serotoninergic innervation in muscles of *Eisenia fetida* was investigated using immunohistological staining and *in vivo* electrochemical measurements.

The circular muscle layer has a richer 5-HTergic innervation than the longitudinal one. The relative density of immunoreactivity in the circular muscle layer is 2.73±1.0%. Differential Pulse Voltametric (DPV) measurement with carbon fiber microelectrodes in this muscle layer produced a 272.5±15.0 nA peak current. These values in the prostomium were lower, 1.02±0.2% and 135.0±5.8 nA. Using prerecorded calibration curves these values corresponded approximately 320 μmolar and 158 μmolar 5-HT concentrations, respectively.

In the visceral muscles of the pharynx and the gizzard 5-HTergic nervous elements appear as single fibres or small fibre bundles. The occurance and arrangement of the 5-HTergic fibres are similar to the ones observed in the somatic muscle. The density of the 5-HTergic fibres, as well as the values of DPV measurements clearly reflect differences of the 5-HT concentration. In the gizzard, densitometric and electrochemical evaluation gave 1.28±0.6% and 137.5±12.6 nA values, respectively, while 1.12±0.6%; and 122.5±5.0 nA were obtained in the pharynx. These peak height values corresponded to about 161 μM and 158 μM concentrations, respectively. According to our present data we conclude that the DPV method could be a good technique to determine the changing serotonin content in various *in vivo* physiological conditions.

POSTNATAL EXPRESSION PATTERN OF DOUBLECORTIN (DCX) IN SOME AREAS OF THE DEVELOPING BRAIN OF MOUSE

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Doublecortin (DCX) – a microtubule-associated protein – is expressed in migrating and differentiating neurons throughout the central and peripheral nervous system during embryonic and postnatal development. Expression of DCX remains high within neurogenic regions of the adult mammalian brain, i.e. rostral migratory stream (RMS) and newly generated neurons in the adult dentate gyrus (DG). We compared the postnatal expression pattern of DCX from P2 to P22 in the RMS and DG with special emphasis on the cerebellum of mouse by immunohistochemistry. Weak expression of DCX was detected in the RMS at P5 and become gradually stronger during the second postnatal week and reached its strongest expression by P18-P22. Moderate DCX immunostaining was present in the DG at P8, its marked expression – characteristic of newly generated neurons in the adult DG – appeared only by P22. The earliest expression of DCX was found at P2 in the premigratory zone of the external granule cell layer in the cerebellum, and remained rather strong throughout the whole period while granule cell precursors were present. Besides descending granule cell precursors, upward migrating precursors of molecular layer interneurons exhibited also DCX immunostaining: these bipolar neurons were arranged in the sagittal plane and built up transitory migratory streams during the second postnatal week (especially at postnatal day P11/P12). During the third postnatal week, their number gradually decreased and finally disappeared. Expression of DCX was detected not only in bipolar (migrating) neurons, but also in remodeling dendritic processes during their settlement, reflecting on the role of DCX in postnatal neuronal migration and differentiation in the developing cerebellar cortex too.

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INDUCTION PATTERN OF MMP-9 AND -2 IN A MODEL OF ABSENCE EPILEPSY

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Genetic models of human absence epileptic seizures allow to investigate molecular mechanisms of seizures. Spike-wave discharges (SWDs) of WAG/Rij strain is a good model to study. The number and duration of SWDs increase with age and show circadian pattern. Induction of gelatinases (MMP-9 and -2) has been described in focal epilepsy models correlating with sustained depolarisation and cell loss. In our previous study, 4-aminopyridine induced seizures caused strong MMP-9 induction without massive cell loss suggesting the possibility that MMP-9 induction is not necessarily coupled with cell damage but could participate in protective mechanisms preserving neurons from death. Thus, to study the role of these MMPs in absence epilepsy is a real need so we examined the pattern of MMP-9 and -2 in WAG/Rij rats. We measured MMPs by zymography in typical periods of seizure genesis (seizure and non-seizure). We found that the daily pattern of MMP-9 induction show correlation with the appearance of seizures. Furthermore, we investigated the effect of intraperitoneal LPS injection enhancing the number of SWDs on increase of MMP-9 levels. In SPRD rats LPS treatment had weaker effect on MMP-9, than in WAG/Rijs. Comparing physiological gelatinase levels in young and old rats we found that high amount of MMP-9 in young brain is sustained in old WAGRij rats but not in SPRDs. These high MMP levels in young rats can be explained by it's role in brain development. Our results suggest that the MMP-TIMP system and the related protein network in WAG/Rijs is really sensitive and might be part of some protective mechanisms that prevent the extensive damage of the brain caused by abnormal neuron activities in absence epilepsy.

NEUROPROTECTIVE EFFECTS OF VINPOCETINE IN PRIMARY CORTICAL NEURONAL CULTURES

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Vinpocetine (ethyl apovincaminate), a synthetic derivative of the Vinca minor alkaloid vincamine is widely used for the treatment of cerebrovascular-related diseases. One of the possible mechanisms underlying its action is protection against the cytotoxic effects of glutamate.

As glutamate excitotoxicity leads to the disregulation of mitochondrial function, we investigated on primary embryonic cortical neuronal cultures whether Vinpocetine has a direct action on mitochondrial membrane potential. Flow cytometry indicated that 25µM Vinpocetine reduced the decrease of mitochondrial inner membrane potential induced by glutamate treatment.

As Vinpocetine has a binding affinity to the peripheral type of benzodiazepine receptor (PBR) involved in the mitochondrial transition pore complex, the neuroprotective effects of Vinpocetine and of PK11195 and Ro5-4864, two additional drugs with selective and high affinity to PBR, were compared in glutamate excitotoxicity assays. Vinpocetine protected the cells in a 1-50 µM concentration range. PK11195 and Ro5-4864 were also slightly neuroprotective especially in high concentrations, but with different acting profiles. Combining the pretreatment of PK11195 or Ro5-4864 with the administration of Vinpocetine showed that PK11195 or Ro5-4864 increased the neuroprotection by Vinpocetine in a dose dependent manner indicating that the action mechanisms of these drugs may be different.

Taken together, our results do not suggest neuronal PBRs as a direct target for the neuroprotective action of Vinpocetin in culture.

IMPACT OF TRAUMATIC EXPERIENCE ON AGGRESSION

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Post traumatic stress disorder (PTSD) has a multi-causal psychopathology with multiple characteristics depending on the type of the trauma. Main symptoms of PTSD are anxiety associated with traumatic events (avoidance, nightmares) and hyperarousal. Other symptoms like depression, impaired impulse control and violence, can also accompany some forms of PTSD. PTSD associated with violence can be characterized by rather hypo- than hyper arousal, therefore presenting a physiologically different stress disfunction and behavioural change. In our experiments we investigated the effects of different traumatic events (single footshock, single or multiple defeat from a conspecific) on aggressive behaviour of adult male Wistar rats and the accompanying hormonal and neuronal activation. Clarifying the acute and chronical impact of trauma we monitored behaviour either 1 day or 28 days after the traumativ experience. Results suggest that non-aggressive trauma results in lowered aggression both in acute and chronical manner. Social defeat as a traumatic experience induced a different and more complex changes in agonistic behaviour. Activation of HPA axis after trauma showed only a contact-specific elevation (control, psychosocial, fighting respectively), but trauma itself did not influence the hormonal levels. Crucial brain regions controlling aggressive behaviour (hypothalamus, amygdala, prefrontal cortex) showed a similar, contact-specific activation. Our data suggest that different type of the traumatic experience may result in differencies in aggressive behaviour.

SIMULTANEOUS VISUALIZATION OF MULTIPLE ANTIGENS WITH TYRAMIDE SIGNAL AMPLIFICATION USING ANTIBODIES FROM THE SAME SPECIES

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After immunohistochemistry (IHC) began to be used routinely, a number of investigators worked on methods for staining multiple molecules in the same tissue sections or cells. Achieving this goal was not easy, however. One reason for this is that the majority of primary antibodies employed in IHC reactions are raised in rabbits, and recognizing signals from two different rabbit antibodies is not trivial. Thus, all of the protocols described to date have serious limitations. Here we report a simple, quick, and cheap solution to the problem. It has two major advantages over existing methods: First, two or more antigens can be visualized in the same section with fluorescent dyes that are commercially available. Second, since the technique relies on signal amplification, both rare and abundant antigens can be detected.

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CHANGES IN TARGET SELECTIVITY OF CALRETININ-POSITIVE INTERNEURONS IN THE CA1 REGION OF THE EPILEPTIC HUMAN HIPPOCAMPUS

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Calretinin (CR) is expressed in dendritic and interneuron selective inhibitory cells in the CA1 region of the human hippocampus. CR-containing cells were shown to be vulnerable to ischemic and epileptic injury: their numbers decrease and their dendrites become segmented. We aimed to examine the CR-positive terminals and their targets in the control and epileptic human hippocampal CA1 region. Two types of CR-positive terminals can be found: Type 1 originates from the thalamic nucleus reuniens and establishes asymmetric synapses. Type 2 originates from local interneurons forming symmetric synapses. Surgically removed hippocampi of temporal lobe epileptic patients were examined and compared with post mortem control samples. The immunostained samples were analyzed in the electron microscope. In control samples, the majority of CR-positive synapses were symmetric in the strata oriens and pyramidale. In the stratum lacunosum-moleculare mainly asymmetric synapses were detected. Our results show that the relative ratios of CR-containing terminals giving asymmetric synapses increased and those giving symmetric synapses decreased in the epileptic tissue. In controls, 22% of the terminals targeted CR-positive dendrites, whereas in the epileptic samples it was reduced to 8-11%. The number of contacts between CR-positive dendrites also dropped. These results suggest a reduced inhibition and synchronization of dendritic inhibitory cells by interneuron-specific interneurons, which may lead to abnormal plasticity of excitatory inputs.

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EFFECTS OF INTRAAMYGDALOID GHRELIN ON PASSIVE AVOIDANCE LEARNING

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The brain-gut peptide acylated-ghrelin (aGhr) known as a potent growth hormone (GH) secretion increasing substance. aGhr has influence on memory and learning processes, too. Its effect is realized partly via GH secretagogue receptor (GHSR) type 1 a. The amygdaloid body (AMY) plays important role in the memory and learning processes. Projections of ghrelinergic neurons were identified in the AMY, and previously we verified that aGhr infused into the AMY caused liquid food intake decrease. The aim of present study was to examine the possible effects of aGhr in the AMY on learning. Male wistar rats were examined in two-compartment passive avoidance paradigm. Animals were shocked with 0.4 mA and subsequently were microinjected bilaterally with 50 and 100 ng aGhr, 30 ng GHSR antagonist D-Lys3-GHRP-6 (ANT), ANT+ 50ng aGhr (dissolved in 0,15 M sterile NaCl /0,4µl) or vehicle into the AMY. Fifthy ng aGhr significantly increased the latency time, the 100 ng and the ANT alone were ineffective. The effect of 50 ng aGhr was eliminated by the ANT pretreatment. Effect of 50 and 100 ng aGhr were also investigated on spontaneous motor activity in openfield test (OPF) and on anxiety in elevated plus-maze(EPM). In OPF the number of crossings and the distance moved, in EPM the time spended on closed and opened arms were recorded during 5 min observation periods. There were no any alterations in these parameters in aGhr treated rats compared to controls. Our results suggest that in aversive situations intraamygdaloid aGhr enhances learning processes and memory. It is a specific effect, because it was eliminated by ANT pretreatment and can not be explained by any changes of spontaneous motor activity or anxiety.

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MORPHOLOGICAL LINK BETWEEN CHOLINERGIC AND GONADOTROPIN-RELEASING HORMONE NEURONS IN THE RAT BRAIN

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The central regulation of gonadal functions is attributable to luteinizing hormone releasing hormone (LHRH) producing neurons located in the preoptic area of the rat brain. The neurosecretory axons terminate in the median eminence where they release LHRH into the hypophysial portal circulation. A wide variety of neurotransmitters, including acetylcholine (ACh), modulates LHRH cell functions. ACh effectively stimulates LHRH release *in vitro* from mediobasal hypothalamic fragments at nanomolar concentrations (Richardson SB. et al. 1982). In order to reveal the structural correlates of the cholinergic regulation of the LHRH system, immunocytochemical double labeling was performed in male Wistar rats.

Cholinergic fibers were revealed using antibodies against either the ACh-synthesizing enzyme, choline acetyltransferase (ChAT; Chemicon, AB144P) or against vesicular acetylcholine transporter (VAChT; Sigma, V5387) which takes up ACh into synaptic vesicles. For detection of LHRH, a polyclonal antibody (LR-1, a gift from Dr. R. Benoit) was used. At both light and electron microscopic levels, ChAT- and VAChT-immunoreactive (IR) boutons were directly apposed to the dendrites, and less frequently, to the perikarya of LHRH neurons. In some cases, asymmetric synapses were observed between the juxtaposed profiles.

These findings indicate that central cholinergic pathways are capable of regulating LHRH neurons via forming direct connections.

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REGENERATION AND PEPTIDE PROFILE CHANGES IN THE CENTRAL NERVOUS SYSTEM OF *EISENIA FETIDA*

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Some earthworm species have enormous capability to restitute their losted body segments. In this process, commonly named "regeneration", certain structures (e.g. neoblasts, central nervous system) and several chemical substances (e.g. hormons, neurotransmitters, growth factors) are believed to be involved, however, main steps of regeneration remained unknown yet.

This study focuses on the effects of the central nervous system (CNS) to the caudal regeneration of *Eisenia fetida* applying rigorous experimental protocols (conventional light and electron microscopy) to follow the kinetics of caudal segment restitution. In addition, the changes of the peptide profiles of the ventral nerve cord (VNC) were observed by means of SELDI-TOF Mass Spectrometry in normal and in damaged VNC and in regenerated ganglia.

In our investigations the dynamics of regeneration of intact worms were compared to those ones, where (1) the brain, (2) the first 3 segments (3) and the circumpharyngeal connectives were removed, by counting regenerating segments. The results showed that the effects of all these kinds of operation stimulate the caudal regeneration.

SELDI-TOF Mass Spectrometry analysis of control and severed ventral nerve cord showed that only a distinct peptide group, characterized by 1-5 kDa mass intervals, was up regulated in the damaged ganglia suggesting their role in regeneration.

Our results prove that (i) CNS of earthworms synthesizes several peptides that have influence on the caudal regeneration, further (ii) brain products have putative inhibitory effect on segment formation. Identification of these peptides is in progress in our laboratories.

EXPRESSION AND POSSIBLE ROLE OF GLUTAMIC ACID DECARBOXYLASE-65 (GAD-65) IN THE MIGRATION OF LHRH+ NEURONS DURING MOUSE DEVELOPMENT

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LHRH (luteinizing hormone- releasing hormone) neurons originate from the olfactory placode around gestation day E10,5 in the mouse and migrate along the vomeronasal nerve towards their final destination in the septo-preoptical area (POA).

□-Aminobutyric acid (GABA) is synthesized by two highly homologous GAD isoforms, GAD-65 and GAD-67, which differ in sub-cellular localization and kinetic properties. It has been shown that GAD-67 affects the migration and positional diversity of the embryonic LHRH neurons. Here we have examined the expression of GAD-65 in the migratory LHRH neurons in wild-type and transgenic mice, and followed the migration of LHRH neurons in GAD-65 knock-out mouse embryos.

Our results show that GAD-65/GFP is prominently expressed at earlier and intermediate migratory stages and is down-regulated shortly before settling in the POA. The level of GAD65 gene expression is very low in LHRH neurons migrating on the vomeronasal axons as revealed by in situ hybridization, but becomes detectable in the ventral forebrain in E14,5 mouse embryos. Distribution of LHRH cells along the migratory pathway is different in the GAD65 *knock-out* mice compared to the wild type, indicating that GABA synthetized by GAD-65 enzyme in LHRH cells of the nasal compartment may increase their speed of migration, which is in line with the proposed inhibitory role of GABA on migration of LHRH neurons. Moreover, GABA synthetized by the GAD65 enzyme may play a role in the guidance afforded by the strenght of attachment between LHRH neurons and vomeronasal axons. As a result, in the absence of GAD65 enzyme the migrating LHRH neurons can be "mis-guided" to ectopic positions.

CHRONIC ROTENONE TREATMENT OF THE INTACT SNAIL *LYMNAEA STAGNALIS* DECREASES THE TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND INCREASES THE DOPA-DECARBOXYLASE IMMUNOREACTIVITY IN THE CNS

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In gastropods the central pattern generators producing rhythmical movements during feeding and locomotion are commanded by dopaminergic elements and modulated by the serotonergic system. The muscular tone is also modulated by both 5HT and DA. We tested the effect of rotenone on the pond snail *Lymnaea stagnalis*. In experimental animals the rotenone effect is related to selective impairment of the dopaminergic functions in the CNS.
Chronic exposure of the intact snails for 1-8 days in 0.5 µM rotenone resulted their impaired behaviours. The animals displayed severe postural abnormalities (due to their decreased muscular tone) as well as gradually decreasing spontaneous locomotory activity. The feeding rate (the number of bites/minute of the feeding animal) similarly showed a progressive decrease in 0.5 µM rotenone until the 4th day, when all treated animals stopped feeding. In the CNS of animals exposed to 0.5 µM rotenon for 7 days HPLC assays revealed a decreased level of dopamine both in the buccal and subesopageal ganglia, while the serotonin content decreased only in the subesophageal complex of the CNS. Immunocytochemical study revealed the loss of tyrosine hydroxylase labelling in dopamine-containg elements (including the giant dopaminergic neuron in the pedal ganglion) but no change in serotonin-immunostaining in the CNS. Moreover, rotenone treatment evoked a marked increase of aromatic amino acid decarboxylase immunoreaction in both the serotonin and tyrosine hydroxylase immunostained central neurons.

Our results suggest that the abnormal body posture and behavioural impairments (decreased locomotion and feeding) of the animals during rotenone treatment are due to selective inhibition of the dopaminergic functions. Therefore the probably still intact serotonergic system result a serotonin dominance in muscular modulation, causing a general relaxation of the muscular system.

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EFFECTS OF OXYGEN TENSION ON THE SURVIVAL AND DIFFERENTIATION OF NEURAL STEM CELLS

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Previously we demonstrated that the fate of implanted stem cells was modified by the host environment. Brain lesions, causing long-lasting ischaemia (hypoxic conditions), were suspected to influence the survival and differentiation of exogenous stem cells. Therefore, we examined the fate of GFP-4C neural stem cells in experimental environments with different oxygen tensions. The effects of *hypoxic conditions* were investigated, on retinoic acid (RA)-induced or non-induced neural stem cells. Under *in vitro* hypoxia (1% O_2, 6 hours), the viability of non-differentiated GFP-4C cells did not decrease. After induction, differentiating neural cells needed higher O_2-supply. The consequences of *hyperbaric oxygen* treatment (hyperoxia) were studied *in vivo*, on cold lesioned host animals implanted with GFP-4C cells. For lesions, a modified model of Klatzo et al. was applied. For implantation, GFP-4C neuroectodermal stem cells were used (Demeter et al. 2004). Lesioned and normal mice were treated with hyperbaric oxygen (2.5 ATA 100% O_2 for 90 min), each day for one-week, either before or after implantation. The host animals were sacrificed 2 and 6 weeks after the implantation. In lesioned, hypoxic cortex, GFP-4C cells survived for a long period (>2 months), but did not differentiate to neurons and only sporadically to astrocytes. In animals treated with hyperbaric oxygen, an increased rate of differentiation was detected: the implanted cells survived for an equally long period, but formed both neurons and astrocytes.
The data indicate that neural stem cells can survive and proliferate under hypoxic conditions, but require higher O_2 tension for neural tissue type differentiation.

LONG -TERM CONSEQUENCES OF LOW FREQUENCY ELECTROMAGNETIC FIELD EXPOSURE IN RATS: A CHRONIC STRESS STATE?

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It is believed that different electromagnetic fields have some effects on biological systems, ranging from damaging to curing. The aim of our present work was to study the long term consequences of low frequency electromagnetic fields (LF-EMF, e.g. above trasformers) with a special focus on the development of chronic stress.

Adult male Sprague-Dawley rats were exposed to LF-EMF (50Hz, 500μT) for 5 days, 8h daily (short) or for 4-6 weeks, 24h daily (long). Anxiety was studied in elevated plus maze test (EPM), whereas depression like behaviour of the long-treated group - was examined in the forced swim test (FS). Some days after behavioural examination, the animals were decapitated while in resting state and organ weights, blood hormone levels as well as ACTH precursor POMC mRNA level from the anterior lobe of the pituitary were measured.

Both treatments were ineffective on somatic parameters, namely non of the changes characteristic to chronic stress (body weigth reduction, thymus involution and adrenal gland hypertrophy) were present. The usual POMC elevation was also missing. The hormonal stress-reaction was also similar in control and short term exposed rats, although long-term EM-exposure induced a mildly reduced stress reactivity together with an enhanced open arm time on EPM (anxiolysis). The long-term treatment also induced a depressive-like change with longer floating time in the FS test. An enhanced blood glucose level was also found after prolonged EM-exposure.

Taken together, chronic EM-exposure does not seem to be a source of chronic stress for the rats. Long-term exposure is needed for any symptoms to develop. Although some of these changes are positive (anxiolysis), depressive-like symptoms and metabolic disturbances also appeared.

FIRING PROPERTIES OF CA1 HIPPOCAMPAL NEURONS DURING CHOLINERGICALLY INDUCED FAST NETWORK OSCILLATIONS IN SUBMERGED SLICES

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Gamma-frequency (30-70 Hz) oscillations are a prominent feature of hippocampal network activity and have been suggested to contribute to encoding and retrieval of memory. However, the precise function and the cellular mechanisms of these fast network oscillations remain largely unknown. The aim of this study was to characterize the behaviour of distinct types of neurons during cholinergically-induced fast network oscillations in the CA1 region of the hippocampus. Action potentials and synaptic currents of CA1 neurons were recorded during carbachol-induced (20 μM) gamma-oscillations in horizontal mouse hippocampal slices. Half of the pyramidal cells (PCs) and one third of the interneurons (INs) were significantly phase-coupled to the local field oscillation. Phase-coupled PCs fired near the minimum of the field oscillation (Φ_{AP}= -2.34±0.1; n=9; phase measured in radians), whereas phase-coupled INs tended to fire later in the cycle (Φ_{AP}= -1.46±0.1; n=8). The dominant phasic input to phase-coupled PCs was inhibitory, whereas phase-coupled INs received strong phasic excitation. This data combined with our previous results from the CA3 region of rat hippocampus, suggests a model whereby the firing of CA3 PCs excites both CA3 and CA1 INs that fire at latencies indicative of monosynaptic excitatory connections. In contrast, the spike timing of CA1 PCs is controlled primarily by inhibition.

These findings are consistent with in vivo recordings, suggesting that carbachol-induced fast network oscillations in vitro closely resemble hippocampal gamma-oscillations in the behaving animal.

CONSEQUENCES OF PERINATAL (±)MDMA EXPOSURE IN RATS

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Objective: Methylenedioxymetamphetamine (MDMA, ecstasy) is a very popular recreational drug. The aim of the present study was to examine the consequences of (±)MDMA exposure during the gestation period in the offspring Methods: Pregnant female Wistar rats were treated with (±)MDMA (15 mg/kg sc.) on the 5th, 12th and 19th day of the pregnancy. The offspring was separated on the 21st postpartum day. Offspring of female rats treated with physiological saline served as control. Body weight, food consumption, locomotor activity in novel surroundings, learning ability in shuttle-box, muscle strength and intensity of conditioned place preference (CPP) evoked by (±)MDMA treatment were measured.
Results: 1.) The birth weight of (±)MDMA-exposed offspring was smaller and this body weight lag remained even at adulthood, together with a significant difference in food consumption. 2.) Male offspring showed increased locomotor activity in novel surroundings, other differences, however, were not observed. 3.) (±)MDMA exposed female offspring showed enhanced number of escape failures in the shuttle box, decreased muscle strength in wire suspension test and increased sensitivity to place preference-inducing effect of (±)MDMA .
Conclusion: Our data prove that (±)MDMA exposure during the embryonic state results in long-term physical backwardness and behavioral differences concerning locomotor activity, learning capacity and enhanced sensitivity to reinforcing effect of (±)MDMA. This latter data can be considered as higher risk to abuse liability.

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MULTIFUNCTIONAL BIOPOTENTIOSTAT FOR FAST IN VIVO NEUROCHEMICAL MEASUREMENTS

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The use of voltammetry for the analytical detection of electroactive compounds is an established method. In neurophysiology, the common aim is the accurate measurement of bioactive compounds in the brain, *in vivo*. However, measurements of rapid (sub-millisecond) concentration-changes and simultaneous recording of neuronal activity is still a methodological issue. In this study, we developed a universal biopotentiostat for combined neurochemical and electrophysiological measurements using carbon fibre microelecrodes. Our system is based on a there-electrode voltage clamp arrangement with a limitation of the current passed through the active electrode to protect the living tissue. The machine works in fast scanning cyclic voltammetry (FSCV), amperometry, or external input modes. The internal voltage ramp is generated by a PIC18F2550 IC and a MAX 532 D/A converter. Signal levels can be adjusted in five steps between +-2.5V, with a speed of 100-1000 V/s, 10 Hz. Data processing, is synchronized to a start TTL signal before the onset of the voltage ramp. For the detection of current changes at the electrode tip, the measuring voltage is compared and the current-voltage converted signal of the nonlinear load of the electrode input. During the voltammetric measurement, the start TTL signal works as the gating signal for blocking the high impedance input of the built-in multiunit amplifier. In the intervals of the FSCV ramps (90 ms/cycle) the same TTL signal blocks the input of the current-voltage converter amplifier to avoid the presence of any external current source and enable the recording of bioelectric signals around the neurons. The properties the device is tested with various biogenic amines and in complex experimental conditions, *in vivo*.

THE EFFECTS OF MAST CELL DEGRANULATION ON NEURONAL ACTIVITY IN THE THALAMUS OF NORMAL AND OVARIECTOMIZED RATS.

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Mast cells (MCs) are immune cells of peripheral origin. However, they can also be located in the central nervous system, mostly in the thalamus (TH). MCs secrete several bioactive chemicals, which influence the surrounding neurons. We previously showed that MC degranulation alters neuronal activity in the TH. In the present experiment, we investigated the distribution of MCs in the rat brain and the estrous cycle dependence of MC degranulation in normal and ovariectomized (OVX) animals. For histological identification of MCs we used acidic toluidine blue. Four-barrelled microelectrodes were used for combined microiontophoresis of MC activator C48/80 and extracellular single unit recording. Morphological results revealed that there are few MCs in the brain in proestrus and diestrus (10-20 cells), while in OVX animals, cell numbers varied between 14-28 (diam=14.286±4.36). In normal young females, MC numbers were btw. 1874-6900 (cell diam=13.265±2.856) and MCs were located mostly in VPM, Po, CM, DLG, LPLR, MCPC, MGV nuclei of the TH. The effects of MC activation on neuronal activity showed clear oestrus-cycle dependence. The action of C48/80 was mostly inhibitory in proestrous or estrous (51/108, 47.2%). In metestrous, C48/80 increased the firing activity of neurons (32/45, 71%). In diestrous, C48/80 did not change the activity of neurons either in normal animals or in the OVX subjects (19/19, 100%, and 13/15, 87% resp.). Data suggests that there is strong correlation between MC numbers and the effects they exert on neuronal activity in the rat TH. Further investigations will clarify the biochemical nature of MC-caused changes of neuronal activity in the brain.

PHYSIOLOGICALLY ACTIVE *ACONITUM* ALKALOIDS ALTER NEURONAL FIRING ACTIVITY IN THE FOREBRAIN OF THE RAT

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In traditional medicine, diterpenoid alkaloids of the *Ranunculaceae* family are widely used analgesic and antirheumatic agents. However, their pharmacological action in the mammalian brain is not yet defined. In the present study, we (i) isolated *Aconitum* alkaloids with potential biological activity from the Hungarian flora, and (ii) examined their neurophysiological effects on neuronal firing activity in the rat forebrain, *in vivo*. Nine alkaloids were isolated (from *A. vulparia, A. anthora* and *A. toxicum*) by means of pH-gradient extraction and multiple chromatographic purification. Four-barrelled microelectrodes were used for combined extracellular recording of spontaneous neuronal activity and ionthoporetic injection of *Aconitum* alkaloids together with various known neuronal receptor agents.

We recorded extracellular single unit activity of 212 neurons during 590 drug-ejection trials in area of the hippocampus (HIP) or thalamus (TH). Four of nine tested alkaloids had prominent effects on neuronal activity *in vivo*. Delcosine (DEL), neoline (NEO) and songorine (SON) mainly decreased (34%, 50%, 68% respectively), and the septentriodine (SEP) mainly increased (74%) the spontaneous firing activity of tested neurons. In addition, mostly hippocampal neurons responded to SEP (excitation, HIP: 100%, TH: 53%), NEO (inhibition, HIP: 90%, TH: 17%) and SON (inhibition, HIP: 69%, TH: 68%) treatments.

The present results demonstrated that mammalian forebrain neurons are responsive to treatments with aconitine alkaloids *in vivo*, indicating their rapid, transmitter-like action in the CNS.

THE EFFECTS OF VARIOUS NEUROPROTECTIVE COMPOUNDS ON DIFFERENT RETINA DEGENERATION MODELLS

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Several retinal degeneration models are described in the literature. Similar degenerations are induced by different diseases. PACAP, urocortin (URO) and diazoxide (DIAZ) are neuroprotective in animal models of different brain pathologies. We investigated the neuroprotective role of these compounds in two rodent model systems: monosodium-glutamate (MSG) induced retinal degeneration (Exp #1) or chronic carotid occlusion (CCO) (Exp #2).

Wistar rat pups were either treated MSG (s.c.) on P1, P5 and P9, or adult rats were subjected to CCO while we injected a neuroprotective substance (DIAZ, URO or PACAP) intraocularly in the right eye. In both cases animals were sacrificed after 21 days and the retinas were processed for histology.

The MSG injection on P1 or P5 did not cause discernible alteration at the light microscopic level. When retinas were treated with 3x MSG, the IPL disappeared and the inner nuclear and ganglion cell layers fused. The CCO led to a severe degeneration of all retinal layers.

PACAP treatment significantly ameliorated CCO effects and the MSG-induced retinal damage. Significant neuroprotection could be achieved in DIAZ and URO treated retinas. The order of neuroprotective efficiency was DIAZ > PACAP > URO in Exp #1, while the order was URO > PACAP > DIAZ in Exp #2.

Our present results may have clinical implications in reducing glutamate-induced excitotoxicity or ischemic retinal degeneration in ophthalmic diseases. The potency of the above drugs differs in counterfeiting degenerative effects in different models.

COMPLEX CHEMOSENSITIVITY OF LIMBIC NEURONS IN THE RAT AND MONKEY FOREBRAIN

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The nucleus accumbens (NAcc) and the mediodorsal prefrontal cortex (mdPFC) are known to be involved in the central regulation of feeding. To characterize complex chemosensory attributes of the neurons here, extracellular single neuron activity was recorded in the NAcc and mdPFC of anesthetised Wistar rats and alert rhesus monkey by means of tungsten wire multibarreled glass microelectrodes during microelectrophoretic drug administrations and gustatory stimulations.

More than the fifth of all neurons tested in the NAcc and mdPFC changed in activity in response to microelectrophoresis of D-glucose. The two subtypes of these glucose-monitoring (GM) cells showed differential topography in the NAcc: the excitatory type neurons were identified predominantly in the core, whereas the inhibitory type cells were found mainly in the shell region. Gustatory stimulations effectively modulated neural activity in both limbic regions (appx. 60% of all units in the NAcc and 40% in the mdPFC). One third of mdPFC GM cells, whereas more than 80% of accumbens GM neurons also displayed taste responsiveness. Firing rate of half of all gustatory cells, however, more than 70% of the GM taste neurons were modulated by DA. Microelectrophoretic application of D1 or D2 receptor antagonists suspended taste elicited neuronal responses in several neurons.

These data indicate that endogenous and exogenous chemosensory signals densely converge in the limbic forebrain. By processing complex chemosensory information, GM neurons appear to play distinguished role in the central feeding control.

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ANXIETY OF NOVELTY SEEKER/AVOIDER RATS: THE EFFECT OF ALFA-2 ADRENOCEPTOR ANTAGONISTS

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High responder rats to novelty (HR), those having higher locomotor scores in a novel open field can be connected to the human trait of sensation seeking. These rats also tend to choose new environments when they are given a free choice. Anxiety is closely related to the elevation of norepinephrine (NE) level in the medial prefrontal cortex (mPFC). The alfa-2A receptor antagonist BRL44408 (BRL) increases extracellular NE levels by inhibiting presynaptic receptors, while imiloxan (IMI) antagonises postsynaptic alfa-2B receptors, and let NE act on alpha-1 receptors, both having anxiolitic effects. In the present experiment, we searched for differences in the baseline behaviour of HR and LR (low responder to novelty) rats in a general anxiety model, the elevated plus maze (EPM), and investigated their performance after the administration of BRL or IMI in the mPFC. Our results show that several components of the EPM behaviour differed between HR and LR rats: number of rearings, open arm entries, and time spent in the open arms were higher in HRs, showing decreased anxiety of the group. BRL acted as an anxiolitic in HRs, but it was ineffective in LR rats. IMI increased the anxiety of HRs, but none of the doses changed the behaviour of LRs.These results reveal major distinction in the NEergic function of the mPFC of different novelty seeker rats, probably due to different receptor distributions. In HR rats, lower NE and serotonin levels were detected, that may result in increased sensitivity of receptors. Therefore, lower doses of the antagonists can be effective in HRs, but only higher doses are potent in LR counterparts. Our experiment gives further evidence for the importance of considering individual differences in the use of therapeutic drugs.

DSM-IV BASED VALIDATION OF AN ANIMAL MODEL OF PTSD

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Post-traumatic stress disorder (PTSD) is a severe psychiatric disorder that develops in a significant part of human population undergoing traumatic life events. It is generally believed that the inadequacy of treatments is due to a deficient understanding of the pathomechanisms of PTSD, but the available models are often found inadequate. This work aims to present a DSM-IV based analysis of a widely used animal model that in our experiments appeared to resemble core clinical symptoms of PTSD. Traumatic stress experience was induced by a single exposure to intense (3mA) footshock in rats. Traumatized rats showed increased fear responses and prolonged corticosterone response during and after trauma. Upon later exposure to the traumatic context, rats displayed increased freezing and escape jumps, resembling DSM-IV criterion ‘intense psychological distress’. Exposure to the stressful context was also associated with marked autonomic hyperarousal (‘physiological reactivity’). Traumatized rats showed diminished locomotion in different types of novel situations (‘diminished participation in significant activities’) and marked social avoidance in the social approach-avoidance test (‘detachment of others’). Aggression towards a smaller intruder was lowered and paralleled by low autonomic arousal (‘restricted range of effect’). Day/night rhythm of traumatized rats’ heart rate was flattened and they showed hyper-vigilant behavior when faced with a novel object. These symptoms lasted for at least 28 days. These data suggest that the long-term behavioral and autonomic effects of a single exposure to electric footshocks sufficiently fulfill requirements of each criterion of DSM-IV to classify it as an animal variant of PTSD.

ANALYSIS OF INTERACTION BETWEEN MEDIAL SEPTUM AND HIPPOCAMPUS

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Hippocampal theta, a highly regular, 3-10 Hz oscillatory pattern, is coupled to different stages of information processing in the hippocampus. The medial septum (MS), through the reciprocal connection with the hippocampus is a key regulator of hippocampal theta genesis. To reveal the mechanisms of theta-frequency synchronization of the septo-hippocampal network, we attempted to analyze the interaction between the MS and the hippocampus by different approaches.

We recorded EEG in the pyramidal layer of the CA1 region of the dorsal hippocampus and concurrently single unit activity in the medial septum using the juxtacellular technique in urethane anesthetized rats. In order to determine the direction and the temporal dynamics of septo-hippocampal interaction different mathematical methods were employed: circular statistics and information theoretical approach. In order to analyze similar types of signals the interaction between MS and hippocampal units were also accomplished.

Based on our analysis the recorded MS neurons could be divided into two major groups. Theta-bursting neurons with some non-bursters composed one group which "drove" the hippocampus during hippocampal theta as opposed to non-theta states. In the other group including only non-bursting cells hippocampus was observed to be the "leader" during both non-theta and theta hippocampal EEG patterns. In both groups the degree of interaction increased significantly in response to the formation of hippocampal theta. Two neurons not belonging to the above groups a septal dominance over hippocampal activity concerning the direction of interaction was detected.

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MAP KINASE IS ACTIVATED IN CORTICOTROPIN-RELEASING HORMONE (CRH)-CONTAINING NEURONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) FOLLOWING BACTERIAL LIPOPOLYSACCHARIDE (LPS) ADMINISTRATION

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While stress and bacterial infection up-regulate the hypothalamic-pituitary-adrenal (HPA) axis, the precise cellular mechanisms underlining activation of hypophysiotropic CRH have not been fully elucidated. Since LPS increases cfos expression in the PVN and mitogen-activated protein (MAP) kinases increase expression of cfos via phosphorylation of the transcription factors Elk-1 and CREB, we raised the possibility that MAP kinase may be linked to the activating effects of endotoxin on CRH neurons. In the present study, therefore, we determined the effect of LPS on the phosphorylation of MAP kinase (ERK) in the PVN. In saline treated controls, isolated weak phospho-MAP kinase immunoreactive neurons were observed in the PVN. However, a dramatic increase in phospho-MAP kinase immunoreactivity in the PVN was noticed 2 h after LPS administration, which then gradually declined to baseline levels 12 h following injection. By double-labeling immunofluorescence, all CRH neurons in the PVN contained phospho-MAP kinase 2 h after LPS. We conclude that LPS rapidly increases the phosphorylation of MAP kinase in CRH neurons of the PVN, suggesting an important role for this enzyme in stress-induced activation of the HPA axis. We propose that the transient increase in MAP kinase phosphorylation following LPS administration may result in the phosphorylation of CREB and ultimately may be responsible for increased CRH gene expression in association with infection.

CLUSTERING OF A-TYPE POTASSIUM CHANNELS AT INTERCELLULAR JUNCTIONS IN THE RAT MAIN OLFACTORY BULB

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Voltage-dependent K^+ channels comprise the most diverse family of voltage-gated ion channels. The variability in their molecular structure is paralleled by the numerous neural functions in which these channels play an important role.
In this study we investigated the subcellular distribution of Kv4.2 and Kv4.3 subunits in the rat main olfactory bulb (MOB) and asked whether they were evenly distributed on the somato-dendritic surface of the neurons or whether an uneven distribution could increase the complexity of their functional roles. In the granule cell layer, inhomogeneous cell-surface distribution of the Kv4.2 subunit was detected in ~10% of the granule cells. In this layer, a prominent clustering of the Kv4.3 subunit was also detected in $GABA_A$ receptor □1 subunit immunopositive short-axon cells. EM immunogold localization revealed that these subunits are highly concentrated in non-synaptic contact sites between neurons in a cell-type specific manner. In the external plexiform layer, clusters of gold particles were found in mitral cell lateral dendrites contacting either other mitral cells or granule cell apical dendrites or spines. In the glomerular layer, prominent Kv4.2 subunit clustering was apparent on some external tufted cells. A subset of calretinin immunopositive juxtaglomerular cells exhibited inhomogeneous labeling for the Kv4.3 subunit in their processes, which formed calyx-like structures around the somata of other periglomerular cells. Our results reveal a complex, cell type-specific inhomogeneous cell-surface distribution of A-type K^+ channels in the rat MOB, suggesting a potential role in intercellular interactions and olfactory information processing.

NEURAL MECHANISMS OF GLOBAL ATTENTIONAL MODULATION

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Attending to a specific feature of an object increases neural sensitivity for this feature globally, throughout the visual field. Here we use event-related fMRI in human observers to characterize the effect of such global attentional modulation in retinotopically mapped visual cortical areas.
On one side of fixation, two moving-dot apertures were presented and each aperture contained two orthogonal directions of motion (e.g. leftward and upward motion in one aperture, rightward and downward in the other). Observers monitored the speed of the dots moving in one direction in one of the two apertures. We then measured the BOLD response evoked by an additional to-be-ignored unidirectional motion stimulus presented on the other side of a fixation.
In all visual areas tested – including area V1 - the BOLD response was larger when the ignored stimulus matched the currently attended direction compared to when it matched any of the unattended directions. In some areas the BOLD response was lowest when the ignored stimulus matched the irrelevant direction of motion that was presented in the same aperture as the target compared to one of the other unattended directions.
These results provide evidence that global attentional selection mechanisms modulate visual information processing throughout the visual cortex. Our findings also suggest that the facilitatory and inhibitory mechanisms of global attentional selection have differential effects on neural processing in different visual cortical areas.

NOVEL DRUG CANDIDATES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) was labeled the „silent epidemic" of our century. Several hypotheses try to understand the cellular-molecular mechanisms that lead to AD. The amyloid cascade hypothesis focuses on toxicity of β-amyloid peptides, the tau hypothesis emphasizes the important role of the tau protein in stabilization of microtubules. According to the newest theory the re-activation of cell division cycle leads to cell death by aposklesis in ageing neurones. Mitogenic stimulation is starting by hypoxia, synaptic loss and overproduction of amyloid precursor protein (APP). The neurotoxic β-amyloid peptide Aβ 1-42 will be produced from the alternative cleavage of APP and this compound seems to be the most important factor for maintaining the irreversible events (neuronal death) during the progression of AD. Compounds reacting with Aβ 1-42 by neutralizing the toxic effect of the peptide or supporting its metabolytic cleavage by proteases are drug candidates of AD. We have found a group of peptides and peptidomimetics that can bind to the surface of Aβ aggregates and prevent interaction with cell membrane proteins. These compounds show neuroprotective effect in different *in vitro* and *in vivo* tests (MTT-assay, electrophysiology, etc.). The first compound Leu-Pro-Tyr-Phe-Asp-amide (LPYFDa) is a very potent neuroprotective agent and served as a lead for drug design. Peptidomimetics were synthesized and tested, two of them (P29 and P59) show very low toxicity, high neuroprotective effect and pass through the BBB. P29 and P59 are drug candidates for treating AD.

EFFECT OF RAPHE-STIMULATION ON THE ACTIVITY OF IDENTIFIED HIPPOCAMPAL INTERNEURONS RECORDED IN VIVO

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The hippocampus has a key role in several brain functions. The hippocampal network exhibits various behavior-dependent activity patterns manifested as oscillations with characteristic dominant frequency ranges. Among the various neuromodulators regulating hippocampal activity serotonin has the largest diversity of receptors expressed specifically by certain neuron classes. The aim of our study is to uncover the intricate complexity characterizing the effect of serotonin on the hippocampal network. To this end we recorded the responses of neurons in the hippocampus of anesthetized rats to the 1 Hz / 1 mA (pulse width: 1 ms) stimulation of the median raphe nucleus (MRN), the main source of the serotonergic innervation of the hippocampus. After the recording the neuron was labeled juxtacellularly and processed for immunocytochemistry for identification.

Three types of responses were observed: i. short, < 10 ms latency, fast decaying facilitation with >50 success rate indicating monosynaptic connection in case of 5 neurons; ii. long, > 50 ms latency, long duration (> 10 ms) facilitation exhibited by 2 neurons; iii. long-lasting (> 50 ms) inhibition recorded in 2 neurons. From the first group three neurons fired phase coupled to the ongoing CA1 pyramidal layer theta. Also form this group 3 cells were successfully labeled of which 2 were proved to be CCK immunopositive one located in the CA3 pyramidal layer while the other on the border of the CA1 stratum radiatum and lacunosum-moleculare.

Our preliminary results imply that the serotonergic system by exerting temporally focused, highly efficient influence on the hippocampal network, may contribute to the formation of the various hippocampal oscillations in a more complex way than previously thought.

DISTRIBUTION OF TYPE 1 CANNABINOID RECEPTOR (CB1) IMMUNOREACTIVE AXONS IN THE MOUSE HYPOTHALAMUS

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Type 1 cannabinoid receptor (CB1) is the principal endocannabinoid receptor in the brain. CB1 plays critical role in the regulation of hypothalamic functions. However, the distribution of CB1-containing axons in the hypothalamus is essentially unknown. Therefore, we have analyzed the distribution and ultrastructural characteristics of the CB1-immunoreactive (IR) axons in the mouse hypothalamus using an antiserum against the C-terminal 31 amino acids of the mouse CB1. We found that CB1-IR axons innervated densely the majority of hypothalamic nuclei, except for the suprachiasmatic and lateral mamillary nuclei where only scattered CB1-IR fibers occurred. CB1-IR innervation of the arcuate, ventromedial, dorsomedial and paraventricular nuclei and the external zone of the median eminence corroborated the important role of CB1 in the regulation of energy homeostasis and neuroendocrine functions. Ultrastructural studies to characterize the phenotype of CB1-IR fibers established that most CB1-immunoreactivity appeared in the preterminal and terminal portions of axons. Analysis of CB1-IR synapses in the paraventricular and arcuate nuclei detected approximately equal numbers of symmetric and asymmetric specializations.
In conclusion, the study revealed the dense and differential CB1-IR innervation of most hypothalamic nuclei and the median eminence of the mouse brain. At ultrastructural level, CB1-IR axons established communication with hypothalamic neurons via symmetric and asymmetric synapses indicating the occurrence of retrograde signaling by endocannabinoids in hypothalamic neuronal networks.

MODELING OF FIELD POTENTIALS FROM AREA CA1 OF THE RAT HIPPOCAMPUS

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Several extracellular electrophysiological methods have been developed in order to understand the activity of neural networks (electroencephalography, electrocorticography, multielectrode recordings, etc). Although these methods can be considered already as classical, the relation between the recorded potentials and the underlying neuronal activity is not yet completely understood.
Extracellular potentials originate from complex interactions between many factors, e.g. spatial distribution of current sources, distribution of positive and negative electrical charges (dipoles), time course of the events and complex geometric and conductive properties of the extracellular space.
Based on three-dimensional reconstructions of rat hippocampal pyramidal neurons, a model of the extracellular potentials has been developed. Synaptic stimulation, electrotonic spread of the excitation in a passive dendrite and the mechanisms underlying action potential generation has been studied. In a second phase the model was extended to dendrites with active conductances. Simplifying the extracellular space to an infinite homogenous and isotropic medium, it was given a quantitative account of the extracellular effect of the postsynaptic potentials and action potentials. The generation of dipoles has been described. The potentials generated by active and passive dendritic trees were compared.

DIFFERENT EFFECTS OF ABETA1-42 OLIGOMERS AND FIBRILS ON AMPA RECEPTOR FUNCTION IN VIVO

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The underlying cause of Alzheimerâ€™s disease (AD), a progressive neuropathological disorder is supposed to be the accumulation and aggregation of a misfolded protein, amyloid beta (AÎ˛). One of the earliest clinical symptoms of AD is the loss of short-term memory and subtle cognitive impairments that eventually progress to serious global dementia. Works in AD models show that AÎ˛1-42 damages synaptic plasticity in the hippocampus, a brain region which suffers a great impact during AD. Continuous modulation of synaptic NMDA and AMPA receptor efficiency is widely considered as a cellular mechanism of learning and memory, which is greatly impaired in AD. Despite the extensive research in this topic, the exact mechanisms by which AÎ˛ alters synaptic function remains to be clarified. AÎ˛1-42 may undergo a fibrillogenic process, under which distinct aggregational structures and forms arise from small oligomeric assemblies to larger protofibrillar species. Some studies suggested distinct biological actions for amyloid assemblies in different aggregational state. Hence, we prompted to examine the effects of oligomeric and fibrillar AÎ˛1-42 on the NMDA and AMPA evoked neuronal firing *in vivo*. The aggregation states of the solution used were verified by different methods. Transmission electromicrographs and quasi-elastic light scattering (QLS) recordings demonstrated that the applied protocols resulted in oligomer or fibrillar amyloid assemblies respectively, which remained stable for the time interval of the electrophysiological measurements. By utilizing these protocols, we obtain consistent and reproducible results for AÎ˛1â€“42 structure, aggregation property and biological activity. Here, we demonstrate different effects of AÎ˛1-42 oligomers and fibrils on postsynaptic function. Both aggregational forms enhanced NMDA evoked neuronal responses, however, fibrillar species ablated, while oligomers enhanced AMPA induced firing. These results suggest the utmost importance of characterizing the AÎ˛ solution used in electrophysiological experiments.

MORPHOLOGICAL EVIDENCE SUGGESTING A ROLE OF AGRP AS AN INVERSE AGONIST AT MC4 RECEPTORS IN THE HYPOTHALAMIC PVN

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AGRP is believed to antagonize the effect of α-MSH at melanocortin receptors. To provide anatomical evidence to support this possibility, we studied whether AGRP fibers terminate in close proximity to α-MSH fibers on the surface of melanocortin 4 receptor- (MC4-R) expressing neurons in feeding related centers of the hypothalamus, by performing triple-labeling immunofluorescence and confocal microscopy on hypothalamic sections of MC4-R-GFP transgenic mice. In the mid level of the paraventricular nucleus (PVN), GFP neurons were heavily innervated by AGRP fibers, but contacted by far less α-MSH fibers. In some cases, adjacent AGRP- and α-MSH-immunoreactive (IR) varicosities were found on the surface of MC4-R cells, but in general, AGRP and α-MSH fibers terminated separately on these cells without apparent close associations between them. In the lateral part of the caudal PVN, a population of GFP-IR neurons was contacted only by AGRP-IR fibers. In the dorsomedial nucleus (DMN), the majority of GFP neurons were heavily innervated by both AGRP- and α-MSH-IR fibers, but these fibers terminated separately from each other on target neurons. We conclude that in the PVN MC4-R neurons are contacted by far more AGRP boutons than α-MSH boutons, and in general, AGRP varicosities are not associated closely with α-MSH boutons on MC4-R neurons. Thus, we propose that AGRP may act independently from α-MSH in the hypothalamus in support of its potential action as an inverse agonist on MC4-R.

INTRAPERITONEALLY ADMINISTERED PENTAPEPTIDE DEFENDS AGAINST ABETA1-42 INDUCED NEURONAL EXCITATION IN VIVO

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Amyloid-beta peptides (Aβ1-40, and Aβ1-42) have determinative role in the pathogenesis of Alzheimer's disease (AD). Inhibition of the misfolding and aggregation of soluble Aβ species by peptide based molecules seems to be promising in the therapy of AD. In our former study, several pentapeptides proved to be protective against the N-methyl-D-aspartate (NMDA)-response enhancing effect of Aβ1-42 *in vivo*. One of them, the most effective Leu-Pro-Tyr-Phe-Asp-amide (LPYFDa) was tested in this work under other circumstances, because it was administered intraperitoneally, not directly into the brain.

We have utilized *in vivo* extracellular single-unit electrophysiology combined with iontophoresis on hippocampal CA1 neurons of Wistar male rats. LPYFDa was able to block the Aβ1-42 induced neuronal excitation. A temporal window have been observed, within the compound was effective. We should assume that LPYFDa penetrates the blood-brain barrier (BBB) and resists to proteolytic enzymes.

We applied blood-brain barrier permeability study with tritium-labeld LPYFDa in order to visualize, that the peptide reaches the brain. It showed, that [^{3}H]-LPYFDa readily crosses the BBB. These data shows that peptide based molecules may serve as leads for the design of drug candidates for the therapy of Alzheimer's disease.

RECEPTOR BINDING AND G-PROTEIN ACTIVATION BY NOVEL NON-MAMMALIAN ENKEPHALIN PENTAPEPTIDES

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Enkephalins are closely related endogenous pentapeptides of brain origin and they have morphine-like pharmacological effects. In mammals and in the human there are two enkephalins: Leu- and Met-enkephalin differing only at the C-terminus; Tyr-Gly-Gly-Phe-Leu and Tyr-Gly-Gly-Phe-Met respectively. In our study we investigated two, novel non-mammalian enkephalin pentapeptide and their receptor binding properties. These new non-mammalian enkephalin pentapeptides are Ile^5-enkephalin and Phe^5-enkephalin; of which were deduced from African clawed frog (Xenopus laevis) and African lungfish (Protopterus annectens) brain cDNA sequences. These enkephalins where biochemically tested using rat brain membrane preparations. Two assays were used: radioreceptor binding assay and G-protein activation assay. Both of the new peptides turned out to be moderate affinity opioid agonist ligands in these biochemical experiments. Phe-enkephalin found in the fish has unexpectedly low affinities toward mu- and delta sites, but exhibits moderate affinity toward the kappa- opioid receptor.

TEMPORAL ORDER OF SYNAPTIC FILTERS: IMPLICATIONS FOR F → D OR D → F PROCESSES

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In this study we raise the question of interchangeability of synaptic filters. We consider phenomenological short term synaptic depression and facilitation models available in the literature. Then we assume conditions, where the nonlinear synaptic filters can be approximated by linear differential equations. We change the order of the differential equations and examine the conditions to provide the same result in the reversed order for all initial pairs of states and inputs. In order to do so, we use Laplace-transforms which transform time functions to frequency dependent functions of a complex variable. We stress that even in a simple linear system filters cannot generally be applied in arbitrary order without changing the result, thus the order of the presynaptic and postsynaptic filter processes in a neural network might be important.

BEHAVIOURAL EFFECTS OF LONG-TERM LOW-FREQUENCY ELECTROMAGNETIC EXPOSITION IN RATS

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Non-ionising electromagnetic radiation (NIEMR) and especially low-frequency electromagnetic fields (LF-EMF) have drawn increasing attention since their level had been increasing in the last decades. Due to the fact that they are not able to break up chemical bonds, it is questionable whether they have any effect on the health and disease of the living systems. Most of the studies have focused on cell-biological, cancer-accelerating and molecular-biological effects, and much less have been done to study more general, non-specific and behavioural changes (e.g. diffuse headaches, dizziness, nausea, bad mood, decreased productivity, etc.) possibly caused by electromagnetic exposition. Our aim has been to use animal models to study whether LF-EMF can cause any such non-specific health effects hardly identified by medical examination though probably having some physiological background.

In these experiments, adult rats had been exposed to a 50 Hz 500 μT electromagnetic field which corresponds to the field generated by city transformers built into houses and offices. A 60 hours LF-EMF exposition caused the decrease of the number of the active and an increase of the number of passive elements in the open-field and smart-box tests. We have not found any significant change in the motivation (novel object test) neither preference tests (taste-aversion, place-preference) refer to any signs of internal discomfort caused by the exposition. Exposition, on the other hand, decreased stress-reactivity (plus-maze) and increased the social competence and dominance in a competition-situation (resource competition test).

To sum it up, although we have found changes in some aspects of the behaviour, our data do not support the general view that LF-EMF causes non-specific health problems.

THE ROLE OF VASOPRESSIN IN DEPRESSION: EFFECTS OF CHRONIC MILD STRESS IN RATS LACKING VASOPRESSIN (BRATTLEBORO STRAIN)

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Objective: Vasopressin (AVP) plays an important role in the hypothalamo-pituitary-adrenal (HPA) axis regulation as well as in stress-related disorders. Our previous studies confirmed that AVP-deficient Brattleboro rats represent fewer depression-like behaviour compared to animals that express AVP. Here we tested the hypothesis that AVP-deficient rats may be more resistant to the development of depression-like symptoms and accompanied HPA-axis hyperactivity after chronic mild stress (CMS).
Methods: Male Brattleboro rats (AVP-) were compared to their littermattes (heterozygous, AVP+). CMS consisted of different mild stimuli (e.g. wet cages, restraint) for 6 week. Elevated plus maze and forced swim test were used for behavioural characterization, while organs and blood for HPA axis parameters were collected from decapitated rats.
Results: In controls CMS resulted in symptoms of chronic stress state characterized by typical somatic (body weight reduction, thymus involution) and endocrine changes (resting plasma ACTH and corticosterone elevation and POMC mRNA elevation in AL). The floating time in forced swim test was enhanced together with a reduced open arm time on elevated plus maze and the daily food intake was also reduced. Unexpectedly, the lack of AVP could not influence any of CMS-induced changes.
Summary: In conclusion, behavioural and endocrine effects of CMS are similar in AVP+ and AVP- animals. The lack of difference might be explained by the compensatory role of overexpression of CRH in AVP- animals.

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EXPLORING DRUG EFFECTS WITH SPECIFIC VOLTAGE- AND PERFUSION PROTOCOLS

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Several drugs have multiple protein targets. When different voltage gated channels are affected by the same drug, it is very difficult to predict the final effect of a drug on the activity of a single neuron, because voltage gated channels interact with each other via the membrane potential. Both anticonvulsants and antidepressants have effects on diverse ion channels, including a voltage- and use-dependent inhibition of sodium channels. Anticonvulsants are known to stabilize the fast inactivated state, and many papers argued, that antidepressants act the same way. We have recently described a novel mechanism of the effects of antidepressants on the sodium-channels: stabilization of the slow inactivated state (Lenkey et al, 2006). Using traditional voltage-clamp protocols the two mechanisms (stabilization of the fast inactivated state with slow drug association, vs. stabilization of the slow inactivated state) cannot be distinguished explicitly.Therefore, we developed specific voltage- and perfusion protocols to address this problem. We tested protocols in voltage-clamp experiments, and also in simulation experiments using a novel sodium-channel model, which can simulate drug effects (Karoly et al, unpublished). Using the protocol that best performed both in voltage clamp experiments and in simulations, different mechanisms of inhibition can be distinguished using electrophysiology only, i.e. without mutagenesis or enzymatic treatment experiments. Protocols of this kind could be useful for high throughput analysis of drugs with sodium channel inhibitory property.

MULTIPLE OBJECT TRACKING IN AMBLYOPIA

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Visual loss in amblyopia is not restricted to reduced visual acuity and deficits in spatial vision. Recently, it was suggested that attentional functions might also be impaired in amblyops. Our goal was to investigate the efficacy of visual attentional selection in amblyopia using the multiple object tracking task, which require selection and tracking of a subset of visual objects in a visual display containing moving identical objects.
We assessed 15 adult patients with unilateral amblyopia and 15 controls on the multiple object tracking task. In our amblyopic group there were anisometropic and/or strabistic patients as well. Both the amblyopic and the control group was tested monocularly and data were analysed separately for the amblyopic and the fellow eye of the amblyops as well as the dominant and non-dominant eye of the controls. There were three testing conditions which differed in the speed of the moving objects.
It was found that patients with amblyopia performed significantly better with their fellow eyes than with their amblyopic eyes at all three speed levels tested. Interestingly, at the highest speed, the performance of the control group was also better when the dominant eye was tested as compared to the non-dominant eye. Although, using ANOVA we found no significant main group effect, amblyopic patients performed systematically worst with their amblyopic eye than controls with their non-dominant eye.
Our results provide evidence that amblyopia affects attentional functions. In amblyopic patients attentional selection is less efficient in the case of visual information conveyed by the amblyopic eye as compared to that originating from the fellow eye.

CONNECTION BETWEEN KYNURENINE AND ARACHIDONATE CASCADE

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Kynurenic acid (KYNA) and L-kynurenine (L-KYN) are the major degradation products of L-tryptophan. L-KYN is present in the blood, brain and peripheral organs. The neuroprotective and cerebrovascular effects of KYNA are mediated by N-methyl-D-aspartate (NMDA) receptors. NMDA receptors are present also on platelets membranes. The cerebral circulation may be regulated by locally synthesized eicosanoids from platelets and the vasculature. We investigated the effect of L-KYN and KYNA on the eicosanoid synthesis of platelets. Platelets were isolated from male Sprague-Dawley CFY rats (n = 5-11), and 10^8 cells/ml TC Medium 199 were incubated with KYNA or L-KYN at 37°C for 5 min, then with 1-^{14}C arachidonic acid (0.172 pmol/ml) for 10 min. The extracted eicosanoids were separated by overpressure thin-layer chromatography, and quantitatively (dpm) determined by liquid scintillation. The synthesis of all COX metabolites was inhibited by both KYNA (10^{-5} M) and L-KYN (10^{-6} M), while the production of lipoxygenase metabolites was not modified. Platelets synthesized significantly less vasodilator COX metabolites both in the presence of KYNA (10^{-5} M: 8473±745 dpm) and L-KYN (10^{-6} M: 8262±243 dpm) vs. controls (14492±1774 dpm). The amount of vasoconstrictor COX metabolites was decreased only by L-KYN (10^{-6} M: 7510±338 vs. 10252±929 dpm in controls). Our results suggest that the cerebrovascular effects of L-tryptophan metabolites may be mediated by altered eicosanoid synthesis.

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BIOELECTRIC PROPERTIES OF DEVELOPING NEUROECTODERMAL STEM CELLS

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The cell biological and molecular characteristics of neural stem cells together with their potential use for cell-based therapies have been intensively investigated. The electrophysiological properties, however, have been only sporadically studied. Understanding the bioelectric properties of undifferentiated neural stem cells and neuronal progenitors is inevitable for getting insight into the physiology of developmental processes.
In the current work the bioelectric features of neural stem cells and committed but still proliferating progenitors were studied. The appearance of different ion currents was also monitored in the course of *in vitro* induced neuronal differentiation.
The neural stem and progenitor cells are gap junction coupled and display large passive conductance. With the advancement of neuronal differentiation, the cells cease ionic coupling and show voltage dependent ion currents. Delayed-rectifying potassium currents are present in the entire period of the *in vitro* induced neurogenesis. Voltage dependent sodium currents appear at an early phase of neuronal commitment, often preceding any morphological changes. A-type potassium current, however, was detected only at the stages of neuronal network formation.
The passive conductance – a feature of non-differentiated stem and progenitor cells - was significantly reduced in the presence of gap junction blockers. Blocking gap junction communication resulted in voltage dependent ion currents in some of the cells formerly displaying only symmetrical passive current.
Early developing neuronal cells are known to contain an elevated intracellular chloride concentration. The passive conductance has an important chloride component besides the potassium ion movement. The mechanisms establishing the high intracellular chloride content, however, are far from clear. Our data suggest that voltage dependent chloride channels may function in neural stem and progenitor cells besides the passive move of chloride through gap junction channels.

THE EFFECT OF SUBTLE CHANGES IN CALCIUM CONCENTRATION ON MITOCHONDRIAL ROS GENERATION

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The effect of intracellular calcium concentration on the generation of mitochondrial reactive oxygen species (ROS) is controversial. Data have been published both about increased and decreased ROS production due to increased Ca^{2+} level. Our aim was to compare the effect of Ca^{2+} on mitochondrial ROS generation using various respiratory substrates. Mitochondrial H_2O_2 generation, Ca^{2+} uptake, and membrane potential ($\Delta\Psi m$) were recorded using fluorescent dyes. NAD(P)H autofluorescence was also measured. Mitochondria were energized in the absence of ADP (state 4 respiration) using glutamate-malate, succinate and α-glycerophosphate (αGP) as substrates. In succinate driven mitochondria 500 nM Ca^{2+} created depolarization and fast oxidation of NAD(P)H with a decrease in ROS production. With αGP, the same [Ca^{2+}] resulted in hyperpolarization, increase of [NAD(P)H] and a corresponding elevation of ROS generation. With glutamate-malate the increased Ca^{2+} level brought almost no change in ROS production, although there was a slight decrease in $\Delta\Psi m$ and the NAD(P)H pool was quickly oxidized. Our studies indicate that the various respiratory substrates generate H_2O_2 through different mechanisms. Succinate generates ROS using the highly $\Delta\Psi m$ dependent reverse electron transport (RET). Ca^{2+} uptake decreases $\Delta\Psi m$, creates unfavourable conditions for RET, thus the ROS generation will be decreased. αGP-dehydrogenase is activated by calcium and able to produce more ROS (see accompanying poster). Using NADH generating substrates there is a drop in $\Delta\Psi m$ and in NAD(P)H level without a corresponding decrease in ROS generation indicating that in this case other factors than $\Delta\Psi m$ and [NAD(P)H] would influence ROS formation.

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ALPHA-GLYCEROPHOSPHATE-INDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN ISOLATED BRAIN MITOCHONDRIA

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Many enzymes are associated with mitochondrial production of Reactive Oxygen Species (ROS). In spite of the plethora of papers the importance of various ROS producing sites and the mechanism of superoxide or H_2O_2 generation are still debated. The present study describes the characteristics of α-glycerophosphate (αGP) induced ROS production in isolated guinea pig brain mitochondria. Mitochondria established membrane potential ($\Delta\Psi m$) and released H_2O_2 parallel with an increase in NAD(P)H fluorescence in the presence of αGP (5-40 mM). H_2O_2 formation and the increase in NAD(P)H level were inhibited by rotenone, ADP or FCCP, respectively, indicating the role of reverse electron transfer (RET) in the ROS generation. The residual H_2O_2 formation in the presence of FCCP was stimulated by myxothiazol, in mitochondria supported by α-GP but not by succinate. We suggest that metabolism of α-GP leads to ROS generation primarily by complex I *via* RET, and in addition, a significant ROS formation could be ascribed to αGPDH. Furthermore, elevation of extramitochondrial calcium concentration increased the αGP-dependent ROS production. Increased [Ca^{2+}] stimulated the activity of αGPDH by decreasing its Km towards its substrate. Increased enzyme activity resulted in higher membrane potential, faster oxidation and increased NAD(P)H steady state. The Ca^{2+}-dependent increase in ROS production was most prominent under resting conditions (no ADP present), but Ca^{2+}-induced elevation of H_2O_2 production could be detected in the presence of ADP as well. Our results indicate that extramitochondrial stimulus-respiration coupling via mitochondrial αGPDH can be associated with increased ROS production.

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UNCONDITIONED CONTEXT DEPENDENT VISUAL PREFERENCE PROCESSED BY SUBTELENCEPHALIC REGIONS IN THE DOMESTIC CHICK (*GALLUS DOMESTICUS*).

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Higher order visual processing such as context dependent shape or colour preference is thought to be regulated by telencephalic centres. Recent studies suggest that subtelencephalic visual systems can function autonomously not only in anurans but also in amniotes such as mammals or birds. In order to study the effect of telencephalic structures on innate preferences toward food stimuli, massive telencephalic lesions were made in young domestic chicks. Two similarly shaped stimuli with different colour (red and green) were presented to the chicks. When the two presented stimuli were either insects or small fruits telencephalectomised chicks preferred small red stimulus, while intact animals preferred green insects and showed no preference toward green or red fruits. However, when the insect and fruit stimuli of the same colour were presented simultaneously, both the intact and the telencephalectomised chicks preferred insect if both stimuli were green and fruit if they were red. This is the very first evidence that subtelencephalic regions of an amniote are able to discriminate and prefer shapes in a colour dependent manner. Our data shows that the context independent red preference represented in subtelencephalic systems is attenuated by telencephalic structures. Such interaction can be useful as a model for further studies to reveal the coordination of innate and learned elements of behaviour and can be helpful for a better understandidng of subtelencephalic components of human blindsight.

THETA SYNCHRONIZATION IN THE MEDIAL SEPTUM AND THE ROLE OF THE RECURRENT CONNECTIONS

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Medial septum-diagonal band (MSDB) complex is considered as a pacemaker for hippocampal theta rhythm. Identification of the different cell types, their electro-physiological properties and their possible function in the generation of a synchronized activity in the MSDB is a hot topic. A recent electro-physiological study showed the presence of two antiphasically firing populations of GABAergic neurons in the MSDB (*Borhegyi et al., 2004*). Other papers described a network of cluster-firing glutamatergic neurons able to generate synchronized activity in the MSDB (e.g., *Manseau et al., 2005*) We give two different models for theta synchronization in the MSDB. In the first one (*ping-pong model*) GABAergic neurons are intrinsic theta periodic oscillators while in the second one (*feed-forward model*) they are of fast firing type receiveing periodic input from local glutamatergic neurons. GABAergic neurons showed bimodal phase-distribution in both models. To test the reliability of our models we studied the response of MSDB neurons to pharmacological modulation of GABAergic synapses in anaesthetized rats. Single units from the MSDB, and hippocampal field potential from the CA1 region were recorded under control condition and after the i.v. application of zolpidem. Zolpidem instantaneously
disrupt theta oscillation in the septo-hippocampal system and inhibited the firing activity of MSDB neurons. In the ping-pong model modification of the GABAergic synapses slowed down the oscillation but did not diminished significantly the firing activiy of the neurons, while in the feed-forward model firing rate of GABAergic neurons decreased with the synaptic strengh.

NEURODEGENERATION, NEUROPROTECTION AND KYNURENINES

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The kynurenine pathway (KP) is the main pathway of the tryptophan (TRP) metabolism, which is primarily responsible for nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). The central substance of this pathway is kynurenine (KYN) produced from TRP via a transition product, formly-KYN, with the aid of TRP- or indolamine-2,3-dioxygenase (TDO or IDO). KYN is a precursor of the neuroprotective kynurenic acid (KYNA) and the neurotoxic quinolinic acid(QUIN). KYNA is formed directly from L-KYN by irreversible transamination and this compound is a broad-spectrum antagonist of the excitatory amino acid (EAA) receptors, which can act primarily at the strychnine-insensitive glycine-binding site of the NMDA receptors. Moreover, KYNA non-competitively blocks the alpha7-nicotinic acetylcholine receptors, and can therefore take part in glutamatergic and nicotinergic neurotransmission. We found that kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. CA3 stimulation-evoked population spike activitiy was recorded from pyramidal layer of area CA1 of the rat hippocampus. In another series of behavioural experiments, water maze and open-field studies were carried out to test the presumed protective effect of KYN+probenecid pretreatment against pentylentetrazol-induced seizures. Furthermore, administration of KYN produced significant increase in the normál corcitcocerebral blood flow (cCBF) in rabbits; the peak values were recorded at the dose of 1 mg/kg (187% at 120 and 150 mins). The cCBF-improving effect of KYN was immediate and highly pronounced alsó in rabbits with carotid occlusion. Pretreatment with either atropiné or L-NAME prevented KYN-induced enhancement of the normál and the ischemic cCBF alike. It is suggested that the cCBF-increasing effect of KYN might be mediated by activation of cholinergic and nitric oxidé pathway. In another experiment densitometric analysis pro ved that, in consequence of 6-OHDA treatment, not only tyrosine hydroxylase (TH) but alsó KAT-I immunoreactivity diminished considerably in the remaining substantia nigra pars compacta neurons in rats. It is hypothesized that biochemical approaches which increase KYNA content of the central nervous system might prevent the deleterious effect of 6-OHDA and, supposedly, alsó the neuronal degeneration characterizing Parkinson's disease.

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COMPARISON OF CHEMICALLY INDUCED (AP-4) GRAND MAL AND INBRED (WAG/RIJ) PETIT MAL- ABSENCE EPILEPSY MODELS

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Our epilepsy models reflect two distinct ways of seizure genesis: the *Wag/Rij* (Wistar Albino Glaxo/Rijswijk) rat strain is an inbred petit mai- absence epilepsy model with thalamo-cortical *spike-wave discharges*; the locally administered K+ channel blocker 4-aminopyridine (4-AP) produce sustained depolarization in the parietal cortical focus and the spread of the seizure to frontal, ocdpital and temporal cortical areas (grand mai epilepsy). Histopathologically, epilepsy can result a very rapid appearance of "dark" neurons that can recover or die (through apoptosis). "Dark" neurons ha ve compacted intracellular structure and their rapid formation during various kind of cellular stress/trauma suggest pre-transcriptional, biophysical modification of their protein composition. Our results show that the appearance of "dark" neurons in the different brain areas was highest at 3 hour post 4-AP application while it was negligible in the WAG/RIJ model. Various electrophysiological methods were applied to study the spatial and temporal kinetics of the spread of the seizures. These measures show that the electrophysiologically disclosed seizure patterns in the two different epilepsy models are not going hand in hand with the histopathologically demonstrated "dark" neuronal changes.

MOLECULAR DISSECTION OF THE GABAERGIC NEUROGLIAFORM CELLS IN THE RAT SOMATOSENSORY CORTEX.

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The postsynaptic effect of cortical neurogliaform cells is characterised by unitary IPSPs on pyramidal neurons mediated by both GABA-A and GABA-B receptors (Tamas et al. 2003 Science 299 1902). This is different from most other GABAergic cells, which evoke unitary GABA-A receptor mediated responses. Neurogliaform cells are recognised by their dense, short distance dendritic and axonal arborisation originally described by Ramon y Cajal. The molecular composition of neurogliaform cells may provide forther clues for their role, therefore we tested them for several molecules using immunocytochemistry. Whole cell patch-clamp recorded non-pyramidal cells in layers 2/3 of the somatosensory cortex were labelled with biocytin for identifying their axonal and dendritic patterns. Neurogliaform cells were identified based on their firing pattern and the axonal arborisation. Identified neurogliaform cells were immunopositive for the chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and nitric oxide synthase. In perfusion fixed cortices, these molecules were co-localised in interneurons showing also strong somatic labelling for alpha-actinin, a previously reported molecular marker of neurgliaform cells in the hippocampus. This combination of molecular cell markers might make it possible to estimate the distribution and numerical density of neurogliaform cells in the cerebral cortex. Another distinct population of interneurons was also immunopositive for COUP-TFII, but several well-known interneuron classes were immunonegative.