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Identical hilar neurons express cocaine- and amphetamine-regulated transcript (CART) peptide in the human and monkey hippocampus

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Cocaine- and amphetamine-regulated transcript (CART) peptide mRNA was discovered in the rat striatum following cocaine and amphetamine administration. Since both psychostimulants elicit memory-related effects, localization of CART peptide in the hippocampal formation may have functional importance. Previous examinations demonstrated different cellular localization of CART peptide in human and in rodents.

In the present study, the expression of CART peptide was examined using immunohistochemistry in the hippocampus of the rhesus (Macaca mulatta), marmoset monkeys (Callithrix jacchus), and in that of the tree shrew (Tupaia belangeri) as well as in humans. Tree shrew is an animal phylogenetically between insectivores and primates that is used for the modelling of human psychiatric disorders, such as depression.

In all the examined species CART expression confined to principal neurons of the hippocampus. Both in humans and in monkeys, mossy cells of the hilar region expressed CART and granule cells of the dentate gyrus were CART-negative. Dense CART-immunoreactive axonal fiber plexus of the associational pathway outlined the inner one-third of the dentate molecular layer. In addition, in the hippocampus of the tree shrew and marmoset monkey a subset of CA3 pyramidal cells were CART-immunoreactive as well. A few interneurons were also observed in the CA1 area.

Our results show that CART peptide expression in the non-human primate hippocampus is identical with that of the human. In addition, the CART immunreactive cells were the same type in the subprimate tree shrew that support its closer relationship to the primates than to the rodents. The difference in the CART peptide expression between primates and rodents suggests that psychostimulants cocaine and amphetamine may induce memory-related effects at different points of the same excitatory circuitry of the dentate gyrus. Since CART peptide is a marker for mossy cells in primates, CART immunohistochemistry make it possible to monitor the impairment of mossy cells in primate models of epilepsy and in other neurological diseases.

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Localization of the CB1 cannabinoid receptor in the chick brain and its implications in passive avoidance learning

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The type 1 cannabinoid receptor (CB1) has recently been in the focus of interest due to its several possible roles in behavior and memory. However, distribution and function of this receptor in the avian brain has not yet been studied. We investigated the immunohistochemical localization of the CB1 receptor in the brain of the domestic chick using polyclonal antibody against CB1 receptor in coronal vibratome sections. To study the role of the cannabinoid system on avian learning, the effect of the CB1 antagonist SR141716A was assessed in a passive avoidance learning task.

Intensely labelled CB1 immunoreactive (CB1+) neurons were present in the ventral tegmental area and the hippocampus, showing an even cytoplasmic staining. In the latter area CB1+ axon fibers were also observed. We found labelled cells also in the lateral septum, where the immunostaining occurred as a cup-like mass surrounding the cell body. Many intensely stained fibers were detected in the arcopallium, a region homologous to the mammalian amygdala. We also found several CB1+ fibers located in the medial striatum and nucleus accumbens. Overall, the distribution of CB1 receptor appeared similar to previous findings on mammalian brains.

Intraperitoneal treatment with SR141716A 30 minutes before passive avoidance training had no effect on the retention of the learning task. Conversely, when the SR141716A was administered after the passive avoidance training trial, 30 minutes before the recall, the chicks showed strongly impaired memory retention as compared with the control animals.

The neuroanatomical observations indicate that the CB1 receptor is abundant in the avian brain areas with a known relevance to learning, i.e. limbic structures and the basal ganglia. The results of the behavioral study suggest that CB1 receptors may be active at the time of the second wave of protein synthesis associated with the consolidation of memory.

Comparative immunohistochemical studies of ependymal features and cytoskeletal proteins in mammalian radial glia and avian ependymoglia

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In the mature mammalian brain the ependyma is intensely immunopositive to cadherin and aquaporine 4. In the immature brain still radial glia lines both the meningeal and ventricular brain surfaces. During maturation, radial glia is replaced by astrocytes and ependymocytes, which have no long radial processes. In parts of the avian brain, however, the ventricle-lining cells preseve their radial processes (ependymoglia). The present questions are i) whether in rats the appearances of intense aquaporine and cadherine immunopositivities correlate with the replacement of radial glia with ependymocytes in lining the ventricles; ii) whether in chicken these substances are localised to the ventricular perikarya, or evenly distributed along the basal processes, too; iii) is there a correlation between their occurrance and the intermediate filaments? Developing rats were investigated from E14 to P10. Their glial architecture was visualized by the immunostaining of nestin, an intermediate filament protein occurring in both radial glia and young ependyma. To detect all the cadherins, polyclonal pan-anticadherin was applied. Nestin-aquaporin4 and -pancadherin double immunofluorescence labelings were also performed. In chicken GFAP was investigated in parallel to the cadherins and aquaporin-4. Both aquaporin-4 and cadherin positivity became intense only in the late intrauterine stage in rats (E17-18 and E19-20, respectively), preceding, however, the disappearance of radial glia. Their appearance however, followed a similar order along the ventricular system as that of the radial glia. In chicken the pan-anticadherin and anti-aquaporin-4 antibodies decorated the ventricular perikarya much intense than the radial processes, although both were immunopositive to GFAP. In both species the spinal cord ependyma, and some circumventricular organs (subcommissural, in chicken paraventricular, subtrochlear) were negative to aquaporin-4. In rats, this phenomenon proved to be secunder in development. Results suggest i) local differences in the ependyma in both development and functions; ii) in mammals the ependymal features appear before the formation of the definite ependyma; iii) in birds the ependymal markers are concentrated to the ventricular perikarya in contrast to the contraluminal processes; iv) there is no correlation between the intermediate filaments and the occurrance of cadherins and aquaporin.

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Effects of three neuroprotective agents on global ischemic brain damage in Wistar and SPRD rats

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Global cerebral ischemia during cardiac arrest, trauma and shock leads to irreversible brain damage. Pyramidal cell death in the hippocampal CA1 region induced by four-vessel occlusion (4-VO) in rats is a well-known model of the ischemic neuronal injury. Some previous papers studied the effect of neuroprotective compounds in a 4-VO model in some rat strains (for example Wistar, SPRD, Fischer 344), however none of them made a comparison among efficacy of the same drug in various rat strains.

The neuroprotective effects of three compounds (NBQX, GYKI-53405 and 7-nitroindazole) were tested in the 4-VO model in both Wistar and SPRD male rats in this study. Transient global cerebral ischemia was produced by a combination of bilateral vertebral artery electrocoagulation and carotid artery occlusion for 10 minutes. Neuronal loss in the CA1 field was evaluated by light microscopy and scored with a use of a point scale.

The competitive AMPA receptor antagonist NBQX (doses: 3x30 mg/kg intraperitoneally at 60, 75 and 85 min following reperfusion) significantly reduced the pyramidal cell death in the CA1 region in both Wistar (47 %; $p< 0.01$) and SPRD rats (91 %; $p< 0.01$), however the effects are different to some degree.

The non-competitive AMPA antagonist GYKI-53405 (30 mg/kg intraperitoneally 30 min following reperfusion) decreased the cell loss only in SPRD rats (49 %; $p< 0.05$).

The nitrogen oxide synthase inhibitor 7-nitroindazole (2x25 mg/kg intraperitoneally at the start of occlusion and at 50 min following reperfusion) moderated similarly the hippocampal neuronal death in both Wistar (40 %; $p< 0.05$) and SPRD rats (31 %; $p< 0.05$).

In conclusion, the two rat strains differently responded to the three neuroprotective compounds. The release of glutamate due to ischemia, density of various AMPA receptor subtypes in the CA1 area of the hippocampus as well as absorption and metabolism of compounds studied may be different in the two rat strains and some or all these factors may be implicated in the diverse effects of these neuroprotective compounds.

Persistent neurotoxic effects of a single dose of MDMA (Ecstasy) on serotonergic axons

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Background: MDMA (3,4-methylenedioxy-methamphetamine, Ecstasy) is a widely abused amphetamine-like psychostimulant with well documented serotonergic neurotoxicity in many species including rats. However, the degree of axonal loss of serotonergic neurons several months after a single dose of MDMA has not yet been investigated.

Objective: We aimed to compare the acute and persistent effects of MDMA on serotonergic axonal loss.

Methods: A single dose of MDMA (15 mg/kg, i.p.) was administered to male Dark Agouti rats. Three days or 6 months after MDMA the animals were intracardially perfused under anasthesia with 4% paraformaldehyde, the brains were removed, embedded in paraffin and sectioned.

The density of serotonergic axons was assessed by tryptophan hydroxylase immunohistochemistry. The axonal density in the frontal cortex, caudate-putamen and hippocampus CA1 was quantified by morphometric image analysis.

Results: MDMA caused considerable decreases in serotonergic axonal densities 3 days after MDMA treatment. There were marked differences in the degree of denervation (significant treatment x brain area interaction, $p<0.01$), with significant decrease in the frontal cortex (-39%, $p<0.05$) and CA1 region of the hippocampus (-55%. $p<0.01$), but not in the caudate-putamen (-36%, $p=0.13$). Six months after MDMA, partial recovery of densities were attenuated (treatment x time interaction: $p=0.06$), significant decrease compared to saline-treated animals were found only in the CA1 region (-39%, $p<0.05$).

Conclusion: These data provide evidence that MDMA causes serotonergic axonal degeneration. However, important regional differences exist, suggesting different vulnerability of serotonergic-axons towards MDMA. Furthermore, a partial axonal regeneration may occur several months later, but the degree of regeneration varies between brain regions.

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Properties of unitary GABAergic IPSCs recorded in the apical dendrites of CA1 pyramidal neurons

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Anatomical studies have described inhibitory synaptic contacts on apical dendrites, and an abundant number of GABAergic synapses on the somata and proximal dendrites. The number of inhibitory contacts dramatically decreases with distance from the soma, but local electrophysiological characterization of these synapses is missing. We directly recorded the dendritic GABAA receptor-mediated inhibitory synaptic input in adult mouse hippocampal CA1 pyramidal neurons.

Unitary GABAergic events were evoked using localized applications of a high osmolarity (~600 mosm) solution near the dendritic whole-cell recording pipette. Glutamatergic synaptic events were blocked by kynurenic-acid (3 mM) leaving picrotoxin-sensitive IPSCs. We measured the amplitudes, and kinetic properties of mIPSCs at the soma and three different dendritic locations. The amplitudes of mIPSC recorded at these sites were as follows (soma: 14.6 ± 1.5 pA; at ~50 μm: 15.3 ± 1.8 pA; at ~120 μm: 14.4 ± 1.5 pA; at ~200 μm: 17.9 ± 2.1 pA). The 20-80% rise times and the 50% decay times of local mIPSC were independent of the location of the synapses: (Trise: soma: 501 ± 20 μs; at ~50 μm: 442 ± 34 μs; at ~120μm: 481 ± 32 μs; at ~200μm: 504 ± 43 μs; T50decay: soma: 7.4 ± 0.3 ms; at ~50 μm: 7.1 ± 0.6 ms; at ~120 μm: 6.6 ± 0.4 ms; at ~200 μm: 6.1 ± 0.3 ms). The frequency of mIPSCs was 5 Hz at the soma, in contrast to <0.5 Hz at dendritic sites, where it could be increased to 10-20 Hz and 6-10 Hz by the hyperosmotic stimulation protocol. Our data show a considerably less distance-dependent scaling of inhibitory events than that seen for EPSCs in the same neurons, consistent with the idea that dendritic IPSCs may have restricted local effects.

Kinaesthetic control of motor learning and performance

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In daily life, we come across many tasks that require reaching to, manipulating, and displacing objects. The control of such seemingly simple skills is rather complex. Notwithstanding this issue has received considerable attention in the relevant literature, further analysis of the relationship between variability of starting positions and endpoints is needed to clarify the mechanisms for controlling these movements. Our intention in the present series of measurement was to describe the learning and execution of simple goal-directed movements from the starting position to a predetermined endpoint with kinaesthetic localisation. Subjects were 140 older (11-17 years) children, and 30 elite athletes. Different motor tests were used. 1. Walking 10 m in a straight line to the endpoint. 2. Drawing a 10 cm long straight line. 3. Drawing 15 cm long zigzag line both in horizontal and vertical plane. 4. Learning to reach to a point being 20 cm, 40 cm, and 60 cm distant from the starting position. The first attempt of all of these tests was executed under visual guidance, and then five trials were done without visual information. The errors both in the distance and the direction were measured. The results show that (1) there are no significant differences between basketball players, handball players, and weightlifters in errors of distance and orientation of walking to the unseen endpoint. (2) There is a tendency to improve accuracy of drawing kinaesthetically guided straight and zigzag lines with age. (3) Greater errors occur in drawing unseen zigzag lines both in vertical and in horizontal plane then in drawing straight lines. (4) The errors in distance are generally smaller then those in orientation in drawing without visual information. (5) The errors in distance were stops mainly before the endpoint, while no significant difference was found in orientation between errors to left or right side. (6) Elite basketball players produced small errors in kinaesthetically guided distance learning. The errors in distance increased after exercising at 70 % target pulse. Our results show that cooperation of the afferent visual and kinaesthetic information during the first trial is unable to prolong the accurate execution of the same goal-directed movements in the lack of visual information. The errors both in distance and direction increase with the complexity of the movements. However, the development of the motor abilities results in improvement of kinaesthetic guidance both in learning and execution of goal-directed movements.

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Diazoxide and dimethyl sulphoxide reduces chronic cerebral hypoperfusion induced glial reaction in the rat white matter

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Chronic cerebral hypoperfusion has been previously shown to result in cerebral white matter lesions during aging or dementia. Diazoxide, the mitochondrial ATP sensitive potassium channel opener, was found to prevent chronic hypoperfusion-induced glial changes in the gray matter, but its effect in the white matter has not been investigated yet.

Chronic cerebral hypoperfusion was induced by permanent bilateral common carotid artery occlusion in anesthetized (400 mg/kg chloral hydrate, i.p.) male Wistar rats (n=18). Diazoxide (5mg/kg) or its solvent dimethyl-sulphoxide (DMSO) were administered i.p. (0.25 ml) on 5 consecutive days following the surgery. Sham-operated animals served as control for surgery, and non-treated rats as controls for treatments (n=18). Thirteen weeks later the animals were sacrificed, and astrocytes and microglia were labeled immunocytochemically (GFAP and CD11b, respectively) in 20 µm thick coronal brain slices. Astrocytic proliferation and microglial activation were evaluated by quantitave morphometry in 3 white matter regions: the internal capsule, the corpus callosum and the optic tract .

Neither the occlusion nor diazoxide treatment altered the astrocytic proliferation and the microglial activation in the internal capsule. Cerebral hypoperfusion enhanced astrocytic proliferation in the optic tract, whilst diazoxide in DMSO (but not DMSO alone) decreased the baseline GFAP signal in the corpus callosum. Cerebral hypoperfusion induced microglial activation in both the corpus callosum and the optic tract. Diazoxide (in DMSO) treatment prevented microglial activation after occlusion in the corpus callosum, while in the optic tract DMSO alone showed a similar effect.

Our results suggest that the optic tract is selectively sensitive to cerebral hypoperfusion. Diazoxide and DMSO treatment can suppress glial activation in the hypoperfused white matter areas. Since glial proliferation can be regarded as an indirect marker of ischemic neural degeneration, the treatments applied here may moderate ischemic damage in the brain.

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Structural and functional diversity of external tufted cells in the rat olfactory bulb

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Olfactory sensory neurons of the nasal epithelium project their axons to the glomeruli of the main olfactory bulb where they form synapses on the apical dendrites of the principal cells (mitral/tufted). The glomeruli are the first processing station of the olfactory pathway, where in addition to sensory input, complex interactions occur between principal cells and a variety of juxtaglomerular neurons. Juxtaglomerular cells (neurons with cell bodies located around the glomeruli) consist of external tufted (ETCs), periglomerular, and short-axon cells. To understand the cellular and synaptic mechanisms of olfactory information processing, the intrinsic properties and synaptic connectivity of these nerve cells need to be deciphered. ETCs possess a complex apical dendritic tuft similar to mitral cells. The electrical and morphological properties of ETCs have been investigated previously, however, little is known about how the morphological and functional characteristics correspond to each other.

In the present study, we have investigated the active and passive electrical properties of ETCs using somatic whole-cell patch-clamp recordings in acute rat olfactory bulb slices (ages: P28-P58). During the electrophysiological recordings, the cells were filled with biocytin for post hoc morphological reconstruction and analysis. ETCs exhibited large variability (coefficient of variation=SD/mean) in their passive electrical properties, such as their input resistance (mean±SD: 259.8 ± 160.9 MΩ, n=31, CV=0.62) and membrane time constant (21.5 ± 15.5 ms, CV=0.72). Based on their responses to depolarizing current injections, ETCs displayed a large diversity regarding their supra-threshold electrical properties such as the amplitude and time course of spike after-hyperpolarization (CV=1.7); spike amplitude- and frequency-adaptation (CV=2.2); mean (CV=0.69), SD (CV=1.1) and maximum (CV=0.85) of the inter-spike-interval distributions. Furthermore, we also tested the intrinsic sub-threshold resonance properties of the cells, according to which the cells also showed a large heterogeneity (peak resonance ranging from 0 to 10 Hz). Morphological analysis of the functionally characterized cells indicated a large diversity in the number, length, and arborisation patterns of the apical tufts, as well as the presence and location of secondary dendrites. Principle component and cluster analysis of these data will be conducted to examine how the physiological and morphological diversity correlates.

The present findings demonstrate that ETCs form a functionally and morphologically diverse population of neurons. We suggest that this diversity is the result of cellular and molecular specializations developed to fulfill specific functional roles in transmitting and processing information at the first stage of the olfactory pathway.

Role of vasopressin in the activation of the hypothalamo-pituitary-adrenal axis during repeated morphine withdrawal induced chronic stress: changes detectable by molecular biological methods

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Nowadays drug abuse is a serious social problem. Repeated administration of increasing doses of morphine is known to induce chronic stress-like changes in rats. In chronic stress situations the regulatory role of vasopressin (AVP) in the hypothalamo-pituitary-adrenal axis (HPA) is thought to be more prominent. Thus, we tried to confirm the the role of AVP in the maintenance of chronic hyperactivity of the HPA during repeated morphine treatment. Natural AVP mutant Brattleboro rats were compared to heterozygous controls. Increasing doses of morphine was injected twice daily for 16 days and animals were killed by decapitation 4h or 16h after the last dose. The CRH mRNA in nucleus paraventricularis hypothalami (PVN) and amygdala, AVP mRNA (detectable even in the AVP deficient homozygous animals) in PVN and the POMC mRNA in adenohypophysis were measured by semiquantitative in situ hybridisation method. The serious withdrawal (16h after the last injection) was able to induce significant elevation in the CRHmRNA level both in PVN and amygdala. The lack of AVP per se increased the CRH mRNA levels on both area very much. Both type morphine withdrawal induced elevation in the CRH mRNA level in PVN of AVP deficient rats with no further increase when compared to AVP deficient, saline treated animals. The CRH mRNA level in amygdala failed to show any changes in AVP deficient rats comparing to normal, control animals. The AVP mRNA was increased in repeatedly morphine treated animals (4h after the last injection) without changes in 16h withdrawal and AVP deficient groups. The POMC mRNA in the anterior lobe of the pituitary was significantly elevated in both morphine treated group (4h or 16h after the last injection). The lack of AVP had no influence on these elevations. Taken together we confirmed that repeated morphine treatment causes a severe chronic stress state with increased CRH and AVP mRNA levels in PVN and elevated POMC mRNA in the anterior lobe of the pituitary. The elevation of CRH mRNA levels in the absence of AVP under basal conditions can be the result of a compensation, which could maintain a normal POMC mRNA level as well as normal hormone levels in AVP deficient animals. The reserved high CRH mRNA level in PVN and POMC elevation in anterior lobe of the pituitary in AVP mutant rats suggest that AVP does not play a crucial role in the chronic hyperactivity of the HPA axis during repeated morphine withdrawal. The elevation in AVP mRNA in PVN 4h after morphine injection suggest that the role of AVP could be more important during the acute phase of a stress reaction. The absence of the CRH mRNA elevation in the amygdala 16h after last morphine injection in AVP deficient animal suggest that AVP can play a role in the withdrawal-related emotional processes, but this effect could have other pathways than the PVN-related one.

Electric-shock experiments support the gel-to-gel phase-transition theory of the formation of "dark" neurons (whole-cell ultrastructural compaction)

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Background information: Formation of "dark" neurons is a frequent concomitant of various neurological diseases. It is long known that its essence is a potentially reversible, rapid reduction of the distances between all ultrastructural elements (compaction) inside the affected neurons. For the explanation of their enigmatic features, a recent paper has combined a hardly-known physical phenomenon (gel-to-gel phase transition) with a minority opinion of the physical state of the living cells (gel-like state). The present poster demonstrates and discusses the results of two experiments that strongly support this theory.

Methods: At the end of transcardial perfusions with ice-cold physiological saline for 30 min. or with isoosmotic potassium chloride for 5 min., but immediately before perfusion fixation, condenser-discharge electric shocks (350 V, 500 μF) were administered to rats through surface electrodes (4x4 mm2) pressed onto the temporal muscles of the scalped skull.

Results: Light microscopy revealed massive shrinkage, hyperbasophilia and type-III argyrophilia in somata, dendrites and axons of a proportion of neurons randomly scattered in an otherwise non-impaired environment. In the electron microscope, dramatic compaction of undamaged ultrastructural elements and markedly increased electron density were observed in the affected somata, dendrites and axons, and aggregation of the nuclear chromatin with a pattern other than that characteristic of apoptotic cells. These morphological features display a high degree of similarity to those previously observed following the in-vivo administration of similar electric shocks.

Conclusion: This surprising fact questions whether the ultrastructural compaction induced by the condenser discharge electric-shock either in vivo or post mortem is a result of any cascade of enzyme-mediated processes. On the other hand, the gel-to-gel phase-transition mechanism of the ultrastructural compaction proposed previously by a study that compared the morphological damages in neurons after the in-vivo and the post-mortem administration of a special kind of head injury, is supported by the results found here.

Photoreceptor distribution in retina of the spadefoot toad (Pelobates fuscus)

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Pelobates fuscus is a fossorial species which is active mostly at night. Thus, it is often prayed on by some owl species. Vision may be an important aspect of selection. In this study our objective was to study the photoreceptor types of spadefoot toad and its distribution in the retina. The animals were collected from nature with the permission of the Danube-Drava National Park. We used light-, fluorescence- and electron microscopic methods in our experiments. We made 1 µm thick sections of resin embedded material and stained it with toluidine blue. Based on the light microscopy, we identified two types of rods and one type of cone. Because of the unusual structure of the cone (i.e. lack of a clear oil droplet) we made immuno labeling with primary antibody against cone opsin (COS-1) to demonstrate presence of the cones. We determined the ratio of cones and rods in five location (dorsal, nasal, ventral, temporal, central) of the retina. We found that about 10 % of the photoreceptors were cones and there was only a small difference among location within the retina. We found most of the cones in the central part of the retina and least in the ventral part. Retinal distribution of the cones were examined in wholemount using immunocytochemistry and the results supported the light microscopy observations. Current results indicate the presence of a single cone type. To verify this finding, and also because of the unusual cone structure observed in 1 µm thick section, we decided to study the cones in the electron miscroscope. We did not find oil droplet in the cones which are presents in all the anuran species studied formerly however there is a dense material in the place of the oil droplet in the inner segment. To our surprise we found 3 morphologically distinct rod types, one minor and two major rods.

Dendrodendritic and dendrosomatic connections between the oculomotor and trochlear motoneurons of the frog

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The prey-catching and predator avoiding are important behavioural patterns for survival of the animals. They require complex and coordinated activity of different muscles including the external eye muscles. As in other vertebrates, frogs have six classical external ocular muscles innervated by the oculomotor, trochlear and abducens nerves; their nuclei are located in the dorsomedial nuclear group of the brainstem. In our earlier experiments we have described that the caudal part of the oculomotor nucleus is continuous with the rostral part of the trochlear nucleus and a dendritic bundle of the oculomotor nucleus running into the trochlear nucleus and distributed among the motoneurons. Similar organization of the trochlear dendrites was detected in the oculomotor nucleus.

This study tries to reveal the existence of direct contacts between the oculomotor and trochlear motoneurons. The experiments were carried out on frog, Rana esculenta. The trochlearis and oculomotor nerves were cut, and their proximal stumps were labeled with retrograde fluorescent tracers (nIII: Rhodamine binding dextran amine /micro ruby/; nIV: Fluorescein binding dextran-amine) simultaneously.

By using of confocal laser scanning microscope we could detect a large number of connections in both nuclei. In the oculomotor nucleus, 85% of trochlear dendrites formed dendrodendritic connections, the rest of dendrites (25%) were engaged in dendrosomatic connections. We could describe similar results in the trochlear nucleus: 77% of oculomotor dendrites appeared in dendrodendritic and 23% in dendrosomatic contacts with the motoneurons of the trochlear nerve. To study the fine structure of the connections between oculomotor and trochlear dendrites and perikarya we have applied cobalt-lysine and cobalt-cloride to the contralateral roots of nIII and nIV in the same animal. The retrogradely labelled structures were studied with electron microscope. We could not detect chemical synapses between the oculomotor and trochlear motoneurons.

Our results suggest that the dendrites and perikarya of the nIII and nIV are in close apposition, and their co-activation – responsible for vertical eye-movements – is probably mediated by non-synaptic pathways.

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Angiotesinerg mechanisms in the central nervous system regulating salt intake and fluid balance

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The zona incerta (ZI) and the renin-angiotensin system in the brain plays a definitive role in the regulation of fluid balance, thirst and hunger for salt. From the circumventricular organs, sorting information of the homeostatic state of the perifery, as well as from brain structures inside the blood-brain-barrier, angiotensinerg axon-terminals converg on the surface of the ZI. Angiotensin receptors (AT1, AT2) were also found in this brain region. Earlier, in our experiments angiotensin II and III (AII, AIII) microinjected into the ZI were proved to have dipsogen effects, but via different receptorial mechanisms.

In the present experiments AII (100 ng) and III (200 ng) microinjections into the ZI of rats have been studied on drinking water and salt solution (0.15 M) in two-bottle test procedures during the consequent 60-min-daily-drinking period. The volume consumed were determined in every 5 min all along the experiments. The dipsogen and salt hunger inducing power of the two angiotesins were compared to vehicle treated rats. After, angiotensin receptor antagonists on AII or AIII induced fluid intake were also tested. Before drug injection, preoperatively and postoperatively, animals were habituated to the two-bottle test paradigm. Neither neophobia nor preference was experienced for the first time of introduction of the salt solution. From day two of habituation animals prefered water to sodium solution all the time, when not treated. In the first experiment AII increased animals' preference to salt solution compared to water insomuch, that water intake of AII treated rats dropped to 50% compared to the veh treated animals during the first 35 min drinking period. In the second experiment AIII also increased sodium intake, compared to the veh injected animals, but not to the expence of water intake. AIII injected animals ingested the same amount of water as the veh injected ones. Considering the antagonist pre-treatments in the third and fourth experiments, animals were injected by 90 ng Losartan, an AT1 antagonist or 180 ng PD 123319, an AT2 antagonist, respectively. Both AII and AIII increased sodium consumption. The effect of AII could be blocked both by losartan and PD 123319. On the other hand, the effect of AIII could not be blocked by losartan, but by the PD 123319.

The effects of AII, AIII, Losartan, PD 123319 on salt hunger have not been tested in the ZI. The finding that salt intake increased after AII or AIII injections and it could be blocked differently by the antagonists in two-bottle tests suggests that AT1 and AT2 receptors play partially different roles in the regulation of salt and water intake int he zona incerta.

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Repeated 4-aminopyridine seizures reduce parvalbumin content in the medial mammillary nucleus of the rat brain

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Parvalbumin containing fast spiking neurons play a crucial role in synchronizing the activity of excitatory neuronal circuits in the brain. Alterations of parvalbumin content in these neurons can affect their spike characteristics and, ultimately, may increase the susceptibility of neuronal circuits to epileptic seizures. In the present study, we examined whether repeated 4-aminopyridine-induced seizures modify the regional parvalbumin contents in the rat brain.

5 mg/kg 4-aminopyridine was injected intraperitoneally in adult rats, controls received the solvent. Animals were sacrificed at 3 h after a single acute treatment, or following repeated, daily treatments of 12 days.

In situ hybridization indicated significantly decreased parvalbumin mRNA level in the medial mammillary nucleus (77%) at 12 days. The decrease of parvalbumin content in the medial mammillary area revealed by Western blotting was also significant (20,1%). The number of parvalbumin-immunoreactive neurons did not change in the medial mammillary nucleus, although the intensity of the staining did show a visible decrease.

The results reveal the downregulation of the transcription of the parvalbumin gene and the decrease of parvalbumin synthesis in medial mammillary nucleus neurons in response to experimental seizures, a process possibly contributing to the amplification of epileptic activity in the cerebral cortex.

The effects of corticotropin-releasing factor and urocortins on striatal dopamine release induced by electric stimulation

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Corticotopin-releasing factor (CRF) is the major physiologic regulator of the hypothalamic-pituitary-adrenal (HPA) axis and it also functions as a neurotransmitter in the central nervous system (CNS) coordinating the endocrine and behavioral responses to stress. Urocortin I (Ucn I), Urocortin II (Ucn II) and Urocortin III (Ucn III) are structurally similar but pharmacologically different members of the CRF peptide family. The actions of CRF and Ucns are mediated by two different types of G protein-coupled CRF receptors, CRFR1 and CRFR2. It has been shown that CRF produced a dose-dependent increase in dopamine synthesis antagonized by alpha-helical CRF (9-41). In the present study superfusion system was used to investigate the effects of CRF and Ucns on rat striatal DA release following electric stimulation. The involvement of CRF receptors was studied by pretreatment of striatal slices with specific CRF antagonists. CRF (100nM) and Ucn I (100nM) increased significantly the tritiated DA release from rat striatum. Ucn II (100nM) and Ucn III (100nM) were ineffective. Pretreatment of striatal slices with CRFR1 antagonist Antalarmin (100nM) inhibited considerably the DA-release induced by electric stimulation and influenced by CRF and Ucn I. Pretreatment with CRFR2 antagonist Astressin-2B (100nM) was ineffective. These results suggest that CRF and Ucn I mediates striatal DA release through the activation of CRFR1. Ucn II and Ucn III are not involved in the striatal DA activation induced by electric stimulation.

Repeated 4-aminopyridine seizures moderate parvalbumin and Fos-B gene expression in the rat cerebral cortex

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Epilepsy is a chronic disease, characterised by recurrent seizure activity. In human case histories, the first symptom towards a long-lasting pathological change is often an acute, unprecedented convulsion. We investigated the long-term effect of repeated, brief seizures in animal experiments, and tried to map the spread of neuronal hyperactivity with immunohistochemical methods. Inducible transcription factors can be used as the immunohistochemical marker of increased neuronal activity. The aim of our study was to map the Fos-B expression following repeated 4-aminopyridine- (4-AP) induced generalised seizures in the rat neocortex and hippocampus. We also aimed the neurochemical characterization of the activated neuronal population, by means of parvalbumin immunohistochemistry.

In the present study, we administered 4-AP to male Wistar rats once in every day during a 12-day period. The initial dose of the convulsant (4.5 mg/bwkg) has been gradually raised in order to elicit the seizure activity. The control animals received the solvent of 4-AP (physiologic saline) in the same volume. On the day 12, 1 hour after the last 4-AP-injection, the animals were perfused; and Fos-B and parvalbumin double-labelling immuno-histochemistry was performed on frozen coronal brain sections. The immunoreactive cells were counted (Fos-B-positive cells and parvalbumin-positive cells alone and Fos-B + parvalbumin double-positive cells).

Our data show a significant increase in Fos-B expression in all the studied allo- and neocortical areas. The number of parvalbumin-positive cells was constant; the number of double-labelled cell population was higher in the studied areas, however not in a statistically significant manner.

According to literature data, Fos-B is considered to be a cellular marker of long-term changes. No change occurred in the number of the parvalbumin-positive inhibitory cell subpopulation, nevertheless, these cells showed an increased Fos-B gene expression activity in this chronic model, in allo- and neocortical areas, as well. This phenomenon emphasises the role of the inhibitory cell population in the neuronal circuits participating in the cerebrocortical seizure activity.

Role of multimodal target-determination in the functional neurosurgical treatment of movement disorders

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Introduction

Advances in image-guided stereotactic surgery, microelectrode recording techniques and stimulation technology have been the driving forces behind resurgence in the use of functional neurosurgery for the treatment of movement disorders. Stereotactic deep brain ablations and deep brain stimulation (DBS) techniques are most common procedures in ameliorating the symptoms. In this report, we describe the methods used to localise the stereotactic targets and we present the characteristics of deep brain activity in different brain structures.

Material and methods

Over the past ten years 347 stereotactic procedures were performed for movement disorders at our department. The patients with different kind of disabling -(parkinsonian PD), essential (ET), multiple sclerosis (MS), olivopontocerebellar (OPC), or post-traumatic (PT)- tremors resistant to pharmacological therapy underwent microelectrode-guided stereotactic thalamotomy or DBS. The lesions and the DBS electrodes were directed at the ventralis intermedius (Vim) nucleus of the thalamus. For patients with idiopathic Parkinson's (PD) disease pallidotomy in globus pallidus pars interna (Gpi), or thalamotomy, or –since DBS technique is available at our department– subthalamic nucleus (STN) DBS implantation was perform according to the symptomatology. Patients with dystonia underwent pallido-, or combined pallidothalamotomy according to the efficacy of relief of symptoms.

For target determination three main modality were used. The morphological target was assigned on magnetic resonance imaging (MRI) on T1 or T2 weighted images, which were referenced to the Schaltenbrand and Wahren stereotactic atlas. Microrecording of the targeted area was then performed. Spontaneous and evoked multiunit and single-unit neuronal activity was recorded at various sites along the trajectory. The recorded pattern was depended on the investigated nucleus. The neuronal noise was high in Vim, STN, and Gpi, and diminished when the electrode left the nucleus or entered the electrically silent white matter. The test stimulation of the targeted area was resulted in relief of pathological movements.

Results

The result of surgery was evaluated according to internationally standardised clinical rating scales and also tremorometry. Significant improvement was proved in clinical symptoms according to rating scales, tremorometry and on video, as well. Operative morbidity was not dysabling in case of ablative surgery and not permanent in DBS cases.

Conclusion

Multimodal target-determination makes functional stereotactic surgery safe and efficient for the treatment of movement disorders. The precise multimodal target-assignment is an essential tool in avoidance of surgical morbidity.

Behavioural effects of non-ionising irradiation in rats: Animals treated as adult

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Although there are plenty of research data on the effect of non-ionising electromagnetic radiation (NIEMR) on the cell-physiology, only a few studies deal with the behavioural and non-specific effects (e.g. dizziness, headache, ill-being, nausea, etc.). Since these non-specific effects are difficult to study in humans, we have initiated a research project in which multiple animal tests are used to study the phenomena as well as possible mechanisms in their complexity. In this poster, we report the first attempts in which an electromagnetic field generated by a coil, mimicing domestic transformers and their field, have been used. We hypothetised that the behaviour of irradiated rats will differ from their control peers in many respect.

Some of the rats were irradiated in a large coil (500 mT) which could host a plastic box with a perforated cover in which the rats could move and behave freely but where neither food nor water had been available. Another group received irradiation by a small coil (100 mT) placed under the cage or box during the experiments.

First, basic activity of the animlas was characterised by an open-field test and by a motimeter recording, of which the former described the pattern of the behaviour whereas the latter was rather suitable for activity measures. Open-field tests were run either right after or during the irradiation, whereas activity measures always followed irradiation.

The second group of tests detected preference changes i.e. whether the animals avoided certain places (in this case the area above the coil) or certain stimuli (i.e. taste) associated with the irradiation.

Finally, the third group of tests reported here delt with more complex, social behaviour. The social interaction test detects changes in the behaviour in a situation and place strange for both peers whereas in the territorial test one of the rats is the host and the other is an intruder. In our experiments, the social partners were alike (i.e. both irradiated or both controls) whereas in the territorial test the inrtruder was treated and the host was a stranger.

As far as the preliminary results show, we have not been able to detect statistically significant differences among the control and irradiated groups. However, conclusive results will only be available after processing all data since no single experiment alone is expected to yield usable data. To observe presumably mild and non-specific effects, one need to run complex, multivariate testing and analysis that may find differences if there were any.

Regulation of tritiated NA release from rat hippocampal slices by presynaptic P2X receptors

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Regulation of tritiated NA release from rat hippocampal slices by presynaptic P2X receptors Experiments on rat hippocampal slices were carried out in order to explore whether the release of noradrenaline from hippocampal terminals of central catecholaminergic pathways can be modulated through P2- receptors. The slices were preincubated with tritiated NA (2,5 μCi/ml) for 45 min. P2 purinoceptors agonists ATP, ADP and α,β - meATP elicited concentration dependent tritiated norarenaline efflux with the following rank order of agonist potency: □,□-methyleneATP > ATP ≥ ADP.

The widely selective P2 receptor antagonist PPADS (30μM) significantly, but partially decreased the response evoked by ATP (10mM). NF023 (10μM) and an antagonist of the P2X1 receptor the NF449 (100nM) also reduced ATP-evoked tritiated NA outflow. On the other hand, suramin (300μM) and the P2X7 receptor selective antagonist BBG (100nM) were without effect and the P2Y1 receptor selective antagonist, MRS 2279 was also ineffective to modify ATP (10mM) - induced tritium outflow. Accordingly, 12h pretreatment of the slices with the Gi protein inhibitor pertussis toxin (2,5 μg/ml) did not significantly modify ATP - evoked NA release. Using primers specific for cDNAs of most the cloned P2X and P2Y receptor subunits, RT - PCR analysis revealed that mRNAs encoding P2X1, P2X2, P2X3, P2X4, P2X6, P2X7 and P2Y1 subunits were expressed in the brainstem containing catecholaminergic nuclei projecting to the hippocampus.

In summary, the rat hippocampal terminals of central catecholaminergic pathways are equipped with ionotropic P2X-like receptors, whereby the release of NA could be positively regulated. The potent action of α,β-meATP, the sensitivity of the effect of ATP to 30μM PPADS, 10μM NF023 and low pH (6, 5) are consistent with the activation of homomeric P2X1 or P2X3 receptors. The NA release appears to have non - vesicular origin, and a direct Na^+ efflux through the receptor - ion channel complex could be the subcellular mechanism responsible for it.

Neuroendocrine and metabolic challenges recruit distinct functional domains in the rat hypothalamic paraventricular nucleus

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Hypothalamic circuitries integrate multimodal signals essential for survival. Combining voluntary sucrose ingestion model as a manipulation of caloric status leading to obesity, and adrenalectomy that is known to activate the hypothalamic-pituitary-adrenal axis by removing the glucocorticoid feedback signal, here we provide evidence on an interaction of energy metabolism and the central stress system in a long-term scale. Adrenalectomized (ADX) or sham operated adult Wistar rats allowed to drink 1M sucrose solution, 0.5% saline and water for a period of 3 weeks, then sucrose were withdrawn in a set of animals for further 3 weeks. ADX resulted in an increase in saline, and decrease in sucrose consumption indicating effect of mineralocorticoids on electrolyte, and glucocorticoids on carbohydrate balance. To study central effects of this caloric and steroid manipulation, gene-expression of corticotropin releasing hormone (CRH) and arginine-vasopressin (AVP) in the hypothalamic paraventricular nucleus (PVN) was analysed by isotopic in situ hybridization histochemistry. In parallell to an elevation of plasma ACTH, ADX resulted in an increase in both CRH ($F(1,22)=8.9887$, $p=0.0066$) and AVP mRNAs ($F(1,22)=32.8034$, $p<0.0000$) in the medial dorsal parvocellular (mpd) compartment of the PVN. Chronic sucrose consumption also elevated significantly both stress-related neuropeptide's mRNAs (CRH: $F(1,22)=6.358$, $p=0.0194$; AVP: $F(1,22)=5.3300$, $p=0.0378$) in the hypothalamus and increased circulating levels of the fat-derived hormone leptin. In contrast to the ADX-induced vasopressin expression that was evident in the hypophyseotropic (mpd) area, sucrose-induced elevation of AVP mRNAs was predominantly localized in the autonomic projecting (medial ventral parvocellular, mpv) domain of the PVN. Our present data reveal a common CRH response to adrenalectomy and sucrose, and a differentially regulated expression of parvocellular AVP in the absence of glucocorticoids and during chronic high-calory intake.

Nucleus accumbens subregions: hodological and neurochemical analysis in domestic chick

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The nucleus accumbens (NAc) plays a crucial role in addiction, motivation, reinforcement of behaviour, and in decision-making (escape, offense, freezing) which are important in the survival of the species. In mammals, NAc consists of two subdivisions: NAc shell (NacS), which is rich in calbindin (CB), calretinin (CR) and neuropeptide Y (NPY) immunoreactive elements; and NAc core (NAcC), which is poor in these markers. These subregions differ in their projections, too. For example, NacS, but not NacC, receives noradrenergic input from the nucleus tractus solitarii (NTS). The organization of the NAc has not been extensively studied in birds, in particular, we wished to examine the precise anatomical location of NAc in relation to the formerly defined medial striatum (MSt). According to earlier experiments in our laboratory, the NAc occupies a ventromedial field of MSt, and it is larger than previously thought: in rostral section levels the NAc expands more laterally, whereas in caudal levels it is situated more dorsally and laterally.

The distribution of NPY, the calcium binding proteins parvalbumin (PV) and calbindin D-28k was investigated by immunohistochemistry in the subregions of NAc of ten-day-old domestic chicks. We found that NAc is neurochemically separable into two subregions: one of them being rich in CB and NPY immunoreactivity but poor in PV, lying along the ventral border of MSt (presumed NAcS). The other subregion is rich in PV but the immunoreactivity to CB and NPY is low. The latter subregion occupies the dorsomedial part of MSt in rostral levels, whereas in caudal levels it is gradually shifted in a laterodorsal direction toward central MSt (presumed core region).

Following injection of the anterograde tracer biotinylated dextran amine (BDA, MW:10000) into the NTS, marked fibers and terminal fields were observed in the presumed shell, but not the presumed core region, or in the rest of medial striatum (MSt).

Our findings suggest that the described subdivisions of avian NAc are likely to be homologous to the mammalian NAcS and NAcC. Thus, the organization of NAc and related systems may also reflect similarities of functional roles.

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Retinal processing - capturing the essence in real-time

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The mammalian visual system analyzes the world through a set of spatio-temporal channels. The organization of these channels begins in the retina where a dozen filters extract different dynamic features from the visual scene. Our goal is to capture the essence of these retinal filters and implement it in a real-time environment.

The work is based on neuro-physiological measurements. The different channels excitatory, inhibitory and spiking patterns are available from biological measurements. Our modeling approach is neuromorphic. The primary motivation is to derive an algorithmic skeleton from the measurements and to make possible the on-line parameter changing. The model is tuned for half a dozen different channels with half percent spatial-temporal error.

We are reporting results about functional characteristics of a few channels following the recent discovery of this multi-channel retinal processing. In particular, we have been found qualitatively different maps of a video clip in the Local Edge Detector channel (LED), the Sluggish channel and the On transient channel.

The target platform is a stand-alone vision-computer, called Bi-i. The Bi-i is an of-the-shelf device that is capable to sense and compute our mammalian retina model in real-time. The system computes four retina channels with useful spatial resolution in video real-time. The easy on-line control is solved with faders and rotary controls.

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Event-related brain potential correlates of adaptation to faces and body parts

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Prolonged exposure to an individual face or an image of a body part leads to adaptation and will bias the perception of subsequently presented test faces or body part images.

The goal of the present study was to uncover the mechanisms of neural plasticity underlying such high level figural adaptation using ERP. As our stimuli, we used computationally derived morphs of female and male faces as well as morphed female and male hands. Behavioural effects of a 5 second adaptation to either a specific face or hand stimulus (or as a control condition to their Fourier randomised version) were measured using face or hand gender discrimination task. In our ERP experiments we measured the effect of face and hand adaptation on the parito-occipito-temporaly peaking N170 component of the ERP (recorded from 23 channels, positioned according to the 10-20 system).

Our behavioural results showed a strong category-specific adaptation to both faces and hands. We found no cross-category adaptation effects; i.e. adapting to a face did not affect hand gender discrimination just as adapting to a hand image did not affect face gender discrimination. Our ERP measurements revealed that both adaptation to faces and hands significantly increases the latency and decreases the amplitude of the N170 component (compared to the adaptation to the Fourier randomised images) in the case when the test and the adaptor stimuli are from the same category but not when they are from different categories. There were no category-specific adaptation effects on the P100 components of the ERP responses.

To conclude, we suggest that high level configural adaptation is reflected in the N170 components of the ERP responses.

Immune-challange-induced ERK1/2 phosphorylation in gonadotropin releasing hormone (GnRH) neurons in vivo

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It is well established that immune-challenges can attenuate or disrupt the reproductive capability and influence the release of cytokines and immunoglobulins during immune responses have been implicated in this endocrine aberration. The principal regulatory neuron of fertility, the GnRH neurons, may play a key role in the immune-challenge-induced aberrant regulation as they express cytokine receptors. Since the critical intracellular downstream signal of the activated cytokine receptors is the phosphorylation of ERK1/2, in the present study we investigated whether an immune challenge can alter ERK1/2 phosphorylation in GnRH neurons.

We set up in vivo experiments for immune challenge, where a T cell-dependent B cell response was induced by KLH-FITC administration in female mice. We detected the ERK and phosphorylated ERK1/2 (pERK1/2) in GnRH neurons by means of quantitative double labeling fluorescent immunohistochemistry. We also investigated the gonadal steroid dependence of this response comparing the effect of the immune challenge in sham operated and gonadectomized mice. In order to test the efficacy of the immune response IgG and IgM production was measured using ELISA and number of plasma cells was detected by ELISPOT method.

Fluorescent immunohistochemistry revealed pERK/12 immunoreactivity in cytoplasm and nucleus of GnRH neurons and showed that ERK immunoreactivity restricted to cytoplasm of GnRH neurons. While the ERK expression of GnRH neurons was not sensitive either to immune challenge or to gonadectomy, the KLH-FITC induced a four fold increase in ERK1/2 phosphorylation in GnRH neurons. In contrast KLH-FITC administration failed to have an effect on ERK1/2 phophorylation in GnRH neurons in gonadectomized mice. Interestingly, our findings also demonstrate that the level of ERK1/2 phosphorylation was significantly higher in gonadectomized comparing with sham operated non-immune-challenged mice. The ELISPOT and ELISA measurement confirmed a strong immune response since KLH-FITC administration significantly increased the levels of IgM and IgG and number of plasma cells in these experiments.

Taken together, our data demonstrate a massive ERK phosphporylation in the GnRH neuron following T cell-dependent B cell response. Our findings also suggest that gonadal steroids may play critical role in the immune-challenge-induced ERK phosphorylation in GnRH neurons.

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Long term effects of single traumatic experience in rats

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Traumatic stressors lead to the development of post-traumatic stress disorder, which affects approximately 3% of the population. The treatment of this disorder is still difficult; and laboratory approaches are also deficient. We have studied the long-term effects of single electric shocks in rats with the final aim of developing a novel and more appropriate model for studying this disorder in the laboratory. Rats exposed to 10 mild (0.8 mA) or strong (8 mA) electric shocks developed a marked social avoidance that lasted at least 28 days. In the rats exposed to strong shocks this was associated with a marked locomotor suppression in novel environments but not in the home-cage. The shocks induced a marked increase in plasma glucocorticoid levels, but this effect disappeared rapidly, and glucocorticoid secretion profiles were similar in control and shocked rats over the month following the shocks. In contrast, autonomic stress responsiveness changed markedly. Both situational reminders and the shocking environment induced a strong and lasting increase in the heart rates of shocked rats four weeks after the single shock exposure. The heart rates responses of control rats were markedly smaller. Heart rate responses to an aggressive encounter were also markedly increased by shock exposure. In addition, the aggressiveness of rats was also affected four weeks after shock exposure. We conclude that electric shocks induce marked and long lasting changes in the behavior and autonomic responsiveness of rats. These changes are in many respects similar with those seen in post-traumatic stress disorder.

Food intake behaviour: Are 'extrahomeostatic' processes really extrahomeostatic?

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Homeostasis is one of the most frequently used basic terms in physiology and biology. Recently, however, a lot of new data have emerged that seem not to be in concert with Cannon's principles: in freely behaving organisms 'milieu interne' is far from being stable nor are any of the so far observed variables. Theoretical and practical considerations suggest that the only stable feature of the living organisms is integrity, which requires permanent adaptation i.e. frequent changes of all vital functions: variostasis.

Multiplicity of the regulatory mechanisms and of the target functions results in a multitude of complex activities both internal and external, minute after minute. Contrary to the general belief, actual or possible internal imbalances (needs) activate not only visceral compensatory mechanisms but many behavioural actions of different sort. Food intake, foraging behaviour or taste preference, however, have been so far regarded as 'extrahomeostatic' as opposed to hormonal changes and visceral reflexes termed as homeostatic. Our analysis shows that in many cases behavioural changes are superior to visceral reactions and seem to be more adaptive, too. Though according to Cannon's principle, decreased sugar availability has to be compensated by emptying the stores (i.e. glycogenolysis), both theoretical consideration and experience suggest this would be one of the last things the organism should do. Instead, visceral and behavioural mechanisms switch on to replace the lost sugar and save the stores for emergency situations when glucose-surge is a must. Changes of the taste preference (alliesthesia), selective hunger and satiety, as well as learned feeding patterns help to save the organism's integrity very effectively, frequently much before actual internal needs arise (anticipatory behaviour). Our results show that most of the internal signals initiating behavioural changes are non-conscious but if immediate action is needed, they may reach consciousness in which case they seem to be mostly unpleasant. It seems now evident that most of the visceral stimuli reaching consciousness are alarm signals, usually initiating protective or diseases behaviour whereas normal homeostatic signals have only indirect effect mostly of affective character (e.g. pleasant or unpleasant).

Models of the expectancy-based placebo-effect

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According to our theoretical considerations, placebo-effects have at least two different mechanisms: conditioning and expectancy. Whereas the former has been established a long while ago and many–human as well as animal – models have been created, the latter hasn't been substantially modelled so far. The aim of our present work is to set up appropriate models of the expectancy-based placebo effect. Though it is very difficult to exclude conditioning or other forms of learning from a placebo-study, a model should be regarded as satisfactory if it is - at least - more than that: expectancy must be evidently present.

Our first human studies have shown that disorders, in which – as opposed to those of organic origin - psychological factors play a significant role in the aetiology, are good models of the expectancy-based placebo effect which proves to be a useful tool in therapy and is specific to these groups. A typical example of this model is the food-allergy versus food-aversion study in which the latter group was successfully treated with placebo whereas the former resisted. The curative potential of the placebo-treatment seems to be very high, presumably comparable to those of the cognitive and/or suggestive therapies. In fact, we suggest that expectancy-based placebo-effect shares many features with the two latter. Other studies with different functional disorders have also been initiated recently. The alcohol-consumption study (see Nagy et al. at this Conference) seems to be a good human model, too.

Contrary, however, to the numerous animal models of the conditioned placebo-effect, we have been unsuccessful as yet to find or create a good animal model of the expectancy-based placebo-effect. Anecdotic accounts of doctor-patient relationships in animals told by veterinarians suggest that most of the animals are unable to foreseen the consequence of the doctor's action and hence to anticipate getting better. Any animal models we have tried to set up so far proved to be either impossible or essentially based on conditioning. It is still not clear whether apes and/or dogs were able to use expectancy in their behaviour, but no data has been found by this time. The authors therefore welcome any suggestions for how to create expectancy-based animal models. Just to make this request more challenging, the authors disclose and admit that their hypothesis regarding this question is the contrary: expectancy-based placebo-effect is typically human and hence no satisfactory animal model exist.

Rapid behavioural effects of glucocorticoids

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Glucocorticoid hormones are released from the adrenal gland in response to a number of physical and psychosocial challenges and modulate behavioural responses through various mechanisms. The variety of behaviours affected in different situations raise, however, questions regarding the specificity and roles of glucocorticoids in controlling behaviour. Earlier we demonstrated that corticosterone injection enhanced territorial aggression in a resident-intruder test of rats very rapidly within 2-7 min via non-genomic mechanisms. To elucidate the specificity and context dependency of this rapid behavioural effect, in the current study we assessed the acute behavioural consequences of glucocorticoids in non-social challenging situations and in a more complex social setting that approaches the natural situation of rats. As non-social challenging situations, we assessed the rapid behavioural effects of glucocorticoids in the elevated plus-maze (EPM) test, which measures three different kinds of behavioural responses: locomotion, anxiety-like behaviours, and risk assessment. The acute inhibition of glucocorticoid synthesis decreased risk assessment, but did not affect locomotion and anxiety-like behaviours. Corticosterone administration increased risk assessment, without affecting locomotion and anxiety-like behaviours. Moreover, plasma corticosterone levels measured immediately after testing strongly correlated with the intensity of risk assessment. The effects of corticosterone were rapid, as occurred even when the hormone was injected 2 min before behavioural testing. In addition, the effect was resistant to protein synthesis inhibition. These data demonstrate that glucocorticoids are able to increase specifically risk-assessment behaviours by non-genomic mechanisms novelty-related, non-social challenging situations. The acute effects of glucocorticoids were also investigated in a social setting, in which three individually marked and unfamiliar male rats were placed into a cage for 15 days. At the end of this period the acute consequences of corticosterone injection were investigated. Corticosterone injected animals exhibited more aggressive interactions compared to untreated and vehicle injected animals. Our results suggest that glucocorticoids are able to induce specific and context dependent behavioural adjustments to meet immediate requirements set by the challenge.

The influence of slow cortical oscillations on the firing of rat zona incerta neurons

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The external source of GABAergic inhibition of dorsal thalamic nuclei has traditionally been attributed to the thalamic reticular nucleus, The zona incerta (ZI) has recently been identified as being an inhibitory pathway that selectively innervates relay cells in higher order thalamic nuclei. ZI effectively reduces the spontaneous activity in the thalamus and exerts strong feedforward inhibition on the peripheral evoked responses of relay cells.

ZI receives a prominent excitatory input from the layer V of several cortical regions suggesting a cortical modulation of cellular activity. To establish the correlation of firing pattern with global cortical rhythms juxtacellular recordings and labellings were performed in the rat ZI under different anaesthetic conditions with concurrent EEG monitoring. Firing pattern of ZI neurons were compared to the activity of thalamocortical cells, known to be strongly modulated by cortical oscillations.

During desynchronized EEG states (light urethane anaesthesia, n=24, or neurolept analgesia, n=9), almost all ZI cells were characterized with a single action potential mode. The frequency of irregular or tonic unit activity in different cells varied widely between 0.2 Hz and 30 Hz and displayed no correlation with the cortical gamma waves. The position of ZI neurons (dorsal, ventral, rostral, caudal ZI) showed no relation to its firing frequency.

During spontaneously occurring or ketamin induced slow cortical waves (1-3 Hz) the activity of most ZI neurons was significantly reduced and became phase locked to the “up states” of the cortical rhythm. Interestingly ZI neurons with lower than 1Hz initial activity increased their firing rate in a way to follow the cortical rhythm. ZI neurons never displayed high frequency bursts activity, which characterize thalamic relay cells. The degree of correlation between cellular activity and the EEG were comparable in relay cells and ZI neurons.

The dendritic trees of ZI neurons were quite extensive (up to 1mm), spanned several ZI sectors in the mediolateral and anteroposterior direction but were compressed dorsoventrally. This morphological arrangement is ideal to integrate simultaneous activity arising in distant cortical regions, especially during synchronized EEG activity.

Since ZI receives no thalamic feedback our data suggest that the rrhythmic neuronal activity in ZI is driven by the neocortex. This enables ZI to convey rhythmic GABAergic signals in relation to the major thalamocortical network patterns.

Changing of the immunoreactive nerve fibers in the root of the tongue in diabetes mellitus after nerve growth factor treatment

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BACKGROUND. AIMS: Peripheral neuropathy is a common complication of diabetes. Our previous studies showed that the density of the neuropeptide containing nerve fibres increased, supposing that the neurotrophic factors might be responsible for these changes. Therefore, our aim was to investigate the effect of one of the neurotrophic factors (nerve growth factor=NGF) on the distribution and density of the different immunoreactive nerve fibers in the root of the tongue of streptozotocin induced diabetic rats. METHODS: Four- week-diabetic animals were treated by NGF for three days (50µg/0,2 ml saline/days). Vasoactive intestinal polypeptide (VIP), neuropeptides (NPY), substance P (SP), nitric oxide synthase (NOS) and tyrosine beta-hydroxylase (TH) immunoreactive (IR) nerve elements were detected by ABC immunohistochemistry. RESULTS: The quantitative analysis of the density of the different neuropeptide containing nerve fibres showed that the IR nerve fibres were found in all layers with different density. After four weeks of diabetes the number of the IR nerve fibres increased significantly, especially the SP IR ones ($p<0,05$). After administration of NGF the number of all investigated neuropeptide-containing nerve fibres decreased significantly. Even the number of the IR nerve cell bodies decreased after this treatment, their density was even less than that of the control. CONCLUSIONS: These results suggest that the NGF treatment might have reverse effect depending on the level of intrinsic neurotrophic factors. In control animals (in the literature) NGF enhanced the number of nerve fibres, however in our experiments NGF treatment caused reverse effect.

Experimental trans-ventricular infection of the central nervous system of rats with neurotrop viruses

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The neurotrop viruses are taken up by axon terminals then expressed in the infected neurons and they are transferred through synapses to reach other neurons. These virus-infected neurons can be visualised with immunocytochemical techniques. It has been show, however, that non-neuronal elements, like ependymal cells are also frequently infected.

The aim of this study was to answer how ependymal cells could be infected and the intraventricularly injected neurotrop viruses where, when and how enter the central nervous system.

Two strains of Pseudorabies viruses (PRV) (Bartha, BDG) were injected into the lateral ventricles of rats. The animals were sacrificed with transcardial perfusion of Zamboni's fixative solution 23, 30, 45, 48, 51, 71 hours after the injection.The brains were removed and serial frozen sections were cut. PRV immunohistochemistry was performed with a polyclonal goat anti-PRV antibody.

Several infected ependyma cells were observed 23 h after inoculation of the virus. They were located in the wall of cerebral ventricles from lateral ventricle to central canal with ipsilaterally dominance.

By 30 hour postinoculation time many infected cells were detected in the circumventricular organs: organum vasculosum laminae terminalis, subfornical organ, area postrema. Several special ependyma cells (tanycytes) became labelled with PRV at the same time.

A fairly high number of infected neurons were found in the mesencephalon, exclusively in serotonin synthetizing neurons in dorsal raphe nucleus and in nucleus centralis superior. These neurons provide a dens network of fibers on the ependymal surface in the third ventricle (so-called supraependymal serotonergic terminals).

Forty five-48 h after the virus injection those neuronal cellgroups that are connected with circumventricular organs, raphe neurons and tanycytes, became secondarily labeled with PRV. They were presented in the supraopticus, the paraventricularis, and the arcuatus nuclei, the nucleus of the solitary tract and in several catecholaminergic cell groups, like the locus coeruleus or the substantia nigra.

At later periods of time (51-71 h after injections) many third- and fourth-order labelled neuron were observed in the infralimbic cortex, lateral hypothalamus and in the lateral tuberomamilaris nucleus. Finally, the virus was transferred to other neurons and appeared even greater fields of central nervous system that the animals can not further survive.

In summary, the present study clearly demonstrated the entrance and the further spreading of neurotrop virus in the rat brain after intraventricular injection. These areas (ependyma cells, CVO, tanycytes, supraependymal serotoninerg terminals) form entering gates to central nervous system.

A tecto-thalamo-cortical pathway providing sensory information to the basal ganglia

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Basal ganglia play and important role in the sensorimotor coordination of motion in all mammals. While the participation of the basal ganglia in the organization of motor control is widely studied, not much is known on the origin of sensory information in the process. Generally, the cortico-striatal pathway is regarded as the main sensory input to these structures.

Here we provide evidence for a separate tecto-thalamo-cortical pathway that bypasses the primary thalamic relay nuclei providing sensory information to the basal ganglia. We survey our morphological and physiological studies that proved the existence of a formerly unknown suprageniculate-anterior ectosylvian thalamo-cortical loop that processes multisensory information from the superior colliculus. The multimodal receptive field properties of these neurons support the notion of a sensory pathway separate from the primary, unimodal ones.

Further, morphological analysis showed a powerful pathway running from the thalamic suprageniculate nucleus towards the caudate nucleus. Based upon these observations we conducted physiological studies that found the sensory propeties of the caudate nucleus and the substantia nigra chracateristically similar to those found in the structures of the tecto-thalamo-cortical pathway.

These morphological and physiological studies provided evidence for the notion that the sensorimotor function of the basal ganglia is served by a pathway that processes tectal, multimodal information through a thalamo-cortical loop.

Receptor binding, functional and morphological studies with a new delta opioid antagonist, Tyr-Tic–(2s,3r) beta-MePhe-Phe-OH

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The highly specific delta opioid antagonist TIPP (Tyr-Tic-Phe-Phe-OH) was modified by building in a conformational constrain to obtain a more stable analog. The resulting compound, Tyr-Tic-(2S,3R)beta-MePhe-Phe-OH was used in comparative analyzis in rat, wild type and delta opioid receptor knock-out (DOR-KO) mouse brain membranes to investigate the yet hypothetical heterogenity of the delta opioid receptors. [3H]Tyr-Tic-(2S,3R)beta-MePhe-Phe-OH with a specific activity of 53.7 Ci/mmol was prepared with catalytic dehalogenation. Saturation binding experiments yielded in a dissociation constant ,Kd of 0.28 ± 0.001 nM and receptor density, Bmax of 155 ± 6.6 fmol x mg protein –1 in rat brain. The binding affinity was decreased in the presence of Na+ which result confirmed that the new ligand is an antagonist. There were fewer binding sites with higher affinity in wild type mouse brain membranes. No specific binding was detected in membranes of the DOR-KO mouse brain. In accordance with this result, no labeling was seen in receptor autoradiography studies in DOR-KO brains after 3 months exposure. In contrast, intense labeling was observed with [3H]Tyr-Tic-(2S,3R)bMePhe-Phe-OH in brain regions known to contain delta opioid receptors at high density. The specificity of the binding sites of [3H]Tyr-Tic-(2S,3R)beta-MePhe-Phe-OH was further evaluated in competition assays. While mu and kappa specific ligands showed poor affinity, the prototypic delta ligands Ile5,6-deltorphine and naltrindol displaced the radioligand with high affinity. Interestingly, unlabeled Tyr-Tic-(2S,3R)beta-MePhe-Phe-OH displaced more binding than the former two delta ligands in mice but not in rats. Naltrindol and Tyr-Tic-(2S,3R)beta-MePhe-Phe-OH also differed in their ability to antagonize the stimulating effect of the delta agonist DTLET in mouse brain. These results further support the notion that there are different subtypes of the delta opioid receptors. The new, stable, potent peptide antagonist ligand combined with transgenic animals is a good tool for future studies of opioid receptor heterogeneity.

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Network state-dependent operation of excitatory synapses in the hippocampus

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The activity of hippocampal pyramidal cells (PC) varies depending on the behavior of the animal and on the accompanied network state. During hippocampal theta activity, CA1 PCs show asynchronous activity and fire at a very low frequency. In contrast, during sharp-waves PCs increase their firing frequency over 100-fold and many cells fire synchronously. In the present study, we addressed how the presynaptic activity of CA1 PCs influences the precise operation of their output synapses.

We performed whole-cell voltage-clamp recordings from oriens/alveus interneurons (IN) in acute slices of juvenile rats (14-18 days-old). Asynchronous presynaptic activity was mimicked by activating only a single PC by performing paired-recordings, whereas highly synchronous presynaptic firing was imitated by extracellularly stimulating the axons of ~50 presynaptic CA1 PCs. During paired recordings, short trains of presynaptic APs at 50 Hz evoked facilitating unitary excitatory postsynaptic currents (uEPSC) in oriens-lacunosum-moleculare and in some oriens-bistratified cells. The number of release sites between the recorded cells was independently determined by quantal analysis and by electron microscopy. Our results showed that short-term facilitation of uEPSCs was the sole consequence of an increase in the release probability (Pr) with no alterations in the quantal size. The lack of multivesicular release or intersynaptic crosstalk/spillover was also supported by our kinetic analysis. A ~5-fold increase in Pr from the beginning to the end of the AP train had no effect on the decay time constant of uEPSCs (beginning: 2.5±0.3 ms, mean±SEM; end: 2.5±0.3 ms, n=11). In contrast, when EPSCs in oriens/alveus INs were evoked by extracellular stimulations, a slowing down of EPSC decay times was observed during the stimulus trains (beginning: 2.6±0.2 ms; end: 3.7± 0.4 ms, n=16), suggesting crosstalk or spillover between neighboring synapses. This hypothesis was further tested by using low- and high-affinity competitive glutamate receptor antagonists. The application of the low-affinity antagonist γDGG (0.5 mM) reversed the slowing of eEPSC decay times (from 3.6±0.5 ms to 2.3±0.2 ms, n=9) and resulted in a significantly smaller block (52±3% vs. 63±3%, n=11) of the peak amplitude of eEPSCs under high Pr conditions. These effects were not observed with the high-affinity antagonist CNQX (1 μM), demonstrating that the effects of the low-affinity competitive antagonist are likely to be due to Pr-dependent alterations in the synaptic glutamate concentration profile.

Our data demonstrate that the synaptic glutamate concentration transient varies depending on the Pr during synchronous high-frequency activity of PCs, but synapse-independence is preserved when CA1 PC cells fire asynchronously.

Extrareticular GABAergic system: a conceptually new type of inhibition in the thalamus

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Higher order thalamic nuclei are characterized by a prominent excitatory input originating in layer V of the neocortex. Morphological and physiological properties of this input are similar to the peripheral afferents suggesting a cortical instead of a peripheral drive of these nuclei. Recently we described a novel GABAergic afferent system that exerts powerful inhibitory effect selectively on higher order thalamic relays and originates outside the well-known thalamic reticular nucleus (nRT), hence termed "extrareticular" GABAergic pathway.

In this study first we systematically compared the morphological properties of the GABAergic terminals originating in the nRT and in the "extrareticular" anterior pretectal nucleus (APT) in the same higher order thalamic nucleus. Next we examined the consistency of morphological features in extrareticular GABAergic terminals originating in other sources.

Reticular terminals were small to medium sized (0,8-2 □m) and frequently contacted more than one postsynaptic target (up to three). The number of release sites per target was usually one. In contrast, APT terminals were large (2-6 □m) and always contacted a single target via multiple release sites (up to 13). nRT terminals showed random target selection, the vast majority of the terminals contacted thin, distal dendrites, whereas a minority innervated thick, proximal ones in proportion to their occurrence in the neuropil. APT terminals, however, preferentially innervated proximal dendrites (diameter larger than 1 □m) and ignored the abundant thin dendrites. Multiple rows of puncta adhaerentia (filamentous contacts) were common in extrareticular, but absent in nRT terminals.

GABAergic terminals in the thalamus originating in the zona incerta, ventral lateral geniculate nucleus, substantia nigra pars reticulata, and entopeduncular nucleus were similar to APT terminals in size, number of release sites, postsynaptic target selection and the presence of filamentous contacts.

The profound difference among reticular and extrareticular terminals suggests a division of labour between the two types of GABAergic pathways. On the other hand similarity among GABAergic terminals originating in different extrareticular nuclei indicates similar rules of operations. This suggests that GABAergic mechanisms will be qualitatively different in first order and higher order nuclei. Selective extrareticular GABAergic control of relay cell activity will result in effective, presumably state dependent gating of thalamocortical information transfer in higher order but not in first order relays.

Viral vectors

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Pseudorabies virus (PRV) is a non-human pathogen neurotropic herpesvirus. Strain Bartha (PRV-Ba) and its recombinant derivatives are widely used as tract tracers to ro reveal synaptically-linked neural circuits. The major challenges in tract tracing studies are as follows:

(1) We must be sure that the obtained labelling pattern shows functionally related neurons. That is, spreading of the tracer virus should be restricted to synapses. We chose two strategies to solve this problem. (1a) In one system with a specific mutation we both reduced the virulence of PRV-Ba and altered its spreading characteristics. Due to the reduced virulence of the virus, the immune system has enough time to isolate the afflicted cell and protect viral release from the cell. (1b). Another approach is based on the deletion of glycoprotein (g)D gene of PRV-Ba. Deletion of gD gene disables the virus to infect neurons from the intercellular space (with the excpetion of the first infection), but its ability to spread accros sysnapses is retaind.

(2) In order to being able to physiologically characterize infected neurons, the virus must have very low virulence. We have generated a virus strain with this properties by eliminating the virion host shut-off genes, which is a major virulence factor.

(3) A novel possibility for monitoring the activity of neurons is the utilization of activity markers. We inserted the synaptopHluorin gene to the PRV-Ba genome. SynaptopHluorin was generated by rendering green fluorescent protein pH sensitive and by fusing it with VAMP, which resulted in anchoring synaptopHluorin to the inner surface of synaptic vesicles. Upon releasing the content of vesicles to the synapric cleft, the synaptopHluorin molecule gets to outer surface of the cell membrane where the pH is close to 2 pH higher, which results in the sudden brightening of the activity marker.

Study on th effect of lamotrigine in different in vitro seizure models

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Lamotrigine is an anticonvulsant effective in the treatment of different types of epilepsies. It has been suggested that the main mechanism of action of lamotrigine is the inhibition of glutamate release through blockade of voltage sensitive sodium channels. There are conflicting reports on wether inhibitory transmission is enhanced by this anticonvulsant. It can be assumed that lamotrigine also exerts effect on calcium channels.

The above mentioned effects were demonstrated in different seizure models in vitro, in brain slice preparations from different brain regions, such as hippocampus and amygdala. In our present experiments we compared the effects of lamotrigine on spontaneous and evoked seizure dischrges developed in rat cortical slices in the presence of different convulsants.

Experiments were carried out in cortical slices prepared from young adult, male Wistar rats. 4-aminopiridine, magnesium-free solution and bicuculline were applied to evoke epileptiform activity. Spontaneous and stimulation evoked field responses were analysed in the presence of different convulsive agents. The stimulus intensity dependence and the effect of paired-pulse stimulation on the peak-to-peak amplitude were compared in the presence and absence of lamotrigine. Signals were digitized and evaluted using the Analsys program (Experimetria, Budapest, Hungary).

The pattern and time course of the evoked responses changed significantly in the presence of the different convulsants. A long-lasting second component appeared after about 15-20 min incubation. Lamotrigine (50 and 100 μM) applied for half an hour into the perfusion solution had practically no effect on the first components of the evoked response but significantly dercreased the second component. The effectiveness, however, was different in the case of the different convulsants. The 4-aminopyridine induced epileptifirm acivity was the most sensitive to the action of the antiepleptic agent, however, bicuculline induced spontaneous activity was the most stable and proper for analisys.

These data indicate that our in vitro seizure models are suitable for a comparative analysis of anticonvulsant drug effects.

Flow cytometry-based measurement of Tau phosphorylation in double-transfected hGSK 3β/hTau-ECR cell line.

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The neuropathological features associated with Alzheimer's disease (AD) include the presence of intracellular neurofibrillary tangles (NT) in defined regions of the brain. NT consists of paired helical filaments which contain mainly a hyper phosphorylated form of microtubule-associated protein Tau. Glycogen synthase kinase-3β (GSK-3β) plays pivotal role in the regulation of Tau phosphorilation.

Stably transfected human (h) Tau and hGSK-3β ECR cell line was generated to obtain a robust in vitro cell-based assay to quantify changes in Tau phosphorylation. EcR-293 as control and hGSK-3β/hTau-transfected cells were induced with 1 μM muristeron-A for 48 h. Cells then were harvested, fixated (1% paraformaldehide) and further processed for immunocytochemistry. Dual color analysis was carried out on FACScan (Becton Dickinson) flow cytometer. Primary antibodies were rabbit anti-human-phospho-Tau (pSer396 and pSer202) [Sigma, 1:100] and monoclonal mouse anti-Tau [Sigma, 1:100]. Secondary antibodies were FITC-conjugated anti-rabbit IgG and PE-conjugated anti-mouse IgG [Sigma, 1:100]. Mean fluorescence values (arbitrary fluorescence unit) were obtained from gated populations and ratio of phosphorylated Tau and pan-Tau was calculated.

As a result of the induced gene expression, pSer396 site specific immunofluorescence values rose from 94.9+11.1 to 533.9+56.8. This 4.8times elevation in phosphorylation enables us to evaluate the potential inhibitory effect of GSK-3β inhibitors on Tau phosphorylation at Ser396 site. We have measured Ser202 site specific phosphorylation as well, but it proved to be less extensive (382.1+38.9 AFU) accordingly the pan-Tau / pSer202 ratio value was also reduced (15.8+2.0).

The selective GSK-3β inhibitor SB-415286 dose dependently inhibited GSK3β overexpression-induced Tau phosphorylation. The IC50 value for was 0.13 μM at the Ser396 site and 43.5 μM at the Ser202 site. The marked difference between the effectiveness of the reference compound points to the difference between the sensitivity of the two specific phosphorylation sites. This new methodological approach was also validated by the conventional Western blot analysis.

Immunhistochemical characterization of the neurons in the filum terminale

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The chemical character of the neurons of the filum terminale of adult Wistar rats and young kittens (15 and 60 days old) was studied with imunohistochemistry. Animals were perfused in deep anesthesia and the spinal cord with the filum terminale was removed. Fifty µm thick transverse and longitudinal frozen section were prepared from the filum terminal of the rats, while 60 µm thick Vibratome cross sections were cut from the filum terminale of the cats. Neurons were ubiquitously detected with neuronal nuclear protein (NeuN). Calretinin, nitric oxide synthase (NOS) and galanin was shown in the neuronal perikarya while substance P (SP) labeled nerve fibers using immunohistochemistry. Glial cells were labeled with glial fibrillary acidic protein (GFAP) immunreaction. The distribution of the immunostaining was analyzed with the aid of confocal laser miscroscopy.

GFAP immunreactive components showed a radial distribution between the surface of the filum terminale and the central canal. The ependymal lining of the the central canal remained unstained, but fine GFAP positive processes could be seen between the adjacent ependymal cells in the cat filum terminale.

NeuN labeled small and medium size neurons around the central canal, some of them were immediately adjacent to the ependymal layer. Calretinin-, NOS- and galanin-positive neurons were of various size and possessed dendritic arborizations of various dimensions and orientation. Observations in the filum terminale of the cat suggested structural differences between the gray matter of the filum terminale and that of the spinal cord. The gray matter in the filum terminal was densely interwoven with GFAP positive glial processes, while the GFAP positive processes were hardly detectable in the gray matter of the spinal cord. This observation underscores our earlier suggestion (1) that “nerve cells in the filum terminale may represent neurons in an early phase of commitment and differentiation.”

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Visualization of neuroendocrine and metabolic interactions in the rat brain

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Homeostatic control of metabolism and energy balance depends on central nervous system structures and mechanisms. In the present study, we addressed the relationship between systems related to food intake, neuroendocrine and autonomic regulation and the metabolic state of the individual. Since there is a strong relationship between glucocorticoids and energy balance we combined two models: 1) adrenalectomy that activates the hypothalamic-pituitary-adrenal axis by removing the glucocorticoid negative feedback signal, and 2) voluntary sucrose ingestion as a strong manipulation of caloric/metabolic state. Adrenalectomized (ADX) or sham operated adult Wistar rats were allowed to drink 1M sucrose solution, 0.5% saline and water for a period of 3 weeks, then sucrose were withdrawn in a set of animals for further 3 weeks. Consumption values, body weight gain were measured daily, and ACTH and leptin levels in the serum were assessed by RIA. Neuronal activation was followed by immuncytochemical detection of Fra2, a member of the Fos-family responsive to chronic or repeated stimuli. ADX resulted in a marked increase in serum ACTH and decrease in leptin levels, and induced strong Fra2 expression in the medial dorsal (mpd: hypophyseotropic) and ventral (mpv: autonomic) parvocellular part of the hypothalamic paraventricular nucleus (PVN). Chronic sucrose consumption significantly elevated circulating levels of leptin, and induced Fra2-immunoreactivity in the mpv subdivision of the PVN, but not in the mpd. In addition, sucrose also increased the number of Fra2 immunopositive profiles in the hypothalamic dorsomedial nucleus and in the central amygdala. Our results demonstrate that endocrine and caloric manipulations produce distinct activational patterns in the CNS. Furthermore, Fra2 seems to be a suitable marker to reveal chronic cellular activation e.g. in response to neuroendocrine and metabolic challenges.

Effects of ghrelin on spontaneous and "open-field" behaviour

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In order to shed further light on the physiological role of ghrelin in the central nervous system, in the present experiments the effects of ghrelin on spontaneous and exploratory motor activity were tested in an open-field apparatus and by a biotelemetric monitoring system (Mini Mitter, USA) in the latter. Rats were implanted with a transmitter that detected their locomotor activity, and different doses (0.5-5 mcg) of ghrelin were administered to them intracerebroventricularly (icv.). Administration of ghrelin caused a significant increase in the locomotor and rearing activity in "the open-field" apparatus, while only the dose of 5 mcg evoked a significant increase in spontaneous locomotor activity recorded by telemetry. To determine the mediation of the motor action of ghrelin, corticotropin releasing hormone (CRH) antagonist, the dopamine antagonist haloperidol, the nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine-methyl ester (L-NAME) or the serotonin 5-HT(2) antagonist cyproheptadine were administered to the rats. The motor responses were diminished by the preadministration of the CRH antagonist and haloperidol, while L-NAME and cyproheptadine did not prove to be effective in reducing the ghrelin-evoked behavioural response in the open-field test. Further haloperidol, being administered 30 min before the peptide treatment, transiently and significantly reduced spontaneous locomotor activity. Present data suggest, that in the behavioural responses evoked by ghrelin, both CRH release and the action of the dopaminergic neurons of the subcortical motor system might be involved.

Experimental therapy of diffuse axonal injury

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Traumatic brain injury (TBI) evokes diffuse (traumatic) axonal injury (TAI), which contributes to morbidity and mortality. Damaged axons display progressive alterations gradually evolving to axonal disconnection. In severe TAI the tensile forces of injury lead to a focal influx of Ca2+, initiating a series of proteolytic processes wherein the cysteine proteases, calpain and caspase modify the axonal cytoskeleton, causing irreversible damage over time postinjury. Although several studies have demonstrated that the systemic administration of calpain inhibitors reduces the extent of ischemic and traumatic contusional injury a direct beneficial effect on TAI has not been established to date.

The current study was initiated to address this issue in an impact acceleration rat-TBI model in order to provide further evidence on the contribution of calpain-mediated proteolytic processes in the pathogenesis of TAI, while further supporting the utility of calpain-inhibitors.

A single tail vein bolus injection of 30mg/kg MDL-28170 was administered to Wistar rats 30min preinjury. After injury the rats were allowed to survive 120 min when they where perfused with aldehydes. Brains were processed for immunohistochemical localization of damaged axonal profiles displaying either amyloid precursor protein- (APP-) or RMO-14- immunoreactivity (IR) both considered classic markers of specific features of TAI.

Digital data acquisition and statistical analysis demonstrated that preinjury administration of MDL-28170 significantly reduced the mean densities of damaged RMO-14- as well as APP-IR axonal profiles in the brainstem fiber tracts analysed.

These results further underscore the role of calpain-mediated proteolytic processes in the pathogenesis of DAI and support the potential use of cell permeable calpain-inhibitors as a rational therapeutic approach in TBI.

Programmed cell death of interneurons in the embryonic chick lumbosacral spinal cord

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Naturally occuring cell death is a well described phenomenon, common to many parts of the central nervous system including motorneurons in the ventral horn of the spinal cord. A great deal of experimental evidence indicates however, that there are some regions of the central nervous system in which programmed cell death is insignificant or does not occur at all. For example, an apparent absence of cell death among spinal cord inteneurons has been reported. In the present experiment, we have reinvestigated this issue by detecting activated caspase-3, a new and sensitive marker for apoptotic cell death, by immunocytochemical techniques. Here we demonstrate for the first time that, in contrast to previous reports, a substantial number of spinal interneurons within the dorsal and ventral gray matters die during embryogenesis. Our results suggest that programmed cell death of inteneurons is initiated at embryonic day (E8), and completed by E20 in the lumbosacral spinal cord of the embryonic chick.

Locating the psychophysiological basis of the placebo effect: the gate control model and the anterior cingulum

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Earlier we suggested a model describing the placebo effect that since than has undergone many modifications based on the new results. The proposed complex bio-psycho-social model synthesizes the so far constructed approximately 50 different theories of the placebo effect and discusses the biological, psychological and social acpects in a common framework.

In our model, a distinction is made between the placebo effect based on conditioning and that based on expectation: whereas the first can be completely explained by our knowledge of conditioning, the mechanism of the second is almost unknown (yet). To explain the expectational placebo effect on a psychophysiological basis, we assume the presence of two "gates" (nervous inhibitions) that have emerged during the evolution. The first gate filters the information coming from the vegetative nervous system to the orbitofrontal region (bottom-up). The second gate filters the instructions going from the mind to the visceral system (top-down). We suppose that the temporary opening of the second gate might be responsible for the expectational placebo effect: discrete instructions from the mind can reach directly the vegetative nervous system. Social interactions and/orsuggestions might have a role in opening the second gate. In addition, we assume that there is a genetically determined disposition to let the gate being opened.

Latest data suggest that the upper gate should be somewhere in the anterior cingulum which works as a "switch" or some kind of a "translator" between the neocortex and the vegetative system. Human language has the potential to describe situations and states that are not real (e.g. "I get a headache from this person"); if these thoughts were handled as instructions for the vegetative system that could lead to fatal consequences, thus there was a need for a neural inhibition between the neocortex and the vegetative system. This inhibition might be modified by the limbic system (emotional states). The strength of the inhibition might differ from person to person, in addition to the genetically determined "mighty 33 %" placebo respondence.

In this poster we describe the current state of the model, and the empirical studies designed and launched to test the validity of the model. Since it is impossible to completely overview all the areas covered by the model, the authors welcome any suggestions or criticisms concerning the model and the empirical research.

Quantitative EEG in Alzheimer's Disease: Spectral and Complexity Changes

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The aim of the present study was the investigation of the changes of the linear and non-linear characteristics of EEG in Alzheimer's Disease (AD) to be able to to develop a sensitive and specific method for the early diagnosis of AD.

Seven mild to moderate probable AD patients participated in the study along with eight healthy elderly control subjects. The EEG recordings were performed according to the 10-20 international system (19 channels, bandpass: 0.1-48 Hz, A/D rate: 1000 Hz). Relative frequency spectra of the EEG in different frequency bands (gamma; 25-35 Hz, beta: 14-25 Hz, upper alpha 11-14 Hz, lower alpha: 8-11 Hz, theta 4-8 Hz, delta: 0.5-4 Hz) were analyzed. Besides this conventional procedure complexity measures were also calculated on the same data. The Omega-complexity (reflecting the degree of linear spatial synchrony) and Nonlinear Synchronization (the likelihood of dynamic, nonlinear spatial synchronization) were analyzed. The EEG was recorded for 2 min during eyes-closed and eyes-open conditions.

We found a significant increase in the delta band ($F(1,13=4,62$, $p<0,05$) and decrease of the upper alpha band ($F(1,13)=7,63$, $p<0,037$) in the AD group in both the eyes open and eyes closed conditions. A strong tendency was seen regarding the increase of theta power ($F(1,13)=3,17$, $p<0,09$) and decrease in the gamma band ($F(1,13)=3,77$, $p<0,071$) in the AD group. We found no significant Group (AD vs. Control) x Side (Left vs. Right hemisphere) or Group x Condition (Eyes Open vs. Closed) interactions. Thus, no difference was observed between the two groups with respect to the effect of eyes opening.

The analyses of complexity showed a robust increase of signal complexity in the AD group. Omega increased significantly in most bands (theta, delta, lower alpha, gamma, $F(1,13)=7,39$, $p<0,017$; $F(1,13)=4,96$, $p<0,044$; $F(1,13)=5,19$, $p<0,040$; $F(1,13)=6,46$; $p<0,024$ respectively) both in the eyes open and eyes closed conditions. Synchronization showed congruent changes: it decreased significantly in the delta and gamma bands ($F(1,13)=4,2$, $p<0,05$, $F(1,13)=4,75$, $p<0,048$), and marginally significantly in the theta and both alpha bands ($F(1,13)=3,07$, $p<0,10$; $F(1,13)=3,29$, $p<0,092$ respectively).

Our data confirm the results of the literature concerning the „slowing" of the EEG (increase of slow and decrease of fast bands of the EEG) in AD. The remarkable EEG-complexity changes in AD are probably due to the detorioration of cortico-cortical and thalamocortical connections, a characteristic feature of this disease. With the progressive loss of neurons and synapses local and remote synchronization declines in AD. Our methods seem to be sensitive for the documentation of this phenomenon.

The effects of NR2B NMDA receptor subunit overexpression on the migration of cerebellar granule cells in vitro

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During the development of nervous system, internal and external factors interact to regulate the migration of postmitotic neurons to their final place. Signalling pathways coupled to changes in intracellular Ca++ levels are known to influence cell migration, leading to a positive correlation between intracellular Ca++ fluctuations and the speed of cell translocation.

NMDA receptors (NRs) are involved in several physiological and pathological processes, partly due to their high Ca++ permeability. Based mainly on studies using cerebellar model systems, NRs are known to influence neuronal migration as well. NRs are heteromers of NR1 and NR2 (2A-2D) subunits, displaying distinct spatial and temporal expression patterns.

In the developing cerebellum, migrating cerebellar granule cells (CGCs) express NR2B subunits which are replaced by the NR2C subunit at the time of neuronal circuit formation. The replacement of NR2B by NR2C subunit is impaired in NR2C-2B knock-in mice expressing NR2B instead of NR2C. This leads to NR2B expession in adult cerebellar granule cells and to the conservation of a subunit composition normally found in young, migrating granule cells.

To address how altered NR subunit composition affects cell survival and migration, in vitro dissociated granule cell cultures were prepared from wild-type (WT) and NR2C-2B cerebella. Glutamate-induced excitotoxicity tests and Ca imaging experiments revealed increased NR2B expression in mutant CGC cultures. Time-lapse videomicroscopy showed that migratory period and average speed of CGCs were increased in NR2C-2B cultures, while growth cone motility was not affected by NR2B overexpression.

In order to decide whether migratory changes are due to increased amount of NR2B or to the lack of NR2C subunits in mutant CGCs, the combined use of subunit-specific antagonists and NR2C knock-out animals are also planned.

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Identification of interneuron types and probable dentate granule cells in extacellular multiunit recordings from the dorsal hippocampus of behaving rats

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Identification of functional classes of interneurons and principal cells of the dentate gyrus are a fundamental prerequisite for the analysis of the function of the local hippocampal neuronal networks in various behaviorally relevant circuit operations. To this end, we made multi-channel extracellular recordings from the dorsal hippocampus of behaving rats. We were able to discriminate principal cells and functional classes of interneurons according to various physiological criteria and local field potential dependent discharge patterns. By inspection of the spike autocorrelograms for interneurons, first we separated the cells into theta-modulated and theta-independent subtypes. With this separation, our interneuron sample of 105 cells consists of 75 theta-modulated and 30 theta-independent cells. The two groups were also separate in many other statistical parameters we examined. The 75 theta-modulated interneurons were further separated into 4 groups according to their firing phase relationship to the theta oscillation. Sharp wave-associated discharge patterns were also calculated if continuous sleep recording were available for the interneurons. In contrast to what one could expect from recent juxtacellular recording studies (Klausberger et al, 2003, 2004) the examined interneurons showed divergent sharp wave-associated discharge patterns inside the 4 theta phase firing groups. In the dentate gyrus we identified some cells with very low firing rate (<0.1 Hz), low firing rate cells (<1.0 Hz) with small discontinuous place fields and relatively higher firing rate cells (>1.0 Hz; <3.0 Hz) with homogeneous place fields. We propose that these cells are “silent” granule cells, multiple granule cells firing together, and granule cells with real place fields, respectively. This work was supported by MRC and OTKA F 034513.

Plasticity of somatostatin, somatostatin binding sites and somatostatin sst2A receptor in the hippocampus of patients with temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is often characterized by morphological alterations of the hippocampal formation (hippocampal sclerosis) together with profound phenotypic changes in different hippocampal interneuron types. A subpopulation of hilar somatostatin (SRIF) interneurons undergo degeneration in patients with hippocampal sclerosis. However, surviving SRIF neurons exhibit a marked increase in their axonal projection site, the outer molecular layer of the dentate gyrus, suggesting that surviving neurons might re-innervate the distal dendrites of granule cells. Since SRIF has hyperpolarizing effects, axonal sprouting of surviving SRIF neurons might modulate granule cell hyperexcitability. While plastic changes of SRIF interneurons were widely studied in TLE, the status of SRIF receptors is still lacking. Therefore we first characterized and measured the density of 125I-SRIF binding sites in controls (n=12) and epileptic patients (n=14). We demonstrated that the SRIF binding sites in the human hippocampus belong to the sst2 receptor subtype, and that density of sst2 receptor binding sites is significantly decreased in the CA1-3 fields in TLE patients. In the dentate gyrus, significant decrease was observed in the outer molecular layer, but not in the inner molecular layer. In accordance with autoradiographic findings, immunohistochemical analysis revealed a loss of sst2A receptor immunoreactivity in CA1-3 fields as well as in the outer molecular layer of the dentate gyrus. Our results suggest that whereas in the CA1-3 fields the decrease in sst2A receptor density is principally due to hippocampal sclerosis, in the outer molecular layer of the dentate gyrus it might represent a local receptor down-regulation due to chronic SRIF exposure. This phenomenon might contribute to granule cell hyperexcitability and consequently enhanced seizure activity.

Neuromorphological evidence for the existence of glutamatergic innervation of neuropeptide Y and pro-opiomelanocortin-derived peptide containing neurons in the hypothalamic arcuate nucleus of the rat

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The hypothalamic arcuate nucleus is a heterogenous cell population containing a number of neurochemically different cells, among others neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) derived peptide expressing neurons which are involved in the regulation of feeding and energy homeostasis, release of hypophysiotropic hormones, sexual behavior and thermogenesis. There is a dense plexus of glutamatergic fibres in the arcuate nucleus. The aim of the present studies was to examine the relationship of these fibres to the NPY and POMC neurons in the cell group. Double label immuno-electron microscopy was used. Glutamatergic elements were identified by the presence of vesicular glutamate transporter 1 (VGluT1) or 2 (VGluT2) (selective markers of glutamatergic elements) immunoreactivity. A significant number of VGluT2 immunoreactive terminals were observed to make asymmetric type of synapses with NPY and with □-endorphin (a marker of POMC neurons) immunostained nerve cells of the arcuate nucleus. About 15% of VGluT2 synapsing terminals established asymmetric synapses with NPY positive cells and more than 40% of VGlut2 positive terminals formed synapse on □-endorphin positive neurons. VGluT2 positive perikarya were also observed, part of them contained also □-endorphin. Nerve terminals containing both VGluT2 and □-endorphin were demonstrated in the cell group.VGluT1 fibers were not detected under the electron microscope. Our observations demonstrate that glutamatergic terminals form asymmetric type of synapses on NPY and POMC-peptide-containing neurons of the arcuate nucleus. These findings indicate that glutamate acts directly on NPY and POMC nerve cells of the cell group.

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Cyclic changes in synaptic connectivity in the arcuate nucleus

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Androgens and estrogens are known to affect the number of synaptic inputs in different neuronal populations of the central nervous system. In the arcuate nucleus a rapid reduction in the number of axo-somatic synapses was described between proestrus and estrus which subsequently returned to baseline level at metestrus. More recently we have demonstrated that the estradiol induced remodelling of axo-somatic inputs to arcuate neurons is specific, because not all of the synapses are affected. A quantitative postembedding immunocytochemical analysis revealed that the administration of a single dose of 17 ß-estradiol resulted in a significant and specific decrease in the number of GABA-immunoreactive axo-somatic synapses in ovariectomized rats. In the present study we have examined the changes in the number of axo-somatic, axo-dendritic and spine synapses during the oestrus cycle of three-month-old rats.

The animals were killed by intracardial perfusion with 4% glutaraldehyde in 0,1 M phosphate buffer in the morning (between 10 and 11 AM) of proestrus, estrus, metestrus and diestrus days and processed for normal embedding in Durcupan. Postembedding immunostaining for GABA was carried out and the numerical density of axo-somatic, axo-dendritic and spine synapses was determined by the unbiased disector method.

Our data show that there are cyclic changes in the synaptic pattern of the arcuate nucleus. On estrus day we could observe a significant decrease in the number of GABAergic axo-somatic and an increase in the density of excitatory spine synapses. These values return to the normal level on metestrus day. All other subpopulation of synapses remains unchanged. The morphological changes are temporally correlated with the neuronal activity in the arcuate nucleus, which may indicate that the modifications in the numerical balance of inhibitory and excitatory synaptic inputs are associated with cycling changes in firing frequency.

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Relationship between skill entropy classification and motor performance

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Early studies of structural adaptations of vertebrate bodies regarding to comparative movement biomechanics among fishes (Gray, 1957), horses (Hildebrand, 1960), birds (Storer, 1952) and humans (Napier, 1967) revealed the main skeletomuscular structural and functional differences of the investigated biological systems. Therefore it is interesting that up until recent years very few studies have been published about the effect of the integrated function of the neuromuscular and skeletomuscular systems on the performed skill (Cordier et al.,1994; Mitra et al., 1997) From one hand this fact is understandable because to investigate such a complex entity and its interaction with the performed skill parametric and nonparametric statistical procedures give limited possibilities to answer particular research hypotheses. The authors of this study intended to find a method with the help of entropy classification that would allow researchers, rehabilitators, and neurologists to assess the level of the performed movement based on strictly continuous kinematic variables. Hr: There will be a relation between skill dependent total body system entropy and sport branch specific success. Fifteen highly trained professional athletes participated in the study. Their mean age, height and weight were (M±SD) 24.2±4 year, 83.1±8.6 kg, 186.5±5.2 cm, respectively. Each subject was Olympic or World Champion assuring that the performed skill is very high in quality. An Ariel Performance Analysis System (APAS) was used to record and to digitize the co-ordinate values for eighteen body points (Dempster, 1955) at a nominal 60 Hz sampling rate. Whole body and segmental entropy values were calculated from displacement data that were correlated to a rank order that described lifetime sport branch specific success among the subjects. Results of Kendall Tau correlations indicated significant relation (Kendall's τ=0.9) between H(A) and Success Rank Order at a $p<0.05$. In accordance to this result, the following conclusion can be drawn: In case of sport specific skills executed by world-class athletes the total entropy value of the movement system defined by the sum of 64 displacement entropies is a 90% probability indicator (predictor) of the sport success (as criterion) calculated as detailed the above. In general the result allows the authors to underline the fact that movement entropy could be a very useful tool for practitioners to objectively assess movement development and condition.

Non-linear dynamic behavior in specialized human movements

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Brisson and Alain in 1996 revealed evidence that common optimal movement pattern does not exist in cases, when subjects try to reproduce the best experimental subject's motor performance according to its kinematic template. Their findings and the fact of anthropological and physiological variability among humans underlines the hesitation of accepting the search for a general descriptor of optimal motor performance in linear domains. The purpose of this study was to test for characteristics of non-linear dynamics behavior in highly skilled human subject's whole body movement systems. Hr: The dynamics of the displacement time series of movements performed by world-class athletes is non-linear and chaotic. Fifteen subject's sport branch specific movements were recorded, digitized and analyzed with an APAS. Retroreflective markers were used to mark 18 different segments on each subject. Correlation dimension (D) and Lyapunov exponent measure (L) differences between original displacement time series and phase randomized surrogate data were tested with t-test of independent samples. Significant differences between L (Loriginal vs. Lsurrogate ; 6.37-1.75 vs. 1.45-0.26) and D (Doriginal vs. Dsurrogate ; 3.30-3.13 vs. 2.25-2.24) in case of each movement and the fact that L for original time series were outside of the 10 surrogate data generated for each movement (6.37-1.75 vs. 1.45-0.26) indicate characteristics of chaotic behavior in the investigated different movement systems. The non-linear behavior of human movement coordination has already been established at the neural level (Alexander and Globus 1996, Newell 1998) thus the disclosed results are just reassurances of human movements functioning as dynamic complex systems at the mechanical realization level also. It is also important to recognize that for the objectiveness of experiments performed on human motor performances hypothesis testing statistical procedures and analytical methods should carefully be revised because their validity does not extend to the non-linear dinamics and chaotic domain.

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GABA and its receptors in the reticular thalamic nucleus

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Parvalbumin immunoreactive large calyciform terminals, synapsing with large dendritic bulbs, and described by us in the reticular thalamic nucleus of the rat, were formerly erroneously identified by numerous authors as perikarya, containing a large immunonegative nucleus. In addition to proving the terminal character of these calyciform endings, herewith we present immunocytochemical evidence that these terminals contain GABA. The GABAergic, parvalbumin immunoreactive calyciform terminals synapse with dendritic bulbs of unknown origin. Reconstruction of the GABAergic, parvalbumin immunoreactive calyciform complexes revealed that somato-dendritic synapses, described earlier by us, can be transformed into dendro-dendritic synapses in the course of ontogenetic developments. Immunocytochemical investoigations revealed that receptors GABA A-alpha3 and GABA A-alpha6 are present on the surfaces of the relatedt post-synaptic elements and these receptors characterize also the further course of post-synaptic dendrites in the reticular thalamic nucleus. Demonstration of c-fos immunoreactivity after electrical stimulation support neurophysiological studies suggesting inhibitory function of the reticular thalamic nucleus that seems to play an outstanding role in determining the level of attantion versus distraction, mainly in the field of nociception, to be forwarded finally to the cerebral cortex.

Spectral and complexity characteristics of the EEG in patients with unilateral ischemic stroke

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Objective: The goal of this study was to compare the EEG of patients with ischemic stroke to that of an age-matched control group, using both spectral and complexity indices. The patient group was heterogeneous regarding the localization and extension of the lesion.

Methods: EEGs (19 channels, A/D rate: 1000 Hz) were recorded in 15 patients with unilateral ischemic stroke (8 females; age: 60.4 years, S.D.: 6.54) and in 11 healthy subjects (6 females; age: 62.8 years, S.D.: 9.08) during eyes-open and eyes-closed (2-2 min) conditions. Spectral analyses (power spectrum, relative spectrum, symmetry index) and complexity analyses (synchronization likelihood (SL), Omega complexity) were performed on artifact-free epochs (2048 data points) in the following frequency bands: delta 0.5-4 Hz, theta 4-8 Hz, alpha1 8-11 Hz, alpha2 11-14 Hz, beta1 14-25 Hz and beta2 25-35 Hz. The statistical analyses were carried out for each and every index by 2x2 (condition x group) ANOVA in each frequency band and by Discriminant Analysis (DA) with stepwise method.

Results: There was no significant main effect of group factor, or significant group x condition interaction using the results of spectral analyses as dependent variables of ANOVA. The mean SL of the theta band was significant lower in the patient group ($F(1.24)=4.945$; $p=0.036$). The mean Omega complexity of the theta band was significant higher in the patient group ($F(1.24)=11.941$; $p=0.002$) in both conditions.

Conclusion: The DA was more effective when performed with complexity indices, compared to when spectral ones were used. The complexity indices were more sensitive than spectral indices. Since the theta band plays an important role in large-scale connections we assume that damage of these connections cause complexity and synchronization changes in this frequency band. Because of the heterogeneous patient group, differences compared to the control group indicate global changes in the EEG caused by stroke, which appears to be independent on the localization of the ischemic lesion.

Interaction between nicotine and PACAP

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Activation of cholinergic pathways by nicotine and nicotinic agonists has been shown to elicit antinociceptive effects in a variety of species and pain tests. The possible influence of pituitary adenylate cyclase activating polypeptide (PACAP; 250 ng i.c.v.) and Theophylline (1 mg/kg i.p.) on the antinociceptive responses following acute nicotine administration was investigated in intact mice. Heat-radiant tail-flick test was used to assess antinociceptive threshold.

Our results clearly show that nicotine is able to induce a dose-dependent antinociceptive effect. As previously reported, i.c.v. administration of PACAP alone had no effect on pain sensitivity but in a dose of 250 ng, it significantly diminished the acute analgesic effect of a single dose of nicotine (6 mg/kg i.p.). Theophylline pretreatment also significantly diminished the nicotine induced analgesia.

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Acute stress responses in vasopressin deficient Brattleboro rat

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The central regulatory component of the hypothalamo-pituitary-adrenal axis (HPA) is the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) both originating in the nucleus paraventricularis hypothalami (PVN). We studied the role of AVP in the HPA axis regulation during acute stress using the natural AVP deficient mutant Brattleboro rats. Homozygous diabetes insipidus (di/di) rats were compared to heterozygous (di/+) animals. Different acute stress paradigms were used and blood samples were collected through the vena jugularis or through decapitation. Adrenocorticotrop hormone (ACTH) and corticosterone plasma levels were measured by radioimmunoassay. The stress-induced ACTH elevation was significantly impaired in the absence of AVP after 10 min novelty stress or during iv egg white injection, but was not changed after ip hypertonic saline. The corticosterone elevation was similar in all studied stress situation in AVP positive and negative animals. These data suggest that AVP plays a stressor specific role in the HPA axis regulation. One can suppose that a high intensity stressor can overcome any effect of AVP deficiency but that was not the case as the immune challenge (egg-white injection) was quite a strong stimulus but the lack of AVP was able to reduce the ACTH elevation markedly. Different stressors can activate different vasopressin-containing neurones in the brain and probably not the parvicellular PVN is the most important source for that vasopressin, which is playing a role in HPA activation.

Genetic polymorphism and Attention Deficit Hyperactivity Disorder; in silico drug design strategy to discover novel dopamine transporter inhibitors

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Attention deficit hyperactivity disorder (ADHD) is the most common behavioral disorder of childhood. Genes involved in the synthesis, metabolism and transport of monoamine neurotransmitters have been related to the heritability of ADHD. Transporter-assisted uptake of dopamine (DAT), noradrenalin (NAT) and serotonin (SERT) have been drug targets for several neuro-psychiatric disorders including ADHD. Involvement of the dopaminergic system in the aetiology of ADHD has been suggested since the symptoms can be successfully treated with methylphenidate, a potent blocker of DAT, and significant increases in DAT in ADHD patients has been demonstrated. As specific aims of a project focusing on the role of genetic polymorphism of dopaminergic neurotransmission in ADHD the present study was undertaken: (i) to further investigate the possible association of genetic polymorphism of the 3' untranslated region (UTR) of DAT1 gene with ADHD; (ii) to discover novel DAT inhibitors using in silico drug design strategy. No statistically significant difference was found in allele frequencies between the control (N=604) and ADHD-patient (N=64) groups. In transmission disequilibrium test (TDT) 87 ADHD family trios have been tested and no preferential DAT1 variable number of tandem repeats (VNTR) allele transmission was found. Using in silico drug design tools a biased compound set (35 cpds) was selected from a large discovery library (> 200, 000 cpds) synthesized at ComGenex. Based on characteristic structural elements of known inhibitors of DAT, we first selected 116 compounds representing 13 chemical core structures by using similarity algorithm and Example Mediated Innovation for Lead evolution (EMIL) database. Then we applied Absorption-Distribution-Metabolism-Excretion-Toxicity (ADMET) filtering by MetabolExpert and HazardExpert softwares. The remaining 35 compounds were tested for DAT inhibition at 10 μM conc. using striatal synaptosomes from rat brain. Twelve compounds were active inhibitors of DAT (> 90 % inhibition), indicating that the in silico selection procedure was effective. Out of the 12 compounds 2 inhibited DAT also at 100 nM conc. with no or low inhibitory potency on SET or NAT. Conclusions: Our data do not support a possible association between the polymorphism of UTR VNTR of DAT1 and ADHD. The application of in silico drug design tools resulted in a very high hit rate (12 out of 35) in the primary in vitro screen for DAT-inhibition. These two compounds are considered as leads for optimization and further development.

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Behavioural effects of non-ionising irradiation in rats: Animals treated as juvenil

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Groups studying biological effects of non-ionising irradiation usually focus on carcinogenic and other disruptive processes. Recently, however, the possibility has been raised that in addition to (or instead of) these cellular effects, non-ionising irradiation have a more general effect on the organism, presumably of behavioural nature. The aim of the present program has been to address these problems by experimental and observational methods that make it possible to study behavioural effects as well as the complex physiological mechanisms lying behind.

One of the most striking problems of non-ionising irradiation is the exposure of the children and youngsters. Although no conclusive evidences have surfaced (as yet) either against or for the long-lasting effects, this possibility at least warrants for caution. This is why treating young animals has been in the center of the research project of which some preliminary data are reported here.

Subjects were young Long-Evans rats irradiated as young just after weanling for 12 days by an electromagnetic field generated by two large coils. Rats received 2 hours irradiation with 500 μT every day. We hypothesised that if there were any effects of the exposure to the behaviour, it may be detected in the adulthood, thus the rats were tested for long-term effects as adults.

Several features of the behaviour have been investigated: basic behavioural pattern (open-filed, motimeter activity) in which we tried to describe the general motility, movement patterns, distribution of the activity in time and space and the changes of some characteristic behavioural elements. Another series of experiments was designed to study social behaviour including territorial as well as social interactions. Finally, we have started to study anxiety and also possible memory impairments in the exposed population. In all these experiments, littermates of the exposed rats served as controls thus we have an age- and gender-matched control group.

Though it is too early to say any conclusion of these still ongoing studies, we may already say that no major effects, i.e. dramatic behavioural changes have been found. However, our preliminary results still allow for the possibility that some non-specific effects might be found.

Embryogenesis of transmitter systems in a model invertebrate (Lymnaea stagnalis, Gastropoda, Mollusca)

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Embryonic development of different chemical signaling systems, with special attention to aminergic (serotonin [5-HT], dopamine [DA], octopamine [OA], histamine [HA]), peptidergic (FMRFamide-related peptides [FaRP], Mytilus inhibitory peptide [MIP]) and nitrogen monoxidergic (NO) neurons, was investigated in the pond snail Lymnaea stagnalis, applying immunocytochemistry combined with biochemical (HPLC) and/or pharmacological-physiological assays.

Embryogenesis of the different signaling systems can be classified according to the timing of appearence, duration of development and spatial distribution. Four major events can be distinguished. i) Extraganglionic neurons, developing from early (E30%), veliger stage in both anterior and posterior positions, display transient phenotypes (5-HT, DA, FaRP, MIP) and disappear by the end of the embryogenesis. These cells can be involved in morpho- and gangliogenesis. ii) Other populations of neurons, containing 5-HT, FaRP and MIP, respectively, appear within the different ganglia of the CNS by E35-50% stages, and are characterized by the continuous increase of cell number until hatching. It is suggested that these neurons participate in gangliogenesis and establishing early interneuronal connections. iii) Small subsets of DAergic and OAergic neurons can be observed within the CNS from the late stages (E75-85%) of embryogenesis. Development of these aminergic neurons can well be correlated with HPLC-assayed concentration levels and maturation of locomotor, feeding and respiratory behaviors. iv) Early (E50%, DA, FaRP, MIP) and late (E75%, NO) development of signaling systems of sensory elements at the periphery is suggested to be connected to gangliogenesis and plastic events. NOergic regulation of the development of adult-like behaviors is supported by pharmacological-physiological tests as well. v) The HAergic system represents an intermediate way of development, by appearing early, at E50% stage, but finishing embryogenesis with altogether 14 HAergic cells. It is followed by a rapid increase of labeled cell number and HA-concentration at both central and peripheral level during postembryogenesis, suggesting a decisive regulatory role of the HAergic system when Lymnaea starts free-living juvenile life.

According to ultrastructural investigations, unspecialized axo-axonic and axo-somatic membrane contacts dominate in the CNS during almost the entire length of embryogenesis. At the periphery, the first muscle cells can be detected only by metamophosis (E50%), meanwhile close but unspecialized neuro-muscular contacts are to be found first by E75% stage, which is the time of the formation of adult-like behavior, such as gliding and feeding. These observations emphasize the dominance of non-synaptic, modulatory processes during embryogenesis at both central and peripheral levels.

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Role of the TRPV1 receptor in subacute airway inflammatory model of the mouse

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Pro-inflammatory neuropeptides released from the activated peripheral terminals of capsaicin-sensitive, transient receptor potential vanilloid 1 (TRPV1)-expressing, primary sensory neurones induce local neurogenic inflammation in the innervated area. Meanwhile, somatostatin (SOM) exerts systemic anti-inflammatory action. The aim of the present study was to examine the role of the TRPV1 receptor in airway inflammation using receptor gene-deficient mice.

Subacute pneumonitis was evoked by intranasal administration of E. Coli lipopolysacharide (LPS, 60 ul, 167 ug/ml) in TRPV1 knockout (KO) mice and their wild-type counterparts (WT). Respiratory parameters of unrestrained animals were measured with whole body plethysmography (Buxco). Since inflammation is accompanied by airway hyperresponsiveness, bronchoconstriction was induced by inhalation of 50 ul muscarinic receptor agonist carbachol (carbamoyl-beta-methyl-choline) in increasing concentrations (5.5, 11.0, 22.0 and 44.0 mM) for 1.5 min 24 h after the induction of inflammation. A calculated parameter called Penh (enhanced pause: ((expiratory time/ relaxation time)-1): (max. expiratory flow/ max. inspiratory flow)), which refers to the airway resistance was detected during 15 min after each carbachol stimulation. Plasma and lung SOM concentrations were measured with RIA at the end of the experiment. Histological examination of the lung was performed and composite inflammatory score was calculated. Myeloperoxidase (MPO) enzyme activity in the lung, as a quantitative indicator of inflammation, was measured with spectrophotomety. In a separate group of WT mice, the SOM receptor antagonist cyclo-somatostatin (C-SOM, 3x100 ug/kg i.p.) was injected.

Carbachol-induced bronchoconstriction, inflammatory changes (perivascular/peribronchial oedema, leukocyte infiltration, Goblet cell hyperplasia and alveolar macrophage infiltration) and MPO activity were significantly greater in the TRPV1 KO and C-SOM-treated groups. Plasma and lung SOM concentrations markedly increased after LPS administration in WT, but not in KO mice.

These results provide evidence for a novel type of endogenous counter-regulatory mechanism, which is mediated by SOM released from sensory nerve terminals into the circulation in response to TRPV1 receptor activation. It inhibits airway inflammation and consequent bronchoconstriction.

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The effects of neuropeptides on food intake in rats after subdiaphragmatic vagotomy

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A role for afferent fibers of the abdominal vagus has been suggested in many situations (satiety, hunger, feeding-related metabolic/thermal changes, fever, etc.). The development of satiety or hunger may involve various neuropeptides, besides vagal afferent fibers. In satiety, cholecystokinin (CCK) has effects on the abdominal vagus and in the hypothalamus. In fasting, both hunger and the hypometabolism may involve vagal afferents and central neuropeptides. In fever similar afferent vagal and neuropeptide mechanisms may also be presumed to participate. Vagal damage may influence such neuropeptide-related mechanisms and phenomena. In the present experiments male Wistar rats of about 350-450 g were used. Under ketamine-xylazine anesthesia a total subdiaphragmatic vagotomy was performed (with excision of a few mm-s of the vagal trunk). Postoperatively the animals were anorexic, and they needed special care (temperature, fortified food, sweet fluid, etc.). They still lost about 20% of their body weight and the mortality rate was over 10%. However, after the second postoperative week they started regaining the lost weight. 6 weeks after vagotomy a guide cannula was surgically implanted into the lateral cerebral ventricle (ICV) for later injections. Food intake following injection of various neuropeptides ICV or intraperitoneally (IP) was investigated: the 3-h cumulative food intake and the 30-min fractional body weight changes were assessed. ICV injection of 10 μg NPY induced smaller and slower food intake in vagotomized rats than in control ones of similar body weight. The re-feeding after 24-h or 48-h food deprivation (which supposedly depends on endogenous NPY-activation) also became delayed and smaller in vagotomized than control rats. Similarly to changes in NPY-effectiveness, the demonstrated normal anorexigenic effect of 100 pg ICV leptin (given 30-min prior to measurements) was missing in vagotomized rats during their re-feeding after a 24-h fasting period. In contrast to this, an IP dose of 5 μg CCK effectively antagonized the re-feeding after a 48-h fasting period (as if acting centrally), similarly as in control rats. Apparently, centrally acting substances exerted weaker effects than in controls, possibly due to efferent innervation abnormalities. Besides, complete vagotomy was followed by suppression of hunger signals, which is interesting since capsaicin-induced isolated damage of the afferent fibers resulted in suppression of satiety signals.

Cerebroprotective effect of talampanel, a non-competitive AMPA-antagonist in chronic focal cerebral ischemia model in rats

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Background and aim of the study: The beneficial effect of the anti-epileptic talampanel was previously shown in acute focal cerebral ischemia models in rats and mice. The compound (6 x 2 mg/kg i.v.) reduced the infarct size by 49% (in rats) and 44.5% (in mice) after 1 h ischemia and 24 h recirculation. The aim of present study was to test the therapeutic potential of talampanel under clinically more relevant conditions, in a chronic model of stroke with not only morphological but also functional assessments.

Methods: Three groups of male CDBR rats (sham operated, ischemic control and talampanel treated) were used for the experiments. Transient middle cerebral artery occlusion (tMCAO) was produced by intraluminar filament technique. On the day of the operation the animals were treated with talampanel (10 mg/kg i.p.) or vehicle six times

(30 min, 1.5 h, 2.5 h, 3.5 h, 4.5 h and 5.5 h post-occlusion). The functional deficit was measured on the days 1, 2, 3, 4, 7, 15, 22 and 29 after the tMCAO. Survival rate, body weight, neurological status, locomotor activity, spontaneous rotation, muscle strength, motor coordination, balancing and reaction time following forepaw stimulation were monitored. At the end of the 30-day observation period the brain tissues were histologically analysed.

Results and conclusions: Due to the restitutive capacity of the rat brain a spontaneous improvement of motor deficit was observable in several behavioural tests during the 30 days in the ischemic control animals. Talampanel treatment improved the survival rate, the stroke-induced rotation, the failure of the motor coordination, the balancing disturbances and the prolongation of the reflex time at every timepoint comparing with the ischemic control. The effects were statistically significant in rotometer, rotarod and beam walking tests. Analysing the size and the severity of the infarcted area of the brain tissue no remarkable differences were found between the vehicle and talampanel treated animals. In conclusion our results indicate that talampanel is able to attenuate the long-term neurological and motor dysfunctions following stroke in rats. However, a spontaneous recovery of the behavioral functions was detectable in the control animals as well. The complex functional monitoring applied in the present study is unique in the literature and may serve as a predictive approach for a possible clinical investigation.

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Subiculum-Temporal Cortex Interactions During Spikes And Seizure.

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The hippocampus plays an important role in the generation and maintenance of paroxysmal activity in the temporal lobe epilepsies (TLE), which constitutes the majority of the focal epilepsy cases in humans. Altered excitatory, inhibitory signaling and massive synaptic reorganization of inhibitory and excitatory networks was found within the epileptic hippocampus and temporal cortex. Despite these findings the generation and propagation mechanisms of paroxysmal activity in TLE is still under debate. In vitro electrophysiology studies suggest that the subicular region of the human hippocampus might have an important role in seizure generation.

To elucidate the local and long range neuronal network interactions during interictal spike-wave complexes and seizures in the hippocampus and lateral temporal lobe, microelectrode arrays (multielectrodes) and clinical subdural strip electrodes were implanted in epileptic patients undergoing anterior temporal lobectomy under general anesthesia. Two 24 channel multielectrodes, separated by 5mm were inserted into the subiculum and an 8-contact strip was placed on the pial surface bending over the temporal pole reaching temporobasal structures. Electrical stimulation of the strip contacts were used to elicit hippocampal responses. In order to minimally disturb the medial-lateral pathways, the hippocampus was reached through an incision in the fundus of the junction of the superior and medial temporal gyri.

Spontaneous subicular spikes were highly synchronized across the two recording sites. Two types of CSD profiles were observed, one with initial sink in the pyramidal layers, and the second type with initial sink in the apical dendrites. Cortical spiking showed variable pattern, multiple spike foci. Comparing subicular and cortical spiking activity, only about 5% of the events coincided, limited data showed that cortex was usually leading.

Single shot (0.2ms, 5-15mA, 0.5Hz) electrical stimulation of the strip contacts located in the basal temporal area, close to the pole elicited strong response from the subiculum. Onset latencies varied between 4 and 40ms depending on the strength and location of the stimulation. Solitary afterdischarges were originated in the cortex, having comparable onset latency in the subiculum. The CSD profile of the evoked spikes was markedly different from the spontaneous spikes.

Train (50Hz, 2sec) stimulation elicited self sustained afterdischarges both in the subiculum and in the cortex. As the seizure-like activity developed, the subiculum and cortex became highly synchronized. CSD profile of the self sustained activity was very similar to the evoked subicular spikes.

Based on our preliminary data we conclude that in the interictal state both the subiculum and cortex are able to generate spikes independently, however the self sustained seizure-like activity arises in cortico-hippocampal networks.

The effects of rhythmic environmental factors on the chicken pineal gland in vitro.

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Chicken pineal gland is an appropriate model for investigating biological rhythms, since it continues the melatonin (MT) secretion in a circadian rhythm for several days even in vitro. MT seems to have a fundamental role in controlling of circadian rhythms in vertebrates. However, limited number of data are available on the mechanisms of the synchronization and on the responsiveness to short (occurring in periods or cycles of less than 24 hours) light or temperature rhythms of the rhythmic MT secretion.

To collect more data on this area, dynamic in vitro, 4 day long bioassays (perifusion analysis) were performed on chicken pineals. The illumination and the temperature of the perifusion columns were changed periodically at specific time points of the rhythm. MT concentrations of the effluent medium were determined with RIA.

Our results indicate that the circadian MT rhythm can be entrained with short rhythmic pulses of light and temperature. However, in most of our experiments, the short rhythm was combined with the signs of the normal circadian rhythm. Also, when the system was restored to constant temperature and continuous darkness, the 24 hour period time returned.

It is concluded: (1) the period time of rhythmic MT secretion can be modified in a wide range with the modified rhythms of temperature and illumination, and (2) the elements of the chicken pineal clock can possess different sensitivity for the periodic alterations of the environmental factors.

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Three-dimensional visualization of GABA-immunoreactive neural structures in the central nervous system of invertebrate model-animals: a mapping study

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By means of a whole mount immunocytochemistry developed in our laboratory the number, pattern and geometry of GABA-immunoreactive (IR) neural structures were identified in the central nervous system (CNS) of lumbricid earthworm, Eisenia fetida and Lumbricus terrestris (Annelida, Oligochaeta). Except a small variance of labelled structures in the supra- and suboesophageal ganglion the morphology and pattern of stained perikarya and nerve fibres proved to be the same in both species investigated. This study gives experimental evidences that GABA acts as neurotransmitter in (i) few motoneurons, (ii) certain sensory pathways, namely in ventrolateral and ventromedial sensory longitudinal axon bundles, and in largest amount in (iii) fine-fibred polysegmental interneuronal tracts. The latter structures show repeating pattern in each ventral nerve cord ganglion: behind the first segmental nerves and at the level of the third ones two groups of heavily stained neurones located and their processes (both ipsi- and contralateral) form four bundles of interneuronal tracts that run close to the dorsal giant axons without interruption from the terminal ganglion to the suboesophageal one. The supraesophageal ganglion possesses an extremely rich GABA-IR fibre-network located in both the anterior and posterior commissural parts. The former part mainly consists of processes of the own large ganglion cells of the ganglion, while fibres of the latter one arising from the ventral nerve cord. To clear the functional role of the GABA-IR interneuronal tracts in locomotion, escape and withdrawal reflexes of earthworms is in progress in our laboratory.

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Spatial and temporal visual properties of the neurons in the feline suprageniculate nucleus (Sg)

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One of the structures that provide sensory information to the cortex bypassing the primary thalamic sensory relay nuclei is the suprageniculate nucleus (Sg) of the posterior thalamus. Earlier physiological studies found multimodal (visual, auditory and somatosensory) characteristics in Sg. The aim of this study was to estimate the spatial and temporal frequency properties of the neurons. These data could provide information on the function of these thalamic nuclei in the behaviour of the animals.

Spatial and temporal visual sensitivity was studied in 102 neurons of the feline suprageniculate nucleus. To study the spatio-temporal characteristics of the cells, we used computer-controlled drifting sinusoidal gratings. Extracellular single-unit recordings were performed in halothane-anesthetized (0.6 %) immobilized, artifically ventilated cats.

Most of the cells were strongly sensitive to the direction of drifting gratings. All the neurons displayed responses for rather low spatial frequencies and the majority of the units responded optimally to high temporal frequencies. Thus, the neurons in Sg closely resemble the spatial and temporal response properties of neurons in the cortex along the anterior ectosylvian sulcus (AES) or those in the deeper layers of the superior colliculus.

Our results support the notion that a separate tecto-suprageniculate-AES/insular pathway takes part in the processing of sensory feedback of motion.

Estrogen modulates potassium currents and expression of the Kv4.2 subunit in GT1-7 cells

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Pulsatile secretion of gonadotropin-releasing hormone (GnRH) is essential in the process of reproduction. Frequency of the secretion is under the negative feedback effects of estrogen and correlates with the firing rate and oscillation in the intracellular calcium concentration of the GnRH-producing neurons. Therefore, phenomena influencing the firing rate can modify the pattern of hormone secretion. The frequency of the action potentials is partially adjusted by voltage gated potassium currents such as A- and K-type currents. We therefore investigated relations between estrogen treatment and function of the A- and K-type potassium channels in GnRH-producing immortalized hypothalamic neuronal cells (GT1-7). Whole cell clamp recordings showed the presence of the A-type potassium current after treatment of 24 h, but absence of the current after 8 h. However, the current could still be evoked after 48 h. The amplitude of the K-type current decreased with time. Treatment of the cells with a culturing medium containing both estrogen and the estrogen receptor blocker Faslodex (ICI 182,780; 1 μM) abolished occurrence of the A-type current. Quantitative real-time PCR data demonstrated that expression of the Kv4.2 subunit of the A-type channel was low at 0 h, 0.5 h, 2 h and 8 h estrogen treatment (15 nM), elevated after 24 h treatment and decreased when estrogen was present for 48 h. Our results show that potassium currents could be targets of the negative feedback effects of estrogen.

Functional neurotoxic effects obtained by acute administration of two mitochondrial toxins, 3-nitropropionic acid and malonic acid.

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Mitochondrial toxins are becoming important tools in modelling human neurological diseases. 3-nitropropionic acid (3-NP) is a natural substance found in some weeds, and in foodstuffs infested by certain moulds causing occasional human intoxication with nervous system manifestations. Malonic acid (MA) is also found both in plants and animals. 3-NP causes an irreversible block of mitochondrial succinate dehydrogenase while the effect of MA is reversible.

In the present study, 3-NP and MA were given to young adult male Wistar rats in acute, self-controlled experiments. The animals were anesthetized with ip. urethane, the head was fixed in a stereotaxic frame, the left hemisphere was exposed by opening the skull, and ball-tipped silver wire electrodes were placed on the primary somatosensory and visual cortical areas. From these, spontaneous activity and sensory evoked potentials (EPs) were recorded. From the tail nerve, compound action potentials were recorded with electrical stimulation. After the control period (3 complete set of records) 20 mg/kg 3-NP or 600 mg/kg MA was given ip. and further records were taken to see the immediate effect.

In the spontaneous cortical activity, 3-NP caused a shift to lower frequencies, which effect was not seen in MA-treated rats. The latency of the somatosensory EP decreased after administration of 3-NP or MA but he effect was slight. Its duration showed a decrease-increase trend with 3-NP but only a late increase with MA. The dependence of these changes on stimulation frequency was different with the two substances. Alterations of the visual and auditory EP were less characteristic. In the tail nerve action potential, conduction velocity decreased and frequency-dependent fatigue increased.

The effects of the two substances were not fully alike. The differences suggest that some effects are possibly not due to the mitochondrial toxicity of the substances, which may be relevant for the human disease models.

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Hypothalamic and medial amygdala infusions of urocortin 3 elicit site-specific effects on feeding in rats

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Members of the corticotropin-releasing factor (CRF) family, such as urotensin, urocortin (Ucn1), urocortin 2 (Ucn2), and urocortin 3 (Ucn3) are hypothesized to mediate behavioral, autonomic, and endocrine responses to stress. All these peptides exert their actions through G-protein-coupled receptors, referred to as the CRF1 receptor, the CRF2 receptor (and, in fish, the CRF3 receptor). Central administration of these peptides is anorectic leading to the hypothesis that CRF-like peptides play an important role in the regulation of feeding behavior. Intracerebroventricular (i.c.v.) infusion of human Ucn 2 and murine (m) Ucn 3, two members of the corticotropin-releasing factor (CRF) family with CRF2 selectivity and high affinity, exerts delayed anorectic effects. The ventromedial and paraventricular hypothalamic nuclei (VMH, PVN) and the medial amygdala (MeA) are feeding-related regions in which Ucn 3 immunoreactive terminals or CRF2 mRNA have been observed. To test the hypothesis that these regions were sites of anorectic action for the urocortins, the present studies examined the ingestive effects of mUcn 3 (0, 100, 500 ng) infused into the VMH (n=7), PVN (n=8), or MeA (n=12) of adult, nondeprived, male Wistar rats using a Latin square design. Intra-VMH Ucn 3 had delayed anorectic and hypodispic effects, reducing the quantity (58-73%) and duration of feeding as well as water intake (33-62%) in the 3rd-4th post-infusion hours. Intra-PVN Ucn 3 also reduced the quantity (57-65%) and duration of feeding as well as drinking (32-68%) during this period. Reduced intake resulted partly from a reduction in meal frequency, and intra-PVN Ucn 3 also decreased the within-meal rate and within-bout regularity of eating. In contrast, intra-MeA Ucn 3 did not alter the quantity consumed during 6 hr. Rather intra-MeA Ucn 3 (500 ng) made rats eat more, but smaller and briefer, meals and eat more rapidly within bouts of feeding. Thus, intra-MeA Ucn 3 only altered the manner in which food was eaten. Intra-PVN and -VMH Ucn 3 also differentially modified the quantity consumed. The results support the hypothesis that the hypothalamus mediates the delayed anorectic effects of i.c.v. urocortin administration and suggest site-specificity in the feeding-related effects of CRF2 receptor activation.

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Efforts to find the responsible transmitter in the prolactin release induced by salsolinol

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Dopamine is the generally accepted prolactin inhibitory factor. Salsolinol is a prolactin releasing agent which has specific binding sites in the brain and in the pituitary. A group of compounds affecting L-DOPA decarboxylase activity displace 3H-salsolinol from its binding sites. Therefore the effect prolactoliberin salsolinol was examined as a possible modulator of dopaminergic neurotransmission. In the median eminence there was no indication of the decreased dopaminergic activity as it was measured injecting salsolinol alone, or together with L-DOPA. In the median eminence, neurointermediate and anterior lobe of the pituitary salsolinol and its antagonist 1-methyl-dihydroisoquinoline induced no changes of dopamine or norepinephrine metabolism, which might have explained the consecutive prolactin release or its inhibition. Neither the antagonist nor salsolinol altered the in vitro release of dopamine in the median eminence. When measuring the peripheral norepinephrine release, however, a sharp and significant interaction was revealed. The results point to a possible preferential role of peripheral norepinephrine release in the endocrine regulation.

Diagnostic value of amygdala, hippocampus and total brain volumes

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INTRODUCTION: Volumetric analysis of brain magnetic resonance images (MRI) measures structural changes associated with neurological and neuropsychiatric disorders. In recent years several studies have shown the utility of the volumetric measurement of the medial temporal lobe structures, especially the amygdala and the hippocampus in temporal lobe epilepsy, memory disorders, Alzheimer disease and schizophrenia. AIM: The purpose of this study was to measure and establish our normal values of the hippocampus and amygdala volumes. METHODS: All studies were performed using a 1.0 T Siemens unit with FISP 3D sequence. One millimeter, contiguous coronal scans of 40 healthy volunteers aged 19-26 years were obtained. The regions of interest were outlined using a mouse driven cursor. To determine the anatomic boundaries of the hippocampus and the amygdala the authors followed a generally accepted protocol previously described by C. Watson. RESULTS: The mean right and left hippocampal volumes were 2.12 cm3 (SD = 0.31) and 2.07 cm3 (SD = 0.3) and the mean right and left amygdaloid volume were 1.19 cm3 (SD = 0.19) and 1.2 cm3 (SD = 0.2) respectively. The mean asymmetry between the right and left hippocampus and amygdala was 3.17% and 3.48%, respectively. The inter-observer reliability range (alpha) was between 0.97 and 0.77. Subregional analysis of hippocampus was calculated as well. CONCLUSIONS: Normal volumetric data measured in our study fell in the midrange of the values in the literature. The volumetric analysis of the hippocampal subregions may allow assessment of providing more sensitive determination of the atrophic area, which may be important in epileptic disorders.

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Role of aminergic systems in the regulation of feeding activity and heartbeat in embryos of Lymnaea stagnalis

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In pharmacological experiments snail embryos at E95% stage of development were treated to monitor and quantify the effect of different aminergic (serotonin [5-HT], dopamine [DA], octopamine [OA]) pharmacons on the feeding activity, based on the frequency of mouth opening (radula protrusion and retraction). To monitor the rate of the heartbeat, E70% embryos were treated with the same aminergic drugs but OA, which is yet not present in the embryos. In parallel experiments, the activity of the aromatic amino acid decarboxylase involved in the biosynthesis of monoamines was measured by HPLC method. Effect of inhibitors of the enzyme activity was also investigated. In dose-response experiments, 5-HT enhanced the frequency of heartbeat by 21,51 ± 2,98, whereas DA decreased it by 19,41% ± 3,51 at 1 mM concentration. One of the 5-HTergic agonists, tryptamine had a similar effect to that of 5-HT. Other 5-HTergic agonists decreased the heartbeat in the following rank order (0,01 to 1 mM): 2,5-dimethoxy-4-iodoamphetamin (15.07% ± 3,55) > 8-hydroxydipropylaminotetralin (23.07% ± 2,59) > N,N-dimethyltryptamin-hydrogen oxalate (41,27% ± 6,56) > α-methyl-5-hydroxytryptamine (16,57% ± 1,80). From the 5-HTergic antagonists studied, 7-methyl-tryptamine (0,1 mM, 24,77% ± 5,8) and mianserin (0,5 mM, 23.39% ± 6,03) decreased the heartbeat. DAergic agonists (0,1 to 1 mM) inhibited the heartbeat in a rank order: apomorphine (32,02% ± 7,3) = 4-hydroxyphenethylamine (25.64% ± 4,76) > flupenthixol (antagonist, 95,06% ± 8,59) > 3-hydroxyphenethylamine (18.01 % ± 5,77). Octopamine, applied at 1 mM concentration, decreased the radula activity. The following 5-HTergic pharmacons (0,01 to 1 mM) had an agonistic activity (0,01 to 1 mM): N,N-dimethyltryptamin-hydrogen oxalate (74,24% ± 13,58) > methysergide (69,98% ± 6,22) > 5-HT (84,94% ± 13,33) > tryptamine (77,52% ± 23,04), whereas 2,5-dimethoxy-4-iodoamphetamin reduced the frequency of radula protursion. DA (1 mM, 51,38% ± 7,65) enhanced, apomorphine (0,01 mM, 51,38% ± 7,65) decreased the intensity of radula movement. p-Chlorophenylalanine (a tryptophan hydroxylase inhibitor) decreased the feeding activity. Each aromatic decarboxylase inhibitors inhibited the activity of decarboxylase enzyme. Using L-DOPA as a substrate, the 50% inhibition of the enzyme activity was found at a concentration of 0,53 μM for carbidopa, 7,5 μM for 3-hydroxylbenzyl-hydrazine (mHBH) and 136 μM for α-methyl-DOPA. When the 5-hydroxy-tryptophan was used as a substrate, the enzyme activity was inhibited by 50% at a concentration of 2,86 μM. The present findings suggest that monoamines are involved in the regulation of feeding activity and heartbeat during the embryonic development of Lymnaea.

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Effects of vipocetine on various neuronal ion channels

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Vinpocetine is a synthetic ethyl ester of the Vinca minor alkaloid apovincamine. A therapeutic formulation of vinpocetine (Cavinton) has been used to treat cognitive disorders, especially vascular dementia. The drug has a diverse pharmacological profile that includes phosphodiesterase inhibition and actions at both ligand and voltage gated ion channels. According to recent data peripheral benzodiazepine receptors (PBR) may also be involved in the actions of vinpocetine (Gulyás et al., J. Neurol. Sci., in press). We report here on the action of vinpocetine on delayed rectifying potassium channels, and on the ability of the drug to induce inward current, which can be inhibited by the PBR antagonist PK11195 (PK).

Transmembrane current inducing effect of vinpocetine was investigated on primary cultures of cortical neurons from 17-day-old rat embryos, while potassium channel inhibition was studied in dorsal root ganglion (DRG) cells prepared from 6-day-old rats, using standard whole-cell patch clamp recording.

In 40 out of 60 cells vinpocetine (100 □M) induced an inward current with mean amplitude of 200 pA. This current was insensitive to bicuculline and strychnine, inhibitors of GABA-A and glycine receptors, respectively, but was partially inhibited by APV (a competitive blocker of NMDA receptors) and CNQX (an inhibitor of AMPA/kainate receptors). Surprisingly this current was also sensitive to PK, a potent and selective PBR blocker. This compound concentration dependently blocked vinpocetine evoked current with a maximum effect of about 60%. The IC50 value of PK was 4 nM which is in good agreement with literature data derived from ligand binding experiments. According to these data vinpocetine may have an action on PBRs.

In another set of experiments vinpocetine blocked delayed rectifier potassium current concentration dependently. On the contrary, transient potassium currents were not inhibited. Since Kv2.1 channels, which are also expressed in DRG cells, have been implicated in apoptotic cell death, inhibition of these channels may partially explain the well-documented neuroprotective effect of vinpocetine.

Our present results may help to clarify the mechanism of the pharmacological actions of vinpocetine.

Endocannabinoid modulation of cortical circuits. Linked to anxiety

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Earlier results of our laboratory demonstrate that the mechanism by which both endo- and exogenous cannabinoids interfere with network oscillations and associated cognitive functions is the reduction of basket cell-mediated GABAergic inhibition in the hippocampus, neocortex and amygdala via presynaptic CB_1 cannabinoid receptors. These receptors are selectively expressed by axons of the cholecystokinin-containing, but not by the parvalbumin-containing interneurons. We provided evidence that glutamatergic EPSCs are also reduced by cannabinoids to a similar extent, but this effect remains unchanged in CB_1-knockout mice, thus is likely mediated by a novel cannabinoid receptor. Pharmacological characterization of this receptor revealed that it has a 10-fold lower affinity for the agonist WIN 55.212 compared to CB_1, and can be antagonized by the VR1 receptor antagonist capsazepine (unlike CB_1).

We hypothesised that the conflicting data in the literature about cannabinoid actions on behavior may be explained by the simultaneous modulation of GABAergic and glutamatergic transmission via CB_1 and the novel CB receptor, respectively. We investigated this hypothesis in anxiety tests, the social interaction task and the elevated plus-maze, using wild type and CB_1 knockout animals. In the first experiment, CB_1-knockout and wild type mice were compared, whereas in the second, the cannabinoid antagonist SR141716A (0, 1, and 3 mg/kg) was administered to both CB_1-knockout and wild type mice. Undrugged CB_1 knockout mice showed a reduced exploration of the open arms of the plus-maze apparatus, as well as a more limited interaction with their partner in the social interaction paradigm, thus, this strain appeared more anxious than the wild type. The cannabinoid antagonist SR141716A reduced anxiety in both wild type and CB_1-knockout mice, and the anxiety scores returned to control values in the knockouts. Since SR141716A binds to both CB_1 and the putative novel receptor, our data in the CB_1-knockout mice suggest that the new CB receptor located on glutamatergic terminals is anxiogenic, and selective blockade of this receptor may represent an ideal new target for the pharmacotherapy of anxiety.

An alternative possibility to achieve an anxiolytic effect via increased activation of CB_1 receptors is to prolong the action of endocannabinoids. Depolarization of hippocampal pyramidal cells leads to a CB_1 cannabinoid receptor-mediated short-term depression of GABA release from afferent basket cell terminals, a phenomenon termed depolarization-induced suppression of inhibition (DSI). However, the nature of the endocannabinoid (eC) substance involved is still unknown. Neurons synthesize two major eCs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). AEA and 2-AG are degraded through different mechanisms: AEA by fatty-acid amide hydrolase (FAAH) and 2-AG by monoglyceride lipase (MGL). Here we used the first selective inhibitor of MGL (URB602) and an inhibitor of FAAH (URB597) to examine the role of 2-AG and AEA in DSI. To test the effect of URB602 on DSI, we recorded spontaneous action potential-dependent IPSCs from CA1 pyramidal cells of 15-17-day-old Wistar rats using whole-cell patch-clamp. When URB602 was applied, the recovery of DSI was elongated, and DSI area (measured in the first 30 s after depolarization) increased to 181 ± 21 % (n=9). The FAAH inhibitor URB597 had no such effect when used at a concentration that completely blocks FAAH. The results suggest that 2-AG, but not anandamide, is responsible for inhibition of GABA release through CB_1 receptors during DSI. Thus, if prolongation of CB_1 receptor activation is the target of an anxiolytic pharmacotherapy, the approach should be the development of selective inhibitors of MGL and not FAAH.

Medullary adrenergic neurons contribute to the neuropeptide Y-immunoreactive innervation of hypophysiotropic corticotropin-releasing hormone synthesizing neurons in the rat

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The hypophysiotropic corticotropin-releasing hormone (CRH)-synthesizing neurons in the hypothalamic paraventricular nucleus (PVN) are the principal controllers of the hypothalamic-pituitary-adrenal (HPA) axis. Neuropeptide Y (NPY) has been shown to regulate the HPA axis through the hypophysiotropic CRH neurons. NPY-containing axons densely innervate the CRH neurons in the PVN and centrally administered NPY increases plasma ACTH and corticosterone levels. The two main sources of the NPY-immunoreactive (IR) innervation of the PVN are the arcuate nucleus and the medullary adrenergic/NPY neurons. To elucidate the relative contribution of the medullary adrenergic cell groups to the NPY-IR innervation of hypophysiotropic CRH neurons, triple-labeling immunocytochemistry was performed using antisera against CRH, NPY and phenylethanolamine-N-methyltransferase (PNMT), the key enzyme of adrenaline synthesis. By confocal microscopic analysis, the number of single labeled NPY and double-labeled NPY/PNMT boutons in juxtaposition to CRH neurons were counted. In accordance with previous observations, both NPY and PNMT-IR fibers heavily inundated the CRH neurons in the medial parvocellular subdivision of the PVN. The vast majority of the CRH neurons were innervated by both NPY- and PNMT-IR axon varicosities. Double labeled NPY/PNMT boutons comprised approximately 50 % of all NPY boutons in juxtaposition to CRH neurons. In addition, approximately 82.5 % of the PNMT boutons contained NPY. We conclude that half of the NPY-IR innervation of the hypophysiotropic CRH neurons arises from the medullary C1-3 area. Further studies are required to elucidate the other sources of the NPY-IR innervation of CRH neurons.

The effects of aging on auditory event-related potentials

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Objectives: The effects of aging were investigated on different components of auditory event-related potentials and the CNV.

Methods: Three age groups participated in the study: 16 young (mean age: 22.1 years), 9 middle-aged (m.a: 47.8 years) and 13 elderly (m.a: 66.1 years) adults. Experiment I. was an oddball paradigm. Standard binaural tones (1000 Hz, probability: 80%) and target tones (1100 Hz, probability: 20%) were presented and the subjects had to press a button at the occurrence of the target tones. In Experiment II. subjects performed a go-nogo paradigm. The task was to stop a series of tones by button press when a 1500 Hz warning stimulus was presented, and not to react if this stimulus was a 1000 Hz tone. In Experiment III. 7 or 8 tones were presented and participants had to press a button when only 7 tones were detected. The EEG was recorded according to the International 10-20 system (sampling frequency: 200 Hz, bandpass filtering: DC-30 Hz).

Results: In Experiment I. statistical analysis (ANOVA) showed a location x age interaction for the N2b ($F(4,70) = 4.288$, $p = 0.004$) and P3b amplitude ($F(4,70) = 5.255$), $p = 0.001$). The N2b amplitude had a frontal maximum in the young group but was observed with a central maximum in the elderly and middle-aged groups. The P3b had a frontal and parietal amplitude maximum in the elderly both in Experiment I. and II. ($F(4,70) = 4.887$, $p = 0.001$) while it was seen with a parietal dominance in young and middle-aged adults. In Experiment II. an age main effect was seen for N1 ($F(2,35) = 4.122$, $p = 0.025$) and P3b amplitude ($F(2,35) = 2.845$, $p = 0.072$) and P3b latency ($F(2,35) = 2.760$, $p = 0.077$). The amplitude of the N1 and P3b component was largest in the young group and the latency of P3b was longest in the elderly. The warning stimuli elicited a CNV the amplitude of which was highest in the elderly group, similarly to that observed in Experiment III.

Conclusions: Our data support previous findings concerning the age-related scalp distribution of the P3b and N2b waves. The changes of the N1 and P3b waves indicate the age-dependent changes of information processing. However, if enough time is available, these effects – according to the CNV data – can be compensated in elderly.

Preclinical pharmacology of a new antipsychotic agent EGIS-11150

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Schizophrenia is a complex psychiatric disorder which is characterized by positive symptoms, negative symptoms and cognitive dysfunction. According to the two-syndrome concept of Crow (Crow 1985) the positive or productive symptoms include aural and visual hallucinations, delusions, irrational thought patterns. The negative or deficit symptoms include social withdrawal and blunting of emotional responses. Besides the productive and deficit symptoms, anxiety also frequently occurs in the course of the disease and causes further worsening of the outcome of this disorder.

EGIS-11150 is a new atypical antipsychotic agent developed by EGIS Pharmaceutical Ltd. The compound showed strong antiosychotic activity in a broad range of models of negative and positive symptoms of schizophrenia. It inhibited the conditioned avoidance response in rats (ID50 = 0.7 mg/kg po.) and apomorphine induced stereotypy and climbing in mice (0.2 and 0.06 mg/kg respectively). The PCP-induced social withdrawal is thought to be the best animal test modelling both the positive and negative symptoms of schizophrenia. In this model, EGIS-11150 strongly inhibits the effects of phencyclidine, and its effects in the test are more comprehensive than those of olanzapine. EGIS-11150 is an exceptionally potent antagonist of the locomotor effects of PCP (antagonism at 0.015mg/kg orally in the mice).

EGIS-11150 has affinity for a1 receptors (Ki 0.5 nM), 5-HT2A receptors (Ki 3.1 nM), D2 receptors (120 nM) and 5-HT7 receptors (9.1nM). However, it is unlikely that these effects only are responsible for the antagonism of PCP because a1 and 5-HT2 antagonists do not fully antagonise the effects of PCP even at high doses.

EGIS-11150 shows low cataleptogenic potential in the therapeutic dose range.

These findings indicate advantageous therapeutic properties in man, since cataleptogenic effects are often limiting factors of appropriate antipsychotic treatments.

"Dark" neurons - gel-to-gel phase transition. Heretic explanation of a century-old problem

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Based on the combination of a hardly-known physical phenomenon (gel-to-gel phase transition) and a minority opinion of the physical state of the living cells (gel-like state), this lecture presents a conception capable of explaining all the enigmatic features of the formation and nature of "dark" neurons.

The effects of perivagal or intraperitoneal capsaicin desensitization on postprandial hyperthermia and on endotoxin fever

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Postprandially, metabolic rate increases, often together with body temperature. This may be due to nutrients, stretch, or to hormones related to the feeding process. Some of these signals are thought to be conveyed by capsaicin-sensitive afferent fibers of the abdominal vagus. A role for vagus-mediated signals from the liver has also been suggested to participate in the pathogenesis of endotoxin fever, which relies on increased metabolic rate. Intraperitoneal (IP) capsaicin desensitization has been reported to attenuate the early part of fever as well as some forms of postprandial hyperthermia. Since in such treatments the vagus is not necessarily the main or exclusive target for capsaicin to exert its desensitizing action, direct capsaicin treatment of the vagus was used to determine the role of the nerve in the development of postprandial hyperthermia or fever. In anesthetized Wistar rats a perivagally placed piece of cotton wool, soaked with 1% capsaicin solution and kept in position for 20 min, was used for desensitization, or 2 + 3 mg/kg capsaicin was given IP. A week later either a gastric (1.5 mm I.D.) tube or a jugular (pp10) cannula was implanted and exteriorized at the nape. The gastric tube did not interfere with the normal feeding or other activities of the animal. Later on the semi-restrained rats were placed into a metabolic chamber at thermoneutrality, thermocouples were attached and metabolic rate was measured. In rats with gastric tube, measurements happened 5-7 days after operation, and through the gastric tube 3ml/100 g thick suspension of $BaSO_4$ (or water in controls) was injected, and the measurements went on for 3-4 hours. The fever experiments were performed 3 days after cannula implantation: 10 µg/kg E. coli endotoxin was given intravenously and fever was observed for 7 hours. Both types of desensitization prevented the postalimentary hyperthermia of this model. IP desensitization abolished or attenuated the first part of the normal triphasic fever course, perivagal desensitization did not influence this part (but somewhat attenuated the late third phase of fever, which was thought to be related to efferent vagal functions). Apparently, in the present model of postalimentary hyperthermia capsaicin-sensitive vagal afferents may be indispensable. However, in fever initiation (the first phase of fever) the vagus may have no role, capsaicin acts at some other points (probably at liver cells).

Period2 expression in urocortin 1 cells in the Edinger-Westphal nucleus

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Stress activates the HPA-axis and evokes the daily rhythmic secretion of glucocorticoids from the adrenal cortex. Interestingly, stress regulatory pathways, other then the HPA axis, also operate in a rhythmic manner and exhibit circadian rhythms. The suprachiasmatic nucleus (SCN) of the hypothalamus, expressing Period 1 and Period 2 (PER2) proteins is the primary circadian clock regulating daily rhythms in behaviour and physiology, which are not only regulated by the master clock in the SCN, but also locally by widely distributed populations of clock cells in the brain.

Urocortin 1 (Ucn1), a newly identified member of CRF neuropeptide family, is abundantly expressed in the Edinger-Westphal nucleus (EW), and mediates a variety of stress-induced responses. Recent results on the expression of clock genes, e.g. Per2 outside the SCN suggests that stress-responsive neuronal structures, such as EW Ucn 1 neurons may also modulate specific rhythms downstream from the SCN master clock. Based on these we hypothesized the circadian rhythmic expression of Per2 in E-WN urocortinergic neurons that may consequently elicit a rhythmic modulatory control over the stress response.

In support of this hypothesis, we found a large number of Per 2 positive nuclei in the EW, and using double-label immunflourescence staining confirmed the co-expression of Per2 and Ucn 1 in the EW. However, the question arose as to the nature of possible descending pathways to control the Per 2 expression in EW neurons. The hypothalamic orexin/hypocretin neurons play critical functions in conveying an efferent signal from putative oscillators to various brain centers. We found the co-distribution of orexin/hypocretin with Ucn 1, and observed orexin/hypocritin positive fibers juxtaposed to Ucn 1 neurons in EW. Based on these results we suggest that hypothalamic orexin/hypocretin neurons may play a role in controlling the expression of clock genes in EW Ucn 1 neurons, and thus influencing the rhythmic activity EW Ucn 1. This may elicit a rhythmic modulatory control over the stress response, and play a role in disturbed circadian rhythms in stress-induced disorders.

H1-receptor blockade is not sufficient to prevent systemic allergic reaction induced cellular and transcriptional activation in the hypothalamus

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Histamine released from mast cells plays a well-defined role in the course of allergic symptoms, and H1-receptors are suggested to be involved in the peripheral and central effects of this type immunological challenge. Experimental systemic allergic reactions (e.g. anaphylaxis) stimulate neurosecretory neurons in the hypothalamic paraventricular nucleus (PVN) triggering activation of the hypothalamo-pituitary-adrenal axis, in addition to this other central regions related to autonomic and behavioral regulation are recruited. We assessed the influence of H1-receptor signaling on cellular and transcriptional changes of stress-related neurocircuitry provoked by anaphylactoid reaction. This type of allergic challenge occurs after the first injection of a foreign protein. Twenty minutes prior to the intraperitoneal injection of ovalbumin, adult Wistar rats received two different doses of intravenous H1-antagonist pyrilamine (5, 10 mg/kg). Animals were perfused after two hours of stress-free period, at a time point that is known maximal level of challenge-induced c-Fos protein and CRH mRNA in the PVN. cFos protein, the marker of neuronal activation was revealed by immunocytochemistry, CRH mRNA was visualized by isotopic in situ hybridization. Intraperitoneal injection of egg white produced symptoms of allergy in the periphery, as well as edema, scratching and a significant increase in cFos immunoreactive profiles in the PVN ($p=0.009$) and CRH mRNA levels in the medial parvocellular PVN ($p=0.003$). To study the involvement of H1-receptor system, pyrilamine was administered intravenously. Pretreatment with 5 or 10mg/kg pyrilamine resulted in the attenuation of peripheral signs of anaphylactoid reaction. However, pyrilamine failed to prevent neuronal activation of cFos ($p=0.3$) and transcriptional changes of neuropeptide CRH ($p=0.7$). Our results support the involvement of H1-receptors in the peripheral arm of allergic reactions, but suggest contribution to other mediators and receptor systems in the immune-to-brain communication during systemic anaphylactoid challange.

Circadian changes of glial fibrillary acidic protein (GFAP) immunoreactivity in the rat lateral geniculate nucleus

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The lateral geniculate nucleus was studied for glial fibrillary acidic protein (GFAP) immunoreactivity in adult male Wistar rats to demonstrate circadian oscillations of this protein in the astrocytes of the nucleus. Particular attention was paid to the dorsal subdivision of the nucleus where retinal fibres terminate. Comparative studies were carried out at mid-day and at midnight. Assessment of microscopic preparations was corroborated by thewir surface densitometry.

During daytime, the dorsal subdivision of the CGL was GFAP immunonegative in sharp contrast to the immunopositive ventral subdivision. The IGL separating the two subdivisions was also found immunopositive.

At night, the dorsal CGL subdivisions showed a marked immunopositivity, while the ventral subdivision and the IGL remained invariably immunostained.

Findings suggest that photic stimuli down-regulate GFAP in the dorsal geniculate nucleus. This is an example how an astrocytic marker may serve as an indicator of neuronal activity.

Pharmacological profile of EGIS-10609, a new non-competitive AMPA receptor antagonist

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AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor inhibition has been hypothesized to provide neuroprotective efficacy after cerebral ischemia on the basis of the activity in experimental ischemic models of a variety of compounds with varying selectivity for AMPA over other glutamate receptor subtypes. EGIS-10609 is a new and potent non-competitive AMPA receptor antagonist with 2,3-benzodiazepine chemical structure. The present study was undertaken in order to compare the in vitro efficacy and in vivo neuroprotective effects of EGIS-10609 to those of GYKI 53405.

In patch clamp measurements, EGIS-10609 inhibited the kainite-evoked whole cell currents in cultured rat telencephalon neurones (IC50=3.9 μM), population spikes in rat hippocampal slices (IC50=2.8 μM) and the AMPA-induced spreading depression in chicken retina (IC50=2.7 μM); these in vitro effects of EGIS-10609 were stronger than those of GYKI 53405 (IC50 values were 7.6, 19.7 and 7.0 μM, respectively). EGIS-10609 was protective against maximal electroshock (MES) and sound induced seizures (AS) with ED50 values of 3.9 mg/kg i.p. and 2.3 mg/kg i.p., respectively. The anticonvulsant effects of EGIS-10609 were similar to those of GYKI 53405 (MES ED50=3.7 mg/kg i.p., AS ED50=2.4 mg/kg i.p.). In global cerebral ischemia induced by 3-min bilateral carotid occlusion in gerbils, EGIS-10609 or GYKI-53405 (20 mg/kg i.p.) administered at 45 min after reperfusion, decreased neuronal death at day 4 in the CA1 area of the hippocampus by 45 % and 53 % and attenuated hypermotility by 59 % and 51 %, respectively. Both compounds dose-dependently reduced cerebral infarct size after permanent middle cerebral artery occlusion in mice and rats. In both species, EGIS-10609 showed stronger neuroprotective activity (minimal effective dose, MED=0.03 mg/kg i.p. in both mice and rats, respectively) than GYKI 53405 (MED=3 mg/kg i.p. in mice, MED=10 mg/kg i.p. in rats). Male Lewis rats were treated with guinea pig myelin basic protein for the induction of autoimmune encephalomyelitis and both compounds were administered at 3 mg/kg intraperitoneal dose twice daily for 7 days starting on day 10 after immunization. EGIS-10609 did not reduce cumulative neurological score but improved histopathological outcome while the effect of GYKI 53405 was statistically significant at a higher dose only (10 mg/kg, twice daily).

In conclusion, our results suggest that AMPA receptor inhibition alone may not be sufficient to account for the robust neuroprotective activity of EGIS-10609 against permanent cerebral ischemia. The favourable neuroprotective effect of EGIS-10609 indicates good clinical perspectives in the treatment of a wide range of human central nervous system disorders; EGIS-10609 may be especially suitable for the treatment of human ischemic stroke.

Artificial neural network with a multiprocessor for analyzing the changes of the firing pattern of the central nervous system caused by the psychopharmacons

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In the course of my previous analyses I showed that in the different tissues of a rat certain neurolepticums induced the increase of the enzyme activity of the Glutathione-S-Transferase (GST). The effect of these psychopharmacons (e.g. the synchronization of the firing patterns) and their side effects (e.g. pseudoparkinsonism) are not entirely clarified. The therapeutic efficiency of the metabolits of the xenobioticums which with the help of GST form a conjugate with glutathione (GSH) and their effect on the nerve-function and on the synaptic signal transfer are also vexed questions.

In my further investigations my purpose was to analyze how these compounds effect the firing pattern change of the RAS nerve-cell network of the brain-stem – which can be treated as their point of attack. With the examination of the synchronic firing phenomena between more nerve-cell groups covered accidentally with electrode arrays the effects of the compounds on the collaterals, i.e. on the synaptic signal transfer, can be investigated.

But to the firing pattern analysis of the nerve-cells of the central nervous system a considerable calculation capacity is necessary even on a smaller surface, and this requires a special hardware. The analyzing and mapping of the synchronic firing activity of the neighboring, often collateral nerve-cell groups can only be achieved by a continuous real time sampling. At a 100 Hz sampling frequency, with an A/D conversion of 14 bits a data flow of almost 200 Kbit/s is achieved from 128 electrodes. For the solution of this problem a high capacity artificial neural network - similar to the living nervous system - was accomplished which has a multiprocessor and combines the analogue and digital characteristics (Analog Digital Neural Computer, ADNC). The highly sensitive recorders and stimulators are controlled by five PIC based microcontrollers. The neural network computer records the firing patterns which consist of the analogue action potentials, processes them, can do operations with them (e.g. looks for connections or for synchronic firing cell groups), or through the electrodes it can send back arbitrary firing matrixes to the nervous system in the form of a stimulus. The operations can be transformed to algorithms because of digital characteristic of the system, they can be directed by a PC, and the firing pattern matrixes can be further analyzed on the computer. ADNC approaches the interactions of the computer nervous system in a new way because it interprets the living nervous system as a part of the artificial neural network with an unidentified structure and connection system.

With the help of ADNC the effect of any pharmacon can be analyzed which affects the complex firing patterns of certain areas of the central nervous system.

Environmental temperature influences the development of postprandial hyperthermia

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Following food intake a rise in metabolic rate is accompanied by elevation of body temperature. This is not a simple consequence of calorie intake: in rats it develops also if calorie-free food is consumed or calorie-free substance is injected into the stomach (although the courses of the metabolic/thermal changes are different: they start earlier when a calorie-rich substance is given). Thus, the afferent signals for the postprandial changes may be different. However, the efferent part, the ways of increase in body temperature may be similar. One similarity is that, independent of the substance given, heat loss never decreases, i.e. heat loss does not contribute to the temperature rise, therefore the postprandial hyperthermia depends entirely on the metabolic rise. The excess heat produced this way might, however, be used for other purposes, e.g. for maintenance of homeothermia in the cold. Accordingly, the hyperthermic response to feeding may depend on the environmental temperature. In cold-adapted (CA) and non-adapted (NA) Wistar rats a gastric tube was surgically implanted, exteriorized at the nape and closed. The tube could be kept in place for several weeks, since it did not interfere with the normal feeding activity or weight gain rate. At weekly intervals the rats were fasting for 48-h, then thermocouples were attached, the animals were semi-restrained and were placed into an open-circuit metabolic chamber kept ventilated in a waterbath at thermoneutrality (25-26 ºC for fasting CA and 31-32 ºC for fasting NA rats). In other cases the waterbath was cooler (10 ºC for CA and 28-30 ºC for NA rats). After settling, a thick suspension of calorie-rich substance ("Fast weight gain" /FWG/ formula of body builders), or a calorie-free substance (BaSO4), or water in controls was injected in a volume of 3 ml/100 g, and metabolic rate and body temperature were followed for 4-h. At thermoneutrality, after FWG injection the body temperature rose quickly by about 0.7-0.9 ºC, and more slowly about the same rise was seen after BaSO4 (but not water) injection – metabolic rate rose parallel. At sub-thermoneutral environmental temperatures the metabolic rate was already elevated and neither metabolic rate nor body temperature increased following injection of either FWG or BaSO4. Apparently, the excess heat produced due to postprandial neural and other factors was incorporated in the hypermetabolism that was necessary to defend homeothermia.

The creatine kinase/phosphocreatine system modulates oscillations in WFACs.

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Wide-field amacrine cells (WFACs) are a special population of amacrine cells, easily distinguishable by its outspreading processes in the retina. In these cells,taken from the teleost white bass the interplay between voltage-dependent Ca (ICa) and calcium dependent potassium (KCa) currents generates membrane potential oscillations.The aim of this study was to test the hypothesis that the creatine kinase/phosphocreatine system - which is known to regulate ion channels - is involved in the regulation of the oscillatory behavior of wide-field amacrine cells (WFACs).

Recordings were obtained from WFACs using standard whole cell voltage clamp techniques. We used both current - and voltage - clamp protocols. The cells were always held at -70mV. To study the Ca currents, the cells were bathed in Ringer's containing 2.1mM Ca2+ with potassium and sodium channel blockers. Creatine phosphokinase was applied through the recording pipette with or without MgATP and/or creatinephosphate. Higher concentrations of Ca2+ containing Ringer's (5-10mM) were applied extracellulary, using the DAD-12 superfusing system.

Under control conditions (2.1mM extracellular Ca2+) including creatine phosphokinase+MgATP+creatinephosphate in the pipette solution had no noticeable effect on the oscillations. Application of high extracellular Ca2+ inhibited the oscillations after a transient enhancement. However, in the presence of the kinase+MgATP the enhacement of the oscillations were more pronounced and sustained. Analysis of the ICa revealed a substantial decrease in the amplitude induced by the kinase.

Creatine phosphokinase activity is responsible for the decrease of ICa, consequently lessening the Ca-dependent inactivation of ICa thereby increases the oscillations. The KCa currents are also influenced so that the membrane potential remains in the optimal range for oscillatory behaviour. It is possible that oscillatory behavior is linked to energy metabolism in WFACs. The creatine phosphokinase may play a critical role during transient metabolic increases to adjust conductance of voltage-gated Ca and K(Ca) channels for optional information processing.

Heterogeneity in target selection of individual septo-hippocampal axons

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In vivo experiments from our laboratory suggested that septo-hippocampal PV containing neurons demonstrate two types of firing property during hippocampal theta and non-theta states and fire phase-locked to two different phases of hippocampal theta activity. In the hippocampus PV containing basket cells and O-LM cells also fire at two different phases of the local theta activity. These results suggest, that the septo-hippocampal projection arising from septal neurons is not necessary homogenous concerning its hippocampal targets, but individual projecting cells might show target preference among the functionally distinct hippocampal interneuron populations, depending on their physiological properties (i.e. which phase of the theta cycle they fire).

We injected an anterograde tracer (PHAL) into the medial septum and visualized the septo-hippocampal fibers in the hippocampus to study the target selection of individual septal fibers. Consecutive hippocampal sections were then stained against different neurochemical markers identifying functionally different interneuron populations, such as the parvalbumin containing basket cells and the SOM containing OLM cells and hippocampo-septal cells. Individual axons were traced through section boundaries and their target selection was recorded. This way the possible target preference (bias in the target selection) of distinct septo-hippocampal axons can be established.

We found that septo-hippocampal fibers are heterogeneous both in their morphology and in their target selection. Axons were identified that primarily innervated SOM or PV-containing interneurons. Axons that showed no target selection, i.e. innervated both populations has also been identified. This suggests that, the target selectivity of individual septal neurons is heterogeneous, confirming the presence of functionally different septal neuron populations. The results also suggest, that more than 2 target selective populations might exist.

Behavioural changes in rats following perinatal exposure to drugs of abuse. II. Effect of subsequent drug challenge

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Objective: Development of subsequent drug sensitivity was examined in animals following perinatal exposure to drugs of abuse – morphine and methylenedioxy-methamphetamine (ecstasy, MDMA) – at the age of 3 weeks.

Methods: Male and female Wistar rats were mated, the day sperm plugs were detected was designated embryonic day 0. The pregnant female rats were treated daily with either morphine (10 mg/kg sc.) or (+) MDMA (3 mg/kg sc.) from embryonic day 1 until the 21st postpartum day, when the offspring was separated. This day was considered as the termination of drug exposure. Offspring of female rats treated with physiological saline served as control. Our experiments have been directed to check whether the locomotor activity enhancing effect of a subsequent challenge with morphine or (+) MDMA remains unchanged in the drug-exposed offspring. Since the opioid antagonist naloxone even in a dose of 10 mg/kg does not affect the locomotor activity of adult rats, but induces hypolocomotion in morphine-dependent ones, which is considered to be a withdrawal symptom, the effect of naloxone was also checked. Locomotor activity in novel surroundings was measured in "CONDUCTA Advanced System" (Experimetria Ltd) 48 hours after separation of offspring. The animals were placed into the activity measuring box 1 hour after administration of morphine, 30 min after (+) MDMA and 10 min after naloxone. The observation started immediately without habituation and lasted 90 min in case of morphine challenge and 40 min following the other two treatments.

Results: 1. Hyperlocomotion was observed in both sexes of control animals after administration of 3 mg/kg morphine during the whole observation period. Duration of hyperlocomotion was significantly shorter both in male and female offspring exposed to perinatal morphine treatment.

2. (+) MDMA in a dose of 1mg/kg induced significant hyperlocomotion in male controls but failed to do this in male offspring exposed to perinatal (+) MDMA treatment.

3. Unexpectedly naloxone in doses of 1 and 3 mg/kg induced hypolocomotion both in the male and female controls but did not inhibited the locomotor activity of offspring exposed to perinatal morphine treatment. The inhibitory effect of naloxone was observed in male offspring exposed to perinatal (+) MDMA treatment, but not in the females.

Conclusion: The results indicate that in animals exposed to perinatal morphine or (+) MDMA the sensitivity toward agonists and antagonists changes, the effects of a subsequent morphine, (+) MDMA or naloxone challenge decrease.

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Effects of the 5-HT_{1B} receptor agonist CP94253 in rats treated with MDMA (Ecstasy) 6 months earlier

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Serotonin (5-HT) plays a significant role in several physiological processes including also the control of vigilance. These effects are mediated by several different receptors of 5-HT.

5-HT_{1B} receptors may be autoreceptors, located on serotonergic axon terminals, or they are located postsynaptically as heteroreceptors, on axon terminals of other neurotransmitter neurons where 5-HT_{1B} receptors inhibit the release of other neurotransmitters including GABA, acethylcholine or glutamate. To study the role of the 5-HT_{1B} receptor in regulation of sleep-wake cycle, we recorded EEG, EMG and motor activity in rats after administration of a selective 5-HT_{1B} receptor agonist. In addition, these effects were compared in control and MDMA-pretreated rats to see whether axonal degeneration could interact with these effects.

CP 94253 (2.5 mg/kg), a selective 5-HT_{1B} agonist, or vehicle were administered to Dark Agouti rats chronically equipped previously with EEG and EMG electrodes. The animals received CP 94253 at light onset followed by 24-hour continuous polygraphic recordings. Active wakefulness (AW), passive wakefulness (PW), light slow wave sleep (SWS1), deep slow wave sleep (SWS2), paradoxical sleep (PS), and their circadian patterns were analyzed in the study. In parallel, the same experiment was performed in a group of animals that had been treated with MDMA 6 months before the challenge.

CP24253 markedly increased AW and reduced SWS2 and PS for several hours. Similar effects of CP 94253 on SWS2 and PS were found in animals treated with MDMA six months earlier. However, effects of CP 94253 on active wake were absent in MDMA pretreated rats (significant time x pretreatment and time x treatment interactions, $P<0.01$). These data provide evidence that activation of the 5-HT_{1B} receptor has strong effects on vigilance and circadian patterns. Part of these effects are connected to intact serotonergic axon terminals, others are independent of those. In addition, a single dose of MDMA causes long-term or persistent changes in the serotonergic regulation of circadian rhythm.

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Examination of EGIS-11006 in anxyolytic and antidepressant models

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The piridazinone compound EGIS-11006 has been synthesized as a potential CNS active ligand. Based on the receptor ligand studies, EGIS-11006 showed binding to 5-HT1A and 5□HT7 receptors. EGIS-11006 displayed anxiolytic potential in two animal models of anxiety. In elevated plus maze model, the fear of the animals from open space and height is employed as source of anxiety. Significant increase in the time spent in the open arms or in number of entries into the open arms is considered as anxiolytic effect. In experiments, rats were placed in the central area of the apparatus 1 h after po. treatment and parameters were recorded for 5 min. E-11006 has been found to increase the time spent in open arm in the elevated plus-maze (MED: 0.03 mg/kg po.).

In the mCPP-induced anxiety model (light-dark discrimination), the basic source of anxiety is the illuminated open part of light-dark apparatus. The anxiety level of rats is further increased by pre-treatment with mCPP. The rats can freely move between the lit and dark compartments. Anxiolytic compounds increase the time/activity in the lit area. The mCPP treatment results in significant decrease in the activity of the animals in the lit compartment and this reduction was reversed by EGIS-11006 (MED: 10 mg/kg ip.).

EGIS-11006 displayed antidepressant effect in the Porsolt test (animal model of depression) in DBA mice. The minimal effective dose of EGIS-11006 was 7.5 mg/kg ip.

Sedative effect can be detected by the measurement of spontaneous motor activity of animals. EGIS-11006 treatment produced 50 % inhibition on motor activity of mice in 83 mg/kg po dose. In vivo studies revealed anxiolytic and antidepressant activity with no sedative side effects in the therapeutical dose range.

In summary, the pyridazinone derivative EGIS-11006 is a new anxiolytic compound with antidepressant activity. The sedative effect of the molecule appeared at higher doses than the therapeutic activity. This low sedative potential of the molecule suggests a good therapeutical perspectives in man.

Examination of reaction time and body sways of young adults

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The reaction time (RT) is an important characteristic of both daily motor activities and sports, though it is not often measured. The RT indicates the subject's quickness to respond to an external stimulus (such as light, sound) with a definite motor skill. A special aspect of the initiation of motor reactions is the speed with which a person can respond to proprioceptive afferent signals to maintain the body stability. The purpose of this study was (1) to examine the RT of athlete and non-athlete subjects, and, (2) to analyse the body sways recorded with a stabilometer. Subjects were 34 young adult men, 22 elite basketball players, and 12 university students. The reaction time was measured by a device measuring the delay between the presentations of light or sound stimuli, and presses the stop button. The body sways were recorded with a stabilometer while the subject was standing on it. Descriptive statistics, t-tests, and regression analysis were used for statistical analysis of the data. The RT elicited with sound stimuli was significantly longer for basketball players than for the university students. No significant differences were found between RTs to light stimuli. The body sways of basketball players were significantly higher on both sides compared to the students' body sways. Minor but statistically significant correlations were found between certain body measures (height, BMI, body fat) and body sways. Our results do not support the common believe that well-trained athletes have shorter RT than non-athletes. However, it is possible to suggest that even if university students have less physical training than the elite basketball players, there is no significant difference between the physical fitness of the two groups. Furthermore, the body measures have an effect on the subject's responsiveness.

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Development of the neurotransmitter phenotype during the in vitro differentiation of the NE-4C neuroectodermal cell line

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The correlation between the regional localization of a neuron and the establishment of its neurotransmitter phenotype is clearly evidenced, but the underlying mechanisms are far from clear. The possible interactions of position-regulating genes with those determine the neurotransmitter phenotypes were investigated by an in vitro approach using the in vitro induced differentiation of NE-4C one-cell derived neuroectodermal stem cells.

Non-differentiated NE-4C cells, cloned from the brain vesicles of E 9.5 mouse embryos lacking p53, express several early anterior neuroectodermal markers such as Sox-2, Otx-2 and En-1. The presence of Otx2 and En1 transcripts in undifferentiated cells, however, seems to indicate an early anterior stem cell fate, as it is shown by the comparative results on various embryonic stem (ES) cell lines. A number of more characteristic region-specific genes (see below) are not active in non-induced NE-4C cells, but get activated in various phases of retinoic acid (RA) induced neuronal differentiation. We found significant increase in the mRNA level of both a dorsal (Emx1) and a ventral (Dlx1) forebrain marker, and also in the activation of the midbrain-specific Otx3, the midbrain-hindbrain marker Gata-2/3 and SCL and the hindbrain-specific Gbx2 and Hoxb2. In stem cells re-cloned from differentiated NE-4C cultures, the properties of the mother clone are retained. In their non-differentiated stage, the re-cloned cells do not express Emx2 or Hoxb2, but both region-specific genes will be activated in the course of RA induced neural differentiation. The remarkable activation of all investigated region specific marker genes upon differentiation, shows that NE-4C stem cells are not positionally determined.

Immunocytochemical analyses demonstrated that differentiated NE-4C neurons could display both GABAergic and glutamatergic phenotypes. The key genes of the dorsal forebrain glutamatergic phenotype (Vglut2) and the ventral forebrain GABAergic phenotype (GADs), however, were detected only in cultures of mature neurons also expressing Emx2 and Dlx1.

The data obtained on our NE-4C neuronal differentiation model suggest that the neurotransmitter phenotype – at least the glutamatergic and gabaergic fates– might be determined in conjunction with the establishment of the regional commitment. It means, that non-differentiated neuroectodermal stem cells, which are not committed to any specific brain regions yet, may develop multiple transmitter phenotypes.

Oxygen content of the perfused solution in submerged slice chamber correlates with the stability of network oscillations induced in hippocampal slices

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Gamma-frequency network oscillations (30-100 Hz) occur in the hippocampus during exploratory behaviour. To study the cellular mechanisms involved, several in vitro models have been developed. Here, we report the successful induction of cholinergically-induced persistent gamma oscillations in submerged hippocampal slices.

Gamma-frequency field oscillations were recorded in the CA3 region of rat hippocampal slices on a MED64 planar multielectrode array or using a patch electrode following bath application of 20 μM carbachol. We observed that both the amplitude and the stability of oscillations were very sensitive to the flow rate. With commonly-used flow rates of 1-2 ml/min, only short-lasting (2-5 min) oscillations with low amplitude were observed. Increasing the flow rate to 3-6 ml/min facilitated the emergence of synchronous gamma-frequency oscillations, which remained stable for up to an hour under our recording conditions. To explore a possible mechanism for the effect of flow rate on oscillations, we measured the oxygen content of the bath solution with an optical oxygen microsensor ('optode') placed 50-100 μm above the CA3 region. The amplitude of the oscillations was seen to change in parallel with the oxygen level. No significant oscillation was observed below 2 ml/min flow rate, corresponding to 40% of the oxygen content of the saturated solution under our conditions. In further experiments we compared the spiking activity of interneurons and the properties of inhibitory postsynaptic currents in the pyramidal cells at low and high flow rate. After bath application of carbachol the firing rate as well as the inhibitory transmission significantly increased both at low and high flow rate, an enhancement that lasted for long time only in high flow rate.

Our results indicate that stable network oscillations can be induced in submerged hippocampal slices, allowing detailed cellular studies of oscillatory activity. The increased firing rate and maintained synaptic transmission, both essential for continuous synchronous activity, could be only ensured by the presence of sufficient oxygen content in the extracellular solution.

The antidepressant fluoxetine rapidly triggers pyramidal dendritic spine synapse formation in the rat hippocampus

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The pathomechanism of major depressive disorder and the neurobiological basis of antidepressant therapy are still largely unknown. Recently, disturbed hippocampal activity has been proposed to underlie some of the cognitive and vegetative symptoms of depression, at least in part because of the loss of pyramidal cell synaptic contacts, a process that is likely reversed by antidepressant treatment. Here we provide evidence that daily administration of the antidepressant fluoxetine to ovariectomized female rats for five days induces a robust increase in pyramidal cell dendritic spine synapse density in the hippocampal CA1 field, with similar changes appearing in CA3 after two weeks of treatment. This rapid synaptoplasticity response may represent an early step in the fluoxetine-induced cascade of neuroplasticity alterations that gradually spread across the entire hippocampal circuitry, leading to the restoration of synaptic connectivity and cognitive function of the hippocampus. Modulation of hippocampal spine synapse density may provide a potential mechanism to explain certain aspects of antidepressant therapy and mood disorders, especially those associated with changes in reproductive state in women, that cannot be reconciled adequately with current theories for depression.

Effects of orexin-A microinjections into the bed nucleus of stria terminalis on food and water intake are antagonized by selective orexin-1 receptor antagonist SB334867

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Orexins are expressed by lateral hypothalamic neurons. These neurons project to numerous brain regions including the bed nucleus of stria terminalis (BST). When orexins are applied i.c.v., they increase food and water intake. Orexin effects are mediated by two G-protein-coupled receptors, i.e. orexin-1 (OX1R) and orexin-2 receptor (OX2R), respectively. OX1R is selective for orexin-A (OXA) and OX2R binds both OXA and orexin-B (OXB). In the BST high level of OX1R mRNA was observed. In our previous experiments feeding or drinking related effects of OXA microinjections into the BST were examined. OXA was applied in the doses of 250, 500 or 1000 ng, respectively. After OXA applications liquid food intake (MilkQuick, Nutricia) and water intake increased and effects were dose dependent.

In the present experiments the selective OX1R antagonist SB334867 (Tocris, 1960) in the dose of 100 ng (0.26 nmol) was applied alone or 15 min prior 500 ng (0.14 nmol) OXA (Sigma, 0-6012l) microinjections in order to prove whether the effects of OXA on liquid food and on water intake can be antagonized. Drugs were dissolved in sterile saline and were microinjected bilaterally into the BST in 0.4 ml volume. Four different groups of male Wistar rats were used: vehicle, OXA or antagonist treated and antagonist+OXA animals, respectively. Liquid food intake or water intake were studied in separate experiments. Without deprivation subjects could consume liquid food or water from 9h to 12h every day. After microinjections food or water intake were measured every 5 min for the first 30 min and at the 40th, 60th, 120th and 180th min, respectively. When group differences were compared by ANOVA the results showed that OXA significantly enhanced liquid food consumption and increased water intake. Application of the antagonist alone did not modify intakes. The vehicle treated, antagonist treated or antagonist+OXA groups did not show any significant differences, neither in milk, nor in water ingestion. Our results show that OXA effects on milk ingestion and on water intake can be antagonized by SB334867 and that OXA effects in the BST are mediated by OX1Rs.

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ERP correlates of translation invariant facial adaptation

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Facial adaptation - induced by prolonged exposure to an individual face - can bias the perceived identity of a subsequently presented face. The goal of the present study is to test how presenting the adapter and test stimuli in different hemifields will affect the magnitude of the perceptual facial aftereffect as well as its ERP correlates (i.e. the increase in latency and decrease in amplitude of the N170 component).

Subjects performed a gender discrimination task for peripherically (6 deg) presented facial morphs of upright or upside down presented female and male faces.

Each trial consisted of a 5 sec adaptation period followed by a test face. During adaptation two stimuli were displayed on the two sides of the fixation: within a block they were either both Fourier phase-randomised images (control condition) or one was a Fourier image and the other was a prototypical female face. After 500 msec blank a test face image (chosen from female – male morphed facial image series) was displayed for 200 ms randomly on either side of the fixation. ERP was recorded from 27 channels.Throughout the experiments fixation was controlled by an infrared eye tracking system.

The psychophysical results showed strong adaptation effect both when the adapter and test images appeared on the same side of the fixation (SAME) as well as when they were presented in different hemifields (DIFF), compared to the control condition. However, the magnitude of adaptation was approximately twice as large in the SAME condition than in the DIFF condition. The adaptation effects on the N170 ERP component followed a similar pattern to that found in the psychophysical data. Interestingly, the behavioural and electrophysiological results were essentially the same for upright and upside down presented faces.

Our results provide evidence that facial adaptation consists of two components - one is translation invariant and the other is not - that might take place at different stages of face processing.

Distribution of glutamate-like immunoreactive neurons in the adult and developing nervous system of gastropod molluscs (Lymnaea stagnalis L.)

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Glutamate (GLU) has been known for a long time as a major excitatory neurotransmitter in both vertebrates and invertebrates. In invertebrates, its role was first of all described at peripheral neuromuscular contacts of arthropods (Sansom and Usherwood, Int. Rev. Neurobiol. 32:51, 1991), and in the giant fiber system and chromatophores of cephalopods (Messenger, Invert. Neurosci. 2:95, 1996). In gastropods, GLU-sensitive (Nesic et al., Neuroscience 79:1255, 1996) and GLUergic (Brierley et al., J. Neurophysiol. 78:3408, 1997) neurons were identified in the CNS of Lymnaea stagnalis. GLU-like immunoreactive (LIR) sensory neurons were found in the Aplysia CNS (Levenson et al., J. Comp. Neurol. 423:121, 2000). In the present study, we investigated the distribution and projection pattern of central and peripheral GLU-LIR neurons, providing their first detailed mapping in the adult and developing nervous system of a gastropod, Lymnaea stagnalis. In adults, it was established that i) altogether 45-50 GLU-LIR neurons are present in the CNS, located mostly in the cerebral and pedal ganglia; ii) central ganglia are connected by a bundle of labeled fibers running in the connectives and commisures, and the neuropils are innervated by GLU-LIR varicose fibers; iii) all peripheral nerve roots are supplied with GLU-LIR axon processes; iv) in the region of the foot, lip and tentacle, numerous GLU-LIR bipolar sensory neurons occur, meanwhile the buccal and foot musculature is innervated by varicose labeled processes; v) both the efferent and afferent projections are organized in bundles. In the juvenile Lymnaea, GLU-LIR elements display a similar pattern of distribution to that seen in adults, including the interganglionic bundle system, rich neuropil and peripheral afferent and efferent innervation. By the P3-P4 postembryonic stage, the number of labeled neurons in the CNS is identical with that of the adult. During embryogenesis the following events of the GLU-LIR system are sequenced by time: i) early innervation of the cerebral and pedal ganglia at E50% (metamorphosis) stage; ii) appearance of the first peripheral nerve roots at the E75% stage; and iii) full development of the CNS innervation and the circumpharyngeal bundle system, appearance of the limited efferent innervation of the foot and buccal mass region at E90% embryonic stage. At the same time, right before hatching, GLU-LIR sensory elements are present only in the caudal foot region. The present immunocytochemical observations are the first indicating a wide-range occurrence of GLU in central and peripheral neurons involved in both afferent and efferent functions in gastropods, and providing simultaneously a good basis for the future physiological-functional characterization of the GLUergic members of different networks underlying behaviors, such as locomotion and feeding.

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Alpha-dystrobrevin immunoreactivity in the adult mouse brain; occurence in glial endfeet and in a subpopulation of hypothalamic neurons

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The Dystrophin Associated Protein Complex (DPC) is a membrane-spanning complex of muscle sarcolemma. Mutations of DPC proteins can lead to various forms of muscular dystrophy, including Duchenne muscular dystrophy (DMD). It is currently recognized that DMD patients suffer from cognitive impairment in addition to their muscle disease, which clearly indicates that DPC plays an important role in the brain. The DPC links the extracellular components (matrix or presynaptic ligands) to the actin-based cytoskeleton and anchors various signalling molecules. Within the brain, the composition of this DPC is cell- and development-dependent.

In our previous studies we have localized several members of the DPC within the brain. Here we focused on alpha-dystrobrevin (alpha-DB), a unique dystrophin-related and dystrophin-associated protein, showing strong expression in the developing neurones (Lien at al., Gene Expression Patterns 4. 583, 2004) but decreasing drastically in the adult brain.

Cortical, thalamic and cerebellar neurons were negative for alpha-DB staining, as shown before. However, a selected population of hypothalamic neurons was clearly immunopositive. Using electron microscopy (EM), immunoreactivity was detected at synapses and, within the neuronal somata, in the membranes of the endoplasmic reticulum. This shows, for the first time, the existence of specific alpha- or beta-dystrobrevin-dependent subpopulations of neurons. Double-labelling studies will be used to characterize these alpha-DB immunopositive neurons further.

Prominent alpha-dystrobrevin immunoreactivity was also observed in the microvasculature throughout the brain, as reported before. EM studies revealed the labelling concentrated in astrocyte endfeet. These results agree with the suggested role alpha-dystrobrevin may have in blood-brain barrier.

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In vitro and in vivo pharmacological examination of the selective somatostatin 4 receptor (sstr4) agonist KD5621

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Neuropeptides released from the peripheral terminals of capsaicin-sensitive primary sensory neurones induce neurogenic inflammation in the innervated area: tachykinins like substance P evoke plasma protein extravasation, calcitonin gene-related peptide (CGRP) produce vasodilatation. On the other hand, somatostatin exerts anti-inflammatory and analgesic actions. The aim of the present series of experiments was to investigate the effects of a high affinity, somatostatin receptor 4 (sstr4) selective synthetic agonist, called KD-5621 on sensory neuropeptide release in vitro and acute inflammatory processes in vivo.

Release of substance P (SP), CGRP and somatostatin from isolated rat tracheae was evoked by electrical field stimulation and measured with specific and highly sensitive radioimmunoassay techniques developed in our laboratories. KD-5621 was added into the incubation medium at the beginning of each fraction. Neurogenic inflammation in the skin of the acutely denervated hindpaw of male Wistar rats was induced by topical application of 1% mustard oil and plasma protein accumulation was determined by the Evans blue leakage technique. In chronically denervated hindpaw, 5% dextran-evoked non-neurogenic oedema was measured with plethysmometry and bradykinin-induced plasma extravasation by the Evans blue method. The examined compound was injected i.p. 20 min before the induction of inflammation.

KD-5621 (100-2000 nM) concentration-dependently diminished electrically-evoked release of all the three measured neuropeptides, but did not influence their basal outflow. The EC50 values for the inhibition of the release of substance P, CGRP and somatostatin were 650.58 nM, 1.4433 uM and 11.0202 uM, respectively. It significantly, but not dose-dependently inhibited both neurogenic and non-neurogenic acute inflammatory processes in the dose range of 1-100 ug/kg.

These results suggest that KD-5621 acting on sstr4 effectively inhibits both neurogenic and non-neurogenic components of inflammatory processes, therefore it might provide a novel therapeutical target for the treatment of several inflammatory diseases.

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Seasonal dependent changes of monoamine levels during food ingestion in the snail, *Helix pomatia*

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In hungry motivated snails feeding is executed by cyclic feeding movements. In gastropods, motivation is raised in the CNS while patterned output for the cyclic feeding movements is generated by central pattern generator (CPG) located in the buccal ganglia. The cyclic feeding movements for both biting and swallowing are executed by separate muscles of the buccal mass and the esophagus which muscles are governed by buccal motoneurons. Both 5HT and DA proved to be effective in the modulation of the feeding program in different gastropod species. The giant cerebral 5HTergic neurons, the MGC in *Helix*, proved to be the extrinsic member of the feeding CPG and its activity correlated well with the feeding arousal in different gastropod species. Identified buccal DAergic neurons proved to be intrinsic member of the feeding CPG and their activity was able to initiate feeding cycles. We have observed that at spring both DA and 5HT levels increased during food intake in the CNS whereas during digestion DA level returned to the control whereas 5HT level decreased under the control level. Contrary to the CNS, in the buccal ganglia which contain the feeding CPG, only 5HT level increased during food intake which markedly decreased at satiation.

In this study we measured the DA and the 5HT levels by HPLC in the CNS and the buccal ganglia during food intake at different seasons. Moreover, the activity patterns of the MGC from starved and feeding animals were tested.

During spring and summer when the animals are active, HPLC assay of DA and 5HT showed that the levels of 5HT markedly increased during food intake in the CNS and the buccal ganglia whereas remained unchanged in the feeding muscles. DA levels increased in the CNS but decreased in the buccal ganglia and the esophagus. However, during autumn and winter when the animals prepare themselves for hibernation, the food intake evoked the decrease of both 5HT and DA levels in both the CNS and the buccal ganglia, whereas satiation increased both DA and 5HT levels. Recording from the MGC in isolated CNS preparation from starved and feeding animals during the different seasons showed that feeding increased the tonic firing frequency of the MGC at any season. However, the firing frequency of the MGC was low during the late autumn and winter.

These findings suggest that during spring and summer the external and internal feeding stimuli increase the activity of DA and 5HTergic neurons of the CNS and additionally they increase the synthesis of DA and 5HT in their central and peripheral segments. However, during autumn and winter the feeding stimuli do not increase the synthesis in the peripheral axon segments therefore the required amount of monoamine on the periphery is covered by a central pool of monoamine resulting in a decrease of the central monoamine level.

DOPA-decarboxylase immunocytochemistry showed that the central DA and 5HTergic neurons and their distal axon segments in the buccal mass and the esophagus were immunostained. However, during autumn and winter we failed to show immunostaining in the distal axon segments. These findings support our suggestion that during autumn and winter the required amount of monoamines in the peripheral organs is covered by a central monoamine pool resulting in a decrease of the central monoamine level.

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Developmental changes of PACAP and VIP expression in the chicken

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Pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal polypeptide (VIP) are two closely related peptides that bind to homologous G protein-coupled receptors, VIP/PACAP receptor I (VPAC1R) and VIP/PACAP receptor II (VPAC2R), with equally high affinity. PACAP also binds to a specific PACAP type 1 receptor. Recent reports suggest that, in common with VIP, PACAP is also involved in the development of the nervous system. To further elucidate the functional activities of these peptides, the present study aims to clarify the appearance and localization of PACAP and VIP in the brain of the chicken during ontogeny.

The immunoreactive peptide contained in the chicken brain was characterized by radioimmunoassay quantification. VIP and PACAP content was determined starting from embryonic day 3 to postnatal life. At all stages investigated, the predominant form of PACAP-immunoreactive material coeluted with synthetic PACAP38. Both VIP and PACAP immunoreactivity appeared early during embryonic life. High concentrations were found throughout the embryonic development, with higher PACAP-concentrations during the first half of incubation time. Consistent with our previous observations, highest concentrations were found in the diencephalon and brainstem. After hatching, both VIP and PACAP levels significantly decreased from day 3 in all studied brain areas. This is in accordance with observations in the mammalian nervous system. The occurrence of PACAP and VIP during embryonic life indicates that these peptides may exert neurotrophic activities also in the chicken nervous system.

Long-term treatment of male and female rats with low doses of the LHRH antagonist Cetrorelix and its effect on the gonadal axis

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High doses of the luteinizing hormone-releasing hormone (LHRH) antagonist Cetrorelix suppress the pituitary-gonadal axis. In order to block endogenous luteinizing hormone (LH) surges in women, Cetrorelix is used in various protocols for in vitro fertilization and embryo transfer, before ovulation is induced with gonadotropins. The profound deprivation of sex steroids by high doses of Cetrorelix has proved suitable for the therapy of sex-hormone dependent cancers. However, in conditions like uterine fibroma, endometriosis and benign prostate hyperplasia, where an incomplete hormone deprivation is indicated, lower doses of Cetrorelix may suffice. The aim of this study was to investigate the effect of a 30-day treatment with a low-dose depot formulation of Cetrorelix on the serum LH and sex-steroid levels in male and female rats. The changes in the expression of LHRH receptor (LHRH-R) protein and in the level of mRNA for LHRH-R were also determined in both sexes.

In both sexes, lower serum LH levels were observed on day 4 after administration. In males, LH returned to control levels by day 10, whereas in females, a rebound LH elevation occurred. Testosterone levels in male rats were decreased up to day 20, but on day 30, the values were similar to the controls. In females, serum estradiol was reduced on day 4; however, by day 10 it returned to normal. Progesterone levels were diminished through the entire period. Pituitary LHRH-R mRNA and LHRH protein levels were not significantly different from the controls.

These results suggest that Cetrorelix in low doses induces only a partial pituitary-gonadal inhibition and might be indicated for the treatment of certain gynecological diseases or benign prostatic hyperplasia.

The possible role of the different endogenous ligands in the antinociception

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Although, several methods and drugs are available for pain therapy, research is continuing in a search for the most appropriate procedure. 'Balanced analgesia' consists of administration of analgesics acting on different system to alleviate pain. The purpose of the present study was to investigate the antinociceptive interactions of endogenous substances – endomorphin-1, andenosine, agmatine and kynurenic acid – acting at different receptors (opioid, adenosine, α2-adrenoceptor/imidazoline and NMDA, respectively) at the spinal level in an inflammation-induced thermal hyperalgesia model in rats.

Materials and Methods

After obtaining institutional ethical approval, intrathecal catheters were implanted into male Wistar rats. After 4 days of recovery nociceptive threshold was assessed by using paw withdrawal (PWD) test. The PWD latencies were obtained before unilateral carrageenan injection, 3 h after carrageenan administration and then in every 10-min interval for 130 min. Dose-dependent effects were determined for all ligands and for their combinations. The drugs were administered at rate of 1 µl/min for 60 min. The area under the curve (AUC) values were obtained by calculating the area during (10-60 min) and after (70-120 min) drug administration . Groups were compared by ANOVA with $P<0.05$ considered significant.

Results and Discussions

Endomorphin-1 (0,1-2µg/min) caused a dose-depedent antihyperalgesic effect during the infusion, but the cessation of administration resulted in a gradual decrease in the PWD latency. Similarly, kynurenic acid (0,1-4 µg/min) also showed a dose-depedent increase in the pain threshold, but motor impairment could be observed at higher doses. In contrast, both agmatine (0.3-3 µg/min) showed very low efficacy. The co-administration of endomorphin-1 with any of these drugs caused a significantly potentiated antihyperalgesic effect. The infusion of the double combinations of agmatine, adenosine or kynurenic acid caused a dose-independent decrease in the thermal hyperalgesia. The endomorphin-1 containing combinations were the most effective in the relieving the thermal hyperalgesia. The effects of the combinations on the normal side were moderate.

Conclusion(s)

In conclusion, while these endogenous substances acting on different receptor systems have low potencies by themselves, the combinations of these ligands caused potentiated antihyperalgesia with decreased side-effects. These data may suggest a new way in the effective pain therapy supporting the significance of the combinatory drug administration of endogenous ligands.

Estradiol modulation of neurogenesis and PSA-NCAM expression in the dentate gyrus of the hippocampus of the adult rat

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Ongoing neurogenesis and maintained expression of the embryonic form of the neural cell adhesion molecule, rich in polysialic acid, (PSA-NCAM) are some of the main characteristics of highly plastic areas in the adult mammalian brain. Both proliferation and survival of newly born neurons in the dentate gyrus of the hippocampus are in close relation with the age and reproductive status of female animals, consequently, estradiol is implicated in the regulation of adult neurogenesis. Moreover, PSA-NCAM is continuously expressed in the granule cell layer of the dentate gyrus.

Our present study aimed to examine the influence of estradiol on neurogenesis in the subgranular layer of the dentate gyrus, and, on the other hand, whether this effect is correlated with the density of PSA-NCAM immunoreactive (IR) granule cells. Wistar female albino rats (n = 20) were bilaterally ovariectomized (OVX), then half of them received seven daily injections of 17 ß-estradiol (OVX+E). On the last day of estradiol treatment, all animals were injected with 5-bromo-2-deoxyuridine (BrdU) to label cells undergoing mitosis. Proliferation of cells was determined in the dentate gyrus of animals sacrificed 1 day after BrdU injection, while survival of the newly generated neurons was studied in rats killed 21 days following BrdU treatment. The density of BrdU labelled cells was not statistically different 1 day after the injection between the OVX and OVX+E groups, but it was significantly reduced after 21 days in the OVX+E group when compared to the OVX animals. The density of PSA-NCAM IR granule cells was also significantly reduced in the OVX+E group when compared to the OVX animals at day 21, and showed no statistically significant change after 1 day of survival.

Our results suggest that high 17 ß-estradiol level does not influence cell proliferation, but leads to decreased survival of newborn neurons in the rat dentate gyrus, and this drop in new neuron density is reflected by a decrease in density of PSA-NCAM IR cells. Thus, PSA-NCAM expression in the dentate gyrus is closely related to the survival rate of newly born neurons.

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Hypophysiotropic thyrotropin-releasing hormone and corticotropin-releasing hormone neurons express vesicular glutamate transporter-2 in the rat

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Thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH) are secreted into the hypophysial portal circulation by hypophysiotropic neurons located in parvicellular subdivisions of the hypothalamic paraventricular nucleus (PVH). Recently, these anatomical compartments of the PVH have been shown to contain large numbers of glutamatergic neurons expressing type-2 vesicular glutamate transporter (VGLUT2).

In this report we presented dual-label in situ hybridization evidence that the majority (>90%) of TRH and CRH neurons in the PVH of the adult male rat express the mRNA encoding VGLUT2. Dual-label immunofluorescent studies followed by confocal laser microscopic analysis of the median eminence also demonstrated the occurrence of VGLUT2 immunoreactivity within TRH and CRH axon varicosities, suggesting terminal glutamate release from these neuroendocrine systems.

These data together indicate that the hypophysiotropic TRH and CRH neurons possess glutamatergic characteristics. Future studies will need to address the physiological significance of the endogenous glutamate content in these neurosecretory systems in the neuroendocrine regulation of thyroid and adrenal functions.

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The role of dendritic spiking in generating rate and phase coding in hippocampal place cells

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Hippocampal place cells represent the spatial position of the animal by firing only in a restricted region of the environment, the place field of the cell. The spike train of the neuron shows both rate and temporal coding: the firing frequency of the neuron increases approximately until the middle and then decreases until the end of the place field whereas the phase of action potentials related to the ongoing theta field potential oscillation decreases monotonically (phase precession). Previously we have shown by analitical calculation and an integrate&fire model how an interplay between a somatic and a dendritic membrane potential oscillation can generate this doubly coding firing pattern. In this study, we verified the validity of this theory in a biologically more realistic model, a two compertmental neuron containing voltage dependent Ca^{2+}, Na^{+} and K^{+} channels; repetitive dendritic spikes triggering somatic bursts were the key elements of the model. Then, we analyzed the effect of dendritic excitation on dendritic spiking by calculating phase response curves. We propose that phase precession appears due to the higher amount of excitation from entorhinal cortex that the place cell receives when the rat is in a particular region of the environment. However, when the rat leaves this region, phase shift continues until dendritic spikes reach the stable phase again; this *free-running phase shift* can be either further phase precession or phase recession, when the direction of phase shift reverses, so that the firing phase of spikes increases. We hypothetise that the functional role of the free-running phase shift might be to provide stability for the temporal code. Finally we investigated the generating mechanism of the recently found property of place cell activity that the firing rate and phase can be uncoupled: while the phase code remaines unchanged, the firing frequency of the animal changes with the running speed of the animal. We found that a speed-dependent input to the soma from the entorhinal cortex can account for this independency of rate and temporal coding in hippocampal place cells.

Tolerance mechanisms in demyelination

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An autoimmune inflammation directed against the myelin sheath of the central nervous system (CNS) is presumed to induce damage of myelin and oligodendrocytes resulting in axonal loss and clinical disability in multiple sclerosis (MS). Here, we examined peripheral and thymic tolerance to CNS antigens in patients with MS and its animal model, experimental autoimmune encephalomyelitis (EAE).

Loss of anti-inflammatory natural killer T cells (NKT cells) characterized by an invariant T cell receptor (TCR) was observed in the peripheral blood of patients with MS during remission. In addition, expression of the invariant TCR could not be detected in CNS plaques of patients with MS, while it was evident in the lesions of an autoimmune demyelinating disease of the peripheral nervous system, CIDP. Thus, a defect in regulatory T cells may contribute to CNS autoimmunity.

The major antigens of the CNS, which can induce an autoimmune CD4+ T cell response resulting in EAE, are proteolipid protein (PLP) and myelin basic protein (MBP). Mice expressing a transgenic (tg) TCR specific for PLP develop fulminant spontaneous EAE reminiscent of MS. When tg mice were back-crossed onto an EAE-resistant genetic background, down-regulation of the CD4 co-receptor on tg T cells resulted in a decreased response to PLP and reduction of EAE. When EAE-susceptible and resistant mice were compared, higher expression of PLP in thymus was evident in resistant mice. These data suggest that thymic expression of a CNS antigen can modulate expression of the CD4 co-receptor on T cells with high affinity TCR, resulting in reduced autoimmunity and tolerance. Peripheral mechanisms can also contribute to tolerance to CNS autoantigens. Using mice bearing a transgenic TCR specific to an altered peptide of PLP, we showed that T cells tolerized to antigen could still respond to different cross-reactive ligands in a hierarchical fashion. Although a higher affinity superagonist ligand broke tolerance, the induced T cell response was characterized by production of anti-inflammatory cytokines. This altered molecular mimicry may thus prevent a detrimental autoimmune response and counter-balance the broken tolerance.

To change the autoimmune response, synthetic amino acid copolymers were designed and examined in a humanized double-transgenic mice expressing the human HLA-DR2 and a human MBP-specific TCR of an MS patient. Novel copolymers reduced EAE by several mechanisms. Beside competing with MBP peptide for the human HLA-DR2, novel copolymers induced an anti-inflammatory non-tg T cell response and prevented up-regulation of human HLA-DR on CNS microglia. Copolymers changed the cellular composition of CNS plaques and altered the expression of several genes examined by gene array.

The effect of diazoxide on the early phase of chronic cerebral hypoperfusion in rats

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Diazoxide (DIAZ) is a mitochondrial ATP-dependent K+-channel opener known to be neuroprotective by its ischemic preconditioning effect. Furthermore, we previously reported that microglial activation in the rat hippocampus could be attenuated by a post-treatment with DIAZ, while spatial learning performance was improved by both DIAZ and its organic solvent dimethyl sulphoxide (DMSO) at 13 weeks of cerebral hypoperfusion.

The aim of our recent study was to characterize the effect of DIAZ on spatial learning capacity and glial activation at an earlier phase (2 weeks) of chronic cerebral hypoperfusion. We also wanted to compare the potential, neuroprotective properties of DIAZ given as pre-treatment and post-treatment in our model of permanent, bilateral common carotid artery occlusion (2VO).

Chronic cerebral hypoperfusion was imposed by 2VO to create cerebral hypoperfusion, or sham operation was performed as control on male Wistar rats (n=59). Diazoxide (DZ) or its inorganic solvent NaOH were administered i.p. (0.25 ml) on 5 consecutive days before or after surgery (n=5-8/group). A week later, the rats were tested in a hippocampus-related learning paradigm, the Morris water maze. Subsequently, the animals were sacrificed and neuronal apoptosis (caspase-3), astrocyte proliferation (GFAP) and microglial activation (OX-42) were labeled with immunocytochemistry in 7 areas of the dorsal hippocampus.

Cerebral hypoperfusion-related learning dysfunction was prevented by pre-treatment but not by post-treatment with DIAZ. Caspase-3 activity was not altered by either 2VO or any of the treatments. Astrocytic proliferation and microglial activation were specifically enhanced (with 25% and 56%, respectively) in the hippocampus CA1 region only in 2VO rats that were post-treated with DIAZ.

In conclusion, DIAZ administered as pre-treatment is suggested to prevent learning dysfunction through its known effect of ischemic preconditioning, however, a post-ischemic application of the drug was ineffective in this respect. Further, DIAZ appears to influence glial proliferation in ischemia depending on the form of treatment (pre-or post-treatment) and the duration of ischemia. However, the exact molecular mechanism and functional significance of these changes remains to be elucidated.

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Novel tipp analogues containing beta-methyl amino acids, biochemical and functional characterization

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The discovery of the prototype delta opioid antagonists TIPP (H-Tyr-Tic-Phe-Phe-OH) and TIP (H-Tyr-Tic-Phe-OH) in 1992 was followed by extensive structure-activity relationship studies leading to the development of analogues that are of interest as pharmacological tools or as potential therapeutic agents (1).

New analogues (Dmt-Tic-erythroBetaMeCha-Phe-OH, Dmt-Tic-threoBetaMeCha-Phe-OH and their C-terminal amide analogues: Dmt-Tic-eBetaMeCha-Phe-NH2 and Dmt-Tic-tBetaMeCha-Phe-NH2) were developed containing structurally modified tyrosine residues (Dmt=2',6'-dimethyltyrosine) in place of tyrosine and beta-methyl amino acids (BetaMeCha) with all corresponding stereoisomers. The potency and selectivity (delta- vs. mu- opioid receptor) were evaluated by radioreceptor binding assays while intrinsic efficacy of these analogues were tested in [35S]GTPgammaS binding assays using rat brain membranes and Chines hamster ovary (CHO) cells stably expressing mu- and delta-opioid receptors.

Analogues showed delta-antagonist selectivity with differences regarding their isomeric forms and those analogues containing a C-terminal carboxamide group displayed a mixed mu-agonist/delta-antagonist profile thus they are expected to be analgesics with a low propensity to produce tolerance and physical dependence. These results constitute further examples of the influence of beta-methyl substitution and C-terminal amidation on the potency, selectivity and signal transduction properties of a peptide as well as they represent valuable pharmacological tools for opioid research.

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Mesures of stereopsis with visual evoked potential in adults and premature infants

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The onset ages for fusion and stereopsis in matured human infants was first studied with electrophysiological technique by Béla Julesz in 1980. The necessity of visual input for the development of the visual cortex was proven in several animal experiments. Determination of the onset ages for stereopsis in premature infants could be an important human experimental evidence to decide whether earlier onset of visual input could speed up the development of the visual cortex or intrinsic genetical programs are more important in the maturational time course for fusion and stereopsis.

In order to study premature infants, the method, elaborated by Bela Julesz, had to be adapted and tested in our laboratory. Dynamic random dot correlogram (DRDC) and dynamic random dot stereogram (DRDS) were presented on the red and green channels of a computer monitor at 1-2 Hz. The timing of the stimulus and the frame rate were controlled very precisely, the electrical scalp response was recorded from Oz-Fpz position, while the subject was viewing the stimulus through red and green goggles. The presence or absence of the Fourier component of the stimulus frequency was determined by T^2_{circ} statistic. In our study 21 young adults were tested with different variation of DRDS and DRDC. The optimal stimulus parameters (i.e. the largest scalp response in amplitude) were determined. Subjects with total stereo blindness and with decreased ability to perceive cyclopean stimuli were also found among the young normal adults. Results of the psychophysical tests on stereo acuity well correlated with the electrophysiological measurements.

Amblyopia accompanies with stereo blindness. Our preliminary experiment on adults demonstrate, that subjects with visus = 1.0 or close to 1.0 on both eyes may have stereo blindness, or impaired stereovision. The ability of subjects to perceive cyclopean stimuli shows individual differences, impaired stereovision is often present in subject with hypermetropic eye, and among subjects with eye vergence problem in their medical history. Our goal is to complete the experiments on premature infants.

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Intrinsic and extrinsic nitrergic neuronal components of the rat medial septum: a correlated light and electron microscopic immunocytochemical study

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Nitrogen monoxide (NO) is a membrane-permeable intercellular signal molecule which is detectable besides endothelial cells in several neuron types of the central nervous system as a co-transmitter. It was shown to play a role among others in synaptic plasticity. In the medial septum/diagonal band of Broca (MS/DB) NO influences the transmitter release from cholinergic and GABAergic projection neurons. Our aim was to study the nitrergic elements of the MS/DB of rat with the help of light and electron microscopic immunochemistry for neuronal nitric oxide synthase (nNOS) and with NADPH-diaphorase histochemistry. We described the types and distribution of nitregric cell types in the MS/DB which were identified mainly as cholinergic neurons. A synaptic target pattern of the nitrergic nerve terminals and varicosities was also established: nNOS-immunopositive terminals established mainly type 1 asymmetrical synapses (75%) but about 1/4 of the nNOS-positive synapses were type 2 symmetrical. The vast majority of their targets in both cases were dendritic shafts and dendritic spines and never somata or axon initial segments. About 23 % of the postsynaptic targets were also nNOS-immunoreactive. The GABAergic nature of the nNOS-positive terminals was proven with postembedding GABA-immunogold labeling. On the basis of fine structure and synaptic target pattern our results suggest that the terminals establishing asymmetrical synapses may belong to local cholinergic neurons forming an intrinsic neuronal network both with each other and with non-cholinergic local neuronal elements. Since the GABAergic cells of the MS/DB are not nNOS-immunoreactive, the origin of double-labeled axon terminals giving symmetrical synapses must be extrinsic. A suspected source may be the pedunculopontine nuclei, but tract tracing studies combined with GABA immunocytochemistry are needed to prove it.

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Neuroendocrine and thermoregulatory actions of ghrelin

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In the present experiments the effects of ghrelin on the hypothalamo-pituitary-adrenal (HPA) system and body temperature were investigated. Different doses of ghrelin (1-5 mcg) were administered intracerebroventricularly (icv.) to adult male rats, and plasma corticosterone was used as an index of the degree of the activation of the HPA system. The body temperature was tested by a biotelemetric monitoring system (Mini Mitter, USA). Icv. administration of ghrelin to rats caused significant increases in plasma corticosterone release and core temperature during telemetric observation. To determine the mediation of the corticosterone response induced by ghrelin, the serotonin 5-HT(2) antagonist, cyproheptadine was administered to the rats. The ghrelin-evoked HPA activation was diminished by preadministration of the serotonin antagonist. To characterize the transmission of the thermoregulatory action of ghrelin, animals were treated with the cyclooxygenase inhibitor noraminophenazone (NAP). The cyclooxygenase inhibitor, applied 30 min after the peptide treatment, transiently and significantly reduced the hyperthermic response. The present data suggest that ghrelin play an important role in the regulation of endocrine response via serotonergic pathway and prostaglandins seem to take part in the mediation of hyperthermic response.

Membrane properties of neural stem cells after in vitro induced neurogenesis by retinoic acid

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NE-4C cells, cloned from primary neuroectodermal cultures of p53-/- mouse embryos (E9) give rise to neurons and astrocytes when exposed to all-trans retinoic acid (RA). Changes in resting membrane potentials (Vrest) and the expression of voltage-dependent K+ and Na+ currents were carried out using whole cell patch clamp technique. To study membrane properties with respect to cell morphology, the cells were filled with Alexa Fluor hydrazid 594 via the patch clamp electrode. In non-induced cells, the majority of cells did not display voltage dependent currents but large passive conductance. Only few cells showed delayed outwardly rectifying K+ (KD) currents. Na+ currents or A type K+ (KA) currents were not detectable. In the course of differentiation, the cultures became heterogeneous and the current pattern well correlated with cell morphology and cell connections. The number of cells expressing KD and Na+ currents was significantly higher in differentiated cultures (RA-Day4-8) than among the committed neural progenitor cells (RA-Day2). Most of neuron-like cells - characterized by positive immunostaining for neuron-specific-tubulin - showed A.type K+ current besides the KD and majority of them displayed TTX-sensitive Na+ currents as well. The passive current pattern was restricted to some neuronally non-differentiated, substrate attached cells.

The responsiveness of GABA was also studied during the RA-induced differentiation. Moiety of both non-induced cells and neuronally committed cells (RA-Day2) displayed GABA-evoked currents, even before the appearance of neuronal processes. The rate of the GABA-evoked responses among the neuronally differentiated cells decreased.

We conclude that non-induced neuroectodermal stem cells and the persisting non-differentiated cells display large passive conductance and some of them show GABA-evoked currents. In the course of neuronal differentiation cells cease their connections, display Na+ and K+ currents and the rate of GABA-evoked responses decreases.

Role of cell to cell connections in the course of in vitro neuron formation

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Fate determination of neural stem cells is governed by signals derived from their microenvironment via multiple forms of contact and humoral cell-to-cell interactions. Contact intercellular communications has been shown to play important roles in the early development of the central nervous system. In the primary germinative layer of the neural tube, clusters of neuroectodermal cells are interconnected by gap junctions (Lo Turco and Kriegstein, 1991). Gap junctions are involved in the control of both, cell proliferation and differentiation. Major components of the cellular milieu surrounding neural stem cells are provided by cells of the astroglial lineage.

NE-4C – a p53-deficient, immortalized neuroectodermal progenitor cell line (Schlett and Madarasz, 1997) – has been used to study the in vitro neuron formation induced either by all-trans retinoic acid or by the presence of primary astrocytes. In recent studies, the gap junction coupling and the importance of gap junction communication between developing cells were investigated.

Based on dye spreading experiments we provide evidence on the neural stem cell/neural stem cell and on neural stem cell/astroglia coupling. In monotypic cultures of non-induced NE-4C cells, large gap junction coupled clusters were found. With the advancement of neuronal development, the majority of differentiating cells ceases gap junction communication with neighboring cells.

Our data demonstrate that gap junction communication plays essential roles in the astroglial induction of neuronal fate in NE-4C neuroectodermal stem cells. Gap junctions are readily formed between stem cells and astrocytes upon co-plating. A permanent blockage of gap junctional communication results in an approximately 30% reduction in the rate of astroglia-induced neurogenesis.

The time course of gap junction formation has been related to multiple parameters of neuronal cell fate determination by NE-4C model cells.

Role of neuro-immune interaction in control of state dependent changes in the brain

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Homeostetic control of temperature and in some extent of neuronal excitability is altered by induction of inflammatory reaction by LPS. As it is already disclosed, pro-inflammatory cytokines as IL-1β are able to induce sleep and also inhibit grand mal epileptic seizures. The regulatory role of IL-1β and some other cytokines as TNF□ or IL-6 in sleep waking cycle is a widely studied field but we know much less about the inflammation related excitability changes in the brain resulting in febrile seizures and altered state of perception cognition under fever. Since a great part of changes in the brain induced by inflammation involves the thalamo-cortical system, we initiated a wide range of studies on an absence epileptic rat strain (WAGRij) in which a tonic gentle hyperpolarization initiates generation of spike-wave discharges (SWD). The calcium spike genesis based SWDs were enhanced on a sleep state change independent manner. The elevation in SWD numbers reached 400% and it was dose dependent but body temperature independent. The cytokine release pattern induced by LPS was the well known profile and it was also dose dependent. As of the molecular mechanism of LPS action on SWD genesis is concerned, one possibility is the IL-1β release from perhaps thalamic reticular nucleus astrocytes. There is another putative way of action of LPS which is the direct effect of LPS on the brain TLR4 receptors but for that the crossing of LPS through the blood-brain barrier should be approved. We found the TLR4 receptor protein both in the thalamus and the cortex. There are data of others supporting induction of TLR4 mRNA expression by LPS. So the TLR4 track of action of LPS can not be totally excluded. Our WAGRij rat model provided a promising possibility for understanding LPS induced changes in sensation and state dependent control of the mammalian brain.

Comparative electrophysiological study of aggregation inhibitors on the neuromodulatory effects produced by beta-amyloid peptide

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Amyloid peptides (Aβ) play a crucial role in the pathogenesis of Alzheimer's disease. The aggregation of Aβ leads to fibril and plaque formation. The inhibition of this process may open an opportunity for the therapy of the illness. It is known that some short forms of the amyloid Aβ(1-42) show β-sheet breaker properties by preventing fibril formation. Here, we studied three new putative aggregation inhibitors: RIIPLa, Kyn-RIIGLa, RVVGVa. All pentapeptides were designed, synthetized and previously tested with MTT bioassay in the Department of Medical Chemistry. In our electrophysiological experiments (field EPSPs were recorded in motor cortical slices) one of the tested aggregation inhibitor (RVVGVa) protected neurons against this attenuating effect of Aβ(1-42).

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Systematic reduction of compartmental neuronal models

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Detailed compartmental models of neurons based on a precise anatomical reconstruction and incorporating a multitude of biophysical mechanisms can provide a fairly accurate description of the behavior of a single cell, but are far too complex to be suitable building blocks of large scale network models. On the other hand, the abstract neuronal models customarily used in network simulations often lack the distinctive characteristics of individual cell types, even though the intrinsic properties of neurons are known to be crucial determinants of network behavior. Thus, it is important to develop relatively simple neuronal models which nevertheless retain most of the physiological hallmarks of individual cell types. However, most such simplified models to date have been developed using entirely ad hoc procedures, which probably result in suboptimal reduced models. Therefore, we have attempted to develop a systematic procedure for finding simplified models which provide an optimal approximation of the behavior of complex compartmental model neurons.

A 455-compartment model of a CA1 pyramidal cell containing 11 different types of active conductance served as the target for simplified models. To measure the similarity of two models, the spatial pattern of the voltage response to synaptic stimulation in different parts of the dendritic tree was compared. Model selection and optimization itself proceeded in two stages. First, a clustering algorithm was run on the response patterns of compartments in the original model in order to determine which compartments are functionally similar to each other and could therefore be combined to form the compartments of the reduced model. Several clustering methods were tested, and the best results were achieved by applying a generalized K-means algorithm to logarithmically scaled data using a city block distance measure. As few as 5 clusters could provide a reasonable approximation, and these clusters corresponded to anatomically well-defined regions of the neuron. Next, the passive and active parameters of the resulting simple compartmental model were optimized. A good fit to the results of the clustering phase and to the original data could be obtained by using the simulated annealing algorithm implemented by the GENESIS neural simulator. These initial results illustrate the viability of our approach, and call for a detailed investigation and comparison of possible methods for the systematic reduction of neuronal models.

Sexually dimorphic expression of oestrogen receptor alpha and beta in the mouse suprachiasmatic nucleus

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Oestrogen has been reported to affect various aspects of circadian rhythm physiology. Since a subset of neurons within the suprachiasmatic nucleus (SCN), the principal circadian oscillator, has been reported to express the oestrogen receptor beta subtype (ERbeta), some of the effects of this steroid on circadian functions may involve direct actions at this site. The present study was designed to establish whether ERalpha and/or ERbeta expression in the SCN differs between male and female mice. Antisera (Z8P for ERbeta; C1355 for ERalpha) directed against C-terminal amino acid sequences of the receptors were employed to detect ER immunoreactivity. In gonadectomised mice, sexual dimorphism was observed in the number of ERalpha and ERbeta immunoreactive cells in the SCN; for both receptor subtypes a significantly higher number was found in the females. In both sexes relatively few cells were immunoreactive for ERalpha; in contrast, six to seven times more expressed ERbeta. Treatment with estradiol benzoate over 24 hours substantially reduced the number of cells immunoreactive for ERalpha or ERbeta in ovariectomized mice. Double-labelling immunohistochemistry identified ER alpha in calretinin-containing cells in the dorso-lateral cell group of the shell region. Unlike ER alpha, ER beta was found in vasopressin and calbindin neurons. The identification of both ER subtypes raises the possibility of differential mediation of oestrogen signals in the SCN. The sexual dimorphism in the expression of ERs may explain some of the previously reported functional and structural SCN differences in relation to sex.

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Anxiolytic-like behavior of AMPA antagonist 2,3-benzodiazepines

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The glutamatergic system has received considerable attention over recent years as potential target for anxiolytic drugs. There appears to be a balance between GABA receptor-mediated inhibition and glutamate receptor-mediated excitation in the amygdala that regulates behavioral responses associated with anxiety. Increase of GABAergic transmission or decrease of glutaminergic transmission leads anti-anxiety effects.

2,3-Benzodiazepines (2,3BDZs) are traditionally synthesised at EGIS Pharmaceuticals. Several compounds of this structural family have non-competitive AMPA antagonist effects. Aim of this study was to test 2,3BDZs in different animal models of anxiety. Independent tests for locomotor activity (spontaneous motor activity, inclined screen) were performed to distinguish sedative effects from anxiolytic activity.

The parent compound GYKI 52466, tested as AMPA antagonist on kainate-evoked whole-cell currents and on hippocampal field potentials proved to be active in three anxiety models, elevated plus-maze test (EPM), light-dark test (LD) and mCPP-induced anxiety model (mCPP) in non-sedative doses. Minimal effective dose (MED) of GYKI 52466 was especially low in EPM (0.003 mg/kg p.o.). GYKI 53405 and GYKI 53655 showed anxiolytic activity in two tests (3 and 1 mg/kg p.o in EPM, 3 mg/kg i.p. in mCPP, respectively) at doses lower than those resulted in sedative effects. NBQX (AMPA receptor competitive blocker) had anxiolytic activity only in one test (EPM). All compounds remained inactive in the drinking conflict model (Vogel).

Our results show that non-competitive AMPA receptor antagonists can profoundly block anxiety-like behavior in rodents independently from their motor depressant activity. However, the utility of AMPA antagonists as new anxiolytics might be hampered by their sedative-like properties at the tested higher doses.

The forebrain glucose-monitoring neural network: multiple roles in the central homeostatic regulation

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Microelectrophysiological invesetigations some 40 years ago demonstrated the existence of so called „glucose-monitoring" (GM) neural cells - with activity changes to increase of the extracellular glucose concentration - in the rat hypothalamus. Since then, similar 'chemosensitive' neurons have already been identified in various regions of the rodent and macaque brain.

To further characterize feeding-associated attributes of these GM cells, complex electrophysiological-neurochemical-behavioral experiments - by means of the multibarreled microelectrophoretic technique - were performed in several forebrain areas (such as the ventromedial hypothalamic nucleus /VMH/, nucleus accumbens, mediodorsal or ventrolateral /orbitofrontal/ cortex /OBF/) in adult Wistar rats and rhesus monkeys. Recently we showed that these „chemoneurons" got specifically activated and - after repeated applications - destroyed by microiontophoretically applied streptozotocin (STZ). Thus, in subsequent behavioral studies, bilateral STZ microinjections were administered into the rat VMH or OBF, and various feeding and metabolic functions were investigated to demonstrate adaptive homeostatic significance of the GM neurons.

In the single neuron recording studies, GM cells were shown to change in activity in response to various microelectrophoretically administered chemicals, as well as to intraoral gustatory stimulations. In addition, GM neurons of the primate forebrain also displayed characteristic firing rate changes during a bar press feeding task.

In the behavioral investigations, a single bilateral microinjection of STZ into either the VMH or OBF was found to result in the development of serious metabolic and feeding deficits. Furthermore, similar microinjection of the primary cytokine interleukin 1beta also elicited homeostatic dysfunctions.

These forebrain chemosensitive cells, by now, are considered to be elements of a hierarchically organized GM neural network along the whole rostrocaudal extent of the brain. The present findings substantiate intimate involvement of the GM neural network in the central regulation of feeding and metabolism, i.e., these chemoneurons appear to be indispensable for the maintenance of an adaptive homeostatic balance for the well-being of the organism.

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The somatostatin receptor expression and somatostatinergic innervations of gonadotrop releasing hormone (GnRH) neurons in transgenic mice

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In spite of the fact that somatostatin (SST) has a critical regulatory role in the hypothalamo-hypophysis system little if any attention has been given to the effect of SST on GnRH neurons, the principal neurons of the hypothalamo-hypophysis-gonadal axis. As a first step in determination of the physiological role of SST on GnRH neurons we have examined the SST receptor (SSTR) expression and the somatostatinergic projections in close apposition of GnRH neurons in male and female mice. In the first experiment, we determined the SSTR2 expression of GnRH neurons using heterozygous SSTR2 knock-out/LacZ knock-in transgenic mice. In these animals the SSTR2 expression on GnRH neurons can be easily quantified using X-gal histochemistry combined with GnRH immunohistochemistry. In the second experiment we investigated the somatostatinergic projections in close apposition to GnRH neurons using GnRH-GFP (Green Fluorescent Protein) transgenic mice. In these experiments the sections were immunofluorescently labelled for SST and the analysis was conducted on a confocal laser scanning microscope (CLSM) followed by the three dimensional reconstruction of GnRH neurons. Our results demonstrated that GnRH neurons express SSTR2 in female and male mice. We found that GnRH neurons of intact female mice expressed significantly higher level of SSTR2 than those of intact male mice. Additionally, the SSTR2 expression of GnRH neurons was decreased by gonadectomy in females but gonadectomy failed to have any effect in males. The CLSM analysis revealed close appositions of somatostatinergic neurons on soma and projections of GnRH neurons and there was no significant difference in the number of appositions on the soma between female and male mice. Our findings show that GnRH neurons express SSTR2 and this expression is sexually dimorphic and gonadal steroid dependent. Our data also demonstrate somatostatinergic appositions on GnRH neurons in both sexes. In conclusion, we suggest here a physiological and sex dependent role of somatostatin in regulation of GnRH neurons.

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Modulation of backpropagating action potential and synaptic stimulation-induced calcium transients by nicotinic receptors in dendrites of hippocampal interneurons.

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In this study we investigated the timing-dependent effect of nonsynaptic transmission mediated by nAChR activation. The facilitation elicited in the early phase of the coincidence of bAP and nAChR activation was made by the extra membrane potential change (by nAChRs) while the depression was created in the later phase of the coincidence by Ca2+ release from intracellular stores.

Also the synaptic stimulation was involved in the nAChR modulation:

In the presence of nAChR activation the synaptic stimulation induced calcium transients were wider and larger suggesting a new type of the modulation of the synaptic stimulation. (The compartments of the pyramidal cell are much more static because they are located geometrically to the spine)

Effect of subtype selective and channel blocker NMDA receptor antagonists on home-cage activity and body temperature in rats (Telemetry study)

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It is well known that NMDA channel blocker ligands like phencyclidine and MK-801 have psychotropic effects in humans and cause behavioural abnormalities in animals, such as motor stimulation. These actions hinder their clinical use as potential medication. It is thought that other types of NMDA receptor antagonists, e.g. subtype selective ligands, may be free from these CNS effects. In this study we investigated the effects of NR2B subunit selective NMDA receptor antagonists on home-cage motor activity and on core temperature in comparison with NMDA channel blockers.

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The studied parameters were monitored by radiotelemetry using a six-channel Mini-Mitter ER-4000 system. Wistar rats of 180-220 g were used.

After the treatment with the NMDA channel blocker MK 801 (0.03-0.3 mg/kg s.c.) and PCP (0.3-3 mg/kg s.c.) hyperactivity and dose-dependent hypothermia was observed. Ifenprodil (3-10 mg/kg i.p.) a polyamine site NMDA receptor antagonist and a preferential non-competitive NR1/NR2B receptor antagonist caused hypoactivity with concurrent hypothermia. Three NR2B subtype selective compound were examined. Dose dependent hyperactivity and hyperthermia were registered after the treatment with Co 101244 (3-30 mg/kg s.c.). Ro 256981 (25-100 mg/kg i.p.) elevated the home-cage activity in a dose dependent manner too, but the body temperature decreased after the higher doses.. CP 101,606 did not modified considerably the measured parameters.

In contrast to the two channel blockers which had similar effects the four NR2B antagonist compounds showed different activity pattern. Althogh actions on other, non-NMDA receptor types are likely to be involved in the behavioural effects of ifenprodile and also cannot be completely excluded in the case of the highly selective antagonists the observed differences suggest that the tested NR2B blockers may have different sites of action on the NR2B subunit. The results also call the attention that even compounds described as selective NR2B antagonists cannot be considered as a pharmacologically homogenous class. The manifested action of a given antagonist may depend on i) the intricate nature of the ligand-receptor interaction, ii) the actual subunit composition of the receptor (see e.g. Chazot et al., 2002) and iii) the regional distribution of the NMDA receptors showing "preference" to a particular ligand.

The antinociceptve interaction of adenosine and kynurenic acid at the spinal level

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Adenosine and kynurenic acid are endogenous ligands acting on different receptors (adenosine, NMDA) with a potential role in nociception at the spinal level. Their antinociceptive effects have already been investigated as monotherapy in different animal models, but only a few studies have reported on their effects on the potency of other drugs. The purpose of the present study was carried out to analyse their effects by themselves and their interactions during continuous intrathecal co-administration in a carrageenan-induced thermal hyperalgesia model in awake male rats.

A paw withdrawal test was used for nociceptive testing. The intrathecal infusion (60 min) of these drugs was administered alone or in combinations (0.01-4 μg/min kynurenic acid and 0.3-3 μg/min adenosine), which was followed by an additional 60-min observation period. For the time-course curves data sets were examined by analysis of variance (ANOVA) with repeated measures; the area under the curve (AUC) values were obtained by calculating the area during (10-60 min) and after (70-120 min) drug administration. The significance of differences between experimental and control values was calculated by using the Fisher LSD test for post hoc comparison.

Kynurenic acid alone caused a dose-dependent antinociception, however it was accompanied by significant motor impairment at higher doses (>0.1 μg/min). Adenosine alone caused a slight increase in pain threshold after the drug administration without any side-effects. However, independently of the applied doses all of the combinations not only significantly ($p<0.05$) increased the paw withdrawal latencies on the inflamed side during and after the infusion but also completely relieved carrageenan-induced thermal hyperalgesia. The combination was almost ineffective on the normal side. The combination of 0.1 μg/min kynurenic acid with different doses of adenosine caused dose-dependent side-effects (motor impairment in 25%), despite the fact that monotherapy with this dose of kynurenic acid did not result in adverse effects.

In conclusion, these endogenous substances that act on different receptor systems have low potencies by themselves, i.e. they produce only modest antinociceptive effects at doses which do not cause adverse effects at the spinal level. However, combinations of these ligands acting on several different receptors/systems can furnish potentiated, dose-independent antihyperalgesia with decreased side-effects.

The effects of GSM mobile phones on the autonomic regulation of the heart in young adults

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We have previously shown that low intensity electromagnetic (RF) fields emitted by GSM mobile phones have influence on single cell unit activity of the rat brain. Dosimetrical measurements have indicated that RF fields emitted by mobile phones, while they are held next to the head, can penetrate deep into the brain tissue and can be absorbed in the ipsilateral hemisphere. Therefore, some elements of the autonomic nervous system can be affected by the RF-exposure. In this study, we investigate whether RF fields have any effect on the regulation of the cardiovascular system. The aim of this study was to test if the exposure produced by a standard GSM mobile phone causes any changes in the cardiovascular functions. The RF exposure dose was comparable to the regular use of the device.

35 young (21-24) adults were tested. Two parallel signals, electrocardiogram (ECG) and surface pletysmogram, were recorded and compared to test heart rate variability (HRV) and pulse rate variability. The ECG signal measurement were taken with disposable electrodes attached to the thorax. Finger arterial pressure waves were monitored by infra-red reflexion surface pletysmograph. There were no differences between standard deviation (SD) values and mean RR intervals in the ECG or pletysmogram so we used the latter in our experiments. The RF exposures were tested in both genuine (test) or sham (control) conditions on two separeted groups. Both groups were first tested in resting position (5min) followed by standing position (sympathetic activation, 5min). Than the RF group were exposed to a 2W, 900 MHz, pulse modulated, continous electromagnetic field for 10 minutes, and the sham group were holding the same phone in the same position without any exposure. Right after this session, the pletysmographic measurements were repeated. Also it was repeated after a (30-50-70 min) recovery period. Blood pressure (BP) was measured in all sessions. 150 individual heart beats were analysed in all sections. We compared the normalised heart rate (HR) discharge, HRV (same as the SD-values), SD/HR, BP and the averaged SD discharge between the two groups in every situation.

There was no significant difference in the values of HR, HRV or BP between the RF and the sham groups. Although the normalised SD discharge (HRV) showed higher deviation in the RF group than in the sham.

In this study we have demonstrated that the RF fields emitted by mobile phones and absorbed by the brain did not have any observable effect on the regulation of the HR and BP in healthy, young adults. However, after the exposure, the HRV-deviation was more pronounced in the RF group than in the corresponding sham group. Based on this observation, we hypothesize, that there is a proportion of the population who can be more sensitive to RF fields. Further investigations should answer the question whether the RF fields have any effect on the heart of senior population or people with cardiac disorders.

MDMA (Ecstasy) causes long-term changes in the expression of the serotonin transporter

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The ring-substituted amphetamine derivative (±)3,4-methylendioxyamphetamine (MDMA) is the active ingredient of Ecstasy tablets which has become a widely used psychoactive drug among young people in Europe and overseas. In the central nervous system (CNS) acute MDMA causes the release of serotonin (5-HT) and, to a lesser extent, dopamine and norepinephrine. In the long term, however, MDMA has a neurotoxic effect and several studies suggest that MDMA causes serotonergic nerve terminal depletion and reduction in 5-HT, as well as in 5-hydroxyindolacetic acid (5-HIAA) concentration and reduces serotonin transporter (5-HTT) density in the brains of rodents and nonhuman primates.

The aim of our study was to seek evidence for these changes at the molecular level several weeks after the exposure to a single dose of MDMA. For this purpose the expression of 5-HTT mRNA was compared in MDMA-treated and control animals in the dorsal raphe nucleus.

Male Dark Agouti rats received either a single injection of MDMA (15mg/kg, i.p.) or vehicle (saline) were decapitated 21 days after the treatment. The expression of 5-HTT mRNA has been demonstrated and measured by in situ hybridization by using a 35S-labeled antisense probe. After drying, sections were apposed to Imaging Plate for 6 days. Averages of 9 slices per animal were included. For quantification of the hybridization signal we used NIH ImageJ software and measured mean grey values over the dorsal raphe nucleus and over a similar size of the surrounding area that did not contain serotonergic cells. Differences measured in grey densities were used for the evaluation and statistical analysis.

High levels of expression of 5-HTT mRNA were found in the dorsal raphe nucleus. Fairly high significant (P=0.01) decrease in the 5-HTT mRNA expression density in the dorsal raphe nucleus in the MDMA-treated animals 21 days after a single injection versus saline-injected rats. Our studies provide evidence that a single dose of MDMA causes long-term changes in the expression of 5-HTT in the dorsal raphe nucleus. This effect is very likely connected to the axonal and terminal lesion of 5-HT neurons induced by MDMA.

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Tactile sensing: sensors and algorhythms

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Tactile sensing is essential for the next generation of robots to perform useful tasks in unstructured environments. The benefits and needs for tactile sensing are demonstrated using a number of laboratory experiments that include combining vision and tactile sensing, gentle grasping, reorienting objects within the grasp and exploration of unknown structures. In living structures the sensing and „processing" parts are closely coupled providing „intelligent sensing".

Artificial tactile sensor arrays are models of the human mechanoreceptors located in the glabrous skin. These sensors transform forces applied to the sensor surface into detectable electric signals. Most commercially available sensors are capable to resolve only the normal pressure applied to the sensor surface while our sensors can measure shear forces too. The 2x2 sensor arrays are made by MEMS technology – the mechanical deformation of micro Si bridges is converted into resistivity change using the piezoresistive effect. The artificial sensors are covered with an elastic layer, like the mechanoreceptors under our skin. This rubber-like substance acts as a first signal processing layer and therefore has a very important role in the signaling pathway. The spatial resolution of the tactile array is 1.5 mm, the working range lies between 0.1 mN and 1 N depending heavily on the elastic cover.

With these sensors, integrated on a Katana robotic arm, we have built up an experimental system, which can measure and store some typical snapshots of topographic flows of pressure field in a tactile action and evaluate these fields by a set of analogic CNN algorithms. The arm is controlled in a closed loop based on the input from the tactile sensors. In these experiments we observed the mechanism of the grasping action of an unknown object and gained information about grasping force direction and distribution, torque and shear stress and object surface properties e.g. friction coefficient.

The localization of type 2 vesicular glutamate transporter (VGluT2) and/or vesicular acetylcholine transporter (VAChT) containing nerve fibres in the hippocampus and colocalization of the two transporters in axon terminals

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There is now fairly strong evidence for the view that the recently discovered VGluT2 is specific to presumed glutamatergic axons and axon terminals localized selectively in various regions of the hippocampus. In addition, the VAChT-immunocytochemistry is an excellent marker for terminal fields of cholinergic axons. VAChT-immunoreactivity visualizes very exactly cholinergic axon terminals probably by virtue of its presence on the membrane of synaptic vesicles accumulating acetylcholine. Using VGluT2- and VAChT-immunocytochemistry at light and electron microscopic level, the fine localization of VGluT2- and VAChT-containing fibre-bounds were examined in various regions of the hippocampus. In addition, applying double-label immunocytochemistry for studying the coexistence of VGluT2 and VAChT in the same axon terminals, investigations with confocal and electron microscopy were processed.

The findings of the light microscopic investigations showed that very dense VGluT2 immunoreactivity was found in fibres accumulated as a strong network on the granular and the supragranular part of the molecular layer of the dentate gyrus. On the other hand, a much less denser fibre bound comparing to the dentate gyrus containing VGluT2-immunoreactivity was observed to be localized continuously on the pyramidal layer of the CA1-CA3 regions of hippocampus. The VAChT immunoreactivity was found to be in fibres accumulated as fibre bounds in a localization similar to the VGluT2-immunolabelled networks. The findings of double immunofluorescent label confocal microscopic experiments, in which VGluT2 and VAChT immunofluorescent labelling were investigated, revealed the coexistence of the two transporters in the same axon terminals.

In our electron microscopic investigations the combination of silver-gold intensification of VGluT2-immunoreactivity with diaminobenzidine (DAB)-immunostaining of the VAChT, evidenced the colocalization of the two transporters in axon terminals both in the regions of the dentate gyrus and the Ammon's pyramidal layers. By these findings we have gathered further information proving that cholinergic axon terminals localized in the dentate gyrus and the pyramidal layers may contain also glutamate as excitatory transmitter.

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Effects of monosodium glutamate treatment on the neurobehavioral development of newborn rats

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Treatment with monosodium glutamate (MSG) during the first 2 weeks of postnatal life is known to cause neuronal degeneration in the arcuate nucleus of the hypothalamus along with retinal degeneration, changes in locomotor activity, learning deficits, feeding abnormalities and growth retardation. However, there are no data on the effects of MSG on the appereance of neurological reflexes and performance in these reflexes during neurobehavioral development of newborn rats. Data on changes in activity are also contradictory: both hypo- and hyperactivity have been reported. The aim of the present study was therefore to investigate the effects of MSG treatment on neurobehavioral development and open-field acitivity of newborn rats.

Newborn rats were treated with 4 mg/ml MSG on postnatal days 1, 3, 5, 9 and 11. Rats were investigated for appereance of physical characteristics and neurological reflexes such as geotaxis, righting, grasp, gait, placing and sensory reflexes. The time to perform righting, geotaxis and gait reflexes was also measured from the day of appereance. Weight was recorded daily. Open-field activity was measured 2, 3, 4, 6 and 8 weeks of age. Motor coordination was tested by inclined board, rota-rod, and foot-fault tests between 2-5 weeks of age.

MSG-treated animals had delayed appereance in air righting, geotaxis, ear twitch and placing reflexes. They performed worse than control animals in geotaxis, righting and gait reflexes. In the open-field, MSG-treated animals were more active only during the first 4 weeks of age, when they had increased time of ambulation, increased speed and rearing and covered more distance. Also, they showed reduced anxiety in the open-field as measured by the time animals spent at the walls. MSG-treated animals performed worse in all examined motor-coordination tests throughout the observation period. The efficacy of MSG treatment was verified by histological examination of the arcuate nucleus. In summary, the present results show that neonatal MSG treatment delays neurobehavioral development, decreases motor coordination and increases activity during the first weeks of postnatal life.

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Analysis and modelling of lateral connections in the cat visual cortex: implications for visual perception

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Psychophysical phenomena such as "grouping" of collinearly arranged and spatially segregated visual cue elements have been assumed to involve iso-orientation selective interactions. On the other hand, a number of phenomena, for example, saliency effects from orientation contrast cannot be explained by sole iso-orientation interactions alone. However, available data are largely based on the functional organisation of populations of cells that cannot take into account inter-individual variability among neurons. In the present study, we analysed this issue by modelling the two-dimensional (2D) distribution (spatial and orientational) of the population data and tested the ability of this model to predict the functional connectivity of single cells.

Methods: Functional mapping of orientation selectivity was carried out using intrinsic signal optical imaging in the primary visual cortex of the cat (n=9). Pyramidal neurons were labelled with extra- or intracellular injections of biocytin resulting in stained populations and individual neurons. The 3D reconstructions of labelled axon terminals of morphologically identified excitatory neurons were aligned with orientation maps. For modelling purposes, spatial and orientation components were estimated, respectively, by (i) a 2D Gaussian centred on the injection site for the population or soma of single cells and (ii) a von Mises function centred to the orientation preference of the injection site or soma. The density of connections was assumed to be proportional with the product of these components. An isotropic Gaussian was added in order to account for non-orientation-specific local connections.

Results: Labelling of a population excitatory connections originating from a single cortical locus showed a typical isotropic distribution locally and iso-orientation specific clustering remotely. The 2D structure of these connections was a good fit to the model (r2=0.65). In contrast to the population data, the 2D pattern of single cell lateral connections was poorly described by the model of spatial and orientation similarity. Moreover, the estimated model parameters varied considerably from cell-to-cell. Nonetheless, by pooling the single cell data (after aligning their spatial position and orientation preference) resulted in a similar distribution to that of the population label indicating that our single cell sample was representative.

Conclusion: The present findings indicate that the functional role of the long-range lateral network should be interpreted with caution. While at the network level horizontal connections display a strong bias towards a common pool of synaptic partners, individual neurons are capable of mediating more complex interactions.

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Is avian choroidal vasodilation regulated by a bisynaptical anatomical circuit?

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In birds, there are two possible homologues of the mammalian nucleus suprachiasmaticus: the medial and the lateral hypothalamic retinorecipient nuclei (MHRN and LHRN). Both of them receive retinal input and express the clock gene pPer2. It has been confirmed that the LHRN projects directly to the nucleus Edinger-Westphal (EW), and this connection is mainly, but not exclusively contralateral (Gamlin et al., 1982). The increase of choroidal blood flow (ChBF) was evoked by electrical activation of the contralateral LHRN and by light stimulation of the observed eye (Fitzgerald et al., 1996). The aim of this study was to clarify the anatomical organisation of this light-induced reflex by a double fluorescent tracing study using Fast Blue (FB) and biotinylated dextran amine (molecular weight: 3 kDa) (BDA).

The fluorescent retrograde tracer, FB was injected into the EW of 7-day-old chick. The other tracer injection (3 kDa BDA) was made into the medial part of the optic chiasm. The animals were transcardially perfused after 7 days of survival. After sectioning, the BDA was visualized with fluorescein (DTAF) conjugated streptavidin. The sections were analysed using a BIORAD confocal microscope.

FB positive cells are remarkably abundant in the contralateral LHRN, and a considerably amount of neurons occur also ipsilaterally. The cells projecting to the EW juxtapose with numerous BDA-containing boutons, moreover, there were cell bodies marked with BDA, too. Considering the high motility of 3 kDa BDA, the short distance between the place of injection and the axon terminals as well as the 7-day-long survival time, it is possible that the tracer diffused transsynapically and was taken up by the postsynaptic cells.

Our findings suggest that there is synaptic connection between the axons originating from the retina and the cells of the LHRN projecting to the EW. Since all, or nearly all neurons in the EW project to the ciliary ganglion (Reiner et al., 1991), the anatomical pathway underlying the light-induced increase of ChBF seems to contain only two synapses in the central nervous system.

Effect of 6-hydroxydopamine treatment on KAT-I immunoreactivity of neurons and glial cells in the rat substantia nigra

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Parkinson's disease (PD), a progressive neurodegenerative disorder, is characterized by a preferential loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC). In one of the commonly used toxin-induced PD models, 6-hydroxydopamine (6-OHDA), a selective catecholamine neurotoxin, induces neuronal damage in rat SNPC. Nerve cells in the SNPC are known to express tyrosin hydroxylase (TH). By means of immunohistochemical techniques, herewith we have shown that dopaminergic neurons in the rat SNPC express also kynurenine aminotransferase (KAT-I) the enzyme taking part in the formation of kynurenic acid (KYNA), the only known endogeneous selective NMDA receptor antagonist and a potent neuroprotective agent. Since KAT-I and TH co-exist in the same neurons of SNPC, we assume that KAT-I exerts a neuroprotective effect on dopaminergic neurons. Another aim of the present studies was to investigate immunocytochemical changes of KAT-I in the rat nigral dopamine neuro system after 6-OHDA injections into the lateral ventricle. Our investigations proved that increasing doses of 6-OHDA produce an increasingly severe loss of nigral KAT-I immunoreactive (IR) cell bodies which parallels with that of the TH IR ones; evidently, even though KAT-I does not prevent death of dopaminergic neurons, it appears that it may slow down disappearence of TH IR, as shown by densitometric studies. Furthermore, we found that under normal conditions, also astrocytes and microglial cells express KAT-I in the substantia nigra; the amount of KAT-I is, however, far less in microglia than in the astroglial cells. After 6-OHDA treatment, the number of both, micro- and astroglial cells increased in SNPC; the possible neuroprotective effect of KAT-I expressed by astroglia might be nullified, however, by the simultaneous presence of microglia.

Mapping cross-modal cortical network with the aid of double retrograde fluorescent tracing in rats

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The aim of our studies was two fold: 1, identifying the areas which send projection to both visual and somatosensory cortices and 2, defining the laminar origin of their connections with the visual- and somatosensory cortices to better understand their interactions in sighted and early blind rats. All the areas studied contained neurons retrogradely labeled either with Diamidino Yellow (DY) or True Blue (TB) after injecting the visual- and somatosensory cortex with the different tracers. Retrograde labeling appeared in a bilaminar pattern in the different areas clearly demarcating the supra- and infragranular layers of the isocortex. The quantitative analysis showed that except for the injected areas retrogradely labeled neurons are distributed similarly in the different cortical areas both following the somatosensory and visual cortical injections. The major difference in the distribution of the retrogradely labeled neurons appeared in temporal association area (TeA), which contained a larger relative amount of neurons in the enucleated group when compared to the control. It was found that a higher proportion of projection neurons was located in the supragranular layers in the TeA after visual cortical injection and, similarly, relatively more neurons project to the somatosensory cortex from the supragranular layers of motor cortex in the control group. This pattern was completely reversed after enucleation. These preliminary findings indicate that tactile and visual modalities are integrated within a common network although with an apparently different hierarchical position of the areas which differs in the sighted and early blind groups.

Rapid functional characterization of sodium channel blockers by fluorimetric measurement of membrane potential in primary cerebellar cell cultures

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Voltage-gated sodium channels (VGSCs) have an essential role in the generation and propagation of action potentials in neurons and several other excitable cells. Drugs modulating VGSC function are being used as local anaesthetics, antiarrhythmics, analgesics, antiepileptics, and could also be useful for the treatment of other disorders (stroke, bipolar disorder, etc.). On the other hand, currently available VGSC blockers mostly have a suboptimal clinical profile owing to their low potency and lack of selectivity. To facilitate the rapid discovery of new VGSC blockers we established a method based on the fluorimetric measurement of membrane potential in rat cerebellar cell cultures. The plant alkaloid veratridine was used to activate VGSCs. Veratridine evoked a relatively slow, dose-dependent depolarization of the cells with an EC50 of 6.4 μM. Drugs known to block VGSCs (TTX, crobenetine, lamotrigine, riluzole, sabeluzole, phenytoin, carbamazepine, lifarizine, flunarizine, lidocaine, tolperisone, etc.) inhibited the veratridine-induced depolarization in a dose-dependent manner. The potency of the blockers was strongly dependent on the dose of veratridine: TTX, for example, had IC50 values of 7, 11 and 28 nM, versus EC50, EC80 and EC100 concentrations of veratridine, respectively. The IC50 values of the blockers examined (versus EC80 concentration of veratridine) were comparable with published data obtained with other methods including electrophysiology. We also examined the possible contribution of several other ion channels, receptors and transporters (voltage-gated calcium channels, ionotropic and group I metabotropic glutamate receptors, Na+/Ca2+-exchanger) to the veratridine response. Selective inhibitors of the proteins mentioned above failed to show any effect on the veratridine-evoked depolarization with the exception of the Na+/Ca2+-exchanger blocking compound, KB-R7943, but this is more likely the result of the direct VGSC-blockade by the drug which has been reported by others as well. In summary we report the establishment of a robust, sensitive assay which – by using 96-well plates and a plate reader fluorimeter – is suitable for the functional characterization of VGSC blockers with medium-to-high throughput.

Neuronal and axonal changes in demyelination

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On the basis of imaging and pathomorphological studies on multiple sclerosis (MS) in addition to chronic demyelination axonal and neuronal injury and loss is supposed to play crucial a role in persistent disability after longer disease duration.

I our former studies (1) we have performed quantitative morphometrical analysis to estimate axonal loss and evaluate axonal changes in cervical spinal cord samples of patients suffering from secondary progressive MS. Completely demyelinated plaques, normal appearing white matter (NAWM) and anatomically identical regions from sex and age matched control cases have been compared. Neurofilament immunostaining was used for identification of the axons.

Highly significant reduction of axonal density (number of axons/mm2) in MS -both in the plaque and in the NAWM- versus the control cases was observed. Axons under about 3.3 □m diameter seemed to be more affected. The intensity of the immunostaining was significantly reduced in the plaque comparing to either NAWM or control.

In further studies (2) we investigated whether protooncogen c.jun was involved in neuronal response to experimental demyelination. Lesion specific expression was observed in nuclei of neurons whose axons transverse the demyelinated area.

Based on our result one can speculate that axonal loss in MS can not only be caused by direct immunological stress, but other process such chronic inhibition of axonal flow followed by neuronal apoptosis may also play a role.

Ultrastrucrural axonal changes in experimental demyelinisation caused by a copper chelator cuprizone will also be presented.

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Entorhinal lesions rearrange afferents, glutamate receptors, increase seizure latency and suppress seizure-induced c-fos expression in the hippocampus of the adult rat

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The left lateral entorhinal cortex was destroyed surgically in Wistar rats, and 40 days later seizures were induced with intraperitoneal 4-aminopyridine injection (5 mg/kg). The EEG analysis of freely-moving animals proved, that the entorhinal lesion increased the latency of the hippocampal seizure significantly and decreased the number of brief convulsions. Following convulsions, horizontal plane frozen sections were subjected to acetylcholinesterase histochemistry, calretinin- and c-fos immunohistochemistry, AMPA-, NMDA- and kainate receptor histoblotting. The number of c-fos-stained cell nuclei in the hippocampus have been used as indicator of the neuronal activation. Sprouting of cholinergic and commissural axons into the dentate molecular layer were observed 40 days after the ablation of the entorhinal cortex, which also decreased the seizure-induced c-fos immunostaining significantly in the hippocampus, compared to the controls. Whilst the level of AMPA receptors did not change, a significant increase of NMDA receptor (NR1 nad NR2B subunits), and KA2 subunit density has been detected in the denervated layers of the hippocampal formation. The GluR1 flop subunit displayed a significant decrease in every layers of the CA1 region. The results not only prove the importance of the entorhinal area in the spread and regulation of hippocampal seizures, but also emphasize the role of the rewiring of afferents and rearrangement of the ionotropic glutamate receptor patterns in the dentate gyrus in the generation of the hippocampal convulsive activity.

Key words: entorhinal cortex – hippocampus – seizure – c-fos – glutamate receptor – immunohistochemistry – rat

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Chronic fasting-induced changes of neuropeptides involved in food intake in the lateral septum of female and ovariectomized rats

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The effect of chronic food deprivation (60 % of the average daily food intake for 1-4 weeks) on the immunohistochemically detectable amount of leucine-enkephalin (Leu-enk), neuropeptide Y (NPY), and galanin (Gal) was studied in the lateral septum. Four groups of young adult Wistar female rats were set. Group 1 had intact female rats fed ad libitum. Group 2 had intact females fasted (60% of the average daily amount of food) for 4 weeks. Group 3 was ovariectomized and after 2 weeks of recovery fed ad libitum. Group 4 was ovariectomized and after recovery fasted as group 2. At the end of each week body weight was measured and blood test was taken and data were collected to prove the undernutrition in groups 2 and 4. Sixty µm vibratome sections obtained from brains after perfusion with 4 % paraformaldehyde were processed by the conventional protocol of pre-embedding NPY-, Leu-enk- and Gal-immunohistochemistry. The incubated sections from control and treated animals were photographed, and then computer-aided densitometry was carried out using Scion Image for Windows.

Ovaryectomy alone caused an increase in the immunocytochemically detectable amount of Leu-enk and NPY whereas the density of Gal-immunopositive elements was decreased. However, the amount of NPY was only slightly, whereas that of Leu-enk was significantly increased. The effect of fasting alone in the intact females resulted in equivocal density changes, probably due to the effect of periodically fluctuating level of female sexual steroids. The effect of fasting and ovaryectomy in group 4 was similar to that of measured previously in intact males except Gal, where an immediate decrease was detected after the first week of fasting.

Our results confirm previous data suggesting that LS belongs to brain areas influencing food intake and energy balance since the expression of the examined 3 neuropeptides showed characteristic changes as a consequence of food deprivation.

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Electrophysiological followment of various neurosurgical treatment to control tremor

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Ablation and deep brain stimulation have been proved to be effective treatments for tremor. The effects of only DBS implantation have been studied thoroughly on tremor characteristics.

The aim of the present study was to evaluate any possible link between the postoperative changes concerning the characteristics of resting hand tremor in Parkinson's disease (n=28) and essential tremor (n=5) and the type and target of 44 surgical treatments (ablation: 32, DBS: 12). Short-term and long-term tremor reduction, irregularity calculation and power spectra analysis were performed from accelerometric data acquired 2 days and 3 months after the surgery with comparison of original tremor measured 2 days before operation.

After effective surgical treatments significant tremor reduction, increase in frequency and irregularity were detected accompanied by power spectral alterations. Not the type and target of intervention, but the etiology of tremor determined the size of changes. Besides, comparing the short- and long-term tremor reduction, a significant increase was recorded after three months despite no clinical worsening was observed except in 4 cases. These 4 interventions were considered to be ineffective and clinically tremor recurred, besides the analysis showed no change in tremor characteristics.

Our results suggest that the presence of uniform postoperative changes in the morphology of rest tremor power spectrum, increase in irregularity and frequency-shift could be due to attenuation of pathological oscillators and might be immediate indicators of the effectiveness of neurosurgical treatments relieving tremor.

Changing estrogen level modifies mast cell-neuron interactions in the rat thalamus

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Although mast cells (MC) are immune cells of heamatopoetic origin, they are revealed in the central nervous system of many mammalian species. In the rat brain, they are located mostly in the thalamus, but their function is not defined yet. In our earlier work, we were able to demonstrate that thalamic MC can modify the firing activity of neurons. In the present study, we asked the following questions: 1. Whether the degranulation of thalamic MC have any effect on neurons in different developmental stages of female rats? 2. Could serotonin, histamine or releated antagonists influence the effects of MC on neurons? 3. Is there any correlation between the effects of MC and the stage of the estrous cycle.

Seven-barreled extracellular micropipettes were used for recording neuronal spiking activity and for microiontophoretic application of MC degranulator Compound 48/80 (C48/80). After the effects of C48/80 were compared with the action of iontophoretically applied serotonergic or histaminergic agents. To determine stages of the estrous cycle, we analysed vaginal smears, wich were stained with papanicolau stain orange G6 and papanicolau A,.

C48/80 did not modify the firing rate of cortical or hippocampal neurons (no MC are found here), however it caused excitation (69% in females, 11% in males), or inhibition (11% in females, 33% in males) in thalamic neurons, possibly due to MC activation. We found differences of neuronal responses in young (6-8 weeks) or adult females. In young females 68% of thalamic neurons responded with excitation to C48/80, while in adults 79%. With electronmicroscopical immunocytochemistry we found serotonin in MC granules, and adjacent neurons. Since serotonin is not localized in thalamic neurons, the products are of MC origin. H1 and H2 histaminergic antagonists could not affect the neuronal responses to MC's stimuli. Female rats in different stages of the estrous cycle responded differently to iontophoretic C48/80 administration: proestrous, no data; estrous, inhibition or no effect; metestrous, excitation; diestrous, no effect.

MC can strongly influence the firing activity of thalamic neurons. These modifications seem to be gender-specific and depend on the current stage of the estrous cycle in females. In earlier reports it has been shown that MC number is the lowest in estrous in the thalamus, and it was increasing in metestrous and diestrous. Therefore, the increasing number of MC in the thalamus often causes excitation, while low number of MC does not modify rarely might hiperpolarize neurons.

Changes in mitochondrial membrane potential and Ca(2+) concentration during epileptiform activity in individual mitochondria

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Several lines of evidence suggest that mitochondrial dysfunction contributes to the patophysiology of epilepsy and other neurological disorders. Mitochondrial calcium ion load during recurrent seizures might alter mitochondrial membrane potential and consequently decrease the proton motive force. By using electrophysiology combined with confocal laser scanning microscopy based imaging, we show for the first time that epileptiform activity under low-[Mg2+] condition results in mitochondrial calcium ion fluctuations and concomitant changes in the mitochondrial membrane potential.

Filamentous and granular mitochondria were identified in the soma and dendrites of patch-clamped CA3 cells in hippocampal slice cultures using the mitochondria-specific, voltage-sensitive dye rhodamine-123 for monitoring mitochondrial membrane potential changes. Interictal activity was associated with asynchronous, short (~10 s) mitochondrial depolarisation restricted to a few mitochondria within dendrites. By contrast, robust, homogeneous rhodamine-123 release into the cytosol was observed during seizure-like events (SLEs), indicating synchronous depolarisation of a large part of mitochondrial compartment, which lasted for several minutes. This was critically dependent on mitochondrial calcium ion uptake and extrusion, as inhibition of the mitochondrial calcium ion uniporter by Ru360 and the electrogenic calcium/sodium ion exchanger by CGP-37157, but not the mitochondrial permeability transition pore inhibitor cyclosporin-A, prevented the SLE-associated mitochondrial depolarisation.

The calcium ion dependence of the mitochondrial depolarisation suggested enhanced calcium ion cycling across mitochondrial membranes during epileptiform activity. By using mitochondria-specific, calcium-sensitive probes, rhod-2 and rhod-ff and spatial frequency filtering-based image analysis, we revealed short lasting (~10 s) fluctuations in mitochondrial calcium ion concentration that become synchronised upon transition from interictal activity to SLEs without net mitochondrial calcium ion accumulation. These findings support the hypothesis that mitochondrial calcium ion cycling during epileptic activity results in disturbance of mitochondrial function and consequently contribute to the seizure-induced cell death.

Facilitation of spike-wave discharge activity by LPS in WAG/RIJ rats

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Interaction between inflammation reaction induced fever and control of neuronal excitability is a critical point in understanding the crosstalk of immune system and brain functions. Extremely high fever and high sensitivity of subjects for fever could result in seizures and severe disturbances of brain function.

To induce experimental inflammation and fever in rats we applied different doses of lipopolysaccharide (10, 20, 50, 100, 350 μg/kg LPS injected ip.) of Gram-negative bacteria. The genetically epileptic WAG/Rij rat strain generating high voltage spike-wave discharges (HVS or SWD) dose dependent manner responded with an increase of number of SWDs to LPS injection. Elevation in SWD number was 200-400%. In the case of 10 and 20 μg/kg dose of LPS the increase in SWD number was significant only in 30-90 and 210-270 min after injection. At 50 to 350 μg/kg doses, the SWD number increased in each hour after injection, only the levels of significances changed by the applied dose of LPS. The highest elevation in SWD number was 400%, observed in 90-150 min after injection of 50μg/kg LPS. Applying higher doses of LPS (100, 350 μg/kg), the elevation of SWD number was not increased with the doses, even the elevation in SWD number were smaller (about 300%) than in the case of lower doses (50 μg/kg). LPS induced increase in SWD number was also verified on old Wistar rats performing SWDs.

Low dose of LPS (10 and 20 μg/kg) increased the body temperature with 1-1.5 °C. Medium dose of LPS (50-100 μg/kg) resulted in a biphasic change in body temperature and high dose of LPS (350 μg/kg) decreased the body temperature. Consequently, increase in SWD numbers at different doses of LPS did not correlate with the body temperature changes; it was an increase in SWD number at all doses studied.

The competitive N-methyl-D-aspartate (NMDA) receptor antagonist AP5 (2-Amino-5-phosphonopentanoic acid) were injected ip. with LPS to establish the effect of AP5 on SWD numbers. Low dose of AP5 (40 mg/kg) and LPS (20 μg/kg) showed an enhancing interaction when they were applied in combination.

Our data reveal a functional connection between epileptic activity and immune reaction and suggests common cellular targets of epilepsy and LPS induced inflammation reaction.

Organization of the 5-HTergic and GABAergic system during the embryonic neurogenesis of Eisenia fetida (Oligochaeta, Annelida)

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Organization of the 5-HT- and GABAergic systems was studied during the embryonic neurogenesis of Eisenia fetida, applying immunocytochemistry and HPLC. Neurogenesis was divided into 10 stages, starting by the appearance of the first immunoreactive neurons (E1), and finished by the hatching (E10). Development of the labeled elements started in two organization centers, namely from E1 in the rostral and from E3 in the caudal part of the embryo.

The first immunopositive neurons in the brain (CG) were GABAergic (1-1 cells), while 5-HTergic neurons appeared here only from stage E5 (3-3 cells). In the subesophageal ganglion both neuron types can be observed from stage E2. In the rostral part of the developing ventral nerve cord (VNC) the earliest born neurons were 5-HTergic (1-1 cells; E3) located centrally in the developing ganglia. These cells became the members of the rostro-ventromedial cell groups. In E4 GABAergic cells appeared in the rostro-ventrolateral part of the ganglia, while further 5-HTergic cells in the caudo-ventromedial part. During embryonic neurogenesis the number of labeled neurons gradually increased in all ganglia of the central nervous system but it did not reach that observed in adults.

In the CG the axonal processes of both cell types were gradually organized into commissures. The fibres in the ventral ganglia (VG) run contralaterally or ipsilaterally, forming nerve tracts organized in lateral and medial position. 5-HTergic lateral tracts appeared from E3, while the medial ones from E4. In this stage GABAergic fibres connected to the medial, then, from E5 to the lateral tracts. Some of the labeled axon collaterals leaved the tracts, and entered first two, later from E6 three pairs of segmental nerves. Both 5-HT and GABA innervation of the periphery can be observed from E3-E4.

In the second organizaton center located in the caudal part of the body only GABAergic neurons appeared in E3. During neurogenesis the 5-HTergic and GABAergic system of the VNC displayed different levels of development: ganglia located in the rostral body segments contain more stained neurons and fibers, than those situated caudally. The stomatogastric nervous system organized from E3, when first fibres appeared in the pharyngeal plexus. Labeled neurons of the stomatogastric ganglia can be seen first at the time of hatching. According to the HPLC measurements, the changes of 5-HT content during embryogenesis correlated with the alteration of the cell number.

The Edinger-Westphal urocortin 1 neuronal system: a potential central core mechanism in adaptation to stress

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Stress is the non-specific response of each organism including human, to any threatening demand, that over time results in wear and tear of the body, leading to diseases, such as cardiovascular pathologies and major depression. Therefore, adaptation to stress is a biological necessity to survive. Many of us succeed, but some of us fail. What could go wrong?

The stress adaptation mechanism has two crucial phases of operation. The so-called initiation and the recovery phase. A commonly accepted dogma in stress physiology states that an imbalance between the initiation phase (IP) and the recovery phase (RP) of the stress adaptation response underlies certain forms of stress-related body and mental diseases. Therefore, the stress-induced activation of IP and RP requires a timely and spatially balanced recruitment of the corticotropin releasing factor 1 (CRF-R1) and 2 receptors (CRF-R2) by the members of the CRF neuropeptide family. The activation of the hypothalamo pituitary adrenal axis (HPA) releases CRF that triggers the IP via the CRF-R1. In the RP urocortins are released, and activate the CRF-R2. Urocortin1 (Ucn1), highly expressed in the Edinger-Westphal nucleus (EW), however, has a unique characteristic in this stress adaptation cascade; it is promiscuous for the so-far known CRF-Rs, although it shows higher affinity for the CRF-R2. In addition, a large body of evidence has been collected that Ucn 1 contributes to the IP via CRF-R1, as well as mediates the RP by activating the CRF-R2.

We and others have recently obtained evidence that the EW is central in governing the recovery phase of the stress response. It does not only respond to various acute and chronic stressors, but also to the administration of various psychopharmacons (anxiolytics and antidepressants) as well as to ethanol. Based on these data we hypothesized that the EW Ucn 1 neuronal-system represents a central core mechanism in the mammalian brain that is crucial for the balanced activation of the IP and RP, and consequently for a successful adaptation to stress. It may also be a new possible target for the development of novel treatment strategies in anxiety and major depression.

In the present lecture I will first overview the recent knowledge on stress adaptation physiology, and address in detail the nature, functioning and regulation of the so-called Edinger–Westphal Ucn 1 neuronal system.

Two types of mu opioid receptor-1 expressing cells in the rat spinal subtantia gelatinosa

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Morphine and its analogs are potent analgesic substances. One of their major targets are the neurons in the spinal subtantia gelatinosa (SG) region expressing μ-receptors. Neurophysiological studies demonstrated that more than 40% of SG neurons responded to μ-receptor agonists. Detailed immunocytochemical mapping of mu opioid receptor-1 (MOR-1), however, identified only 10% of SG neurons as MOR-1 positive. These latter neurons have dense staining for MOR-1 receptor outlining the soma and dendrites as well.

We have tried to reconcile this discrepancy by using a combined morphological and electrophysiological approach. Responses to the μ-opioid agonist DAMGO were recorded with biocytin filled patch-electrode from SG neurons in rat spinal cord slices obtained from young (3-4 week old) animals. DAMGO evoked a membrane hyperpolarization in 37% (n=30) of the recorded cells. Among these neurons two groups could be identified: 63% responded with a relatively small (2.4-2.9 mV) and 37% with large (8.0-13.9 mV) hyperpolarization. Following the electrophysiological recording slices were fixed, resectioned and reacted with antibodies to reveal MOR-1 receptors. Using confocal microscopy we observed that neurons with large DAMGO-induced hyperpolarization tended to be the ones described previously as MOR-1 cells with dense receptor population in the membrane, in contrast, those neurons with small DAMGO-induced hyperpolarization had only a few patches of MOR-1 labeling.

We conclude that there might be two subpopulations of MOR-1 cells in the SG of the rat spinal cord with different receptor densities and response capabilities.

Astroglia-stem cell interactions: dynamics of astroglia-induced neurogenesis

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In the present study we show that astroglial cells instruct non-committed, immortalized neuroectodermal stem cells (NE-4C cell line) to adopt a neuronal fate, while they fail to induce the neuronal differentiation of embryonic stem cells (ES) under the same culture conditions. Time-lapse microscopic tracking and subsequent analyses of cell motility showed that astrocytes do not support the spreading and migration of the non-committed neural stem cells in „contact co-cultures". The hindered motilty results in the formation of clonally proliferating stem cell aggregates. Neuron formation takes place inside these stem cell assemblies. Differentiating progenies can leave the stem cell clusters, as astroglial cells selectively support the migration of postmitotic neuronal precursors. Also, astrocytes induce aggregate formation, and neuronal cell fate commitment of NE-4C in „non-contact co-cultures" as well, where the cells communicate only through the culture medium. The latter finding suggests that astrocytes release soluble factors responsible for the neurogenesis-inducing effect. Our data indicate, that astrocytes help to establish a microenvironment where stem cell/stem cell interactions can develop and the sorting out of the future neurons can proceed [1,2]. This work was supported by Hungarian Science Research Funds OTKA F038110, OTKA T034692 and OTKA T034995.

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Selective mitochondrial damage in nitrergic enteric neurons in rats after chronic alcohol consumption

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The neurotransmitter nitric oxide (NO) in the central and peripheral nervous system is generated by the conversion of L-arginin to citrulline by neuronal nitric oxide synthase (nNOS). In the enteric nervous system nNOS has been shown to be involved in various gastrointestinal diseases. Our previous results showed that in the enteric nervous system of rats after chronic alcohol consumption the activity of nNOS, the amount of nNOS protein and the number of nNOS-positive cells were significantly decreased. These results prompted us to study the ultrastructure of the intestinal wall of chronic alcohol-treated rats after preembedding immunostaining using a nNOS specific primary antibody.

Adult Wistar rats were used, receiving 15% ethanol solution for drinking for 2 months, the average daily alcohol intake was 15-17 g ethanol/kg body weight. The control group received water. The intestines were fixed with 2% glutaraldehyde and whole mount preparations were made revealing the myenteric neurons. The whole mount preparations were immunostained with nNOS antibody and processed for electronmicroscopy.

We found selective mitochondrial damage in the small intestine of chronic alcohol-treated rats. Most of the mitochondria were swollen and disrupted in nNOS-immunoreactive enteric neurons. Smooth muscle cells with disrupted mitochondria were occasionally seen. The mitochondria of epithelial, interstitial or endothelial cells seemed structurally intact. In immunostained neurons the DAB-nickel chloride precipitate was predominantly localized to the membranes of disrupted mitochondria.

The mitochondrial damage might lead to the overall imbalance of energy metabolism in nitrergic neurons of chronic alcohol-treated rats, thereby promoting both apoptotic and necrotic cell death. The mitochondrial localization of nNOS brings forth an interesting question. There has been accumulating evidence for the existence of mitochondrial NOS (mtNOS) having the same cofactor and substrate requirements as other forms of constitutive NOS. mtNOS has been shown to react with nNOS antibodies and has been identified as the alpha isoform of nNOS. Production of NO by mitochondria has important implications in the onset of apoptosis through peroxinitrite formation. The observed immunoprecipite possibly resulting from mtNOS further supports the hypothesis that the reason behind the decreased nNOS activity is apoptotic cell death in enteric neurons of rats after chronic alcohol consumption.

Co-expression of recombinant human GSK-3β and tau in HEK-293 cells

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β protein and paired helical filaments (PHF) composed of hyperphosphorylated tau in neurofibrillary tangles (NFT). Tau is a group of microtubule-associated proteins expressed predominantly in axons. The function of tau is influenced by its phosphorylation status. Hyperphosphorylated tau has a reduced affinity for microtubules (MT) leading to the instability of the cytoskeleton that may trigger neuronal degeneration. Thus the regulation of tau phosphorylation seems to be an important factor in the pathogenesis of AD. In vitro studies have shown that glycogen synthase kinase-3β (GSK-3β), a serine/threonine protein kinase, plays an important role in the regulation of this process.

To examine the function of GSK-3β in tau hyperphosphorylation we developed a cell-based assay using HEK-293 cell line. This cell line provides a model in which tau phosphorylation can be investigated. HEK-293 cells were co-transfected with human GSK-3β and human tau cDNAs using the inducible mammalian expression vector pIND/Hygro and pIND/Neo, respectively. Cell colonies resistant for the selecting agents were picked and tested for hGSK-3β and htau mRNA as well as protein expression using quantitative RT-PCR and flow cytometry based immunocytochemistry, respectively. According to our results, there was a good correlation between mRNA levels and protein immuno-reactivity. Protein expression in the generated clones was three to five fold over the basal level. One clone named H11 exhibited the highest GSK-3β and tau levels; therefore this clone was chosen for investigation of tau phosphorylation using phosphorylation-site specific anti-tau antibodies.

In H11 cells pre-treated with the inducing agent muristerone A (MuA), a significant increase in tau phosphorylation at specific GSK-3β-dependent phosphorylation sites (Ser 199-202 and Ser 396-404) was detected. Immunocytochemical analysis revealed a dose-dependent up regulation of tau hyperphosphorylation with increasing concentration of MuA. In addition, cells pre-treated with the GSK-3β inhibitor LiCl resulted in reduced phosphorylation of tau. Thus, this cell line stably and inducibly co-expressing hGSKβ and htau proteins can be a useful tool to investigate the effect of GSK-3β inhibitors on tau phosphorylation.

Age- and hormone-related changes in the expression of GAP-43 in the olfactory bulb

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GAP-43 (also known as F1, B50, neuromodulin, pp46) is a membrane bound phosphoprotein and putative growth-associated protein abundant in axons, but not dendrites, during brain development. GAP-43 levels are elevated in the brain during the period of neurite outgrowth, extension and synaptogenesis, with a subsequent decrease by 90% with brain maturation. In the perinatal period GAP-43 is expressed in all neurons, then its expression becomes progressively restricted such that by maturity most neurons no longer express detectable levels, although GAP-43 expression is still moderately high in the adult entorhinal cortex, and strikingly high in the adult hippocampus and olfactory bulb.

In the present study we aimed to examine the age- and hormone-dependent changes in GAP-43 expression in the rat olfactory bulb, a brain area with constant synaptic remodeling. By western blotting we could demonstrate that the level of GAP-43 is decreasing with age, in sexually matured animals its expression is lower in males. There is a well-defined cyclic change in intact females with higher values on proestrus day. In ovariectomized animals 17 ß-estradiol increased GAP-43 expression with a peak after 6 hours of hormone injection. GAP-43 immunoreactive structures could be demonstrated in olfactory glomeruli, the staining intensity reflected the changes shown by western blotting.

The results support the notion that GAP-43 gene expression is sexually dimorphic. Moreover, the hormonal regulation of GAP-43 may account for its putative role in structural remodeling and functional plasticity of the nervous system.

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Ca^{2+}/calmodulin kinaseIIα expression in mouse central nervous system during pre- and postnatal development

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The purpose of the current study was:

- generation of a transgenic mouse model, which provides an easy way to identify the principal glutamatergic neurons in the central nervous system

- to follow and characterize the GFP expressing cell-types during development

The principal glutamatergic neurons are characterised by the presence of the Ca2+ /calmodulin kinase (CaMKII) enzyme, which is the major postsynaptic density protein. This kinase is highly concentrated in brain, where it is thought to play important roles in the synthesis and release of neurotransmitters, ion channel regulation, structural modification of the cytosceleton, axonal transport, synaptic plasticity and gene expression. In mammals, four closely related isoforms of CaM kinase II termed α, β, γ and are encoded by distinct genes, each gene shares 80-90% nucleotide sequence identity. While the γ and subunits are ubiquitously expressed, the presence of the α and β subunits is restricted to the nervous system. For creating the transgenic construct we used the promoter region of the CaMKII alpha subunit gene fused to the coding region of eGFP. The 5`flanking region of the CaMKIIα gene contains highly conserved domains with regulatory role, and has been previously shown to confer brain specific expression.

Four transgenic lines were established, in which the presence of the transgene was shown by Southern blot analysis. GFP expression was detectable in entire embryos and sections, with the use of UV light, as well as by GFP immunocytochemistry. Previous papers have described a low level of CaMKIIα expression in newborn mice which increases considerably in the first three weeks. In our lines however the expression was already detectable during embryonic development, which has not been reported in the literature so far. To test if transgene expression resembles that of the endogenous gene, CaMKIIα mRNA levels were assayed during development by semi-quantitative reverse transctiption-polimerase chain reaction (RT-PCR) and the CaMKIIα protein was visualized by immunocytochemistry.

The CaMKIIα-GFP transgenic mice could serve as ideal model for further anatomical, electrophysiological and developmental studies.

On possible functional taxonomies of neural networks

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A goal of definition network-classes may be the traditional demand of prediction: describe the structure based on dynamics or vice versa. One is an observed or supposed item, the other one is deduced from the first. This classical project is in a part naive, moreover ill-posed because without or even with further fixed concepts it cannot be solved unequivocally and in a single canonical way. Moreover, the concept of function is dependent also on extra-net objects which control or which are controlled by those nets. Present aims of taxonomy differs Linnean tradition, albeit it implies comparative neurobiological connotations too. It is different also from the trendy technical classification of network-learning-mechanisms attributed to neural nets during last decades and resulting in new technical network metaphores. Recent approaches based on random-graph-theory provide some understanding of neural networks en gros but tell very few or nothing about specific functional-stuctural relationships.

The present attempt to networks defines two graphical objects: [1#] the structure of nets are 2-edge-coloured,

2-node-coloured digraphs, while [2#] the dynamics of nets is defined automatically as soon as state-transition functions are attributed to nodes. These dynamical graphs are described by the system of their transient-forest and attractors.

The two colours of edges correspond to autoactive and autosilent units of net, while the two classes of edges are for representing excitatory and inhibitory influences. Detailed dynamical phenomena like temporal behaviour, adaptation etc. can be included into atomic state transition laws attached to nodes. This determines both state-space and transitions of states.

Independent modification laws or non-autonomous behaviour can usually be superimposed to the previous prescription. Also, the interpretation of units or their nets is arbitrary.

The number of possible structures or dynamics is enormous, however their classes can be enumerated. One specific goal is just their invention, listing, enumeration and selection cases irrelevant or interesting for neurosciences. These actual enumeration problems require sophisticated group-theoretical tools, like e.g. an analysis of automorphism groups of neural network structures.

Cases of functional predictions and limits are presented. Various units were applied. E.g.: regular patterns of special chaotic spiking neurons is demonstrated. Also, examples of various kinds of spontaneous synchronizations or desynchronization are easily designed.

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Mathematical model for neuro-mechanical control of limb movements

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We present a neuro-mechanical concept and mathematical model that aims to compute the angular changes in the joints of a limb knowing the stimulation pattern of motoneuron pools and biomechanical characteristics of the system. The general model is being developed (Laczko, Walton & Llinas 2003,2004) for establishing relationship between motoneuron activity and the muscle forces underlying joint rotations and for simulation of limb movements.

The motor command for each muscle is modeled as a sequence of stimulation pulses. In each time interval, the command for each muscle is a single element that is the number of action potentials arriving from the muscle's motoneuron pool. Using this command, the model generates angular motions in the joints. We study how the firing rates of motoneuron pools of flexor and extensor muscles are associated with certain angular changes in the joints. The model considers muscle force-length, muscle force - neural stimulation frequency, muscle force - shortening velocity relations, geometric and inertial properties of the limb segments, muscles and tendons. Sensory-motor transformations are included by simulation of the Gamma loop and the gravitational force is imitated.

We address the inverse problem facing the issue that there are an infinite number of motoneuron activity patterns that can implement the same movement. We compute firing frequencies of motoneuron pools that result planned angular changes in the joints of a limb. We approach the emerged inverse problem assuming that one flexor-extensor muscle pair articulates each joint. Each muscle spans only one joint and one of the pair of the muscles is activated at a given time.

This model is a research tool that is applied for discerning hidden properties of neural motor control. Using the model we simulate human leg movements. We show that the computed extensor activities are higher than flexor activities for single joint movements and the model leads to angular movements that require minimal total work from the muscles.

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An oscillatory hierarchy controlling neuronal excitability and stimulus processing in auditory cortex

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Electroencephalographic (EEG) oscillations are hypothesized to reflect cyclical variation in the excitability of neuronal ensembles, with particular frequency bands reflecting differing types and spatial scales of brain operations. Interdependence between the gamma and theta bands suggests an underlying structure to the EEG spectrum, and there is also evidence that ongoing activity influences sensory responses. However, there is no unifying theory of EEG organization and the role of the ongoing oscillatory activity in sensory processing remains controversial. This study analyzed laminar profiles of synaptic activity and action potentials, both spontaneous and stimulus-driven, in primary auditory cortex. We find that - 1) The EEG is hierarchically organized; delta (1-4 Hz) phase modulates theta (4-10 Hz) amplitude, and theta phase modulates gamma (30-50 Hz) amplitude. 2) This Oscillatory Hierarchy controls baseline excitability and action potential generation, as well as stimulus-related responses in a neuronal ensemble. We propose that the hierarchical organization of ambient oscillatory activity allows auditory cortex to structure its temporal activity pattern so as to optimize the processing of rhythmic inputs.

The antiepileptic action of a putative glutamate receptor antagonist in vitro

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Despite recent developments in anticonvulsive drug therapy, the available medicines can effectively control seizures only in about two third of the epileptic patiens. Therefore search for new drugs – favourably acting on new targets – is needed. A novel putative Gluergic antagonist (Szárics et al., 2001, Mol. Pharmacol. 59, 920) and anticonvulsant (Lasztóczi et al., 2002, Neuroreport 13, 351), a quinazolone alkyl carboxylic acid derivative (Q5) was now tested against seizures evoked in hippocampal slices from rat pups (P9-P13). To elucidate the mechanism of action, the effect of Q5 was explored in measurements of binding and function.

Q5 was bound to [3H]-Glu-labelled sites in brain tissue homogenates containing resealed plasmalemmal vesicles (Szárics et al., 2001, Mol. Pharmacol. 59, 920). Q5 did not interact with AMPA, kainate and NMDA receptors and Glu transporters. However, Q5 partially antagonised Glu-induced [35S]GTPgammaS binding, suggesting action on a metabotropic Glu receptor.

Q5 (0.2 and 0.5 mM) suppressed seizure-like events (SLE) recorded from the CA3 region of rat hippocampal slices perfused with low-[Mg2+] ACSF in 60 % and 80 % of cases, respectively. By comparison, the competitive (0.01 mM CNQX) and non-competitive (0.1 mM GYKI 52466) AMPA receptor antagonists reduced the duration of SLEs to 61 ± 5 % and 51 ± 5 %, respectively, but did not completely block them.

Q5 (0.5 mM) reduced the frequency (38 ± 9 %), but not the amplitude of spontaneous EPSCs and both the frequency (21 ± 3 %) and the average amplitude (47 ± 7 %) of spontaneous IPSCs in CA3 pyramidal cells. The action potential-independent miniature IPSCs remained unaffected by Q5. These data indicate an effect on a constitutively active receptor that regulates the excitability of both pyramidal cells and interneurones. Therefore we tried to mimic the effect of Q5 by different metabotropic Glu receptor ligands. Specific antagonists of metabotropic Glu receptors, including group I (0.5 mM AIDA), group I/II (1 mM (R,S)-MCPG) and group III (0.1 mM CPPG) did not affect spontaneous IPSCs. Also, Q5 did not reverse the effect of a broad-spectrum metabotropic Glu receptor agonist, t-ACPD (0.03 mM) on interneuronal activity. By contrast, t-ACPD (0.05 mM)-induced intracellular [Ca2+] enhancements in putative interneurones of strata oriens and radiatum were reduced by 0.5 mM Q5.

The above findings suggest Q5 acting at a metabotropic Glu receptor, pharmacologically not characterised yet. Data indicate a strong anticonvulsive action of Q5 even under conditions when complete blockade of AMPA/kainate receptor mediated excitation is ineffective. This Q5 action may be linked to its ability to reduce the overall excitability of both principal cells and interneurones.

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Immunohistochemical localization of cocaine- and amphetamine-regulated transcript peptide in the central nervous system of the frog xenopus laevis

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The distribution of cocaine- and amphetamine-regulated transcript peptide (CARTp)-like immunoreactivity was only studied, until no, in the CNS. In mammals, CART peptides occur, among others in brain areas that control feeding behavior. The distribution of CARTp immunoreactive structures in the CNS of the amphibian Rana esculenta and the rat showed differences in the visual system, olfactory bulb, preoptic area and the motor nuclei. We assumed that these differences may be related to the different feeding behavior of the two species. The feeding activity of the African clawed frog, Xenopus laevis, is similar to that of the rat. It feeds continuously, irrespective of the season.

The aim of the recent study, was to map the occurence of CARTp in the Xenopus brain and compare it to Rana, that does not feed in winter. In Xenopus, immunoreactive cells and fibers were very sparse in the olfactory bulb. Stained cells occurred in the nucleus accumbens, amygdala, medial pallium, septum, striatum, preoptic nuclei, anterior, central and ventromedial thalamic nuclei, the lateral geniculate nucleus and the hypothalamus. The neurohypophysis showed intense immunostaining. In the mesencephalon, many cells were stained in the Edinger-Westphal nucleus, the superficial layers of the tectum and the laminar and magnocellular nuclei of the torus semicircularis. In the periventricular layers of the tectum, intense fiber staining was characteristic. In the rhombencephalon, cells were stained in the raphe nuclei, central gray, nucleus of the solitary tract and the vicinity of motor nuclei. Neurons of the motor cranial and spinal nerves were surrounded by CARTp positive grains. In the spinal cord, preganglionic cells and beaded axons were stained. Differences were found between Rana and Xenopus in the distribution of CARTp in the olfactory bulb, diencephalon, visual system, preoptic area and the mesencephalic tegmentum. Some of these differences may be related to the feeding behavior of these animals.

Homeostatic regulation, hunger and addiction

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The role of homeostatic regulatory processes is to maintain the integrity of the organism. However, feeding behavior is much more than calory intake and its regulation is not restricted to only a viscerosensory – humoral feed-back operation. Hunger drive will lead to feeding if the appropriate environmental incentives are present, and primary and secondary (learned) cues will guide the organism to find the food reward. Feeding behavior is controlled through continuous monitoring and integration of endogenous chemical information and exogenous sensory signals. Exogenous chemical cues that guide food intake are primarily used in various instinctive and learned feeding actions. The chemical senses (taste and smell) are also utilized in detecting possible sources of foods and fluids. Gustation in particular is the final judge in decision concerning the acceptability of a potential food implying that its analysis must not only identify a chemical compound but is also has to evaluate the physiological consequences (for energy homeostasis) of its ingestion.

On single neuron and behavioral levels our experimental evidences in rats and rhesus monkeys show that amygdaloid and prefrontal mechanisms are involved in detection of taste, smell and the texture of food and hedonic evaluation of flavour. In the rat, dopamine depletion of the prefrontal cortex results in changes of ingestive and rejective responses to different taste solutions, and rejective responses can also be detected to glucose solution. Limbic and prefrontal dopaminergic mechanisms are important in stimulus – reward reinforcing associations including feeding and drug addiction. On the basis of our HPLC microdialysis experiments it has been revealed that after food deprivation feeding induces dopamine release and it can be modified by changes of blood glucose or insulin levels. It is supposed that under pathological conditions rewarding consequences of dopamine release may result in disturbances of normal regulation of feeding and changes in hedonic evaluation of food leading to the appearance of bulimic episodes and/or development of obesity.

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Toxic effects of three insecticide agents on cortical and peripheral electrophysiological parameters in rats, given in double combinations during phases of ontogenesis

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Most of the insecticide agents, used in modern agriculture and insect control, act on the nervous system. Due to their, incomplete target specificity, humans with occupational, environmental, food-borne etc. exposure can suffer neurotoxic damage. Several insecticides with partly or fully different way of action are in use, and the resulting combined exposure may induce novel effects in the nervous system with a number of unanswered questions.

In this study, three insecticides were used: the organophosphate dimethoate (D), the carbamate propoxur (P) and the pyrethroid cypermethrin (C). These were given in DP, DC and CP combinations, 1/25 LD50 each, to female Wistar rats by gavage; from day 5 to 15 during pregnancy, or that plus for the 4 weeks of lactation, or that plus for 8 weeks to the male offspring after weaning. Control rats received distilled water. In their 12th week of life, the young males were prepared for electrophysiology in urethane anesthesia. Spontaneous and evoked activity of the somatosensory, visual and auditory cortical areas, and conduction velocity and absolute and relative refractory periods of the tail nerve were measured.

On the spontaneous cortical activity, the effect of the CP combination was the weakest. A clear difference was seen between the effects of treatment during pregnancy only and that continued in postnatal life. The combinations DP and DC, given during pregnancy, increased low-frequency and decreased high-frequency cortical activity, in all three areas recorded. DC increased delta, and decreased beta1 and gamma activity, DP increased also theta and decreased alpha. Given during pregnancy, lactation and the post-weaning period, delta activity was found decreased by DC and DP treatment, and theta, by all combinations. Gamma activity was higher in the CP and DC groups.

Latency was the parameter of the evoked responses most altered by the insecticides. Given during pregnancy only, the CP combination caused no significant alteration in the latency of the somatosensory, visual or auditory evoked response, while DC caused a stronger, and DP a weaker but significant, lengthening. With increasing exposure time (including lactation or lactation and the post-weaning period) the lengthening was more expressed and significant with each insecticide combination. The effects on the tail nerve had a similar trend.

The results indicate that the effects of these combinations are partly dependent on the developmental period, and that the interactions in the neuro-functional outcomes can be of various strength. Data of this kind may contribute to better mechanistic background of reglation of insecticide use.

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Inhibition of sodium channels by monoamin reuptake inhibitor antidepressants

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Monoamine reuptake inhibitor antidepressants have been shown to cause a use-dependent inhibition on sodium channels, which effect is considered to be responsible for some of the side effects. Although the phenomenon was observed, no detailed study was performed on the kinetic mechanism of this effect.

We addressed the question whether the inhibition may also be important in the therapeutic action of these drugs by assessing the extent of inhibition at neuronal sodium channels at different activity patterns, and deriving a kinetic model for the drug action on sodium channels.

We studied the tricyclic antidepressant desipramine and the selective serotonin reuptake inhibitor fluoxetine on the native sodium channels of cultured hippocampal neurons by whole-cell patch clamp electrophysiology.

Both desipramine and fluoxetine caused an effective use-dependent inhibition of sodium channels in the supposed therapeutic concentration range. The extent of inhibition was extremely dependent on the holding potential, IC50 values were 120.50 mM and 77.42 mM at –150 mV, while 0.72 mM and 2.15 mM at –60 mV for fluoxetine and desipramine, respectively. The mechanism of inhibition was found to be different from the one that was proposed based on the similarity of the use-dependent inhibition by anticonvulsants and antidepressants: Most importantly the slow inactivated rather than the fast inactivated conformation was stabilized by the drugs.

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Pain processing in spinal cord lamina I; differential activation of heterogeneous neuronal ensembles for stimulus characterization

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As the first relay of peripheral nociceptive input and the convergence point of supraspinal nociceptive modulatory action, the spinal cord stands out an important pain integration center. Both disynaptic or multisynaptic loops connect bidirectionally the spinal cord dorsal horn with its multiple tragets in the brain. Nociceptive input transported by the ascending branch of these loops elicits at each target modulation of the various functions it carries out, including the control of nociceptive transmission at the spinal level. The functional state of each brain target also plays a modulatory role upon spinal pain transmission.

Pain is an assorted entity differing in its perceptive and reactive aspects as a function of stimulus characteristics. Whether brain descending actions interfere, not only with the amount of nociceptive input carried supraspinally but also with its quality, and in such a case, in which extent this relates with the characteristics of the noxious event, are questions that need to be addressed. Taking the spinal cord lamina I as a simplified model of the structural heterogeneity of the spinal nociceptive output processor, activation of various projecting spinal neuronal populations in different noxious conditions was evaluated by the use of the c-fos approach. The results point to the differential involvement of various structural neuronal groups in the transmission of nociceptive input to different supraspinal targets as a function of stimulus characteristics. It is suggested that the participation of different cell types in the spinofugal nociceptive system is controlled by the targeted areas in a cell type-specific way, and that such a differential control accounts for the characterization of the noxious event.

Behavioral changes in rats induced by subchronic administration of 3-nitropropionic acid

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Neurotoxic substances, gaining access to the human organism from the environment via food, show increasing importance. The herbal substance 3-nitropropionic acid (3-NP) is a mitochondrial toxin. The Complex neurochemical and neurobehavioral alterations induced by 3-NP have features in common with certain multifactorial, chronic degenerative diseases of the central nervous system (e.g. Parkinson's or Huntington's disease). The basis of the progressive functional disorders is neuronal loss in the striatum, hippocampus and thalamus. Central dopaminergic and glutamatergic transmission, playing a part also in behavioral phenomena, seems to be important in the development of the lesions induced by 3-NP.

In the present study, 6 weeks old (ca. 160 g body weight) male Wistar rats were treated, in groups of 10, with 10 (low dose) and 15 (high dose) mg/kg 3-NP ip. every 4th day, altogether 6 times. Controls were injected with saline. From the 3rd treatment on, the body weight of rats in the high dose group was significantly and dose-dependently less than that in the low dose and control groups. The rats' spontaneous motor activity was tested in an automated open field apparatus. After the 6 treatments, was significantly different in both treated groups vs. control in terms of distance run, speed of run, percent of rearings among all movements, resting rate, and number of finished runs. A deficit of sensorimotor gating, tested with acoustic startle response, was also observed in the treated rats. There was, however, no significant difference among treated and control rats in the rota-rod (motor coordination) and climbing tests.

After all behavioral tests, the rats were prepared for electrophysiological recording. Spontaneous and evoked cortical activity from the primary somatosensory, visual and auditory area was recorded. The results verified the existence of central nervous damage, and some of them were in parallel with the behavioral effects.

The results suggested that such a subacute experimental model, with an extended set of behavioral and electrophysiological measurements, is suitable for revealing further details of the phenomena induced by the toxicity 3-NP, leading to central neurophysiological and neuropathological alterations.

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Expression of extracellular matrix molecules during optic nerve regenereation in the frog

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Hyaluronan or hyaluronic acid (HA) is a non-sulphated glycosaminoglycan, found in nearly all extracellular matrix. Its permissive role was proposed during the development and regeneration of nervous system, however, its inhibitory function was also suggested. The regeneration of lesioned optic nerve in the frog is a suitable model to study the possible role of the HA in the central nervous system. In our previous study we have detected an inhomogeneous distribution of HA in the termination areas of the optic nerve showing the most intensive reaction in the nucleus of Bellonci (nB), the lateral geniculate body (LGB), in the superficial layers of the optic tectum (ot) and in the basal optic nucleus (BON). In this work we have examined the qualitative and quantitative changes in the HA staining pattern during the regeneration of the optic nerve in the frog.

In the experiments the right optic nerve of the Rana esculenta was transsected and the cut ends were re-united. After survival periods of 1-13 weeks the HA was detected by using a highly specific HA-probe, the biotinylated hyaluronan-binding complex. On the termination areas of optic fibers a computer assisted image analysis was performed.

In the diencephalon the nB showed an intensive HA reaction at both sides. In the LGB the HA was strongly stained around the neurons of the intact side representing the perineuronal net (PN), while the PN showed a very weak reaction at the lesioned side. No qualitative differences were detected in the HA staining pattern between the two sides of the mesencephalic termination areas of the optic nerve except for the BON related to the lesioned nerve which was negative till the 5th postoperative week. Image analysis revealed quantitative differences in the intensity of HA reaction between the operated and intact sides. The intensity of the HA-reaction in nB and CGL in both sides decreased by 50 % after lesion till 9th week and from 9th week it increased up to 60-70 % in intact and severed side.

Our results indicate that regeneration of the optic nerve in the frog is accompanied by the modification of HA distribution pattern in the diencephalic and mesencephalic optic centres. The negative HA reaction in the BON deprived from the optic nerve terminals may indicate that the HA inhibits the axonal pathfinding. The decreased intensity of HA reaction in the PN of LGB suggests that the desorganization of the matrix assembly may allow the formation of new synaptic connections.

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Interaction between morphine and PACAP

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The cellular mechanisms for opioid tolerance and dependence are still not clarified. We investigated the effect of pituitary adenylate cyclase-activating polypeptide (PACAP) and MK-801 on morphine analgesia, tolerance and withdrawal in mice. Tail-flick test was used to assess antinociceptive threshold. Intracerebroventricular administration of PACAP alone had no effect on pain sensitivity but in a dose of 500 ng it significantly diminished the analgesic effect of a single dose of morphine. PACAP increased the non-associative, chronic tolerance (induced by morphine pellet implantation) and the associative tolerance (induced sc. morphine injections) to morphine and enhanced the naloxone precipitated withdrawal jumping. Theophylline pretreatment enhanced the effect of PACAP on morphine analgesia but did not affected PACAP effects on tolerance and the withdrawal.

MK-801, a non-competitive NMDA receptor antagonist, had no effect on chronic, non-associative tolerance but decreased the associative tolerance. Development of associative tolerance was significantly retarded by a combined PACAP and MK-801 treatment.

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Selective inhibition of MGL and FAAH indicates a key role for 2-AG but not for anandamide in hippocampal depolarization-induced suppression of inhibition

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Depolarization of hippocampal pyramidal cells evokes short-term depression of GABA release from afferent basket cell terminals (depolarization-induced suppression of inhibition; DSI). DSI is mediated by CB1 cannabinoid receptor located on presynaptic axon terminals, however, the nature of the endocannabinoid (eC) molecule involved is still unknown. Neurons synthesize two major eCs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). AEA and 2-AG are degraded via different enzymatic mechanisms: AEA by fatty-acid amide hydrolase (FAAH) and 2-AG by monoglyceride lipase (MGL). Here, we used newly developed selective inhibitors of MGL and FAAH to examine the role of 2-AG and AEA in DSI.

Through screening and structural optimization, we identified the compound URB602, which inhibited rat brain MGL (IC50 = 28+4 microM, n =3), but had little effect on FAAH (IC50 = 204+33 microM) or CB1 binding (IC50. > 10 microM). In cultured neurons, URB602 (100 microM) increased 2-AG levels, but not that of anandamide. To test the effect of URB602 on DSI, we recorded spontaneous action potential-dependent IPSCs from CA1 pyramidal cells of 15-17-day-old Wistar rats using whole-cell patch-clamp. DSI was evoked by depolarization from –60 mV to 0 mV for 1 s. This stimulus decreased charge transfer by 61 ± 3 % (mean ± S.E.M., n=22), which then gradually returned to control level in 15-25 s. When URB602 (100 microM) was applied, the recovery of DSI was elongated, and DSI area (measured in the first 30 s after depolarization) increased to 181 ± 21 % (n=9). The FAAH inhibitor URB597 had no such effect when used at a concentration that completely blocks FAAH (100 nM; DSI area 106 ± 15 %, n=7). Consistently, electronmicroscopic investigation of the two enzymes revealed exclusive presynaptic localization of MGL, but postsynaptic localization of FAAH. The results suggest that 2-AG, and not anandamide is responsible for inhibition of GABA release through presynaptic CB1 receptors during DSI.

Long-term follow-up study with repetitive transcranial magnetic stimulation (rTMS) in Parkinson's disease

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Several studies have claimed the effectiveness of repetitive transcranial magnetic stimulation (rTMS) in Parkinson's disease. The rTMS therapy has to be repeated regularly to achieve a permanent effect but the side effects of long-term administration of low frequency rTMS are not known. Further, there is no information about its influence on the development of Parkinson's disease (PD). Two different groups of patients with PD were compared in a retrospective study for three years. The first group (A) was treated with drugs, the second group (B) was treated with drugs +rTMS (1Hz, 0,6 Tesla, 100 stimuli per day for 7 days using a round coil). rTMS was repeated at least twice each year for 3 years. Symptoms of PD were assessed using the Graded Rating Scale. Although at the onset of the study Group B patients had greater disease severity and were receiving higher doses of levodopa, this group (receiving rTMS) showed no deterioration in these parameters, whereas those in Group A receiving drugs alone showed a marked deterioration. Hoehn-Yahr (H-Y) stages at the onset of the study and three years later were: Group A: 1,93± 0,75; 3,03± 1,01, Group B: 2,50± 0,83; 2,45± 0,62.The dose of levodopa (mg/day) was at the onset of trial and 3 years later was: Group A: 124,4± 144,0; 555,5± 247,2, Group B: 287,7± 217,1; 333,4± 181,0 (mg/day). The yearly increment in the scores was Group A: 1,308± 0,307 ($p<0,001$), Group B: 0,642± 0,389 ($p<0,1$). Accordingly, this retrospective study usoing regularly repeated rTMS with 1 Hz for 7 days, at least twice yearly for 3 years significantly slowed the development of Parkinson's disease. Unwanted side effects were not observed during the 3 years.

Inhibitory effect of hemicholinium-3 on the presynaptic nicotinic acetylcholine receptor located on the terminal region of myenteric motoneurons.

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Hemicholinium-3 (HC-3), an inhibitor of the choline transporter is commonly used in release experiments, to prevent the reuptake of [^{3}H]choline, thereby improving the sensitivity of the essay. We have previously demonstrated the presence of presynaptic nicotinic acetylcholine receptors (nAChRs) at the terminals of myenteric motoneurons. During these studies we have observed, that the presence of HC-3 significantly influences both ACh release and contraction of the

longitudinal muscle strip preparation. The aim of this study was to investigate the neurochemical background of these effects. Our assumption was, that HC-3 acted on the presynaptic nAChRs, thus we compared the effects of HC-3 with that of mecamylamine, a potent nAChR antagonist.

We examined the effect of HC-3 and mecamylamine on the epibatidine-evoked contraction and release of [^{3}H]ACh on a guinea-pig longitudinal muscle strip preparation in the presence of TTX. This drug was used to inhibit axonal conductance in order to eliminate any interference by somatodendritic nAChRs. We found that HC-3 effectively inhibited the epibatidine-evoked contraction and [^{3}H]ACh release in the submicromolar range (IC_{50} = 0.897 μM and IC_{50} = 0.693 μM respectively), almost as effectively as mecamylamine (IC_{50} = 0.051 μM and IC_{50} = 0.026 μM respectively).

Our data indicate that HC-3 effectively blocks presynaptic nAChRs located on the terminals of myenteric motoneurons. These receptors play an important role in the regulation of cholinergic neurotransmission in the enteric nervous system, therefore our results raise concern over the prevalent use of HC-3 in release experiments, since its usage could make the interpretation of data rather dubious.

The use of mutant pseudorabies viruses for genetic modification of human embryonic spinal cord neurons

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Transplantation of embryonic neuronal tissue into the injured spinal cord is a possible strategy to repair the injured cord. Introducing neuroprotective factors into the transplantable cells is thought to be beneficial to increase the survival and differentiation of the grafted cells. Among various gene delivery systems the viral-based methods have been found to be superior, as they produce the required effects specifically in the target cells.

In the case of rodent neurons the genetically engineered pseudorabies virus (PrV) proved to be a suitable viral vector to deliver foreign genes due to the advantageous features of the virus. The successful infection of rodent cells raised the question whether human neurons can be infected, too.

We examined whether a genetically modified pseudorabies virus with abolished ribonucleotide reductase and early protein 0 genes has the potential to deliver foreign genes into human embryonic spinal cord neurons and these neurons maintain long-term expression after transplantation into the spinal cord.

Our results show that the mutant pseudorabies virus can infect the human embryonic spinal cord cells ex vivo in a high-dose viral suspension, and the virus maintains the gene expression for several weeks. Following grafting the virus infection did not decrease the viability of the transplanted embryonic cells, as they survived and showed differentiation. Grafting of infected human embryonic neurons into the spinal cord of immunodeficient mice induced the infection of the host neurons, while human embryonic neurones grafted into the spinal cord of immuncompetent mice were eliminated within a week and no PrV infection of host neurones were found.

Similarly, direct injection of PrV into the spinal cord of immunodeficient or immunocompetent mice resulted in spreading or elimination of the virus by the host immune system, respectively.

These results suggest in spite of the succesful gene delivery the use of the pseudorabies virus to infect transplantable human cells may be limited by the weakness or the absence of the immune system.

The inhibitory effect of Fast Blue on the Pseudorabies virus infection of CNS neurones

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The Bartha strain of Pseudorabies virus (PrV) can be injected into peripheral or central nervous structures and then effectively infects primary neurones and retrogradely spreads through the synaptically linked neurones. Due to this feature PrV is an appropriate tracer for mapping neuronal networks. Attempts have been made to apply retrograde tracers together with the virus. The question raised whether PrV can be applied together with the retrograde fluorescent tracer Fast Blue (FB). In this study we examined the effect of FB on the PrV infection in lumbar motoneurones at different survival times after virus injection.

The Tibialis Anterior and the Extensor Digitorum Longus muscles of Sprague-Dawley rats were inoculated with the virus (108 p.f.u). After 24 or 48 hours the sciatic nerve was cut, and FB crystals were applied to the proximal stump of the nerve. In control animals the muscles were injected with the virus without FB labelling. The control rats were perfused after 50, 80 and 96 hours, while the double labelled animals were perfused at 80, 96, 100, 120 hours after inoculation.

In control animals motoneurons became infected 50 hours after the injection, and by 80, 96 hours the virus was able to spread to the interneurons. In animals where the sciatic nerve was labelled with FB 24 hours after the virus injection infected motoneurones were not found at 80 h survival time, while after 96, 100 and 120 hours survival some motoneurones and many interneurons were found to be infected. In animals where FB was applied 48 hours after the virus injection at 96 hours survival time the virus infected many (64-72) motoneurones and interneurones.

Our results show that the PrV infection of the lumbar motoneurones can be prevented by the fluorescent dye FB delivered to the primarily infected neurone within 24 hours after the injection, whereas the virus is able to spread to the synaptically linked neurones resulting in productive infection.

Expression of dystrophin-glycoprotein complex in the nervous system of the frog

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The dystrophin-glycoprotein complex (DGC) is a transmembrane signaling complex that plays an important role in the maintenance of connection between extracellular matrix (ECM) molecules and the interior of the cell. The DGC is composed of extracellular alfa-dystroglycan, transmembrane beta-dystroglycan, and a plethora of attached proteins including the intracellular dystrophin, utrophin and syntrophin. The primary extracellular ligands for DGC are the laminins, agrins, biglycan and perlecan. Intracellularly the DGC influences the activity of calmodulin and neuronal nitric monoxide synthase through phosphorylation and dephosphorylation by mitogen-associated serine/threonin kinases. The binding of the DGC to the ECM proteins involves both outside-in and inside-out signaling, similarly to the functional binding of integrins to their ECM ligands.

The expression of the DGC molecules had been mapped so far only in the nervous system of the rat and mouse, in this study we have examined the distribution of dystrophin and beta-dystroglycan in the nervous system of the frog.

The experiments were performed on common water frogs (Rana esculenta). The brain and the spinal cord were removed and immersed into Sainte-Marie fixative and paraffin embedded slides were made. The slides were treated with Dys2 (anti-dystrophin) and anti-beta-dystroglycan primary antibodies. Biotinylated secondary antibodies were used to detect the immunohistochemical signals. By using of confocal laser scanning microscope we could detect the co-localization of the two molecules.

The dystrophin was detected in Purkinje cell and Bergmann glia of the cerebellum. In the brainstem the dystrophin was expressed in the neuropil and in the white matter. The reaction was especially intensive around the blood vessels suggesting the presence of dystrophin in the astroglial endfeet. Dystrophin was expressed mostly in the lateral part of the brainstem, where spinocerebellar and vestibulocerebellar tracts are situated. In the spinal cord the dorsal funiculus was strongly labeled.

Beta-dystroglycan was found in the cerebral cortex, hippocampus and in the olfactory bulb. In the facial and vestibular nuclei, and in the reticular formation the perikarya and proximal dendrites of neurons were strongly labeled. Purkinje cells and Bergmann glia in the cerebellum and motoneurons in the spinal cord also expressed beta-dystroglycan.

In the confocal laser scanning microscope the co-localization of the two antibodies was demonstrated in the perikarya suggesting the intracellular localization of the dystrophin and the intramembraneous localization of the beta dystroglycan.

Our results demonstrated for the first time the presence and inhomogeneous distribution of dystrophin and beta dystroglycan in the nervous system of the frog.

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Subcellular localization of CB1 cannabinoid receptors in the rat basal ganglia

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Endocannabinoids, acting via CB1 cannabinoid receptors are known to be involved in retrograde synaptic signaling. Strong depolarization of the postsynaptic neurons followed by the activation of CB1 receptors suppresses GABA and/or glutamate release, which is termed depolarization-induced suppression of inhibition (DSI) or excitation (DSE). Although both phenomena have been reported to be present in the basal ganglia, the anatomical evidence for these actions has not been revealed. Here we investigate the subcellular localization of CB1 receptors in the striatum (CPu), globus pallidus (GP) and substantia nigra (SN), as well as in the internal capsule, where the striato-nigral and pallido-nigral pathways are located.

In the CPu, CB1 receptors are located both in axon terminals and preterminal segments. However, their distribution is not uniform. In the lateral part CB1-positive axon terminals are relatively abundant and strongly stained, whereas in the dorsal part sparse individual axons forming medium-to-large sized axon terminals were intensely stained for CB1. Most of them form symmetrical synapses. In contrast to the CPu, preterminal axons showed far more intense CB1 immunoreactivity in the GP and SN, while their terminals were very faintly stained. Electron microscopic examination revealed that CB1 receptors were located on the plasma membrane of these preterminal segments of axons forming symmetrical GABAergic synapses with their postsynaptic targets. Non-varicose, thin, unmyelinated fibers in the capsula interna also show strong CB1-labelling, and are embedded in bundles of thick, myelinated CB1-negative axons. The majority of CB1 receptors labelled by immunogold particles are located in the axonal plasma membrane (92.3%), apparently capable of mediating cannabinoid actions. CB1 receptors in this location cannot play a role in modulating transmitter release directly, because the release sites are several hundred micrometers away. They are also unlikely to be on the way towards the terminals, because terminals have very little staining.

Thus, our data suggest that, in addition to the well-know function in DSI/DSE, a novel role can be hypothesized for CB1 receptors in the basal ganglia, possibly in the modulation of action potential conduction along non-myelinated striatonigral axons. DSI or DSE in the substantia nigra and/or striatum/globus pallidus may also involve a CB1 receptor-mediated shunt of action potentials in thin preterminal fibers.

Presynaptic modulation in the spinal cord: orthodoxy and heresy

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Presynaptic inhibition is well established. Classical presynaptic inhibition occurs at terminals of primary afferent axons and is mediated through interneurons that release GABA at axo-axonic synapses. The release of GABA is associated with a depolarisation of the primary afferent terminal (PAD). Although PAD-like phenomena have been recorded from terminals of some classes of interneuron, there is no evedence that axo-axonic synapses are present on interneuron terminals. It has recently become necessary to revise our concept of how information flow in the spinal cord is regulated presynaptically. There are two reasons for this: firstly the acceptance of volume transmission as a mode of signalling in the CNS, and secondly, the discovery that receptors are present not only on primary afferent axon terminals but also on terminals of interneurons. Recently, we examined two classes of receptor that are activated by descending monoamine systems: the α2c adrenergic receptor (α2c –AR) and the 5-HT3 serotonin receptor. The α2c–AR, (unlike the α2A–AR which is present on primary afferents) is found principally on terminals of spinal interneurons. The majority (84%) of these interneurons is excitatory but a small group (11%) is inhibitory. A proportion of α2C-AR-immunoreactive terminals also contain peptides such as enkephalin, somatostatin, neurotensin, neuropeptide Y but they are not present on noradrenergic terminals and thus do not function as autoreceptors. Exctitatory axons that possess the α2c–AR target projection cells in lamina I that are immunoreactive for the neurokinin 1 (NK-1) receptor. Therefore noradrenaline appears to modulate excitatory synaptic transmission from spinal interneurons to projection cells by acting at α2C-ARs; this could be one of the mechanisms that underlie its antinociceptive action. Serotonin 5-HT3 receptors are present on primary afferent terminals and terminals of interneurons. This receptor is found only on excitatory terminals. Like the α2c–AR axons, axons possessing the 5-HT3 receptor also form synaptic relationships with lamina 1 projection cells. However, serotonin probably facilitates excitatory transmission at such synapses and has a pronociceptive action when it acts through 5-HT3 receptors. Thus sensory transmission is not only regulated presynaptically at primary afferent synapses in the spinal cord but is also regulated presynaptically at synapses formed by interneurons which are components of polysynaptic pathways.

Interconnection between neuropeptide Y- and alpha-melanocyte stimulating hormone-synthesizing neurons in the infundibular nucleus of the human hypothalamus

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Peripheral feeding-related hormones, such as leptin, insulin and ghrelin exert their main central effects through the neuropeptide Y- (NPY) and alpha-melanocyte stimulating hormone- (alpha-MSH) synthesizing neurons of the hypothalamic arcuate nucleus. Recent reports have described an asymmetric signaling between these neuronal groups in the rat. While NPY was found to influence alpha-MSH-synthesizing neurons, the alpha-MSH agonist MTII did not modulate the electrophysiological properties of NPY neurons. To analyze the connectivity of the NPY and a-MSH systems in the human hypothalamus, double-labeling fluorescent immunocytochemistry for NPY and alpha-MSH was performed on human hypothalamic sections. Analyzing the sections by confocal laser microscopy, both NPY- and alpha-MSH-IR neurons were embedded in dense networks of NPY- and alpha-MSH-IR axons. NPY-IR varicosities were found in juxtaposition to all alpha-MSH-IR neurons. The mean number of NPY-IR axon varicosities on the surface of an individual alpha-MSH-IR neuron was approximately 9. The majority of NPY-IR neurons were also contacted by alpha-MSH-IR varicosities, although, the number of such contacts was somewhat lower (4 alpha-MSH-IR varicosities/NPY neuron).

The present data demonstrate that these two antagonistic, feeding-related neuronal populations are heavily interconnected in the infundibular nucleus of the human hypothalamus. In contrast to the rat, these connections appear to be reciprocal in the human hypothalamus.

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Role of vasopressin in the activation of the hypothalamo-pituitary-adrenal axis during repeated morphine withdrawal induced chronic stress: somatic and hormonal changes

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Frequently used drugs are morphine and their derived analgesics, which are often used also for medical purposes (perioperative analgesia, cancer-related pain). Opiates are believed to play an important role in the control of the hypothalamo-pituitary-adrenal axis (HPA). The HPA axis is under the central regulation of the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). The role of AVP supposed to come in front during chronic stress. The aim of our study was to examine the role of AVP in the maintenance of chronic hyperacitvity of the HPA during repeated morphine withdrawals. Natural AVP deficient mutant Brattleboro rats were compared to heterozygous control animals. Rats were treated twice daily with increasing doses of morphine (10-100mg/kg, sc) for 16 days. Somatic parameters (body weight, thymus and adrenal weight) and plasma adrenocorticotropin (ACTH) and corticosterone levels by radioimmunoassay were measured. As typical signs of chronic stress the body weight reduction, thymus involution and adrenal gland hypertrophy was present in chronically morphine treated rats, although the absence of AVP could not influence the changes. At the time of the last injection (basal) the corticosterone plasma levels were elevated in morphine treated rats. In the absence of AVP both in control and morphine treated rats higher corticosterone levels were observed. Four hours after the last injections the plasma ACTH and corticosterone level were elevated. The withdrawal (16h after the last injection) could induce a more pronounced elevation. The lack of AVP was able to diminish the elevation in both hormone level at both time-point. Our data suggests that the AVP has no prominent role in the mainenance of the chronic hyperactivity of the HPA axis during repeated morphine treatment, as the somatic parameters and basal corticosterone levels were not changed in AVP mutant animals. The reduced plasma hormone levels at different time-point after the injection suggest, that AVP may play a role in the regulation of the HPA axis during acute stress-induced (morphine treatment and withdrawal) changes.

Expression of hyaluronan and hyaluronan binding proteins during spinal cord development of chicken embryos

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Hyaluronan (HA) is known to play an important role during development. The interaction of HA with HA-binding extracellular matrix molecules (ECM) and cell surface receptors regulates many aspects of cell behavior including cell migration, differentiation and cell adhesion to another cell or ECM. In recent studies we are focusing on hyaluronan, which acts as an autocrine regulator through hyaluronan receptors (CD44 and RHAMM) during signaling pathway and looking for the enzyme that produce the signaling molecule HA.

Our models were chicken embryos collected from 16 to 34 stages of development according to Hamburger and Hamilton (HH). The embryos were immersed in Saint Marie fixative and embedded into paraffin. HA and MNR2 were stained by double labeling immunofluorescence method. CD44 was visualized with immunohistochemistry used an avian specific monoclonal antibody and also analyzed with RT-PCR on whole spinal cord RNA. Hyaluronan synthases (Has) 2 and 3 were examined by RT-PCR and Hyaluronan synthase 1 was identified by immunohystochemistry. All RT-PCR primers are designed for chicken specific.

Strong HA signal was found in those areas of spinal cord that contain neurons expressing MNR2 homeodomen transcription factor, which is known an early marker of the later somatic motoneurons in the lamina IX of spinal cord. The MNR2 positive neurons were found in the intermediate zone of the basal plate in HH23 chicken spinal cord cross section. The intensity of reaction was gradually shifted towards the later lamina IX (ventral motor column) by the time the embryos reached the HH32 stage. We could not detect the CD44 in the MNR2 neurons suggesting that HA signal acts through other than the CD44 receptor. By using RT-PCR we have demonstrated that HA found in the spinal cord of chicken embryos is produced by hyaluronan synthase 2 (Has2).

Co-localization of MNR2 immunoreactivity with the HA reaction in the developing spinal cord of chicken embryo may indicate a permissive role of the HA molecule during the early stage of neuronal development. According our results the HA is produced by has2 in the spinal cord of chicken embryo. This enzyme requires Rac1 small GTPase during lamellipodia formation through CD44 or RHAMM receptor.

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A baclofen insensitive gamma-hydroxybutyrate binding site in the nucleus accumbens

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The nucleus accumbens (NA) may play an important role in the development of γ-hydroxybutric acid (GHB) – a naturally occurring metabolite of γ-aminobutric acid (GABA) – abuse. In many brain areas GHB acts on its own receptor and also binds to GABAB receptors. The presence of GHB binding site in the NA has been shown, but the cellular actions of GHB and the pharmacology of GHB binding are not yet characterized in the NA. Therefore we studied (1) the pharmacological profile of γ-hydroxy[2,3-3H]butric acid ([3H]-GHB) binding to synaptosomal membranes prepared from the striatum and prefontal cortical areas of the rat brain, and to human NA samples; (2) the effect of GHB on intracellular Ca2+ ion signaling in rat NA cells.

Affinity screening of the GABAB agonist, (-)-baclofen, the GABAB antagonist, CGP-55845, GHB and the GHB receptor antagonist, NCS-382 were performed in [3H]GHB displacement measurements. In rat synaptosomal membrane homogenates the competition and saturation experiments showed only one GHB binding site with KD=0,9 microM and a BMAX=8,5 pmoles/mg protein. GHB and NCS-382 completely inhibited [3H]GHB binding (KiGHB:1 microM, KiNCS: 3 microM), but (-)-baclofen and CGP-55845 (1nM-1 mM) did not displace [3H]GHB. A similar profile, complete displacement of [3H]-GHB by GHB and NCS-382, but no inhibition by GABAB agonist or antagonist was found in the human NA samples.

The effects of GHB (0,4-2mM) on intracellular Ca2+ ion signaling were measured in combination with the use of Laser Scanning Confocal Microscopy. NA slices were prepared from neonate rats (13-18 days) and were incubated with the cell-permeable form of the Ca2+ ion indicator dye, Fluo-4AM. In the presence of TTX (1 microM), 0,4mM GHB evoked a transient intracellular Ca2+ ion increase with a range from 149 % to 2594 % (n=12 cells from 5 slices) whereas 2mM GHB with a range from 576 % to 4227 % (n=18 cells from 9 slices). Under conditions, 25 microM baclofen was ineffective. In the presence of CGP-55845 (20 microM), or NCS-382 (300 micorM), GHB still induced intracellular Ca2+ ion transients, whereas the receptor antagonists alone did not evoke any.

Our results exclude the action of GHB on GABAB receptors in rat and human NA samples. Binding experiments indicate the presence of NCS-382-sensitive GHB receptors in the NA, however NCS-382 failed to antagonise the effect of GHB intracellular Ca2+ ion signals. Our experiments also suggest the involvement of Ca2+ ion-sensitive intracellular pathways in the development of GHB abuse.

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Afferent connectivity of the septal area in relation to motivation and learning of domestic chicks

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Lying between the limbic telencephalic and subtelencephalic structures, the septum is involved in the modulation of a number of behaviours (courtship, reproduction, feeding, fear and defense reactions). Little is known on the afferentation of septum in birds. We injected either fast blue or rhodamine coated beads as retrograde tracers in the septal areas of 8 to 10-day-old domestic chicks. Exclusively ipsilateral telencephalic afferents arise from hippocampal centres, ventral pallidum, temporo-parieto-occipital area, dorsolateral corticoid area and arcopallium. The main hypothalamic afferents include the lateral hypothalamic and anterior hypothalamic nuclei and preoptic areas. Fewer afferents arise from the medial hypothalamic and mammillary nuclei. Some of these hypothalamic connections were confirmed by injection of the anterograde tracer Phaseolus lectin in hypothalamic nuclei. The dorsomedial thalamus gives rise to a modest input to septum. Diencephalic contralateral projections were rare. Brainstem projections arise mainly from the ventral tegmental area, pedunculopontine tegmental and interpeduncular nuclei, locus coeruleus, pretectal area, central gray and nucleus linearis caudalis (raphe nuclei). The afferents appear topographically organised: the ventral septum receives more hippocampal afferents than the dorsal septum.

The reciprocal connection with the arcopallium, as well as the strong afferents from hippocampus and ventral tegmental area raise the possibility that the septum may play a role also in learning or motivation behaviours. To test this hypothesis, we ablated the septum of eight 1-day-old domestic chicks bilaterally, after which we trained them by a modified passive avoidance paradigm, using positive reinforcement during pretraining. The chicks did not show any learning impairment either in avoidance or reinforcement tasks. Conversely, learning deficits have been reported following lesions of certain regions projecting on the septum. Thus, the septum is likely involved in other types of behaviours (e.g. spatial or social) as expected from mammalian studies. In conclusion, the organization of avian septal afferents appears anatomically and functionally similar to that of mammals, with subtle differences.

The effects of mechanical stimulation on an isolated intestinal section. A new in vitro method

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The aim of our work has been to investigate how stimuli arriving from the gastro-intestinal tract are processed and what role these stimuli have in the behavioural regulation. In our earlier in vivo experiments, using the Thiry-Vella method, we surgically prepared an isolated intestinal loop into which we could place a balloon from outside. Giving gradually increasing volumes, we found a range were the occurrence of the aversive behavioural elements differed from the expected trend (i.e. the stronger stimulus elicited less aversiveness than expected). We hypothesised that the reason of the differences found in this range might have been due to an irregularity in the response pattern of the gut.

To study this phenomenon, we made Magnus-preparations from the small intestine of the rats and recorded the activity of this in vitro intestine in an organ bath by distending a balloon inserted. Based on the results we suggested that a – possibly local – reflex protects the intestine from being over-distended by changing the compliance in this range.

In the present work we recorded the activity of an intestinal interval distal from the stimulated part in an isolated gut preparation to avoid the superposition of the local volume changes. In this modified Magnus-preparation we could separately study the activity of the circular and longitudinal smooth muscle, respectively, from the same animal quite distal from the stimulation site. Six intensities (i.e. volumes) were applied: 0.05ml, 0.09ml, 0.13ml, 0.17ml, 0.21ml, and 0.25ml. Amplitude, period length and tone of the contractions were measured before, during and after the stimulation.

Results show that the stimulation elicits an immediate contraction of booth the circular and the longitudinal muscles with an amplitude dependent on the stimulation intensity; the two muscular layers, however, react on a different way. Both the immediate reactions to the onset of the stimulus and the long-term (5 minutes) adaptation to the continuous distension showed a similar pattern to that observed earlier. In accordance with our hypothesis, the small intestine shows an adaptation to the mid-range stimuli by changing the compliance of the gut wall. Based on these and other (also human) observations we suggest that this receptive relaxation protects the gut against being over-distended and also decreases the distension-pain we could have detected by the out-of-trend behavioural reactions.

Neuroprotective effects of EGIS-8332, a non-competitive AMPA antagonist, in focal cerebral ischaemia and multiple sclerosis models in rats

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Recent clinical studies have shown that thrombolytic therapy early after the appearance of first stroke symptoms produced clear neurological improvement in patients and these results may accelerate the research of neuroprotective agents. Blockade of the AMPA type ionotropic glutamate receptors has been shown to prevent neuronal loss after ischaemia, trauma or during slowly progressing neurodegenerative disorders by reducing excitotoxic damage due to excessive glutamate release. The aim of this study was to measure the efficacy and therapeutic window of EGIS-8332, a new AMPA antagonist compound with 2,3-benzodiazepine structure, in permanent and transient focal ischaemia tests and also to evaluate its efficacy in a model of multiple sclerosis in rats. For comparison, GYKI 53405, a prototype AMPA antagonist from the same chemical structure, was studied. EGIS-8332 dose-dependently reduced cerebral infarct volume determined using the 2,3,5-triphenyltetrazolium chloride staining when the compound was administered 30 min after permanent middle cerebral artery occlusion (MCAO). The minimal effective dose of EGIS-8332 was 3 mg/kg i.p., while GYKI 53405 was effective at 10 mg/kg i.p. only. Transient MCAO was produced for 1 hour using the intraluminal filament technique and the volume of cerebral infarction was evaluated at 24 hours after occlusion. In comparison with the group treated with vehicle, EGIS-8332 reduced the core (necrosis) volume by 56 % and the total (core+penumbra) volume by 33 % if administered at 2 hours after MCAO. If treatment was delayed efficacy of EGIS-8332 decreased in proportion with time between occlusion and treatment. EGIS-8332 diminished the core volume by 31 % and the total volume by 24 % if administered at 3 hours after MCAO but was ineffective if the animals were treated at 4 hours after MCAO. The effects of GYKI 53405 were similar to that of EGIS-8332 at all treatment times.

Experimental autoimmune encephalomyelitis was induced by treatment of male Lewis rats with guinea pig myelin basic protein, and both compounds were administered twice daily for 7 days from day 10 after immunization. EGIS-8332 reduced cumulative movement disability score by 65 % ($p<0.05$) and partially reversed histopathological damage at 3 mg/kg i.p., while GYKI 53405 attained similar effects at 10 mg/kg i.p. only.

In conclusion, EGIS-8332, a novel non-competitive AMPA antagonist, decreased cerebral infarct size with a wide time-window in permanent and transient focal cerebral ischemia and improved movement disability and histological damage in an experimental autoimmune encephalomyelitis in rats with good efficiency in both models, suggesting that the compound may be a promising neuroprotective agent for further development for the treatment of human stroke and multiple sclerosis.

Altered GABA synthesis in the spinal dorsal horn and its potential effect on primary afferent terminals in Freund`s adjuvant induced inflammation

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Chronic inflammation of peripheral tissues induces substantial and long-lasting changes in various properties of neurons in the spinal dorsal horn resulting in an increased excitability of spinal nociceptive neural circuits.

To estimate the role of inhibitory neurotransmitters in this inflammation evoked neural plasticity we injected Freund's adjuvant in the right hindpaw of genetically modified mice that express green fluorescence protein (GFP) linked to an isoform of glutamic acid decarboxilase (GAD65). Using immunoblot and immunohistochemical methods we found increased expression of GFP and GAD65 in the superficial laminae of the ipsilateral dorsal horn at the level of lumbal 4-5 segments of the spinal cord, although we could not detect a significant change in the number of perikarya labelled with GFP four days after inflammation.

We traced primary afferent fibers terminating in the superficial laminae of the dorsal horn with rhodamine-dextran and stained them for GABAB receptor. A substantial number of these terminals contacted GFP containing neurons and showed GABAB1 receptor immunoreactivity.

To understand the role of the increased synthesis of GAD65 and GABA in inflammation evoked central sensitization further investigation are required. One possible element of altered information processing mechanisms can be the enhanced presynaptic inhibition mediated by GABAB receptor and the consequent decrease in the activation of inhibitory neural circuits of the spinal dorsal horn.

Detection of the mammalian clock gene homolog of Per1 in the chicken pineal gland

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The chicken pineal gland produces melatonin in vitro with a daily periodicity providing a model organ of circadian biological clocks of vertebrates. Molecular clockwork mechanisms usually include a set of transcription factors coded by clock genes. Showing a high degree of phylogenetic conservation, the homologues of several members of the known clock protein families were found earlier in various vertebrate species, including birds.

The drosophila Period-1 (Per1) gene, one of the first clock genes identified, plays a central role in maintaining circadian biological rhythms. Per1 gene was also found in mammals but as an addition, it affects photoentrainment, too. In avian circadian clocks, however, functions of the Per1 clock component remained unknown. Even the evidence of the Per1 gene expression in bird tissues was previously not reported.

Recent study focuses on the detection of chicken transcripts of orthologs for the known mammalian clock gene Per1. Using mouse sequence information, we designed RT-PCR primers to amplify Per1 mRNA fragments from total RNA extracts of chicken pineal glands.

Using the primers CGGAGCTTCTGGGTTGC and CCCAGGGGATGGGACTC (sense and antisense) in RT-PCR reactions, we found a chicken pineal mRNA fragment of about 270 bp, showing similarity both in size and intron content with the mouse Per1 homolog sequence. Our results demonstrate that homologues of the mammalian Per1 clock gene are expressed also in avian tissues.

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Time course of sciatic nerve regeneration in rats: a complex behavioral and electromyographical study

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The aim of the present study was to give a detailed description of the time course of regeneration following sciatic nerve injury using a complex behavioral and electromyographical approach. The sciatic nerve was transected on one hindlimb at the level of the trochanter major. The cut ends of the sciatic nerve were closed in a polyethylene tube in order to promote regeneration. To assess the degree of functional lesion and recovery, numerous tests were performed. During the grid-walk test, animals were placed on a metal grid, 1 m above the floor. The number of foot faults, when the limb fell through the grid, was counted on each side. To test the sensory disfunction, animals were placed on a hotplate, and the time to displace and lick their foot was measured on each side. The sciatic index was assessed by the foot print test. Both hindlimbs of the animals were immersed in blue ink, and rats were allowed to run through a closed corridor with a dark box at the end. Rats were trained 2 times to go to the box preceding the nerve injuries. The floor of the runway corridor was covered with paper, where the animals left a visible footprint. Each footprint was then digitally analysed by ScionImage program. The degree of autotomy was also measured. Functional tests were performed weekly after the injury. Electromyographical measurements were performed daily during the first week following the sciatic nerve injury and then weekly. The present study describes the time course of the functional and electrophysiological recovery and serves as a basis for our future studies with neuroprotective agents that could promote nervous regeneration.

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Neither diabetes mellitus nor acute morphine but acetazolamide administration affect cortical spreading depression related cerebral blood flow changes in rats

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Cortical spreading depression (CSD) is a transient perturbation of the cortical neuronal activity and cortical blood flow (CBF). Although several vasoactive substances of various origins are thought to be implicated in CSD-related transient changes in CBF, the mechanism has never been satisfactorily elucidated. Hyperemia observed during CSD or seizures are, at least in part, mediated through CGRP receptors. The sensory system, including the CGRP containing fibers is strongly affected in diabetes mellitus. We tested the hypothesis that acute morphine exposure and inhibition of the carbonic anhydrase (CA) or experimentally induced diabetes mellitus, would affect the CSD-induced changes in CBF. Male Wistar rats weighing between 250 and 420g were used. Diabetes was induced by a single i.p. dose (65 mg/kg) of streptozotocin (STZ) and the CSD measurements were performed 8 weeks later. In anaesthetized animals CSD were repeatedly induced by topical application of KCl and CSD-related CBF changes were recorded by laser Doppler flowmetry. In diabetic rats (n=5) CSD-induced an elevation in CBF that peaked at 301±31% whereas it was 297±30% in the controls (n=5). In spontaneously breathing rats (n=6), after 3 consecutive CSD morphine was given i.v (1 and 5 mg/kg). At low does of the drug, the CSD-related CBF changes were not different (peak flows: 243±14% vs.238±26% above baseline) from the controls. The significant depression in the blood flow elevation after 5 mg/kg could be attributed to the depressed ventilation because in the ventilated group (n=6) even the high morphine dose did not affect the CBF. After administration of acetazolamide (n= 8, 10 and 20 mg/kg, iv) the CSD related CBF changes were depressed (276±24% in control, and 175±30% and 168±22% after 10 and 20 mg/kg, respectively). Our experiments confirm earlier observations indicating that CSD related CBF changes are intact after impairment of the sensory neuronal function. Our data with the morphine and acetazolamide treatment give new information on the nature of the CSD-related CFB changes.

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Ethanol or Etalon? Placebo effect during alcohol consumption

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Placebo effect is not the privilege of doctors and health care, it is present in our everyday life as well. Most of us know anecdotes that someone was awaken by decaf coffee or has got tipsy from alcohol-free beer. However this phenomenon has not been investigated in details so far thus we designed an experiment to study this phenomenon.

According to our hypothesis, the effect of the alcohol is partially mediated by placebo: it is enough if a person believes that (s)he is consuming alcohol to get into an altered state of mind. (This leads to a further problem: the phenomenon what we nowadays call "placebo effect" is something more complex and, maybe, a better name should be found.)

Subjects were altogether 109 university students. The experiment was conducted in groups of 10-15. Subjects had to drink 4 x 4cl rum with cola separated by ten minutes intervals between each round. Short term memory, standing balance, and subjective self-evaluation of physical, emotional and social state (on visual analogue scales) were measured before the start and after each round. Every second subject had alcohol-free rum aroma instead of rum, though the rum aroma cocktails had similar color, odor and taste as the alcoholic cocktails. All subjects believed that they were having four alcoholic cocktails.

In addition, we conducted the whole experiment with 28 subjects only in a pure cognitive way: they had had to imagine that they are drinking four rounds of 4cl rum with cola and they had to estimate their results in the same tests that were taken with the experimental groups.

Results were shocking and drastic. Though there were some variables that remained constant in both the 'alcohol' and 'aroma' groups (these variables were traits that seem to be unaffected by the alcohol), most variables changed almost in the same way in both the alcoholic and the aroma groups; there was a slight difference only after the fourth round. It seems that these results provide strong evidence that the effects of alcohol consumption in smaller portions (less than three rounds) are mainly due to a placebo effect and are also present in subjects who only believed that they consumed alcohol.

Most strikingly, the results of the cognitive group completely differed from reality: it seems that physical, emotional and social state cannot be estimated cognitively in the case of a complex behavior, and thus placebo effect is supposedly based on unconscious beliefs.

Kynurenine administered together with probenicid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioural study

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The kynurenine pathway converts tryptophan into various compounds, including L-kynurenine, which in turn can be converted to the excitatory amino acid receptor antagonist kynurenic acid, which may therefore serve as a protective agent in such neurological disorders as epileptic seizures. Kynurenic acid, however, has a very limited ability to cross the blood-brain barrier, whereas kynurenine passes the barrier easily. In this study, we tested the hypothesis that kynurenine administered systemically together with probenecid, which inhibits kynurenic acid excretion from the cerebrospinal fluid, results in an increased level of kynurenic acid in the brain that is sufficiently high to provide protection against the development of pentylentetrazol-induced epileptic seizures.

CA3 stimulation-evoked population spike activity was recorded from the pyramidal layer of area CA1 of the rat hippocampus, and in another series of behavioral experiments, water-maze and open-field studies were carried out to test the presumed protective effect of kynurenine + probenecid pretreatment against pentylenetetrazol-induced seizures.

This study has furnished the first electrophysiological proof that systemic kynurenine (300 mg/kg, i.p.) and probenecid (200 mg/kg, i.p.) administration protects against pentylenetetrazol-induced (60 mg/kg, i.p.) epileptic seizures.

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Examination of the novel TRPV1 receptor antagonist JYL1421 (SC0030) in vitro and in vivo in the rat

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The TRPV1 capsaicin receptor/ion channel functions as an integrator molecule of painful stimuli on primary sensory neurons and participates in several inflammatory and nociceptive processes. JYL1421 (SC0030) is a novel TRPV1 receptor antagonist, which has previously been reported to show a broader range of antagonistic activity, higher potency and selectivity than capsazepine on CHO cells. In the present series of experiments we characterized the antagonistic activity of JYL1421 on capsaicin-evoked TRPV1 activation, both in vitro and in vivo using rat models. It concentration-dependently inhibited capsaicin-evoked substance P, CGRP and somatostatin release from isolated rat tracheae, while only the highest concentration (2 microM) resulted in significant inhibition of electrical field stimulation-induced sensory neuropeptide release. Capsazepine, as a reference compound, caused similar inhibition on capsaicin-evoked release of neuropeptides, but markedly diminished the outflow of electrically-induced peptide release as well. Therefore, JYL1421 proved to be much more selective. JYL1421 concentration-dependently inhibited capsaicin-induced calcium accumulation in trigeminal ganglion cells, where capsazepine was ineffective even in 10 microM concentration. Evidence has also been provided for the effectiveness of JYL1421 in vivo. It significantly inhibited capsaicin-induced hypothermia, wiping movements and the decrease of mean arterial blood pressure (Bezold-Jarish reflex) in 2 mg/kg i.p. dose, while capsazepine was ineffective in these models.

Based on these data JYL1421 can be considered a more selective and in most models even a more potent antagonist than capsazepine, therefore it may promote the assessment of the therapeutic utility of TRPV1 blockers.

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Differentiation of genetically engineered stem cells following grafting into the spinal cord

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Embryonic neural stem cells are reportedly used to repair the injured CNS, although the way how they improve various neural deficits is not fully understood. In this study we investigated the capacity of an immortalized stem cell line to differentiate and integrate into the circuitry of the injured spinal cord and possibly replace missing spinal cord populations.

The NE-4C stem cells were originally isolated from the cerebral cortex of p53 knockout mouse embryos and long-lasting expression of green fluorescent protein (GFP) was induced in them. The lumbar motoneurone populations in the spinal cords of host Sprague-Dawley rats was depleted either by avulsion of the L4 ventral root immediately prior to grafting or by neonatal sciatic nerve crush. Various amounts of cultured stem cells were then grafted into the motoneurone-depleted L4 segment of the adult host spinal cords. When ventral root avulsion was performed, the root was gently re-implanted into the host cord.

Transplantation of high numbers of NE-4C cells (5x10E6) induced an unlimited proliferation of the grafted cells in the host cord and the graft destroyed the original cytoarchitecture of the spinal cord. Limited differentiation of the grafted cells was observed along the graft-host interface only, and some of the grafted cells expressed GFP. When lower number of cells was introduced into the host cord (100-1000 cells) the grafted cells differentiated and integrated into the host cord and some of them sent their axons into the re-implanted L4 ventral root. However, grafted cells showed

poor survival when they were grafted into the spinal cord previously depleted by neonatal sciatic nerve crush.

Our studies have shown that embryonic cortical stem cells are able to survive, differentiate and possibly reinnervate peripheral targets following grafting into acutely injured spinal cord and maintain expression of foreign genes for a limited period of time.

CB1 Cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons

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Cannabinoids have been shown to modulate the inhibitory effect of cholecystokinin-containing GABAergic interneurons in the hippocampus via CB1 cannabinoid receptors. Although immunohistochemical studies, using pre-embedding techniques, have demonstrated that these receptors are abundant on GABAergic axon terminals, little is known about their exact location relative to the synapse. Here we used two recently developed antibodies against the CB1 receptor to study this question with the postembedding immunogold method, which allows the quantitative examination of receptor distribution along the axonal membrane, even within the synaptic active zone. CB1 receptor positive terminals target both the dendritic and somatic surface of neurons in the CA1 area of the rat hippocampus. We found no difference between these two populations of terminals either in their CB1 receptor density or in the distribution of receptors on their membrane. Recent studies suggest that endocannabinoids play a role in retrograde signaling at these synapses, i.e. signaling molecules diffuse from the postsynaptic membrane to nearby presynaptic terminals. Therefore, we examined the distribution of CB1 receptors on the terminal membranes. We found that they are rare in the synaptic active zone, but are enriched in the perisynaptic annulus, where they can directly influence synaptic calcium channels. Perisynaptic CB1 receptors represent about one tenth of all CB1 receptors in a terminal. In contrast, CB1 receptors have a lower density on the extrasynaptic membrane of terminals far from the postsynaptic cell. We estimated that these terminals contain exceptionally large numbers of CB1 receptors. An unexpected finding was that the density of CB1 receptors was significantly higher on preterminal axons than on synaptic terminals. These observations suggest that endocannabinoid signaling may subserve roles other than simply reducing transmitter release from axon terminals.

Evidences for a dopamine (DA) regulated adrenocorticotrop hormone (ACTH) secretion from the intermediate lobe of pituitary gland in lactating rats

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The prohormone precursor pro-opiomelanocortine (POMC) is processed to adrenocorticotrop hormone (ACTH) and β-lipotropin (β-LPH) in the anterior lobe (AL), and to α-melanocyte-stimulating hormone (α-MSH) and β-endorphin in the intermediate lobe (IL) of the pituitary gland, respectively. While ACTH secretion is regulated by the corticotrop releasing factor (CRF) produced in the paraventricular nuclei (PVN) of the hypothalamus, the hormone secretion of the IL is under a tonic inhibitory control exercised by the hypothalamic dopaminergic (DAerg) neuroendocrine (NEDA) neurons. Several experimental data suggest that this strict AL and IL specific separation in processing of POMC can change under certain pathological situations. In agreement with this, we have previously observed that plasma levels of α-MSH do not change following DAerg receptor (DA-R) blocker injection. At the same time, it is well known that suckling induces an increase in both plasma prolactin (PRL) and ACTH without having any effect on sympatho-adrenal system. Therefore, the aim of our present studies was to investigate the possible change in the origin of ACTH during lactation using pharmacological manipulation of the NEDA neurons. Plasma levels of α-MSH, PRL and ACTH have been measured using different pharmacological treatments that have well known influence on the NEDA neurons. Inhibition of the biosynthesis of DA by α-methyl-p-tyrosine (αMpT, 25 mg/kg b.w. iv) and/or blockade of the D2-DA receptor by domperidon (DOM, 20 μg/rat iv.) significantly elevated plasma levels of PRL (15,84±2,45 vs. 384,43±88,53 and 40,64±17,37 vs. 567,54±73,01 ng/ml, respectively) and ACTH (104,89±20,09 vs. 489,52±63,19 and 111,53±28,48 vs. 477,30±47,40 pg/ml.), but did not influence plasma α-MSH concentrations. Interestingly enough, the DA receptor agonist bromocryptine (BR, 3 mg/kg b.w. sc) was able to block both the αMpT and DOM induced PRL (3,52±0,67 vs. 3,96±0,76 and 4,82±1,62 vs. 9,14±2,11 ng/ml) as well as ACTH (155,85±30,45 vs. 136,59±22,41 and 186,88±59,89 vs. 282,90±54,51 pg/ml) release, without having any effect on plasma α-MSH concentrations. Our results indicate that NEDA neurons are involved in the regulation of ACTH secretion. It is also suggested that the majority of plasma ACTH is processed in and released from the IL. This change in the source of ACTH may have a physiologically significant meaning in the period of lactation.

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Investigation of light evoked potentials by electrophysiological means during sleep-wake cycle and cortical hypersynchronisation

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Investigation of light evoked potentials by electrophysiological means during sleep-wake cycle and cortical hypersynchronisation

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It is a well known fact that in the case of cortical visual evoked potential the parameters of its components change with the degree of cortical synchronization. This phenomenon is ascribed to the state dependent relay function of the thalamus. Retinofugal projections from the central nervous system have been reported, although the physiological importance of such an input to the retina is questionable because of the low number of axons reaching the retina. Our hypothesis asserts that direct projection from the midbrain dorsal raphe is able to modulate retinal functioning. To test this hypothesis we investigated the changes in the light evoked potentials recorded at three different sites (retina, chiasma opticum, visual cortex) of the visual system in freely moving animals during the normal sleep-wake cycle and cortical hypersynchronization, known as spike-wave discharges (SWDs), the electroencephalographic hallmark of absence epileptic seizures. WAG/Rij rats are genetically determined to produce SWDs of thalamocortical origin lasting few seconds. They are considered to be the pathological amplifications of the normal alpha-spindles.

The area of the first positive component of the electroretinogram, the b-wave (which reflects the K+-siphoning of the retinal Müller cells) did not change significantly during slow-wave sleep (SWS) (113,7±23,5%), paradox sleep (PS) (102,5±11,3%), and SWD (105, 3±26,1%). Both the area of the first positive component (B+) and the first negative component (B-) of the ganglion cell sequence (GCS), recorded from the chiasma opticum changed significantly during SWS (B+ 293,4±38,72%; B- 344,8±54,6%), PS (B+ 190,6±26,8%; B- 182,8±18%) and SWD (B+ 158,4±23,5%; B- 207,2±19,1%). In the case of the cortical visual evoked potential the area of its first positive component (P1) changed significantly during SWS (146,7±21,2%), PS (137,5±20,5%), and SWD (125,9±6,48%). The second negative component of the cortical visual evoked potential changed significantly as well during SWS (129,4±13,0%), PS (125,0±12,1%), and SWD (202,2±22,7%).(The deviations are given in SD. The changes are given in the percentage of the area of the pertinent evoked potential component recorded during wakefullness).

The observed alterations were significant, but not drastic, and can not be the main reason for the loss of vision during normal sleep and spike-wave discharge. However, we can conclude that central efferents modify the visual signal processing at retinal level.

Examination of β-amyloid and other neuroactive chemicals effective on mammalian nervous tissue on Lymnaea stagnalis

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The development of a certain group of the neurodegenerative diseases can be connected to the false processing of proteins in the nervous system. Such a false oligopeptide is the β-amyloid, deriving from false cleavage of the amyloid precursor protein (APP). It is prone to β-aggregation and it is the main factor of Alzheimer's disease pathomechanism.

High-n aggregates of β-amyloid were believed to be very toxic but we know recently that the low-n aggregates are even more toxic. Small aggregates are preferentially in the synapses. Data suggests that soluble oligomers of β-amyloid interfere with synaptic functions, disturbing the learning and memory processes. However, studies on correlation between the aggregate size and synaptic toxicity of Aβ oligomers has not been studied yet.

Thus, we tested the strength of a monosynaptic connection, between the known cerebral giant cell (CGC) and its postsynaptic partner, the B4 motoneuron in the buccal ganglion in Lymnaea stagnalis (pond snail). We also tested the effect of β-amyloid monomers on the resting potential of CGC. In addition we examined the CGC with two-electrode voltage-clamp method. The 1-42-amyloid monomer had a significant hyperpolarizing effect on the CGC, but no changes of synaptic efficiency. The same β-amyloid solutions were tested on hippocampal population-spikes in rats. Solution of mixed aggregates of β-amyloid was applied intra-cerebroventricularly (i.c.v.). β-amyloid solution significantly decreased the amplitude of the responses in the hippocampus verifying the hyperpolarizing effect of amyloid in Lymnea on a mammalian brain. Besides the β-amyloid, we tested the effect of a molecule developed for treating memory failures, in the Lymnaea model. Our experiments show that experiments ran in parallel in vertebrate and invertebrate models can be an effective approach to disclose molecular mechanisms of action of different sized β-amyloid aggregates.

Differential effect of the somatostatin analogue TT-232 on the inhibitory and excitatory postsynaptic currents of CA3 pyramidal cells in vitro

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TT-232, a cyclopeptide analogue of somatostatin, is an anti-tumour drug candidate lacking endocrine side effects, and exerts also systemic anti-inflammatory and analgesic effects. It has been shown that TT-232 bounds to cloned somatostatin receptor subtypes and displaced [125I]-somatostatin specifically bound to hippocampal synaptic membranes with an IC50 value of 0.1nM. Docking experiments suggested its binding to type-1 somatostatin receptor (Simon et al., 2004, Biochem. Biophys. Res. Commun. 316, 1059).

Here we compared the effects of TT-232 (5μM) with the effects of somatostatin (5μM) on different hippocampal activities. Somatostatin reduced both the amplitude and the frequency of pharmacologically isolated spontaneous EPSCs recorded from CA3 pyramidal cells by 28,61±16,7% and 75,8±1,0%, respectively, and also of spontaneous IPSCs by 39,87±10,4% and 52,65±3.0% respectively. In the presence of TTX (1μM), it increased intracellular Ca2+ ion concentration, (12.0±10.2-fold increase of basal fluorescence) in CA3 and hilar cells. The effect of TT-232 on EPSCs was similar to the effect of somatostatin (18,23±6,4% and 75,74±11,9% decrease of the amplitude and frequency, respectively), but it decreased only the frequency of IPSCs by 39.71±3.5% without affecting their amplitude and did not induce intracellular Ca2+ ion changes. Somatostatin decreased the length of low-[Mg2+]-induced seizure-like events by 18,38±12,87% and increased the interval between them by 14,11±17,18%. By contrast, TT-232 reduced both the length and the interval of low-[Mg2+]-induced seizure-like events by 12,94±16,89% and 27,75±1,15% respectively. According to our results TT2-32 activates only a subset of hippocampal somatostatin receptors. TT-232-sensitive receptors control excitatory inputs. Inhibitory inputs, or intracellular Ca2+ ion changes, however, are controlled by TT-232-insensitive somatostatin receptors. The difference in the effect of somatostatin and TT-232 on low-[Mg2+]-induced seizure-like events suggests subtype-specific functions of somatostatin receptors.

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Severity of axonal injury is associated with the energy of injury as well as survival time

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Traumatically induced diffuse axonal injury is evoked by the shearing/tearing forces of injury and significantly contributes to the outcome of the head injured. The impact acceleration rat brain injury model described by Marmarou and coworkers constitutes one of the most widespread models of diffuse axonal injury.

Although the pathobiology of DAI has been extensively analyzed in this model evoking moderate brain injury, the correlation between the energy of the impact and severity of axonal injury was not established. Similarly, the association between the duration of survival and the extent of DAI was not established in mild impact acceleration head injury so far.

The present study was initiated in an attempt to establish the association between the impact /energy of trauma/ and the occurrence of DAI as well as to prove that the magnitude of axonal injury is closely associated with survival time in all energy-levels evoking brain trauma in this model.

In all, 24 WR rats weighing 350-400g were injured with a brass weight of 450g from a height of 50, 100, 150cm with survival times of 30min, 1hr, 2hrs and 6hrs.

Immunohistochemistry with anti-APP as primary antibody (marker of altered axoplasmatic transport) was used to assess axonal damage. Immunopositive axon profiles in the area of corticospinal tract (CSpT) and medial longitudinal fascicle (FLM) were counted using the NIH Image J software. The density of immunoreactive profiles was analyzed and statistically compared to establish significant differences between each treatment group.

The results indicate that the density of APP immunoreactive axonal profiles that is the severity of DAI is positively associated with injury severity in terms of the level of energy evoking TBI. Similarly, in all levels of energy evoking TBI a significant increase could be observed in the density of immunoreactive axonal segments in association with the duration of survival.

These observations indicate the mild head injury is definitely capable of evoking DAI; the kinetic properties of pathological processes appear surprisingly similar regardless the energy-level responsible for TBI

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Functional neurotoxic effects of heavy metal combinations given to rats during pre- and postnatal development

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Heavy metals belong to the well-known pollutants present in the general environment and in workplace settings due to industrial and other activities. There is an amount of data on neurotoxicity of various heavy metals but much less on the consequences of simultaneous exposure by several of them. It is also known that during the periods of prenatal and early postnatal development the nervous system is especially sensitive to toxic influences. The aim of the present study was to investigate certain alterations in the activity of the central and peripheral nervous system of rats, exposed to triple combinations of heavy metals during pre- and postnatal development.

Lead (low dose: 80 mg/kg, high dose: 320 mg/kg, all doses for pure metal), mercury (0.4 and 1.6 mg/kg), cadmium (3.5 and 14.0 mg/kg) and manganese (3.6 and 14.5 mg/kg) were given in aqueous solution by gavage in low and high dose lead-mercury-cadmium (PbHgCd) and mercury-cadmium-manganese (HgCdMn) combination. The three treatment protocols were:

1/ Pregnancy (P) protocol: Pregnant females treated daily from the 5th to 15th day of pregnancy. 2/ Pregnancy+Lactation (P+L) protocol: The dams treated as above plus during lactation from the 2nd day after delivery until separation the offspring. 3/ Pregnancy+Lactation+Post-weaning (P+L+P) protocol: The dams treated as above plus the male offspring treated after weaning for further 8 weeks in a 5 days per week schedule. Each protocol had its own control group, receiving distilled water in the corresponding periods. In their 12th week of life, the young males were prepared for electrophysiology in urethane anesthesia. Spontaneous and evoked activity of the somatosensory, visual and auditory cortical areas, and compound action potential from the tail nerve, were recorded.

In the spontaneous activity, high dose PbHgCd caused increase in the delta and theta, and decrease in the beta2 and gamma bands. The changes were more pronounced in the P+L+P treated rats than in those treated for shorter time. In the high dose HgCdMn groups, the effect on delta activity was absent. In the cortical evoked responses, increase of the onset latency was observed with both combinations, and the change was stronger with longer overall exposure time (P+L+P vs. P+L, P+L vs. P). The conduction velocity of the tail nerve decreased in the treated groups but the changes in the refractoryness of the nerve were not significant.

The results emphasize the need for better protection of potentially exposed humans, in which functional biomarkers, developed on the basis of electrophysiological effects, may have a major role.

Presynaptic properties and postsynaptic targets of peptidergic nociceptive primary afferents that express HCN channel subunit 2 in laminae I-II of the spinal dorsal horn in rats

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Neurons possess a rich variety of voltage- and ligand-gated ion channels, including hyperpolarization-activated and cyclic nucleotide-gated channels (HCN), that are usually activated during membrane hyperpolarization following the termination of action potentials and provide a mixed inward Na+/K+ current that slowly depolarizes the plasma membrane. In our previous study we have demonstrated that HCN channel subunit 2 (HCN2) is expressed by terminals of peptidergic nociceptive primary afferents in laminae I-IIo of the rat spinal dorsal horn. In the present experiment we investigated the presynaptic chemical properties and postsynaptic targets of these HCN2-expressing primary afferent terminals, using double and triple labeling immunocytochemical methods for confocal microscopy. We have demonstrated that most of the HCN2-expressing primary afferent terminals are also immunoreactive for substance-P (SP). These double labeled terminals contact spinal neurons that express neurokinin-1 receptor (NK1-R), the receptor of SP. Investigating the co-localization of HCN2 and vesicular glutamate transporters (VGluT1-3), we found that HCN2 immunoreactivity is completely segregated from VGluT1 and VGlut3, and for some reason the co-localization between HCN2 and VGluT2 was also very weak. We have also demonstrated that only a small proportion of HCN2-IR terminals show positive immunostaining for MOR and GABAB2-R. Investigating the postsynaptic targets of HCN2-expressing primary afferents we found that HCN2-IR terminals frequently contact spinal neurons that express NK1-receptor, calbindin D28k, GluR2 subunit of AMPA receptors and MOR.

Multiple chemosensitivity of feeding-associated neurons in the limbic forebrain

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The nucleus accumbens (NAcc) and the mediodorsal prefrontal cortex (mdPFC), key structures of the forebrain limbic circuitry, are known to play important roles in various homeostatic regulatory functions. Recently discovered special "glucose-monitoring" (GM) neurons here have already been shown to be intimately involved in these mechanisms. Very little is known, however, about feeding-associated chemical characteristics of NAcc and mdPFC neurons, consequently, their 'endogenous' and 'exogenous' chemosensitivities are yet to be defined. In the present studies, therefore, extracellular single neuron activity was recorded in the NAcc and mdPFC of male Wistar rats by means of tungsten wire multibarreled glass microelectrodes during 1) microelectrophoretic administration of various chemicals (such as D-glucose, DA, NA, DA-antagonists, GABA, Ach, IL-1ß) and 2) gustatory stimulations with a standard stimulus array of 6 tastants (orange juice, sucrose, sodium chloride, hydrochloric acid, quinine hydrochlorid and monosodium-L-glutamate as 'umami' substance).

In both regions, more than 10% of all neurons tested descreased or increased in firing rate in response to glucose, thus, they were found to be elements of the forebrain glucose-monitoring (GM) network. These chemosensory neurons displayed differential activity changes to a broad range of neuromodulators tested. Appx. the third of all cells - and a high majority of the GM neurons - were also found to be influenced by intraorally delivered taste stimuli. Our present findings, along with previous data, substantiate a clear overlapping of the exogenous and endogenous chemosensory systems in the rodent forebrain. Results indicate important integrative functions of neurons of the NAcc and mdPFC in adaptive mechanisms of the central feeding and metabolic control.

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Multimodal sensory properties of the neurons in the feline caudate nucleus and substantia nigra

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The basal ganglia are widely regarded as structures involved in sensorimotor co-ordination, but little is known about the sensory background of their function.

We publish here a detailed description of the receptive field properties of excitatory visual, auditory, somatosensory, and multisensory neurons in the caudate nucleus and in the substantia nigra pars reticularis. Altogether 111 caudate sensory neurons and 124 substantia nigra sensory neurons were recorded extracellularly in halothane-anesthetized, immobilized, artificially ventilated cats. The sensory properties of the caudate and nigral neurons were found to be quite similar. The majority of the units were unimodal. We found 30 (27%) visual, 9 (8%) auditory and 31 (28%) somatosensory unimodal neurons in the caudate nucleus and 62 (50%) visual, 6 (5%) auditory and 22 (18%) somatosensory unimodal units in the substantia nigra.. A large portion of the neurons in these basal ganglia was bimodal (33 (30%) in the caudate nucleus and 25 (20%) in the substantia nigra) or trimodal (8 (7%) in caudate nucleus and 9 (7%) in substantia nigra). The visual and auditory receptive fields covered the whole physically approachable sensory field, while the somatosensory receptive fields covered the whole body surface of the animal. The multisensory receptive fields were similarly extremely large. We observed no signs of retinotopic or somatotopic organization among the neurons of the nigro-striatal system.

The particular receptive field properties of the basal ganglia described here suggest that the sensory feedback for motor actions regulated by the basal ganglia might be served by multisensory pathways of tectal origin.

Peripheral vs. central thermosensitivity in cold-adapted and non-adapted rats

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Thermal adaptation status influences many homeostatic mechanisms (food intake, metabolic processes, cardiovascular responsiveness, etc). For analyzing the signals that determine the adaptation status, the thermoregulatory responses were investigated in cold-adapted (CA) and non-adapted (NA) Wistar rats. CA and NA rats were maintained at environmental temperatures of 3-5 ºC and 23-26 ºC, respectively, for at least 4 weeks. In acute experiments their core body temperature (Tc) was measured by thermocouples; an open-circuit metabolic chamber and diaferometer served for assessing metabolic rate. The chamber was immersed into a waterbath, which secured standard environmental temperature and allowed sudden changes of it, as well. Standard thermoneutral temperature was 30 ºC for NA and 25 ºC for CA rats, during exogenous cooling the temperature was 21 or 15 ºC for NA and 15 or 5 ºC for CA rats. For endogenous cooling a pp10 polyethylene loop was preimplanted, which was led through the jugular vein to the inferior vena cava: perfusion of the loop by cold water (ca. 5 ºC) secured endogenous cooling, the severity of cooling was changed by the perfusion speed (2 vs. 5 ml/min by a perfusion pump). Upon 1-h exposure to moderate external cold (21 ºC vs. 15 ºC in NA vs. CA rats), metabolic rate increased by about 50% above the resting level in both groups, Tc decreased about 0.5 ºC in NA but did not change in CA rats. Severe cold (15 ºC vs. 5 ºC in NA vs. CA rats) caused a 100-120% rise in metabolic rate with a greater (about 0.8 ºC) Tc fall in NA and a paradoxical Tc rise (0.5-0.7 ºC) in CA rats. Endogenous cooling by 2 ml/min perfusion enhanced metabolic rate by about 60% in both groups, while Tc decreased by about 0.9 ºC in NA and 0.5 ºC in CA rats. 5 ml/min perfusion in CA rats induced a 110% rise in metabolic rate, together with a 1.5 ºC fall in Tc. The Tc falls were not due to metabolic exhaustion: in NA rats, severe external cold increased metabolic rate so much that such increase should have been enough to maintain normothermia at moderate cold, but the signal/response ratio of the regulation did not allow this. Similarly, in CA rats, the metabolic rise upon 5 ml/min perfusion was so great that it should have been enough to save homeothermia at slower perfusion, but the regulation was similar as in NA rats. The regulatory change causing paradoxical Tc rise upon cooling could only be evoked by exogenous cold signals – their effectiveness changed with adaptation.

Thermal hyperalgesia evoked by a mild heat injury and its pharmacological modulation as measured with a novel increasing-temperature water bath

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Conventional thermonociceptive tests measure the latency of nocifensive reactions (expressed in seconds) evoked by heat stimuli of suprathreshold intensity. In the present study, however, the noxious heat threshold (measured as a temperature in °C) of rats was determined by a newly-developed increasing-temperature water bath. The hindpaw of female Wistar rats was immersed into a container filled with water whose temperature was increased near-linearly at a rate of 24 °C/min until the animal withdrew its paw. This temperature was considered as the behavioural noxious heat threshold which was reproducible upon repeated measurements. After determination of the control heat threshold of the animals thermal hyperalgesia was induced by a heat injury applied to the hindpaw according to the following protocol: under light aether anaesthesia the rat's paw was immersed into a 51°C water bath for 20 seconds. Heat threshold determinations were repeated 10, 20, 30, 40, 50 and 60 min after heat injury.

The nociceptive heat threshold of untreated rats was 43.4+0.4°C. Following induction of heat injury rats recovering from anaesthesia showed no signs of spontaneous pain but upon determining the noxious heat threshold, a 7-8°C drop of the threshold was observed which was maintained for at least an hour. Morphine, diclofenac and ibuprofen injected 20 min after the heat injury dose-dependently inhibited the drop of heat threshold as measured 20-30 min after administration with minimum effective doses of 0.3, 0.3 and 10 mg/kg i.p., respectively. Heat hyperalgesia was also diminished by the somatostatin receptor agonist TT-232 (50-100 microg/kg i.p.). The capsaicin TRPV1 receptor antagonist SC 0030 (2 mg/kg i.p.) inhibited thermal hyperalgesia whereas the lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA, 10 mg/kg i.p.) failed to do so.

It is concluded that the mild heat injury-induced drop of the behavioural nociceptive heat threshold measured by the increasing-temperature water bath is a novel thermal hyperalgesia model in rats. It displays high sensitivity to conventional opioid and non-opioid analgesics as well as the somatostatin receptor agonist TT-232. The mechanism of heat hyperalgesia is likely to involve sensitization/activation of TRPV1 receptors and formation of cyclooxygenase products but does not appear to depend on intact lipoxygenase activity.

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Commissural propriospinal connections between the lateral aspects of laminae III-IV in the lumbar spinal cord

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It has been established that there is a strong functional link between sensory neural circuits on the two sides of the spinal cord. In one of our recent studies we have provided a morphological confirmation of this functional phenomenon, presenting evidence for the presence of a direct commissural connection between the lateral aspects of the dorsal horn on the two sides of the lumbar spinal cord. By using a combination of neural tracing and immunocytochemical detection of neural markers like vesicular glutamate transporters (VGLUT1, VGLUT2, VGLUT3), glutamic acid decarboxylase (GAD65/67), glycine-transporter (GLYT2) and met-enkephalin that are characteristic of various subsets of excitatory and inhibitory neurons, we here investigated the distribution, synaptic relations and neurochemical characteristics of the commissural axon terminals. We have found that the cells of origin of commissural fibers in the lateral aspect of the dorsal horn were confined to the very lateral aspect of laminae III-IV and projected to the corresponding area of the contralateral gray matter. Most of the commissural axon terminals established synaptic contacts with dendrites. The numbers of axospinous and axosomatic synaptic contacts were very limited. We have demonstrated that reciprocal interactions among commissural neurons also exist. More than three-fourth of the labelled axon terminals were immunostained for glutamic acid decarboxylase and/or glycine-transporter, but none of them showed positive immunoreaction for met-enkephain and vesicular glutamate transporters. The results indicate that there is a substantial reciprocal commissural synaptic interaction between the lateral aspects of laminae III-IV on the two sides of the lumbar spinal cord, and that this pathway may transmit both inhibitory and excitatory signals to their postsynaptic targets.

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Drug administration via reverse dialysis during juxtacellular recording of single unit activity in the rat amygdala

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The amygdala can be determined as sensory interface for conditioned stimulus information, such as pain. It is, therefore, important to study the electrophysiology, neurochemistry, morphology and axonal projections of amygdaloid single neurons having role in the sensory processing. Single neurons can be recorded extracellularly and labeled by juxtacellular application of Neurobiotine using high impedance glass microelectrodes. However, the recording is very sensitive to even slight movements of the tissue, so stable recording is not possible during icv drug injection. We present a method for studying the effects of icv administered drugs by utilizing a combination of reverse dialysis and extracellular single neuron recording in the amygdala.

In anaesthetized rats (urethane 1.25g/kg imp.) the guide cannula was moved with a micromanipulator to the ventral part of the lateral ventricle adjacent to the amygdala. The concentric probes consisted of a 1.5 mm long dialysis membrane (o.d. 250 μm) with cut-off molecular weight 35000. A fused silica capillary as inlet was connected direct to Rheodyne model 7125 injector with minimal dead volume. The injector connected to a microinfusion pump, was located outside the grounded recording cage and perfused at 1 μl/min with a Ringer's solution during the whole experiment. The sample loop was completely filled with excess sample volume. Perfusion of drugs with constant concentration can be ensured up to half the loop volume. Solutions of Lidocain (1 %) or Glutamate (0.1mM, 1mM and 10mM) or Ringer as control was perfused. Extracellular recordings were performed using glass microelectrodes (1-2 μm tip diameter, 15-40 MΩ) containing 0.5 M NaCl solution and 2 % Neurobiotine suitable for juxtacellular labeling of single neurons. The electrophysiological equipment consisted of an iontophoretic preamplifier (Supertech Ltd., Hungary), main amplifier PC, Oscilloscopes, and a CED 1401 plus interface. For sensory stimulation foot's pinching was used.

Stable recording of single neurons during continuous reverse dialysis and repeated drug perfusion was possible up to 3 hours. The onset latency of the drug effects was 2 to 10 minutes. Repeated Lidocaine perfusion decreased spontaneous firing rate and changed firing pattern in the neurons. Glutamate (1 mM) increased the spontaneous firing rate and decreased the response magnitude to sensory stimulation. Ringer's solution applied in the same manner did not have any effect on single unit activity. After electrophysiological characterization neurons were labeled by juxtacellular Neurobiotine application.

The present method seems to be useful to avoid artifacts usually caused by liquid injection into the brain tissue. A disadvantage of the technique is the long onset latency of drug effects, so it only can be used for long lasting effects.

Leptin resistance in histamine deficient, histidine decarboxylase (HDC)-KO mice

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Histamine is an important homeostatic regulator, central histamine influences sleep-wakefulness, metabolism and neuroendocrine processes. Histamine is an ackowledged anorectic factor that inhibits food intake and stimulates energy metabolism. The effect of life long absence of histamine was investigated in histidine decarboxylase knock-out (HDC-KO) mice. Adult HDC-KO mice show a metabolic phenotype including obesity, increase in visceral fat depots, hiperleptinemia and disturbed blood sugar regulation. In cold environment histamine deficient animals are unable to mobilize energy. To determinate whether increased leptin gene expression in adipose tissues is responsible for high plasma leptin concentration, we have measured leptin expression in different fat tissues of WT and HDC-KO mice by real-time PCR. The highest leptin expression was found in epididymal adipose tissue, which was 13 times higher than in brown adipose tissue and 3 times higher than in subcutan adipose tissue. However we couldn´t detect significant difference in leptin expression between WT and HDC-KO mice in all adipose tissue samples investigated. Hiperleptinemia is often associated with leptin resistance in obese human patients. To address the issue of leptin resistance, we have compared leptin receptor (OB-R) expression in the hypothalamus and liver of WT and HDC-KO animals. The expression of long (signaling) form of leptin receptor (Ob-Rb) was not significantly different in the two genotypes. In addition, the transcription of two major hypothalamic neuropeptides, the orexigenic NPY and the anorexigenic POMC in the arcuate nucleus was also unaltered in histamine deficient mice compared to wild type animals as revealed by in situ hybridization histochemistry. Further experiments are designed to detect defects in the leptin signal transduction pathway to find out the cause of leptin insensitivity in the absence of central histamine.

Occurrance and the role of G-protein coupled receptor kinase in the desensitisation of mytilus-inhibitory peptide receptors in Helix pomatia

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G protein coupled receptors (GPCR) are regulated via phosphorylation by different GPCR kinases (GRK). The GRK family comprises of seven members (GRK 1-7) and plays an important role in stimulus-dependent receptor phosphorylation and desensitisation of the receptors.. Desensitization is an adaptive mechanism of GPCR, which protects cells against receptor over stimulation. Two types of desensitization have been described: the homologous, who mainly involves GRK's and arrestins and heterologous desensitization involving PKA and PKC, as yet. Mammalian rhodopsin kinase and β-adrenerg receptor kinase (βARK; consists of 689 AA in most species) are the most studied members among known GRK's. βARK is directly activated by a G protein βγ-subunit, thereafter associates with the plasma membrane, where it phosphorylates agonist activated receptor. The βγ-binding domain of βARK 1/2 is located in the plectstrin homology domain (residues 643-670 AA). Here, we report the existence of βARK type kinase in the snail, Helix pomatia. Western blot analysis and voltage-clamp methods were used to demonstrate the presence and physiological role in the desensitization of the βARK. Using an antibody against βARK 1/2 (anti-GRK 2/3, Sigma) we have found that ßARK is expressed in a variety of cells, including neurons, salivary gland, salivary duct and eye. The molecular mass of the βARK was ~80 kDa as estimated by SDS-PAGE (silver staining).We have also shown that protein kinases participate in the desensitization of the mytilus inhibitory peptide (MIP, endogenous peptide family, isolated from molluscan nervous system) receptors. Intracellular injection of anti-GRK 2/3 or PDBu suppressed the desensitization as well as the recovery from desensitization. It is suggested that receptor desensitization was due to the interaction of both GRK's and PKA in the snail neuron. In summary, this is the first report demonstrating the presence of a mammalian type βARK 1/2 in molluscan nervous system and in different peripheral tissues. The physiological role of the βARK in the neuropeptide (MIP)-receptor desensitization was also demonstrated. Our data support the close homology between molluscan and mammalian receptor kinases.

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Functional and morphological study of projection neurons in lamina I of the rat spinal cord

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The superficial laminae (I and II) of the spinal cord are densely innervated by nociceptive primary afferents (many of which contain Substance P-SP) and also receives input from non-nociceptive afferents. Many neurons in lamina I project to brain areas such as thalamus, periaqueductal grey matter (PAG), parabrachial area (LPB) and reticular formation of the caudal ventrolateral medulla (CVLM). 80% of these neurons express the neurokinin-1 (NK1) receptor and most respond to noxious stimuli. Lamina I neurons can be classified into three morphological categories: fusiform, multipolar (also known flattened) and pyramidal cells.

In the present studies we tested the hypothesis that morphology of lamina I neurons in the rat is related to function by examining (1) their afferent input from SP containing primary afferents (identified by the presence of both SP and calcitonin gene related peptide), (2) their projection targets and (3) the up-regulation of Fos following noxious stimulus administered under terminal general anaesthesia.

Projection neurons that expressed the NK1 receptor were found to have a significantly higher density of contacts from SP-containing afferents than those that lacked the receptor. However, among the NK1 receptor expressing neurons innervation density did not differ between the three morphological types. It was found that 85% of projection cells in lamina I could be labelled from either the CVLM or LPB on the contralateral side, and that some cells projected bilaterally. Around 30% of the projection cells project to the PAG and 90% of these were also labelled from CVLM or LPB. Subcutaneous formalin injection, noxious heat and cold induced Fos in many of the projection neurons. For each stimulus the proportion of projection cells with Fos in their nuclei was significantly higher for those with NK1 receptor than for those that lacked the receptor. Within the NK1 receptor expressing group Fos expression did not differ among morphological types following formalin injection or noxious heat. However within this group, noxious cold stimulation caused Fos expression was in 63% of multipolar neurons but only in 19-26% of fusiform or pyramidal cells. Therefore there appears to be some correlation between morphology and function for lamina I projection neurons.

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Anatomy, histology and histochemistry of a ventral nerve cord ganglion in an isopod crustacean Porcellio scaber

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Applying qualitative and quantitative light microscopy, enzyme and immunocytochemistry, extracellular tract tracing (Lucifer yellow and horse-radish peroxidase filling) the organization of a ventral nerve cord ganglion (2nd thoracic ganglion) and its segmental nerves were investigated in Porcellio scaber. Besides the anatomical location and number of neurons further the neuropile organisation some transmitter-specific neurons have also been revealed. The occurrence of GABA, monoamines (serotonin, octopamin) tyrosine hydroxilase, NADPH-diaphorase, neuropeptides (allatostatin, crustacean cardioactive peptide, mio-inhibitory peptide and proctolin) in perikarya and nerve fibres were identified. The relevance of neuropeptide-immunocytochemistry was examined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Similarly to other isopod species three pairs of putative accessory neurons (#1, #2, #3), thought to be innervating the stretch-receptor organ and receptor muscles, were identified in the ganglion that send their processes to their target organs via the third segmental nerves. #1 neurons proved to be NADPH-diaphorase and tyrosine hydroxilase positive while #2 neurons were GABA-immunorective.

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PACAP attenuates the monosodium-glutamate-induced retinal degeneration in the rat

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic neuropeptide with a wide range of effects in the central and peripheral nervous systems. PACAP has well documented neurotrophic and neuroprotective actions in both in vitro and in vivo models of different neuronal injuries. Monosodium-glutamate (MSG) exerts toxic effects in the newborn animals, among others, MSG-treatment leads to retinal degeneration. The aim of the present study was to investigate the possible neuroprotective effect of PACAP in retinal degeneration induced by monosodium glutamate (MSG) in neonatal rats. Preceding the MSG treatment, PACAP (1 or 100 pmol/ 5 µl) was injected unilaterally into the vitreous body on postnatal days 1, 5 and 9. Immediately after the PACAP treatment, pups were treated with 2 mg/g body weight MSG subcutaneously. At 3 weeks of age, rats were sacrificed and retinas were removed and processed for histological examination. Our results show that MSG treatment caused severe degeneration, primarily of the inner retinal layers. The thickness of the entire retina was only approximately half of that of the normal retinas, and the inner nuclear layer seemed to be fused with the ganglionic cell layer, with no discernible inner plexiform layer. Retinas of animals treated with 1 pmol PACAP showed a similar degree of degeneration. However, retinas of rats treated with 100 pmol PACAP showed significantly less damage, with clearly distinguishable inner retinal layers. In summary, our present study shows that local PACAP treatment could attenuate the retinal degeneration induced by the excitotoxic effects of glutamate.

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Dye-coupled connections of the primary afferent fibers in the cerebellum of the frog

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Previous physiological and morphological experiments demonstrated that primary afferent vestibular fibers of the frog established chemical and electrical (gap junctional coupling), synapses on their secondary neurons. When the neurobiotin or Lucifer yellow, indicators of gap junctional coupling, was applied to primary afferent vestibular and dorsal root fibers of the frog a large number of so-called dye-coupled neurons were detected in their termination area including the cerebellum. In this study we have investigated whether the dye-coupled granule cells of the individual vestibular receptors and dorsal root fibers showed somatotopical localization in the cerebellum of the frog.

The experiments were performed on common water frogs, Rana esculenta. Under MS 222 anesthesia the vestibular nerve was approached from a ventral position. The individual branchlets of each receptor and the second, eighth and ninth dorsal root fibers were dissected and labeled separately with Neurobiotin. After 3-6 days the animals were sacrificed and perfused with 2 % paraformaldehyde and 1.25 % glutaraldehyde. The Neurobiotin was visualized on 60 µm sections with ABC reagent and in DAB-nickel solution. The positions of dye-coupled granule cells were reconstructed with Neurolucida.

Application of neurobiotin revealed that the dye-coupled granule cells of the vestibular nerve were predominantly found in the ipsilateral auricular lobe of the cerebellum and in a smaller number in the corpus cerebelli. Neurolucida reconstruction of the granule cells showed that a significant overlap exists between the territories of the individual vestibular receptors. The granule cells related to the anterior semicircular canal and the sacculus were detected throughout the auricular lobe, while the neurons of posterior semicircular canal were located in the caudal and ventral part of cerebellum. Dye-coupled neurons of dorsal root fibers were detected in the ipsilateral corpus cerebelli, only a few cells were detected in the auricular lobe. In case of lumbar segments the neurons were also present in the contralateral side. Labelled cells associated to the cervical spinal segment were located more medially that those cells of the lumbar spinal cord. Similarly to the vestibular receptors, a significant overlap was also detected between the dye-coupled neurons of the cervical and lumbar parts of the spinal cord.

The lack of somatotopical organization of dye-coupled granule cells of the individual vestibular receptors corroborates with the results of previous physiological and morphological experiments showing also a remarkable overlap in the vestibular nuclear complex. The overlap between the dye-coupled neurons of vestibular system and the dorsal root fibers may be the morphological background of the integration of vestibular and proprioceptive stimuli.

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In vitro inhibition of cholinesterases by organophosphates and carbamates in different tissues from invertebrates and vertebrates

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Background: The extensive use of organophosphates (OPs) and carbamates (CBs) as agricultural insecticides has resulted in their widespread distribution in the environment. The primary effect of these compounds is to inactivate cholinesterases (ChEs) in the central and peripheral nervous systems. The insecticides are generally regarded as relatively safe for humans. However, they may enter the blood stream and are subsequently transferred to the body tissues, where they inhibit ChEs.

Objectives: In the present work, the in vitro effects of four of the most widely used OPs (chlorpyrifos, diazonin, fenthoin and malathion) and two CBs (carbaryl and carbofuran) were studied on acetyl- (AChE) and butyrylcholinesterase (BuChE) extracted from different invertebrate and vertebrate tissues, including human.

Materials and Methods: The following tissues were used as sources of a) AChE: human brain, plasma and erythrocytes, rat brain, Drosophila head, and the electric organ from electric eel; and b) BuChE: human plasma. IC50 values were determined in 3-6 independent experiments, using 6-8 different concentrations of each OP or CB. AChE and BuChE activities were measured by the spectrophotometric method of Ellman et al., (1961).

Results: The sequence of effectivity of the inhibitors on the human brain AChE was: carbofuran>carbaryl>chlorpyrifos>malathion>diazonin=fenthoin. The different species yielded different IC50 values for the same inhibitor, e.g. malathion was an approximately 10 times better inhibitor of Drosophila brain AChE than of human brain AChE (9.68 vs 80.6 μM). Carbofuran was a 10 times stronger inhibitor of human brain AChE than of Drosophila brain AChE (0.054 vs 0.59 μM). The human serum BuChE was extremely sensitive to chlorpyrifos (0.026 μM). The human brain and erythrocyte AChE were more sensitive than the AChE in the serum to both OPs and CBs.

Conclusions: The CBs possess characteristic low IC50 levels and are therefore generally more potent than the OPs as in vitro inhibitors of AChE in all the tissues investigated. The different species display different degrees of sensitivity to either OPs or CBs. These insecticides inhibit both AChE and BuChE, sometimes to different degrees. Our results may be of clinical significance in occupational exposure, or accidental and suicidal poisonings due to OPs or CBs in humans.

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Effects of PACAP in animal models of brain pathologies

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic neuropeptide, with several distinct effects in the central and peripheral nervous systems. Among the many different actions, PACAP has been shown to be neurotrophic and neuroprotective both in vitro and in vivo. In the present study we report our recent results about its neuroprotective effects obtained in rat models of different brain pathologies. Among the neurodegenerative diseases, we studied the effects of PACAP treatment in a rat model of Parkinson`s and Huntington diseases. In both models, a local pretreatment of PACAP attenuated the degeneration of the neuronal population induced by 6-hydroxydopamine or quinolinic acid, respectively. Approximately 50% of the subtantia nigra or striatal neurons, respectively, were saved in the PACAP-treated animals. This neuronal protection was accompanied by less severe behavioral symptoms, such as motoric hypo- or hyperkinesia and less pronounced asymmetrical motor signs. This behavioral amelioration could be observed both in the acute postinjury state and in a better behavioral recovery. In a rat model of traumatic brain injury, induced by compact acceleration, PACAP treatment reduced the number of injured axons. We also investigated the effects of PACAP in a glutamate-induced retinal degeneration in newborn rats. It was found that intravitreal PACAP treatment attenuated the inner cellular loss in the retinas of the newborn rats. Taken together, or results further prove the neuroprotective effects of PACAP.

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Effects of PACAP in rat models of different types of brain injuries

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Pituitary adenylate cyclase activating polypeptide (PACAP) was discovered as a hypothalamic peptide that stimulates cAMP production in the pituitary gland. Since its discovery, widespread distribution of PACAP in the central and peripheral nervous systems has been described. PACAP has numerous actions in the nervous system, including neurotrophic and neuroprotective effects. It has been reported that PACAP protects hippocampal CA1 neurons in global cerebral ischemia, it has protective effects in fornix transection, facial nerve axotomy, spinal cord injury and optic nerve injury. In the present study we report our recent results on the neuroprotective effects of PACAP. We investigated its effects in focal cerebral ischemia, in a model of Parkinson`s disease and in traumatic brain injury. In focal cerebral ischemia, induced by the intraluminal filament model, we found that PACAP was able to reduce the infarct size by 40-50% in both permanent and transient occlusion of the middle cerebral artery. PACAP treatment also ameliorated the functional deficits, and reduced the number of apoptotic neurons. In a rat model of Parkinson`s disease, induced by unilateral 6-hydroxydopamine lesion of the substantia nigra, PACAP proved to be a very efficient neuroprotective agent. PACAP-treated animals showed no hypokinetic signs, and the asymmetrical neurological signs recovered by 10 days postlesion in contrast to control animals. The number of dopaminergic cells was 50% on the ipsilateral side when compared to the uninjured side, while it was less than 5% in control rats. In a rat model of traumatic brain injury, induced by compact acceleration, PACAP treatment reduced the number of injured axons. Our results demonstrate that PACAP is an effective neuroprotective agent in various models of brain injuries. The exact mechanism awaits further investigation, but according to presently available data, various factors can play a role, including the antiapoptotic, antiinflammatory and antioxidant effects of PACAP.

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Participation of limbic neurons in the innervation of the stomach

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In our previous experiments using an acidified ethanol induced ulcer model in rats, c-fos immunoreactive neurons were found in various parts of the prefrontal limbic cortex (the anterior cingulate (Aci), prelimbic (PL), infralimbic (IL) and dorsal peduncular (DP) cortical areas). Bilateral transections of the anterior cingulate cortex abolished the gastroprotective effect mediated by delta-opioid receptors in the lower brainstem by interrupting of prefrontal limbic-autonomic connections. Signals from prefrontal limbic cortical areas (PLC) may reach the vagal neurons in the medulla by three different routes: (1), direct projection; (2) projections through the hypothalamic paraventricular nucleus, (3) through the insular cortex or (4) the central nucleus of the amygdala (CNA) could serve as a relay. The existence of a prefrontal-amygdaloid pathway through the cingulate cortex was confirmed by biotinylated dextran amine injections to the Aci. Furthermore, using a transneuronal retrograde tract tracing, pseudorabies virus was unilaterally injected into the CNA. Four days after injections, we found bilateral labeling in all four PLC with an ipsilateral dominance. To improve the existence of a prelimbic-cingulate-CNA pathway, we combined the amygdala virus injections with surgical transsections of the cingulate cortex. After bilateral cingulate transections, we couldn't find virus-labeled cells in the PLC. In the case of transsections ipsilateral to the site of the injections, we found only ipsilateral labeling in the infralimbic and prelimbic cortices. When the transsection was contralateral to the site of the injection, labeled neurons were found bilaterally. These findings indicate that 1) fibers from the IL, PL and probably from DP neurons to the CNA may relayed by neurons in the anterior cingulate area, and 2) the prelimbic-cingulate-CNA pathway is crossing over in the cingulate cortex.

Transfection experiments using antisense locked nucleic acids to study the effect of Bmal1 clock gene on melatonin secretion in superfusion system

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Chick pinealocyte is a self-contained melatonin (MT) rhythm-generating system having an internal clock and photodetectors. It releases melatonin (MT) at night, which is the hormonal manifestation of the dark phase of environmental lighting conditions. Our earlier studies showed that bmal1 clock gene and serotonin-N-acetyltransferase (AA-NAT), the rate-limiting enzyme of MT synthesis are co-expressed in pinealocytes with a circadian pattern. Moreover, a potential binding site for BMAL1 transcription factor (E box element) has been found in the promoter of AA-NAT gene, however, the role of bmal1 clock gene in the regulation of AA-NAT expression is still a matter of debate. In our recent studies chick pinealocytes have been transfected in a superfusion system with two distinct antisense locked-nucleic acids encompassing the start codon of bmal1 mRNA. MT secretion has been screened for 72 hours, and bmal1 and AA-NAT mRNA levels have been measured by RT-multiplex PCR. Our results show that both antisense LNA synthesized for either inducing RNase H activity or blocking the translation machinery decreased significantly MT secretion, whereas an LNA non-complementary to chick bmal1 mRNA had only a negligible effect. Both antisense LNA reduced remarkably the expression of AA-NAT gene, whereas bmal1 mRNA level was suppressed only by the LNA designed for the induction of RNase H activity. Our data provide an evidence for the stimulatory effect of bmal1 and presumedly its protein product (BMAL1) on the gene expression of AA-NAT and the subsequent MT secretion. This study reports for the first time that transfection experiments using antisense LNA can be successfully performed in superfusion system (supported by OTKA T 042713).

Dopamine inhibits nocturnal melatonin secretion in chicken retina through receptors of D2-family

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Dopamine (DA) is the major catecholamine in the retina of various vertebrate species, where it functions as a neuroregulator/neurotransmitter. In birds, both endogenous clocks and photoreceptors are localized in the retina and the pineal gland releasing melatonin (MT) at night, which is the hormonal manifestation of the dark phase of environmental lighting conditions. Retinal MT has been postulated to act as a local neuromodulator inhibiting DA-ergic neurotransmission in the retina. However, little is known about the role of DA in the regulation of retinal MT secretion, and the receptors mediating its effect still remain to be discovered. In our recent experiments the circadian pattern of gene expression of arylalkylamine-N-acetyltransferase (AA-NAT), the key enzyme of retinal MT synthesis was investigated and compared to that of pinealocytes in the same animals. We also studied the role of the two major DA-receptor families (D1 and D2) in the regulation of circadian MT rhythm on an eye-cup model in vitro. AA-NAT mRNA levels were determined by RT-multiplex PCR collecting total RNA samples in every 2 hours, whereas MT content of tissue culture medium was measured by RIA. Our results show that retinal AA-NAT expression exhibits a diurnal rhythm with a 3-fold increase peaking 2 hours after the onset of dark phase which coincides with the elevation of pineal AA-NAT mRNA level. MT secretion also displays an approximately 1.8-fold increase in dark phase in vitro. Quinpirole (1 μM), a receptor agonist of D2-family reduced significantly MT secretion in dark phase ($P<0.05$), whereas it had only a negligible effect on MT secretion in light phase. SKF 38393 (1 μM), a receptor agonist of D1-family proved to be ineffective on MT secretion either in dark or light phases. Our results suggest that DA exerts an inhibitory effect on nighttime MT secretion in chicken retina (reciprocal negative feed-back), which is mediated primarily through the receptors of D2-family (supported by OTKA T 042713).

Chemically labeled neurons in the sacral spinal segments of the cat

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The distribution of two widespread occurring neuropeptides, enkephalin (ENK) and substance P (SP), was mapped in the cross sections of the sacral spinal cord using immunohistochemistry and confocal laser microscope. Young kittens (4 to 60 days old) were perfused in deep anesthesia, the spinal cord has been removed and 60 μm thick Vibratome sections were prepared from the sacral segments.

The marginal zone of the dorsal horn (lamina I) was the only region of the sacral gray matter where both ENK and SP immunoreactive fibers were jointly detected with great intensity. SP immunoreaction was found in the Lissauer's tract, in the intermediate zone and around the motoneurons in the dorsolateral, ventral and ventromedial motor nuclei. Delicate ENK immunoreactive nerve fibers filled the substantia gelatinosa (lamina II) in the dorsal horn. ENK immunoreactive fibers surrounded the central canal and filled the thick posterior commissure. ENK immunoreactive fibers formed a dense arborization around the neurons of the Onuf's nucleus in S1 segment. Two bundles of ENK immunreactive fibers could be traced to the Onuf's nucleus: one along the medial, the other along the lateral border of the dorsolateral motor nucleus. ENK immunoreactive fibers with a minor contribution of SP labeled fibers covered the crescent shaped area of the sacral parasympathetic neurons in S2 segment. Neuronal perikarya were detected with NeuN and anti-cholinacetyltransferase (Chat) immunsera. NeuN immunsera labeled all neurons in the spinal gray matter. Lamina I neurons were densely distributed in a 50 to 80 μm thick bend at the border of the dorsal horn. Neither the Onuf's nucleus nor the sacral parasympathetic nucleus could be delineated purely on the basis of the distribution of the neurons. Motoneurons in all motor nuclei, the neurons of the Onuf's nucleus and the neurons of the sacral parasympathetic nucleus were labeled with anti-Chat immunsera.

Neurons in lamina I are in a unique position to receive the joint and intense innervations from SP and ENK fibers. The differential distribution of SP and ENK suggests two innervation patterns in the sacral motor outflow. Prominent SP innervation with minor ENK contribution characterizes motoneurons supplying trunk and extremity muscles, while prominent ENK innervation with minor SP contribution characterizes neurons supplying the perineal muscles and indirectly the smooth muscles of the pelvic organs.

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Systemically administered glucosamine-kynurenic acid, but not pure kynurenic acid, is effective in decreasing the evoked activity in area CA1 of the rat hippocampus

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The metabolism of tryptophan along the kynurenine pathway (KP) yields several neuroactive intermediates, including kynurenic acid (KYNA), which is the only known endogenous N-methyl-D-aspartate (NMDA) receptor inhibitor; in parallel with this, it is an α7 nicotinic acetylcholinergic receptor (nAChR) antagonist. On the basis of these properties, KYNA might therefore come into consideration as a therapeutic agent in certain neurobiological disorders. However, the use of KYNA as a neuroprotective agent is practically excluded because KYNA hardly crosses the blood-brain barrier (BBB). We recently synthetized a new compound, glucosamine-kynurenic acid (KYNA-NH-GLUC), which is presumed to cross the BBB more easily. In this study, the effects of systemically administered KYNA and KYNA-NH-GLUC on CA3 stimulation-evoked population spike activity in region CA1 of the rat hippocampus were compared. The effect of KYNA or KYNA-NH-GLUC was augmented by probenecid (PROB) (200 mg/kg), which inhibits KYNA excretion from the cerebrospinal fluid. The results showed that, while KYNA administered i.p. or i.v. in doses of 17, 34, 68 or 136 μmol/kg, did not cause any observable change in the animals, 136 μmol/kg KYNA-NH-GLUC (either i.p. or i.v.) resulted in the sudden death of all the animals. The dose of 68 μmol/kg i.v., but not i.p., resulted in a sudden stoppage of breath, but the animals could be reanimated. As small a dose of KYNA-NH-GLUC as 17 μmol/kg i.p. resulted in a reduction in population spike amplitudes; this effect was further augmented by PROB, whereas neither 17 μmol/kg nor higher doses of pure KYNA had a similar effect. The results presented here suggest that KYNA-NH-GLUC passes the BBB much more readily than does KYNA.

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Behavioural evaluation of a simple, non transgenic model of Alzheimer's disease

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It is widely accepted that Alzheimer's disease pathogenesis involves accumulation of aggregated beta-amyloid (Aβ). Our aim was to evaluate whether single intracerebroventricular (i.c.v.) injection of aggregated Aβ can induce a memory deficit in rats, and whether this model can be used for testing neuroprotective drug candidates.

5 µl vehicle (distilled water), Aβ, or Aβ + drug candidate (1:5 molar ratio) was injected i.c.v. in young male Wistar rats. Aβ25-35 (10-3 M) or Aβ1-42 (10-4 M) was aggregated at 37ºC for 4 days. 15 days after the injection, reference memory was assessed in a Morris water maze, and working memory and/or attentional processes were evaluated in a spontaneous alternation task. Anxiety and general locomotory behavior were studied in an open field arena.

The i.c.v. injection of Aβ25-35 induced a slight but significant deterioration of the memory performance in the Morris maze. Such a change was not observed after the i.c.v. injection of Aβ1-42, but it decreased significantly the spontaneous alternation rate. The tested drug candidate restored the performance to control levels. As the behavior in the open field test was unchanged, Aβ effects proved to be specific on memory processes.

These results show that single injection of Aβ alters memory processes, which can be normalized by drugs. Our model is thus relevant, and especially interesting because of its simplicity and because it does not require physiologically unacceptable injection of organic solvents. However, in order to produce more robust alterations, a model involving multiple injections should be tested in the future.

Real time 3 dimensional nonlinear microscopy for the pharmacological study of neuronal circuits and dendritic integration

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It has been a decade since the importance of using nonlinear effects such as two photon absorption for high spatial resolution microscopy was realized [Denk, W. 1990]. Inspite of the fact that pioneers in the field initially adopted this technique for investigation of electric signaling of neurons, it is still a challenge to describe and understand the arithmetics that neurons use to integrate incoming inputs from connected neighboring neurons. The major problem of current nonlinear microscope systems is that the image is typically obtained by scanning the (femtosecond pulse) laser beam in a plane and a 3D image is obtained only after subsequent recording of hundreds of 2D images along the optical axis (z-axis). This procedure typically takes a few minutes being several orders of magnitude longer than the signal transition time (< 1 ms) of a neuron. In order to characterize the arithmetic function performed by a neuron, snapshots on its electric activity in the 3D volume should be taken in those points (such as dendritic spines) that were identified as inputs and outputs of a neural "circuit". Unfortunately, these inputs and outputs lay randomly distributed in the 3D volume that can not be investigated simultaneously by laser scanning microscope schemes.

In this paper we present a novel two-photon microscope scheme being capable of high speed measurement of neural networks or tiny neuronal structures such as spines in a 3D volume of appr. 0.6 x 0.6 x 0.2 cubic millimeter, while keeping the advantages of a convention two-photon microscope (e.g. high spatial resolution and high penetration depth). The basic idea is as follows: after taking a conventional 3D two-photon image of a biological sample, we determine the coordinates of those points (P1, P2 …Pn) in the 3D volume that are to be investigated. During measurement of the neural activity, only these points are sequentially addressed by a high speed acousto-optic (AO) switch combined with a fiber bond (comprising n pieces of single mode optical fibers) and a properly designed optical imaging system.

Dehydroepiandrosterone sulfate (DHEAS) is neuroprotective in a focal cortical cold lesion model1

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Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) are sex hormone precursors. They may function as neurotrophic or neuroprotective factors to protect central nervous system (CNS) neurons against a variety of insults, including excitotoxicity. The present study evaluated the effects of DHEAS in a focal cortical cold lesion model, in which DHEAS was administered (50 mg/kg sc) to a group of young rats one day before and subsequently one hour prior to cold lesion. The focal cortical cold lesion was carried out in the primary motor cortex with the aid of a cooled copper-cylinder placed onto the exposed cortex. After 1 hour, the animals were killed, and the brains were cut into 0,4-mm-thick slices and stained with 1% TTC. Percent hemispheric lesion volume (%HLV) was calculated for each animal. TTC-derived %HLV was significantly attenuated in the DHEAS pre-treated group. In parallel, in vitro electrophysiological experiments were conducted to explore the effects of DHEAS treatment on the synaptic transmission (measured by the amplitudes of field EPSPs) of layer II/III horizontal connections. In our ongoing experiments we are assessing the data from animal groups received both post-treatment of DHEAS and co-application of an aromatase inhibitor, letrozole, with DHEAS as a pre-treatment. The present study suggests that DHEAS may have substantial therapeutic benefit for the treatment of ischemic stroke.

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Voltage-gated K+ channel subunits expressed by the projection cells of the cochlear nucleus. Is there a unique pattern enabling their characteristic firing behaviour?

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The firing behaviour of the neurones is crucially determined by the presence and activity of the voltage gated K+ (Kv) channels situated in their surface membrane. As the subunit-specific channel blockers and primary antibodies targeting the various K+ channel subunits became available, it is possible now to map the Kv subunit pattern of the neurones of interest. In the present work some of the main projection neurones of the rat cochlear nucleus (pyramidal, octopus and giant cells) have been investigated to see whether their unique firing properties could be attributed to a specific distribution of K+ channel subunits.

The basic description of the Kv subunits expressed by the various cells was achieved by using immunochemistry on freshly isolated neurones and in 40-60 um thick free-floating slices. In the cases of the pyramidal cells, functional experiments were also conducted by employing the whole-cell configuration of the patch-clamp technique in combination with subunit-specific K+ channel blockers.

All three cell types showed Kv1.1 and 1.2 positivities, suggesting that they all possess a Dendrotoxin-sensitive, low-threshold current. Kv3.1 positivity was also strong in all three cells types, indicating that they all have a significant repolarizing power responsible for the quick termination of the action potentials. In the cases of the pyramidal cells, the presence of subunits known to be responsible for the genesis of transiently activating K+ currents (Kv1.4, Kv3.4, Kv4.2 and Kv4.3) was specifically studied. Pyramidal cells not only showed strong positivity for all these subunits, but the activity of the Kv3.4 and Kv4.2/4.3 containing channels could also be confirmed by using BDS-I and Phrixotoxin (known to selectively inhibit Kv3.4 and Kv4.2/4.3 containing K+ channels, respectively). Interestingly, when both drugs were applied in the extracellular solution, 60-75 % of the transient current still remained available, suggesting that a substantial portion of the pyramidal transient current is produced by members of the Kv1 family.

Our results indicate that the specific firing pattern of the projection neurones of the cochlear nucleus may not be the consequence of a unique K+ channel population expressed by them, but other factors (synaptic organisation, geometry of cells) must also contribute to the shaping of their specific activity pattern.

Activation and desensitization of the TRPV1 capsaicin receptor by substituted N-oleoylphenylethylamines

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N-oleoyldopamine (OLDA) recently identified in the mammalian brain has been proposed to be an endogenous ligand for the TRPV1 capsaicin receptor. We have investigated the effects of synthetic N-oleoylphenylethylamines (OLDA, 3-methoxy-OLDA, 4-methoxy-OLDA) in vitro on a new stable cell line (HT5-1) expressing a green fluorescent protein tagged version of the rat TRPV1 receptor. The fluorescent receptor allowed purification of HT5-1 cells based on the level of receptor expression resulting in a stable and uniform cell line with several advantages over existing TRPV1 expression systems. Measuring drug induced radioactive calcium uptake in the HT5-1 cell line indicated that the EC50 values of OLDA and 3-methoxy-OLDA were ~50 times higher than that of capsaicin and their efficacy was around 60% of the maximum response of capsaicin, while 4-methoxy-OLDA was ineffective. Fura-2 microfluorimetry measurements of calcium influx upon repeated OLDA , 3-methoxy-OLDA and capsaicin applications showed similar desensitization of the calcium ion transients both in cultured trigeminal ganglion neurons and HT5-1 cells. The in vivo effect of N-oleoylphenylethylamines was determined on the noxious heat threshold of rats by an increasing-temperature hot plate. Intraplantar injection (i.pl.) of OLDA (5 nmol.) markedly decreased the noxious heat threshold and this response was strongly inhibited by the TRPV1 receptor antagonist iodoresiniferatoxin. In mice the nocifensive reaction (paw licking /lifting) evoked by OLDA (50 nmol i.pl.) was significantly less sustained in TRPV1 knockout mice than in wild-type animals. Supp.: OTKA T-034911, T-037523, TS-040753, ETT-326/2003, NRDP1A/0021/2002, NRDP1/047/2001.

Neuropeptide-Y like immunoreactive elements in the retina of the spadefoot toad (Pelobates fuscus)

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In retinal research, experimental animals were Xenopus, Rana and Bufo. Little is knows from species of other genuses. Spadefoot toad (Pelobates fuscus) is a fossorial species which is active mostly at night. One expectation may be that the difference in behaviour can be connected with unusual features of retinal organization. Hence our objective was to study the retinal structure and neurochemistry in this species. Eyecups were made and after fixation we cut 15 μm thick cross section with cryostat and utilized indirect immunofluorescence to localize neuropeptide-Y (NPY) like immunoreactive elements in the retina. We also made wholemount preparations to decide whether the NPY positive cells form a regular, irregular or random mosaic. We have observed two types of NPY-positive large amacrine cells and many strongly immunopositive centrifugal fibres in sublamina 1 of the inner plexiform layer (IPL). Both of the amacrine cells have round soma and wide dendritic field. One amacrine cell type is in the inner row of the INL and project fibres to sublamina 1 while the other is a little further above the IPL and give dendrites to sublamina 2/3. We found 964 immunopositive neurons in one retina. Distribution of the cells were even and the density of amacrines were 22/mm2. The results of the nearest-neighbour analysis shows that the NPY positive cell mosaic has regular spacing (Rn=1.59) and these neurons are evenly distributed in the whole retina. In all frog species studied to date NPY- and tyrosine hydroxylase (TH)-immunopositive elements had similar distribution. However we did not find double labeled elements in Pelobates, similarly to other anuran species. To reveal the connection between the TH and NPY system we currently undertake electron microscopic observations.

Time-related changes of signal system elements in 4-aminopiridine induced epilepsy model

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Epileptic seizures induce a complex change in cellular tuning at the level of signaling system, but the kinetics of that changes are still not properly disclosed. The present study aimed to follow time-related changes of different components of the signal system in 4-aminopiridine induced (4-AP) epilepsy model. This model could exclude the additional signaling procedures induced by motor activity under seizures because it works in halothane anesthesia.

Experiments were carried out on adult SPRD rats. After anesthesia, we initiated epileptic activity with a small quantity of 4-AP (0,3 mg) crystal placed to the occipital cortex for 40 min. The epileptic activity was monitored by EEG. After 40 min 4-AP crystals were eliminated and anesthesia was stopped. Animals were killed at 3, 6, 12, 24, 48 hr after seizures and ipsi- and contralateral hippocampus and occipital cortex were dissected.

The anti-apoptotic phospho-Akt, 14-3-3, phospho-Akt/Akt rate and pro-apoptotic Bad proteins were detected with SDS-PAGE electrophoresis and Western-blot analysis. We applied immunprecipitation to establish Bad and 14-3-3 chaperon colocalization. The amount of caspase-8 was measured with spectrophotometry.

Within ipsi- and contralateral cortex rapid phosphorylation of Akt was detected after 3hr 4-Ap application compared to control samples. In the contralateral hippocampus an elevation in amount of Akt was observed in 3rd h after treatment. A continuous expression of 14-3-3 chaperon was observed in seizure exposed tissue samples collected from hippocampus and cortex. An increase in caspase-8 level was found 30 min after 4-AP treatment.

In the present study, we demonstrated, that seizures triggered the increase in level of phospho-Akt in the cortex which is known to be a prosurvival response. In contrast we observed increase in level of non-phosphorylated Akt in the hippocampus. This augmented level of Akt may be related with dissociation of Bad and 14-3-3. Both in the cortex and in the hippocampus we detected a growing amount of caspase-8 indicating activation of apoptotic signal transduction.

Modular evolution of the avian brain

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Changes of the overall body size can accompany allometric changes in the brain size, whereas it might be expected that selection on some behavioural traits causes size changes only in particular brain structures controlling that behaviour. In this study we carried out factor analyses on brain volumetric data of 28 avian species to assess whether the elements of a functional system could evolve together and independently of the changes in other systems. In order to reveal the correlated changes in the main brain subdivisions we also carried out several multiple regressions on the volumetric data of the medulla oblongata, cerebellum, mesencephalon, diencephalon and a neocortex-homologue region across bird species. Data of species cannot be handled as statistically independent points because of evolutionary relatedness, therefore we applied method of independent contrasts (Felsenstein, 1985). To avoid the effects of the ambiguity of the phylogenetic tree we used the phylogeny provided by Sibley and Ahlquist as well as a phylogenetic tree reconstructed from mtDNA sequences of the species. The method of correct normalisation has been also questioned in the literature, thus we analysed the data by using two different normalisation methods. By applying two phylogenetic trees and two normalisation methods the artefacts of these methods also could be eliminated.

Our results showed that the changes in the size of the elements of one particular pathway occurred in a highly correlated manner, but independently of the changes in other pathways: the parts of the auditory, visual and olfactory system could evolve independently of each other. We also found that nidopallium, mesopallium and Wulst, although not strictly linked functionally, evolved together. Among brain subdivisions we found less evolutionary interdependence in birds than in the mammalian brain as reported by Barton and Harvey (2000). As a significant difference the avian neocortex-homologue region seemed to evolve independently of the other brain subdivisions in contrast to the mammals where correlated evolution of the neocortex, diencephalon and cerebellum had been found. We concluded that the avian brain evolved as a system of relatively independent modules and the independent evolution of the neocortex homologue region could contribute to separation of the telencephalic and subtelencephalic mediating systems, which could increase the behavioural plasticity in birds.

GABA-R-mediated intracellular Ca^{+} signalling in undifferentiated mouse embryonic stem cells

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GABA (g-amminobutyric acid), the principal inhibitory neurotransmiter in the adult nervous system is also synthesized in the mouse embryo, where it exerts depolarizing actions, acting through GABA receptors, thereby raising the intracellular [Ca2+]i . Rise of Cai2+ leads to changes in gene expression, alter neurite outgrowth , activate enzyme pathways and influence neuronal differentiation and survival.

Mouse embryonic stem (ES) cells are pluripotent cells that have the capacity to differentiate into cell types of all three embryonic germ layers. Previous work in our lab has shown that GABA and different components of GABA signalling GABA synthesizing enzymes (embryonic and adult forms of GAD67), GABA transporters (GAT1, and VGAT) and different subunits of GABA-A and GABA-B receptor are synthesized in the mouse ES cells.

In this study we have used Laser Scanning Confocal Microscopy to study the effect of GABA receptor-activation on the [Ca2+]i level of undifferentiated mouse ES cells.

One-day old cultures of mouse embryonic stem cell line R1 were loaded by the cell permeable form of Fluo-3 AM dye. Intracellular Ca+ signalls were measured by using an Olympus BX61WI type confocal microscope. GABA receptor agonists (GABA 1-5 mM, muscimol 30 mM, baclofen 1-25 mM) and the antagonists (bicuculline 30mM, CGP55845 10-30mM) were bath applied. KCl was applied through a pipette positioned adjacent to the clones.

GABA, muscimol and baclofen evoked a transient increase in [Ca2+]i,

the basline fluerescence increases were 11.7+/-8.5-fold,7.6+/-4.2-fold and 10.8+/-9.3-fold, respecively. Muscimol-evoked [Ca2+]i increase was completely blocked by bicuculline. Baclofen induced either one transient increase in [Ca2+]i, or rhythmic [Ca2+]i changes. The former response could be antagonized by CGP55845 while the

rhythmic [Ca2+]i changes were insensitive to CGP55845. Hyperpolarizing GABAergic responses could not be revealed in stem cells, both baclofen and GABA failed to decrease KCl-evoked depolarization.

We could also demonstrate the presence of GABAB-RII and GABAA-b3 receptor subunits in the same ES cell cultures by immunocytochemistry using subunit- specific antibodies. Since the activation of both GABA-A and GABA-B receptors has a depolarising effect that leads to transient accumulation of [Ca2+]i, we suggest that GABA signaling, acting through elevation of [Ca2+]i plays a role in cell-cell communications and/or migration and proliferation during the earliest stages of embryonic development.

Gap junctional coupling between neurogliaform cells and various interneuron types in the neocortex

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Electrical synapses contribute to the generation of synchronous activity in neuronal networks. Several types of GABAergic neurons are coupled to interneurons of the same class via electrical synapses in several brain regions suggesting that synchronization through gap junctions could be limited to homogenous interneuron populations.

We have shown that neurogliaform cells elicit combined $GABA_A$ and $GABA_B$ receptor mediated postsynaptic responses in cortical pyramidal cells. To test whether neurogliaform cells participate in electrically coupled networks, we have recorded from pairs, triplets and quadruplets of cortical neurons in layer 2-3 of rat somatosensory cortex (P18-30). Neurogliaform cells were electrically coupled to other neurogliaform cells (n=7), basket cells (n=6) and members of other interneuron class/es (n=10) showing diverse firing patterns and morphology. Electrical coupling between neurogliaform cells was combined with slow IPSPs in 6 pairs. Neurogliaform cells involved in electrical coupling could elicit slow, $GABA_A$ and $GABA_B$ receptor mediated responses alone (n=89) or in combination with gap junctional potentials (n=13) in other types of postsynaptic cells. In contrast, different interneurons electrically coupled to neurogliaform cells triggered fast IPSPs (n=3) on other interneurons. Electron microscopically verified gap junctions (n=4) were found between proximal dendrites of neurogliaform cells and the soma or proximal dendrites of the coupled interneurons.

Our results suggest that neurogliaform cells are embedded into a widespread network of electrical synapses linking distinct interneuron classes acting on different types of postsynaptic receptor at various regions of target cells.

Description of the somatostatin analogue TT-232 binding crevice in a homology model of somatostatin receptor type 1

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The destruction of somatostatin (SST)-containing cells is the first sign of most central nervous system (CNS) diseases, indicating, an essential role for SST in CNS functioning. Increased CNS level of SST and formation of memory traces may also be related (Nyitrai et al., 2003: Eur. J. Pharmacol. 478, 111). To design specific SST analogues and/or peptidomimetics, however it is mandatory to know the type of SST receptor (SSTR 1-4) involved, and the confirmation of the receptor-ligand complex. When unavailable, it can be disclosed by homology modelling of the receptor followed by ligand docking. A cyclopeptide analogue of SST, TT-232, a promising drug candidate for inhibition of tumour and inflammatory diseases, has been selected for docking (Simon et al., 2004: Biochem. Biophys. Res. Commun. 316, 1059).

SSTR family members contain seven transmembrane (7TM) helices and their x-ray structures are not available, therefore application of homology modelling is required. For SSTR1 homology modelling, transmembrane helices of bovine rhodopsin, the only 7TM receptor structure disclosed so far, are available. By exploring the sequence of rhodopsin helices as template, the binding crevice of TT-232 in SSTR1 can be determined using computational modelling and a subsequent docking procedure. TT-232, showing a highly rigid ring confirmation as revealed in NMR measurements, interacted with the following amino acid residues of SSTR1: Val133, Asp137 (helix 3), Arg197 (helix4), Phe287, Gln291, Asn294 (helix 6), Ser305 and Tyr313 (helix 7). Docking results were validated by binding experiments performed in brain tissue membrane suspensions.

Information on the nature of binding interactions between TT-232 and SSTR1 may help to develop a strategy for the design of peptidomimetics in the future.

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WRA - Wide Range Alignement of EM serial sections

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3D reconstruction of from electromicroscopic (EM) serial sections substantially differs from modeling body parts by linking convoluted planes delivered by CT and NMR. Namely, variations both in relative X-Y position and rotation of the target elements in the adjacent images and also additional problems caused by deformed, deteriorated or missing sections can be overruled only by an aligning paradigm, which exploits the total available information, and results in optimal fitting at an optional level of precision.

Our earlier computer program (DEFLUT) implemented to fit and average digital images of common source, aiming noise reduction (1).

This paper presents a complex computer program to achieve an optimal alignement based on the advanced paradigm of DEFLUT. The present version of WRA performs the precise elaboration of X-Y shift and relative rotation of two consecutive images. The required searching process will be customized by setting four independent parameters which relate the span and density of pixel-scanning basic process. The suspicious searching area should be defined by the user. Optimalization of fitting accuracy versus running time can be achieved by a rather short training period. In the case of sufficient computing capacity and time available, the full-picture fitting and maximal pixel-matching density can improve alignment. The potential precision of Wide Range Alignement, based on complex algorythms is far superior than aligning EM photographs manually with the eyes-wrist-mouse facility (2).

The next program session - Swing - applies the fitting parameters in such a way that one by one transforms the consecutive images into a common coordinate-field resulting in a novel series of pictures, embedding the aligned EM photos.

Finally this block can be fed into the Reconstruct program of Fiala and Harris (2) to create the contours and perform the 3D reconstruction.

All these processes are demonstrated on a basal forebrain NPY+ axonal reconstruction, performed in L. Záborszky's laboratory.

Supported by Hungarian Space Office grant TP 138 to L. Simon and NIH grant 23945 to L. Záborszky.

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Mapping thalamic territories projecting to the visual and somatosensory cortex in sighted and enucleated rats

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Cortical areas are innervated by several thalamic nuclei. While the thalamic regions projecting to the different sensory cortical areas are segregated on the whole, there are territories, especially transitional areas between higher order nuclei where sharp borders cannot be drawn. The major aim of our studies was to investigate the projection of these transitional zones to the visual and somatosensory cortex with the aid of double retrograde fluorescent tracing. The possible role of the transitional zones in cross modal-plasticity is also studied. Visual and the somatosensory cortices were injected parallel either by Fast Blue (FB) or Diamidino Yellow (DY). Retrogradely FB- or DY-labeled neurons clearly segregated in the primer order nuclei dLGN and VB while higher order nuclei contained a mixture of neurons projecting both to the somatosensory or visual cortex in normal rats. Double-labeled neurons were not found. The regions with overlapping retrograde labeling seems to be localized mainly in border regions of nuclei PO, LP and LD as well as VL and VM. This preliminary finding suggests a gradual transition between the higher order thalamic nuclei raising the possibility for the role of these regions in cross-modal plasticity. Studies on enucleated rats are in progress.

EEG theta coherence during crossmodal visual-auditory-somatosensory integration in a multimodal object recognition task in humans

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EEG was recorded in a visual-auditory-somatosensory oddball reaction-time task to study the relationship of cortical crossmodal processing and reaction time. Visual, auditory and somatosensory stimuli were presented alone and simultaneously in four experimental sessions. Target stimuli were applied in the visual modality in order to study crossmodal effects on object recognition. Subjects' task was to indicate the recognition of the target by pressing a button. EEG was recorded from 31 scalp electrodes. Significant decrease in reaction time confirmed that multisensory integration took place in multimodal stimulus condition. Recognition of the target was significantly improved in audio-visual and audio-somatosensory-visual conditions reflected by significantly decreased reaction-time compared to unimodal visual and somatosensory-visual conditions. Analysis of event-related potentials revealed that P300 latency showed clear relationship to behavioral data. Channel phase cross-coherence analysis in the time-frequency plane showed higher coherence in the theta range for audio-visual and audio-somatosensory-visual conditions than that for the other two conditions. Results indicate that audio-visual crossmodal integration is more efficacious in visual object recognitions task than somatosensory-visual integration.

Factors that may control size of propriospinal single fibre EPSPs in lumbar motoneurons of frogs

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Understanding mechanisms that control cell-to-cell impulse propagation in the Central Nervous System is one of the key issues in contemporary neuroscience. Monosynaptic connections between propriospinal axon - motoneuron pairs in the lumbar spinal cord of frogs provide a good model to study this problem, since the mean EPSP amplitudes recorded intracellularly in motoneurons during propriospinal intraaxonal stimulations differed dramatically (range: 0,1 mV to 8,0 mV, n=47). EPSP amplitude differences are accompanied by variations in quantal analysis parameters and average distances of close appositions (presumed synaptic sites) from the soma.

We aimed to study these axon - motoneuron pairs to elaborate further factors that may contribute to the high variability in strengths of these connections.

We used the NEURON software and made high-fidelity passive segmental cable models, based on morphology of 3-D computer reconstructions (NEUROLUCIDA) of the iontophoretically labelled motoneuron dendrites. Synapses were modelled by conductance changes described by alpha functions whose kinetic parameters were estimated by matching the mean experimental EPSPs by the model. Our analysis now extended to six axon - motoneuron pairs; three with “small” (< 1,2 mV) and three with “large” mean EPSP amplitude (> 1,2 mV). Morphoelectrotonic transforms of dendrites were compared to their morphology to explore correlations between positions and functional strengths of synapses. Using EM data, we modelled the dendritic irregularities (thorns) and estimated their total number on these dendrites.

We conclude that 1) Our computer models and preliminary EM data suggest that single and multiple vesicle release at synaptic sites differentiates between axon – motoneuron pairs with “large” and “small” EPSP amplitudes 2) Thorns and non-linear summation may play a major role in discrimination between “large” and “small” EPSPs 3) The size of EPSP remained in the same category (either “small” or “large”) if synapses were redistributed randomly on the dendrites of the same motoneuron 4) Synapses are distributed in the morphoelectrotonic space unevenly and their distributions in geometrical and electrotonic (functional) space are different 5) Geometrical distances of synapses do not always correlate with their functional weights even in passive dendrites.

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Two types of septal choliergic neurons differ in their GABA-B and CB1 cannabinoid receptor expression

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Septohippocampal cholinergic neurons play key roles in learning and memory processes, as well as in the generation of hippocampal theta rhythm. The receptor expression of these neurons for endogenous modulators is still unclear. Here we described the GABA-B 1a/b receptor (GABA-BR) and CB1 cannabinoid receptor (CB1R) content of rat septal cholinergic (i.e. choline acetyltransferase, ChAT,-positive) cells. By using double immunofluorescent staining, we labelled cells either for ChAT and GABA-BR or for ChAT and CB1R. We found that almost two thirds of the cholinergic cells in the rat medial septum (MS) were GABA-BR positive and these cells were significantly larger than GABA-BR negative cholinergic cells. We detected CB1R labelling in cell bodies after axonal protein transport was blocked by colchicine. In these animals about one third of the cholinergic cells were CB1R positive. These cells were larger than CB1R negative cholinergic cells. The analyses confirmed that the size of GABA-BR positive and CB1R positive cholinergic cells were alike, and by using the mirror method we observed that all CB1R positive cholinergic cells were indeed GABA-BR positive as well. All CB1R positive cells were ChAT positive. With retrograde tracing method and double immunofluorescent staining we observed that all septohippocampal cholinergic cells were positive for GABA-BR and at least half of them also for CB1R. These data suggest the existence of two cholinergic cell types in the MS: one expresses GABA-BR and CB1R, has large somata and project to the hippocampus, the other is GABA-BR and CB1R negative and has smaller somata.

Nonlinear and timing dependent input to output processing in single neocortical interneurons

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Neurons receive thousands of convergent synaptic inputs and generate a characteristic output pattern of action potentials, but how neuronal output reflects input activity is not understood. We compared the interaction of identified excitatory inputs in sub- and suprathreshold postsynaptic membrane potentials recording of triplets (n=30) of neurons consisting of two presynaptic pyramidal cells converging onto a postsynaptic fast spiking cell in layer 2/3 of rat somatosensory cortex. Differentially timed excitatory inputs showed close to linear summation at subthreshold conditions. This non-linearity depended on the membrane potential of the postsynaptic cells: slight sublinearity (96.2 ± 2.7 %) was observed at a hyperpolarized membrane potential (–65 mV), which turned to supralinearity (107.7 ± 2.2 %) when postsynaptic cells were depolarized near to threshold (–35 mV). Suprathreshold interaction of the same inputs, however, was highly nonlinear and depended on presynaptic timing. Synchronously activated unitary EPSPs elicited postsynaptic action potentials more precisely than single (2, 5, 10 ms) or asynchronously arriving EPSPs: the distribution of postsynaptic spikes showed reduced temporal jitter and shorter delay relative to presynaptic spikes. Precise spike transmission by synchronous inputs was supported not only by supralinear input summation but also by timing dependent sensitivity of postsynaptic spike generation to preceding depolarization caused by EPSPs. In conclusion, transformation of linear subthreshold summation rules to nonlinear and temporally dynamic spike transmission reflects the sequence and synchrony of inputs and could enhance temporal precision of information flow in cortical networks.

Comparative immunohistochemical studies on different laminin receptors: integrins and dystroglycan-dystrophin complex in glial reaction to to lesion

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Following penetrating cerebral lesions, the basal lamina of glia limitans is reconstructed by the reactive astrocytes, including the post-lesional rearrangement of vessels. Laminin receptors are essential in these processes. Integrins are the most important laminin receptors, and alpha-V and the beta-1 are their most frequently found subunits. Another receptor system is the dystroglycan complex, which contains beside others dystrophins (Dp), either the full-length Dp427 or its shorter variants, e.g. Dp71. Dp71 seems to be their major representative in the brain, and occurs as splice variants, Dp71f and Dp71d. Our previous study demonstrated, that it is the Dp71f, which can be expressed by astrocytes, and its expression increases in glial reactions. The present study compares the immunohistochemical reactivity of dystrophins, dystroglycan and integrin subunits alpha-V and beta-1 in glial reactions following stab wounds in adult rats. The operations were performed in ketamine-xylazin anesthesia. Following postoperative days (POD) 2 to 35, rats were overdosed with ether and perfused transcardially with 4% phosphate-buffered paraformaldehyde solution. Floating vibratome sections were processed for immunohistochemical labeling with fluorescent secondary antibodies. To detect the splice variant Dp71f, a monoclonal antibody (5F3, developed by D. Mornet) was used. The presence of Dp71d, as well as the other variants, including the full-length Dp427 was detected by the monoclonal antibody Dys2 (Novocastra). Glial fibrillary acidic protein, integrin subunits and dystroglycan-beta were also investigated by commercial antibodies. In accordance with the preliminary data, 5F3, but not Dys2 antibody decorated the reactive astrocytes at POD 4, displaying a pattern resembling that of GFAP. Dystroglycan, however, was not detected in the reactive astrocytes, only along the reconstructed (secondary) basal lamina at P0d 12. The intense dystroglycan immunoreactivity visualizing the vessels of the intact brain temporarily disappeared from the territory of the lesion already POD 2. The integrin subunits were not detected in the intact tissue, but alphaV was in the glial reaction (at POD 4), and, temporarily, in the near vessels (POD 4 to 12). Therefore, immunoreactivities of laminin receptors characterise the stages of the glial reaction.

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Repair of the injured spinal cord by transplantation of embryonic motoneurones

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Spinal cord injury results in severe and permanent loss of spinal cord function. To alleviate the patients' and their families' sufferings several experimental approaches have been tried to connect the disconnected spinal cord stumps.

In our experiments a partial hemisection was performed in the cervical 5 (C5) spinal cord segment. The C6 ventral root was avulsed, the gap between the hemisected spinal cord stumps was filled with E13 embryonic motoneurone-enriched spinal cord graft and the C6 ventral root was reimplanted into the graft. Control animals received no embryonic grafts, only the C6 ventral root was reimplanted into the gap of hemisection.

In the grafted animals considerably more retrogradely labelled reinnervating neurones were found than in control animals. Many of these neurones were of garft origin. The grafted animals performed better in the functional forelimb tests than controls. The grafts received various number of fibres from descending fibre tracts, such as the corticospinal tract and the rubrospinal tract. These fibres reached the periphery of the graft, but some of the failed to enter the graft.

According to our results the integrity of the damaged spinal cord can be significantly improved following cervical spinal cord injuries.

Changes in cortical and peripheral electrophysiological parameters in rats obtained by acute administration of 3-nitropropionic acid and MK-801

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Among the known neurotoxic agents, mitochondrial toxins represent a special group. Some of them, like 3-nitropropionic acid (3-NP), have a natural occurrence and occasionally cause intoxication of humans or farm animals. The primary importance of such toxins today is in modelling chronic human neurological diseases, e.g., Huntington's disease. The aim of this study was to observe the functional neurotoxicity of 3-NP, and its potential antagonist MK-801, on the spontaneous and stimulus-evoked activity of sensory cortical areas and on a peripheral nerve in rats in acute and subacute application. It was supposed that the results obtained could contribute to refining of the existing disease models.

In acute treatment, ten weeks old male Wistar rats were given, ip., 10 and 20 mg/kg 3-NP, or 0.05 and 0.1 mg/kg MK-801, and were prepared for recording 24 hours later. In subacute treatment, the rats received 10 and 15 mg/kg 3-NP every 4^{th} day during the 4-weeks period. For preparation, the rats were anesthetized with urethane, and the left hemisphere was exposed by opening the bony skull. Spontaneous and stimulus-evoked activity was recorded from the somatosensory, visual and auditory cortical foci, and compound action potential from the tail nerve.

The acute treatment caused only minor alterations in the functional parameters. In the spontaneous cortical activity, low frequencies decreased and high frequencies increased in the 3-NP treated rats while MK-801 alone had no effect. The frequency dependence in the latency and duration of the somatosensory evoked response was increased both by 3-NP and MK-801. A similar effect was seen in the amplitude of the tail nerve action potential.

The changes caused in the spontaneous activity in the two subacute treatment schemes were partly identical, like decreased delta and theta, and increased gamma activity in the somatosensory and auditory area, or decreased delta and increased beta2 in the visual area. Also, the changes in the parameters of evoked potentials were mostly alike.

The results show that administration of 3-NP caused some alterations in functions independent of the striatum, its classical target. Such alterations can potentially be used in monitoring the development of damage in the disease models based on mitochondrial toxins. The mechanisms of the above effects, e.g. involvement of glutamatergic phenomena, remain to be elucidated.

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Immunesystem transmitted effects of LPS on the epileptogenesis

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Lipopolysaccharide (LPS) is an endotoxin, which is localized in Gram negative bacteria's cell wall – this molecule is recognized by the elements of natural immunsystem in case of bacterial infection and links to TLR4 receptor in the constitution. Primary assumption of the research was that LPS can interfere in EEG synchronization responsibled mechanism of the thalamocortical system, thus it changes the characteristic pattern of EEG during seizure. Our assumption was based on the fact that LPS releases citokines from glial cells and IL-1 powerfully synchronizes EEG and induces sleep. Two strains of chronically implanted individuals of rats were used. WAG/Rij rats are known to be the genetic model of absence epilepsy, which can produce seizures typical High Voltage Spindle (HVS) in EEG. By contrast the Wistar rats are also able to produce HVS, but only some of the very old ones. Using wavelet spectrum analyzes we obtained that LPS interferes with seizure generator mechanisms changing the pattern of spike-and-waves. We used chronically implanted animals with deep electrode in the ventrobasal nucleus of the thalamus. Applying mathematical methodology to detect phase relations of the spindle generation in the thalamo-cortical system we found that LPS decreases the dominance of the frontal cortex in HVS generation and activates the thalamic generators.

Since LPS might penetrate the blood brain barrier in extreme conditions, we tested the presence of TLR4 a known LPS receptor in the brain by Western blot analysis. We detected TLR4 protein in the thalamo-cortical system structures indicating a possibility of direct effect of LPS on thalamic synchronization on IL-1β independent manner. Our data reveals that WAG/Rij model can be very effective in the study of neuro-immune interaction.

Effect of 7-OH-DPAT injected into ventral tegmental area and nucleus accumbens shell on open field behaviour

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Background: The mesolimbic pathway is one of the four dopamine pathways, which originates in ventral tegmental area (VTA) and innervates nucleus accumbens, olfacory tubercule and parts of limbic system. In rodents the mesolimbic pathway plays an important role in locomotion. The nucleus accumbens is rich in dopamine D3 receptors, while relatively fewer number of D2 receptors are present. In VTA and substantia nigra D2 receptors are predominantly expressed. Our experiment was devoted to compare the behavioural effect of the D3/D2 receptor agonist 7-OH-DPAT injected into two different regions of the mesolimbic system.

Methods: Intracerebral double-guide cannula were inserted in VTA or in nucleus accumbens shell (NacS) of Harlan Wistar male rats (weighed 295-305 g). After one week recovery time the animals were bilaterally injected with 30 pmol or 3 nmol 7-OH-DPAT into VTA and 3 pmol or 3 nmol 7-OH-DPAT into NacS. The control animals received distilled water. Immediatelly after the injection the animals were observed in an open field arena. The following behaviours were detected: number of square entries, rearing, total oral movements and grooming time. The localisation of the intracerebral cannula was verified by histological examination.

Results: 7-OH-DPAT injected into VTA caused dose dependent decrease of locomotor activity. In 3 nmol dose non significant decrease could be observed in rearing, grooming time and oral movements, too. Injecting 7-OH-DPAT into NacS caused no significant change in locomotion, rearing and grooming time. However, a moderate increase in the number of oral movements occured after dosing 3 nmol.

Discussion: 7-OH-DPAT has 7-fold selectivity to dopamine D3 over D2 receptors. After local administration into VTA this agonist caused hypolocomotion but this effect was not observed when it was injected in NacS. It suggests that the decrease in movement results from activation of presynaptic (most probably D2) and not postsynaptic dopamine receptors by this low amount of 7-OH-DPAT. Regarding the elevated number of oral movements induced by the intraaccumbal injection a plausible explanation can be the activation of postsynaptic D2 receptors. This assumption is also supported by the literature finding that the mesolimbic dopamine D3 receptors play no role in dopamine-dependent and NacS specific oral movements.

Entorhinal cortex lesion in rats as a dementia model

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Alzheimer's disease (AD) is characterised by the impairment of associative neocortical-hippocampal connections and is initiated in the entorhinal cortex. In the imminent AD there is a deficit in attention, learning and memory functions. The present study aimed at modelling the initial type of AD on rats by examining the major behavioural and morphological changes induced by bilateral excitotoxic lesion of the entorhinal cortex.

Male Wistar rats were divided into three groups: intact control, sham-lesioned and lesioned animals. The lesion was elicited by N-methyl-D-aspartate (NMDA) injections into the medial and lateral entorhinal cortex (3x30nmol at both sides). Behavioural tests were used to measure object recognition in open-field, social attention, and spatial memory function in Morris maze. After transcardial perfusion with paraformaldehyde at postoperative 16th day immunocytochemical staning was performed: nuclear protein of neurons (NeuN), activated astrocytes (GFAP), activated microglia (CD11b) and cholinergic fibre density (ChAT).

The NMDA-lesioned animals showed attention deficit in the novel object recognition and in the social recognition tests. In the course of spatial learning particularly reference memory was impaired. A detailed and a unique way of anatomical quantification could be described in the entorhinal cortex. NMDA caused extensive lesion in the medial and lateral entorhinal cortex represented by NeuN neuron staining. The area of lesion and that of entire entorhinal cortex was measured. Astrocytic and microglial reactions could also be quantified. The impact of lesion was estimated on the density of cholinergic afferentation to the molecular layer of dentate gyrus. Good correlations could be observed between the histological markers of excitotoxic lesion and the altered behavioural parameters.

The behavioural and morphological results demonstrated that the bilateral lesion of the entorhinal cortex serves as a useful animal model for dementia regarding to learning abilities, attention, memory function and social performance. With applying the present model putative neuroprotective and antidementia compounds can be tested and characterised.

A simple image analysis technique for quick densitometry on immunostained ligtht microscopic images

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Computerized morphometry enables quick and numerous analyses onmorphological samples visualized with immunoreactions orhistotechnical procedures. The major features of the positve reactionsare the area and density. Recent personal computers are entirelysuitable for such morphometric tasks. The results of theimmunostaining reactions can be identified at cellular and/or subcellularlevel.In the first step, digitized images are produced. Beside the difficulties oftaking microphotos, the problem of area selection and magnificationcannot be ignored in morphometry. The digitized images should bepreprocessed: grayscale conversion, color depth, resolution, tones etc.should be optomized in the image series. In the next step images aresegmented. During segmentation areas of the image are selected bytheir density. Either in the staining reactions or during taking themicrophotos deviations of the image density may occur. Thesedeviations should be corrected with preprocessing or with comparativereference data taken from negative territories of the same image. If thedeviation of the series is too high manual segmentation can be applied.

Beyond quantitative presentation of data, the benefit of this techinqueis the use of macros developed especially for the given purposes. Withthe application of macros multiple measuremements can be performedon thousands of images in couple of minutes.

Endomorphin II protects against Aβ1-42 induced neuromodulatory effect on CA1 hippocampal neurons in vivo and in motor cortical slices in vitro

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The underlying cause of Alzheimer disease is thought to be the aggregated amyloid fibrils (formed by Aβ1-42 peptide). The toxicity of Aβ1-42 is in correlation with its aggregation properties. Protective pentapeptides (e.g. LPFFD, "Soto" peptide) are known to prevent neuronal toxicity of Aβ. The structure of Endomorphin II (END II; YPFF) shows high similarity to the "Soto" pentapeptide, therefore it might possess some protection against Aβ1-42 excitatory effect on CA1 hippocampal neurons. The protective effect was also confirmed by in vitro MTT assay.

Extracellular, single-unit recordings were taken from the hippocampus. Carbon fiber containing multibarrel electrode assemblies were used to record cellular responses as well as to deliver NMDA, END II, Aβ1-42, and pontamine-sky blue by means of microiontophoresis. Neurons were excited by repetitive iontophoresis of NMDA every minutes for 5 seconds. The spiking frequency of neurons were recorded. After establishing a stable control, END II was co-iontophoresed for 3 minutes, then immediately Aβ1-42 for 1 minute, or a mixture of Aβ1-42 and END II was ejected for 1 minute. The location of the electrode was verified by means of histology.

In other experiments, the amplitudes of field excitatory potentials (fEPSP) elicited by stimulation of horizontal connections were measured in motor cortical slices taken from young rats. The Aβ1-42 was applied locally onto the surface of the slice, which caused a long-lasting decrease in fEPSP amplitudes. Endomorphin II (applied in cocktail with Aβ1-42, in fivefold over excess) protected neurons against this attenuating effect.

END II excited the recorded neurons, raising the possibility, that they were pyramidal cells. END II prevented the NMDA-response enhancing effect of Aβ1-42 only in the mixture form. Therefore END II may exert the protective effect by binding to the surface of the fibrils, and not by binding to the μ-opioid receptor. The potent inhibitor might have potential therapeutical relevance. The neuromodulatory and neurotoxic effect of Aβ1-42 could be prevented by an endogenous tetrapeptide in vivo and in vitro.

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Signaling pathway dependent effects of estrogen on mouse cholinergic neurons

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Estrogen easily passes through the blood brain barrier and alters neuronal functions. Estrogen has a pivotal regulatory role in the reproduction and it has beneficial effects on dementia via the protection of cholinergic neurons in the basal forebrain. Although estrogen is primarily thought to alter the neuronal activity via intracellular estrogen receptors (ERα and β), also exerts idirect effects on neurons modulating signal transduction pathways associated with second messengers such as MAPK, CaMK or PKA pathways. One transcription factor well established to be regulated in a rapid manner by these pathways is the cAMP response element-binding protein (CREB). In present experiments, we have characterized the rapid estrogen effect on intracellular signaling within cholinergic neurons in the basal forebrain *in vivo* and *in vitro*. In in vivo studies, we have determined the expression of CREB and its phosphorylation (pCREB) in cholinergic neurons at different time points after injection of ethyl oleate (vehicle) or 17-β estradiol (E2) to ovariectomized female mice. Whereas, E2 had no effect on number of cholinergic neurons expressing CREB, an increase in pCREB expression was detected 15 min after E2 administration and the levels remained elevated at 4h in medial septum (MS), in diagonal band (DB) and in substantia innominata (SI) but did not change in striatum (STR). Similar to the wild type mice, E2 significantly increased the pCREB-immunoreactivity in ERbeta knock out animals in MS-DB and in SI, but this effect completely absent in STR. To determine, whether the estrogen effect is direct or indirect on cholinergic neurones, we used in vitro slice technics combined with administartion of tetrodotoxin (TTX) to block the action potential and separate the neurones sinaptically. After 15 min of E2 administration, the CREB phosphorilation elevated significantly in MS-DB and in SI, but there were no effect in STR. Summarizing the observations, the estrogen rapidly phosphorylate CREB in adult, female mouse cholinergic neurons *in vivo* and *in vitro*. These effects do not depend on the presence of ERβ in the SI and in the MS-DB. Our in vitro studies demonstrate, that E2 can alter CREB phosphorilation directly acting on the cholinergic neurones. In conclusion, we suggest that the signaling pathway involving, indirect route of estrogen action on cholinergic cells could play an imortant role in cellular mechanisms of estrogen induced modulation of cholinergic neurons.

Release of norepinephrine in the periphery as a possible target of the effect of salsolinol

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A considerable number of evidences suggest that salsolinol, an endogenous tetrahydoisoquinolin is a prolactin releasing factor. It has been shown that the inhibitor of salsolinol induced prolactin release – a synthetic compound: 1-methyl-dihydro-isoquinoline (1MeDIQ) increases the plasma epinephrine and norepinephrine level. Measurements were done to approach the noradenergic activity in the peripheral organs: superior cervical ganglion, stellate ganglion, atrium, spleen. It has been demonstrated that in peripheral organs (atrium and spleen) salsolinol decreases the dopamine content while 1MeDIQ increases it. The norepinephrine/dopamine ratio was increased by salsolinol and decreased by 1MeDIQ. The effect of salsolinol was the most pronounced in the spleen among the organs examined so far. These changes indicate a decreased norepinephrine release due to salsolinol treatment. Results obtained in noradrenaline transporter knock out mice indicate the involvement of this transporter in salsolinol's action, although the decrease of tyrosine hydroxylase activity caused by salsolinol can not be excluded. The results indicate the importance of organ specific release of norepnephrine in respect of regulation of prolactin release. Our studies were supported by OTKA 043370 and 049605 to GMN.

Serotonergic innervation of the avian ventral tegmental area

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The area ventralis tegmentalis of Tsai (AVT) is thought to play a role in memory formation, aversive/ addictive behaviours and stress related visceral responses. In the avian brain, AVT is located in the vicinity of the emerging oculomotor nerves, forming part of the A10 dopaminergic cell group. Several forebrain regions, including the nucleus accumbens, diagonal band of Broca, septal nuclei, bed nucleus of the stria terminalis, hippocampal complex, medial striatum, arcopallium and the avian prefrontal cortex are interconnected with the AVT.

Previous studies in the rat brain have described that AVT neurones project to the dorsal raphe nucleus (DRN) with a certain contingent of the axons being GABAergic. Also, a reciprocal, mainly inhibitory, projection from the DRN has been verified to reach the dopaminergic neurones of the AVT.

The brainstem monoaminergic system of the domestic chicken shows reasonable similarity to that of the mammalian brain, therefore our aim was to detect whether such connections, preferably with an identical chemical nature, exist in the chicken brainstem. The more so, since the accurate identification of the mesopontine serotonergic nuclei is still questionable, to prove the existence of a similar loop would be of great importance.

Free-floating Vibratome sections containing the mesopontine region were selected both from the domestic chicken and zebra finch brains. We employed combined immunocytochemical (tyrosine hydroxylase - TH, serotonin - 5-HT) and enzyme histochemical (NADPH-diaphorase - Nd) techniques to verify the neuronal markers in the avian AVT and presumed DRN.

A profuse serotonergic network has been found in the entire extent of the AVT, forming baskets around both TH immunopositive and negative perikarya as well as Nd reactive cell bodies and dendrites. Only a minor contingent of AVT neurones appeared to colocalize TH and Nd. Nevertheless, the existence of an external nitrergic influence cannot be excluded, since the majority of the 5-HT immunoreactive neurones also express Nd reactivity in the rat DRN. Although the presumed avian DRN homologue, nucleus linearis caudalis, contained fewer neurones colocalizing Nd and 5-HT, the existence of a similar inhibitory loop between the AVT and DRN is suggested.

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The role of neuropeptide Y in complex regulatory changes of energy balance: fasting, cold-adaptation, hyperthyroidism

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Energy balance consists of two intertwined regulatory systems: one serving the balance of energy-containing substances (body weight – food intake/metabolic rate; BW – FI/MR), while the other one serves the caloric balance (body temperature – heat loss/metabolic rate; Tc – HL/MR). MR links them. Both can change its functions in either a coordinated or a compensatory way, in these changes neuropeptides may be involved.

Neuropeptide Y (NPY) is produced mainly in the arcuate nucleus and released primarily at nerve projections in the paraventricular, perifornical and ventromedial nuclei. It has a coordinated anabolic role: it enhances FI and suppresses excess MR towards the basal level. It has no direct influence on mechanisms of HL, i.e. any NPY-related change (fall) in Tc must be due to a poorly compensated metabolic suppression and is not a primary coordinated thermoregulatory phenomenon (anapyrexia).

Deprivation of food (but not water) necessitates an anabolic state: metabolic suppression serves longer survival, while upon re-feeding the animals are hyperphagic. In course of fasting Tc also declines, which is a passive hypothermia. Enhanced NPY activity may explain all components of this pattern, and the NPY production, NPY level and synthesis of NPY-receptors have been shown to increase in fasting. NPY antagonists can attenuate the re-feeding hyperphagia, although reversal of the fasting hypometabolism and hypothermia is more difficult.

During lasting cold exposure the primary target is Tc regulation: the unavoidable HL must be compensated by a commeasurable rise in MR. This must evoke hyperphagia. Although the central NPY production does not increase, the hyperphagic action of exogenous NPY is enhanced as well as the re-feeding hyperphagia after fasting. During central NPY infusion the early rise in FI is accompanied by a rather moderate and transient hypothermia. Apparently, the peripheral cold signals (through the PO/AH region) overwrite the metabolic suppression to NPY, without influencing its consummatory actions.

Substances that primarily affect the regulation of MR, like thyroid hormones, exert a compensatory influence on both FI and HL. However, the compensation is incomplete, a spontaneous hyperphagia develops slowly and the re-feeding hyperphagia is smaller than in controls, although the exogenous NPY can act normally. Apparently, in contrast to cold signals, hyperthyroidism-induced warm signals may attenuate the endogenous NPY activity.

Fasting heterothermia: energy compromise of CNS body temperature control aiding survival

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In adult rats exposed to thermoneutrality five-day-long complete fasting leads to a moderate hypothermia of about 35°C during the day with night acrometron being maintained at normothermic level. Experiments on freely moving mice - with a body mass much lower than that of rats - were carried out to see the modification of core temperature and activity during a lenght of complete fasting that still allowed survival.

C57Bl/6 mice were implanted with intra-abdominal biotelemetry transmitters (MINIMITTER, Series 4000) under general anaesthesia and more than a week afterwards complete fasting for 1 to 3 days was applied. The animals were held in a room of neutral (27-29°C) or cool (23-25°C) temperature under a 12/12 hours light/darkness schedule and with tap water available all times. Body core (abdominal) temperature and activity were monitored in freely moving mice in their own cages both sets of data having been collected every 5 minutes and evaluated by the VitalView software.

A steady state circadian body temperature rhythm developed within one week after transmitter implantation with an amplitude of 1.9 ± 0.1°C and an acrometron of 38.1 ± 0.1°C. At night - but even more during the day - an ultradian oscillation was superimposed on circadian rhythm, both components running virtually parallel with the activity rhythm. One to two-day fasting applied in cool environment resulted in a progressive fall of day minima of body temperature, at the end of the second fasting day approaching or even surpassing 30.0°C. Three-day fasting applied at thermoneutrality led to a more gradual fall of body temperature reaching similary low values. In either case night body temperature maxima remained largely normothermic. On consecutive days of fasting there was a progressive rise in night activity up to 50-100 % above control values.

It is concluded that 2 to 3 days fasting applied in a homeothermic (so-called warm-blooded) species, the mouse, results in an enhanced daily oscillation of core temperature and activity, with day body temperature falling by 7-9°C below the homeothermic values of 37-38°C. A temporary hypothermia of this degree can be regarded as heterothermia when applied for 24 hours. The transient (daytime) abandonment of homeothermia in a nocturnal (nightly-active) species could be the result of an energy compromise allowing normothermia at night apparently as a result of increased physical activity. In turn, high physical activity and movement associated with normothermia at night in a fasting rodent under field conditions of food shortage may allow survival, but the animal has to pay a price in the form of daily heterothermia. The present data provide some evidence missing so far in favour of physical activity (exercise) as a means of thermoregulatory heat production under some pathophysiological conditions such as fasting.

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Morphometric properties of intracellularly recorded cerebellar Golgi cells

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Morphometric properties of intracellularly recorded cerebellar Golgi cells

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Understanding how information is processed by the cerebellar cortex implies a thorough knowledge of the cerebellar circuitry. The inhibitory interneurons of this network have received little attention compared to the granule and Purkinje cells. The aim of our study was to provide quantitative morphometric data which can be used to build detailed compartmental models of the Golgi cells.

Whole-cell voltage-clamp recordings were obtained from microscopically identified putative Golgi cells in rat cerebellar slices. Biocytin injected cells were revealed by the ABC-DAB protocol and were reconstructed using the Neurolucida computerised tracing system.

Golgi cells (n=10) were found in all depths of the granular layer. The cell bodies had rounded or polygonal shape and emitted 4 - 10 radiating dendrites. All cells had apical dendrites penetrating the molecular layer and basolateral dendrites restricted to the granular layer. The axonal plexus could be revealed in only three cases.

The distance along the dendrites from the soma to the most remote dendritic tip was: 327.2±78.7 μm, whereas the maximal polar distance: 262.7±66.6 μm. The average on path distance of all dendritic tips was: 159.9±52.2 μm, and their average polar distance: 123.8±42.8 μm. The ratio of the on path and polar distance (tortuosity factor) was 1.29±0.03, indicating a radial spread of dendrites. The average branch order of all dendritic segments was: 4.67±0.87. The data presented above are for 8 cells. Two out of ten cells had much longer dendritic tree (maximal on path distance: 653.6 and 778.7 μm), therefore they were considered a separate group. The tortuosity factor of the two larger cells was: 1.32 and 1.26; the mean branch order: 4.21 and 5.68, indicating that in spite of the size difference, the overall topology of all cells was similar.

In conclusion the morphometric data obtained is suitable for modelling the spatial integration performed by this cell type.

Behavioral changes after single and simultaneous lesion of the serotoninergic and noradrenergic systems in the rat

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In serious central nervous system (CNS) disorders such as anxiety and depression noradrenergic (NA) and serotonergic (5-HT) pathways have an inevitable role. Nevertheless the exact mechanism by which these monoamine transmitters influence anxiety states, mood and other important CNS functions like memory and learning are not yet fully understood. The way of interaction of these two systems between each other and with other neurotransmitters and neuromodulators is still an interesting field of CNS-researches.

The aim of present study was to evaluate the impact of single and combined lesions of the NA- and 5-HT systems on behavior and hippocampal functions of the rat. Serotonergic and noradrenergic pathways of male Sprague-Dawley rats were chemically lesioned by intracerebroventricular administration of selective neurotoxins 5,7-dihydroxytryptamine (5,7-DHT) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine-HCl (DSP-4) respectively. To investigate anxiolytic-like behavior rats were tested on Vogel lick-conflict test and elevated plus maze, after recovery. In Vogel test, the lesion of either 5-HT- and NA-pathways resulted in slight anxiolysis, the highest but not significant increase of tolerated shocks (28% compared to sham) occured at the double (5-HT and NA simultaneously) lesioned group. On elevated plus maze time spent in the open arms significantly increased after double lesion; the number of entries into the open arms was significantly higher at NA- and double-lesioned rats. These effects indicate strong anxiolytic-like effect of this type of lesion and similar to that produced by 1 mg/kg diazepam in non-lesioned rats. Spontaneous motor activity of the different types of lesions were measured in activity meter utilizing infrared beam interruptions. The results did not indicate significant impacts on the motor activity of the lesions. To evaluate the effects of different types of lesions on learning and memory, long term potentiation (LTP) was measured in CA1 and dentate gyrus regions of the hippocampal slices of lesioned animals. Slight and time-dependent, but not significant changes were seen in the NA-lesioned group.

Our results indicate that the control of anxiety state and the production of LTP requires functional integrity of the 5-HT and NA transmitter systems, and regulation may occur via a common mediator in these CNS-functions, however this theory requires further investigation.

Synchronisation in coupled networks of motor pattern generating neural circuits

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Coordination and synchronization of the activities of oscillatory neural networks is a key requirement for fine-tuned motor behavior. The lobster stomatogastric ganglion (STG) has been one of the prime experimental models of coordination and interactions in such neural networks. In the STG, central pattern generators (CPGs) such as the pyloric and gastric mill networks produce a wide variety of neural interactions. Earlier studies described how these two biophysically and topologically different networks can cooperate. At the same time, the topological and biophysical constraints for coordinated activity between identical CPGs has been less investigated and less understood. In our present study we address this problem by using two intact pyloric networks from two different STG preparations and couple them via artificial synaptic connections. Although pyloric networks of different STGs naturally generate the same motor pattern, their endogenous activities and the phase-relationships of the bursts in their component neurons are different. Hence, coordination of these assemblies requires special constraints for the biophysical properties of the synapses connecting the two circuits. The dynamic clamp method is a valuable tool to study synchronization of such systems. We establish simulated synaptic connections of both electrotonic and chemical kinds between selected neurons of the two CPGs. While electrical coupling of the pacemaker groups of the pyloric networks can synchronize the CPGs, chemical inhibitory connections produce more rubust and stable joint oscillations. As the third type of synaptic configurations, quasi-symmetric connections between the lateral pyloric and the opposite pacemaker neurons provides the best synchronization and the most flexibility. Dynamical properties of the joint networks and their dependence on the connectivity will be discussed.

Changes in mu opioid receptor coupled signal transduction in morphine tolerant chinese hamster ovary cells

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Classical theories of opioid tolerance/dependence include receptor desensitization, internalization/down-regulation and adenylyl cyclase superactivation. All imply that opioid tolerance is a manifestation of a loss of opioid function. However, growing data including the present work challenge the exclusiveness of this formulation. Here we have assessed forskolin stimulated adenylyl cyclase activity in membranes of Chinese Hamster Ovary Cells (CHO) stably transfected with recombinant rat mu opioid receptors (MOR). While DAMGO at 1 μM inhibited adenylyl cyclase activity by 40% in opioid naive cells, its effect decreased by about 50% after 48 hrs morphine treatment indicating the development of tolerance. Inactivation of Gi/Go proteins via pertussis toxin unmasked the ability of DAMGO to facilitate forskolin-stimulated cyclase activity. DAMGO stimulation in tolerant membranes was eliminated following treatment with the Gβγ scavenger QEHA (AC 2 956-982), implying that the stimulation was mediated by the βγ subunits of heterotrimeric G-proteins. CTAP, previously considered to be a neutral mu opioid antagonist, also produced a facilitation of forskolin activated adenylyl cyclase indicating that CTAP exhibits inverse agonist properties in MOR-CHO membranes. CTAP stimulation of the enzyme activity was augmented by chronic morphine. Interestingly, the (augmented) CTAP facilitation of FSK-stimulated adenylyl cyclse activity that is observed in opioid tolerant (but not in naive) membranes was also sensitive to pertussis toxin. This can best be explained by postulating the involvement of Gi-derived Gβγ. These results underscore the multifaceted nature and plasticity of G-protein coupling of mu opioid receptors. We suggest that morphine tolerance reflects a gain of functionality manifested as altered signal transduction beside the well-known loss of function (desensitization).

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Effect of HCN2 channels on synaptic transmission between C/A-delta primary afferents and lamina II neurons of the rat spinal cord

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It has been demonstrated that three types of hyperpolarization-activated cyclic nucleotide-gated cation channel proteins (HCN1-3) are expressed by neurons of spinal dorsal root ganglia (DRG). HCN2 has also been shown to be transported from the somata of DRG neurons to their central axon terminals that terminate in laminae I-IIo of the superficial spinal dorsal horn. Immunohistochemical experiments revealed that HCN2 is widely co-localized with calcitonin gene-related peptide (CGRP), but is also completely segregated from isolectin-B4 binding (IB4), suggesting that HCN2 is mainly expressed in peptidergic nociceptive primary afferents.

In the present study we aimed to investigate the role of these presynaptic HCN2 channels in synaptic transmission between primary efferent terminals and superficial dorsal horn neurons.

Patch clamp recordings were performed in 2-3week old Wistar rat spinal cord slices (4-600□m) with intact dorsal roots (7-9mm). Dorsal roots were electrically stimulated via a suction electrode and EPSPs from cells with monosynaptic C/A□ input (calculated from the latency of the response and the length of the root) were recorded in control conditions and in the presence of an HCN antagonist, ZD 7288 (10□M). We measured the amplitude and kinetic parameters of the EPSPs, and calculated the failure rate of the trials.

In 3 out of 8 cases we observed a reduction in the number of responses triggered by dorsal root stimulation. This effect was reversible in 2 out of the 3 cases. The amplitude of the triggered EPSPs did not show any significant reversible change during the HCN2 antagonist period, apart from the regularly observed depletion of the transmitter content resulting in a "run-down" phenomenon.

Our results suggest that HCN2 primarily increases the reliability of synaptic transmission at the investigated synaptic contacts. This phenomenon might be explained with faster and more efficient repolarization of the terminals expressing HCN2 proteins.

Spatial distribution of Matrix Metalloproteinase-9 activation in 4-Aminopyridine induced epilepsy

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Modifications of extracellular matrix by specific extracellular proteinases are involved in selective tissue remodeling after epileptic seizures. Systemic administration of kainate induced upregulation in expression and enzyme activity of matrix metalloproteinase 9 (MMP-9) but the spread out of MMP9 activation from the epileptic focus is not known. Here we used focal epilepsy model induced by 4-AP (4 –aminopyridine) to investigate the spatial distribution of MMP-9 induction after seizures. We did so because 4-AP model shows ictal end interictal periods as human epileptic events and it works also in Halotahne anesthesia providing a steady state for animals without extensive motor activity. 300-400 g SPRD rats were used in Halothan anesthesia, 0.15-0.3 mg 4-AP crystal was placed on the right occipital cortex for 40 minutes. Fronto-parietal EEG was recorded and MMP levels were analyzed by zymography. Samples were collected from ipsi- and contralateral occipital cortices, prefrontal cortex, hippocampus, thalamus, striatum, cerebellum, brain stem, 6 / 24 h after drug administration.

6 h after treatment, a robust induction of MMP-9 was detected in the epileptic focus and also a more gentle activation was observed in the mirror focus. Increase in MMP-9 was reduced after 24 h. In the left and right prefrontal cortices and hippocampus there was also a slight increase in the level of MMP-9. At 24 h after seizure induction, the lower molecular weight band of MMP-9 zymogram remained, which indicate MMP-9 activation several hours after following increase in proenzyme. In the thalamus, striatum, cerebellum and brain stem no significant MMP-9 activity was detected.

Our data demonstrated that in 4-AP induced focal epilepsy MMP-9 is upregulated and the rate of this process is in correlation with the distance from the epileptic focus. We clearly demonstrated the spread of MMP-9 activation from the focus to the other parts of the cortex.

Homeostatic changes after IL-1b microinjection into the nucleus accumbens of the rat

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The nucleus accumbens (NAcc) is an important part of the neuronal circuit in the limbic forebrain. As a newly recognized, important constituent of the central glucose-monitoring neural network, it is known to be intimately involved in the control of feeding and metabolism. Our previous investigations in the globus pallidus, ventromedial hypothalamic nucleus and orbitofrontal cortex demonstrated important roles of interleukin mechanisms in these above regulatory processes. Our pilot single unit recording experiments verified direct neuronal effects of interleukin-1b (IL-1b) on neural cells in the NAcc. To investigate the homeostatic relevance of these mechanisms, it was examined whether a single bilateral microinjection of IL-1b into the NAcc of adult male Wistar rats -with or without paracetamol (P) pretreatment- can cause alterations in various homeostatic functions. Short- and long-term food intakes (FI), water intakes (WI) and body temperature (BT) were measured before and after the local administration of this primary cytokine into the NAcc. The effects were compared in the following four groups: IL-1b, IL-1b+P, control (CO), CO+P.

Short-term (2h) FI was suppressed by the microinjection of IL-1b and the P pretreatment failed to influence this anorexigenic effect. Although the differences somewhat decreased, these tendencies still remained even in the 24h measurements.

Short-term (2h) WI of the cytokine-treated rats also decreased compared to control animals. P failed to prevent the adipsogenic effect. Long-term (12h and 24h) measurements revealed no substantial WI differences among the groups.

Measuring of core BT 2 hours after the intracerebral microinjection showed significant differences among the groups. A remarkable hyperthermia was found in the BT of IL-1b and IL-1b+P animals compared to CO and CO+P rats.

Our present findings provide the first evidence for intimate involvement of accumbens IL-1b mechanisms in the central homeostatic regulation. Data do not substantiate a direct role of cyclooxigenase mechanisms in the central regulation of food and water intake and BT in the nucleus accumbens. Further experiments are needed to extend our knowledge about cytokine-mediated processes in the limbic forebrain.

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Fine structure of the termination of the temporo-ammonic pathway on CA1 pyramidal cells

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Analysis of the synaptic inputs onto CA1 area pyramidal cells revealed, that the organization excitatory and inhibitory inputs in strata radiatum and oriens (SRO) is considerably different from the organization in str. lacunosum-moleculare (SLM). In SLM, the recipient zone of the direct entorhinal cortex (EC) CA1 projection, the temporo-ammonic pathway (TAP), the excitatory synapses are larger, often partitioned and are frequently located on dendritic shafts, unlike in the case of Schaffer collaterals (in SRO) where the smaller excitatory synapses are exclusively located on dendritic spines. The seemingly more effective excitation in SLM is balanced by a higher inhibitory input (15%) than in SRO (2-5%). Furthermore, while the pyramidal cell dendrites run perpendicular to the afferents in SRO, they run parallel with the EC axons in SLM, suggesting that multiple contacts might be formed between the topographically projecting EC fibers and the CA1 pyramidal cells.

To study the termination strategy of the TAP on CA1 pyramidal cells, we injected the anterograde tracer BDA into the EC to label the projecting fibers, as well as several small injections were made in the CA1 area to label the pyramidal cells. The cells and fibers were then visualized by ABC complex and the sections were embedded in Durcupan.

We reconstructed individual pyramidal cells and the axons that innervated them, from serial LM sections. Axonal varicosities in close appositions with pyramidal cell dendritic shafts or spines were considered putative contacts. We never found multiple, climbing contacts among an axon and a pyramidal cell dendrite. The contacting axons were followed till they left the dendritic field of the pyramidal cells. This way we could also proved that multiple single contacts are not present either. Longer sections of TA fibers were followed from the subiculum or from the alveus. These axons did not form branches en passant, only close to their termination filed. We could also confirm the earlier finding that the projection from the EC to the CA1 area is much more topographical than the projection to the gyrus dentatus (i.e. a small area is innervated in CA1 by axons originating from an EC patch, while the projection is widespread in the dentate gyrus). A sample of the putative contacts has been studied in the electron microscope and the presence of synapses had been confirmed.

Our study shows, that the direct projection from the EC to the CA1 area, the TAP is strongly topographical and forms single synapses on individual pyramidal cells.

Effects of PACAP treatment in a rat model of Huntington disease

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon peptide family with various effects in both the central and peripheral nervous systems. Numerous studies show that PACAP has neuroprotective effects in vitro: it stimulates the growth and survival of neurons, prevents apoptosis and protects neurons against cytotoxicity induced by various agents. In vivo, PACAP shows neuroprotection in global and focal cerebral ischemia, in fornix transection, in facial nerve axotomy, spinal cord injury and traumatic brain injury. Recently, we have shown that PACAP is protective in a rat model of Parkinson`s disease, where treatment with the peptide reduces the dopaminergic cell loss and improves behavioral deficits after a 6-hydroxydopamine lesion of the substantia nigra.

Huntington`s disease (HD) is another progressive neurodegenerative disorder, characterized by severe degeneration of basal ganglia neurons. In the present study we investigated the effects of PACAP treatment in a QA-induced lesion of the striatum, a model of HD. PACAP was given locally, before the unilateral QA injection. Behavioral analysis was performed after 1, 10 and 30 days in an open-field test. Motor activity and asymmetrical signs were evaluated in the control and PACAP-treated groups. Three weeks after the treatment, a catalepsy test was performed by haloperidol administration. Histological assessment of the striatum was done after the behavioral tests were completed. Our results show that PACAP treatment attenuated the the behavioral deficits and reduced the number of lesioned neurons which is a further proof for the neuroprotective effects of this peptide.

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Postinjury administration of pituitary adenylate cyclase activating polypeptide (PACAP) attenuates traumatically induced axonal injury in rats

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Pituitary adenylate cyclase activating polypeptide (PACAP) has several different actions in the nervous system. Numerous studies have shown its neuroprotective effects in in vitro and in vivo experiments. Previously, it has been demonstrated that PACAP reduces brain damage in rat models of global and focal cerebral ischemia. Based on the protective effects of PACAP in cerebral ischemia and the presence of common pathogenic mechanisms in cerebral ischemia and traumatic brain injury, the aim of the present study was to investigate the possible protective effect of PACAP administered 30 minutes or 1 hour postinjury in a rat model of diffuse axonal injury induced by impact acceleration. Two hours after the injury, brains were processed for immunhistochemical localization of damaged axonal profiles displaying either β-amyloid precursor protein- (β-APP-) or RMO-14-immunoreactivity, both considered markers of specific features of traumatic axonal injury. Our results show that treatment with PACAP (100 μg) 30 minutes or 1 hour after the induction of traumatic brain injury resulted in a significant reduction of APP-immunopositive axon profiles in the corticospinal tract (CSpT). There was no significant difference between the number of APP-immunopositive axons in the medial longitudinal fascicle (MLF). PACAP treatment did not results in significantly different number of RMO-14 immunopositive axonal profiles in either brain areas 2 hours after the injury compared to normal animals. The protective effect of PACAP in this model suggests that it could be a promising therapeutic agent in the treatment of traumatic axonal injury.

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Cerebellar granule cells show age-dependent migratory differences in vitro

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Developmental differences between cerebellar granule cells during their migratory period were revealed using dissociated granule cell cultures isolated from 4, 7 or 10 days old (P4, P7, P10) mice. Under all culture conditions, the great majority of cultivated cell populations consisted of those granule cells which did not reach their final destination by the age of isolation. In vitro morphological development and the expression of migratory markers (TAG-1, astrotactin or EphB2) showed similar characteristics between the cultures.

The migration of 1008 granule cells isolated from P4, P7 and P10 cerebella and cultivated under identical conditions were analyzed using statistical methods. In vitro time-lapse videomicroscopy revealed that P4 cells possessed the fastest migratory speed while P10 granule cells retained their migratory activity for the longest time in culture. Cultures obtained from younger postnatal ages showed more random migratory trajectories than P10 cultures. Our observations indicate that despite similar morphological and molecular properties, migratory differences exist in granule cell cultures isolated from different postnatal ages. Therefore, the age of investigation can substantially influence experimental results on the regulation of cell migration.

Comparative analysis of body stability in athlete and non-athlete young adults

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The facts that the human body has a relatively high position of the centre of mass, and a relatively small size of the base of support directed the interest to the control mechanisms of human body stability for a long time now. A special aspect of the investigation is related to the sports activities. A well-balanced athlete should have good coordination and control when performing sports actions. Among the factors that have an effect on the maintenance of balance, the body measures and motor abilities are essential. Therefore, the purpose of this study was to investigate the relationship between body measures and body stability, furthermore, to examine the role of motor abilities in maintenance of static and dynamic balance. Subjects were 62 university students (52 women and 10 men) and 11 elite basketball players (men), age range 17-25 years. The body height, weight, and body fat (%) were measured. The body mass index (BMI) was calculated. A series of motor tests were performed. The equilibrium maintenance was measured with three methods. (1) Balancing on one leg on a beam 50 cm long, 3 cm high, and 3 cm wide. The subject should keep balanced in this position for one minute. Each time the subject lost his/her equilibrium, a new attempt started again. The number of attempts needed to keep in balance was computed. (2) The postural sways were recorded during standing on two legs on the stabilometer for 30 seconds. (3) Walking on a beam 6 m long, 8 cm high, and 4 cm wide. The numbers of step down were assessed. The relation between the anthropometric characteristics and body-balancing movements was calculated by using regression analysis. It was found that the one leg standing and walking on the beam do not differentiate well between the groups. In contrast, the sways on the stabilometer were significantly smaller for women than for men; furthermore, statistically significant differences were obtained from the comparisons of athlete and non-athlete subjects. Moderate but statistically significant correlations were found between body measures (BMI, body fat) and balance, as well as between motor abilities and balance. These results indicate that there is a relatively strong relationship between several body measures and the body-balancing movements during both static and dynamic balance. Furthermore, the motor abilities of well-trained athletes support the maintenance of body posture.

Effect of sodium channel blockers on the windup phenomenon in rat in vivo and in vitro

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Windup is defined as a cumulative depolarisation and increased responsiveness of spinal neurons to repetitive stimulation of the primary afferents. The phenomenon is implicated in the development of central sensitisation of nociceptive pathways, which plays a role in the development of chronic pain states. Thus windup is a suitable model for predicting the possible effectiveness of compounds for the treatment of chronic pain. In this study the effect of sodium channel blockers on spinal neurotransmission was investigated under windup conditions, both in vivo and in vitro.

In our in vivo experiments wide dynamic range (WDR) neurons at the level of L5-L6 segments were excited by electrical stimulation of the ipsilateral sciatic nerve with 16 impulses of 2 ms at 1 Hz (stimulus strength: 3x C-fibre threshold) in anesthetized, spinalised rats. Responses of single neurons were recorded extracellularly, using parylene coated tungsten microelectrodes (Dickenson and Sullivan, 1987), before and after the application of drugs. Both tolperisone and lamotrigine inhibited the windup of dorsal horn sensory neurons dose-dependently, with ED50 values of 9.8 mg/kg and 4.9 mg/kg, i.v., respectively, without affecting responses evoked by single stimulation.

The cumulative motoneuronal depolarization in the hemisected rat spinal cord preparation is suitable for studying windup in vitro. Experiments were performed on hemisected spinal cords isolated from 6-day-old rat pups. Cumulative ventral root potentials were recorded from the L5 ventral root following electrical stimulation of the corresponding dorsal root (exceeding C-fiber threshold) at 1 Hz. Both tolperisone and lamotrigine inhibited the cumulative ventral root depolarisation concentration dependently with IC50 values of 101.2 μM and 46.2 μM, respectively. According to these data, lamotrigine has more potent windup inhibitory activity both in vivo and in vitro, compared to that of tolperisone.

Our results are in agreement with the well-documented effectivity of sodium channel blockers (e.g. lamotrigine, carbamazepine) in chronic pain models and in patients with chronic pain, and provide further evidences on the potential clinical usefulness of tolperisone for pain indication as well.

Post-ischemic administration of diazoxide does not prevent chronic ischemia-related memory impairment, but attenuates microglial activation in the rat brain

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Post-ischemic administration of diazoxide does not prevent chronic ischemia-related memory impairment, but attenuates microglial activation in the rat brain

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A reduced cerebral blood flow has been observed in advancing age and dementia. A similar reduction in cerebral blood flow can be established by a bilateral occlusion of the common carotid arteries of rats (2VO). 2VO has been known to induce neurodegenerative processes in the hippocampus in particular. Furthermore this animal model has been used to test potential neuroprotective agents in ischemia.

Diazoxide is a mitochondrial potassium channel opener with neuroprotective properties when administered before the onset of an acute ischemic event. In this experiment the effect of treatment with diazoxide after 2VO was examined.

To this end, 35 male Wistar rats underwent either 2VO (n=18) or a sham (n=17) operation, followed by an i.p. injection of either diazoxide (0.5 microg/kg in 0.25 ml) or its solvent NaOH (0.25 ml) on five consecutive days. Twelve weeks after the occlusions the rats were trained in the Morris Water Maze, a spatial learning task involving the hippocampus. After the 5 days training, the animals were sacrificed and brain slices were immunocytochemically stained for astrocytes (GFAP) and microglia (CD11b).

Chronic cerebral hypoperfusion was found to significantly impair learning abilities, but treatment with diazoxide did not prevent this impairment. There were no clear effects of chronic cerebral hypoperfusion or diazoxide treatment on astrocyte activation. Chronic cerebral hypoperfusion increased microglia activation in the CA1 region and the dentate gyrus of the hippocampus and in these regions diazoxide treatment after 2VO decreased microglia activation to control level.

In conclusion, although diazoxide is known to be effective when applied before acute ischemia, it appears not to induce any functional improvement when administered after a chronic ischemic event. Although diazoxide appears to prevent microglial activation in ischemia, its mechanism and functional significance remains to be elucidated.

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Phasic and tonic stretch reflex during muscle stretch and vibration

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It is well known fact that under voluntary muscle contraction there is linear relationship between electrical activity of the muscle and the force during ramped isometric contraction. The electrical activity and force generation can be increased by phasic and tonic stretch reflex. In our study we investigated the effect of pretension and pre-activation level on both phasic and tonic stretch reflex and the force production of the muscle vastus lateralis and gastrocnemius muscles. The phasic stretch reflex was evoked by flexing the joint with 5.2 rad/s velocity at a pretension of 20, 40, 60, 80 and 100 % of maximum isometric torque using a special computer aided dynamometer. The subjects were in sitting position and their dominant leg was atteched to the moment arm flexed in 30 degrees of joint angle. Torque and electrical activity (EMG-RMS) of vastus lateralis was recorded during isometric and eccentric contraction. The muscle pretension was developed either slowly or as fast as possible then the muscle was stretched when the adjusted pretension was reached. We found that at slow force development the torque enhancement was lower as compared to the fast force development. Contrary, the electrical activity increased less at fast force development than at slow force development. However, the increase of electrical activity occurred earlier at fast force development than at slow force development.

The tonic stretch reflex was evoked by using whole body vibration. The subjects stood on a vibration platform flexing the hip, knee and ankle joint with different extents. The duration of the vibration exposure was one minute. The vibration frequency was increased from zero to fifty Hz in ten steps. The electrical activity was always elevated for vastus lateralis muscles that increased slightly in the function of the increasing vibration frequency. The greatest electrical activity was estimated at 50 Hz. The electrical activity of the gastrocnemius muscle started to increase at 15 Hz and reached its maximum at 30 Hz then dropped significantly when the vibration frequency increased further. When the muscle tension increased by lifting the heel from the platform, the highest electrical activity was measured at 25 Hz. At 30 Hz the EMG decreased considerably, but it was still greater than the initial level. We concluded that the pretension, activation velocity influence differently the phasic and tonic reflexes.

Behavioural changes in rats following perinatal exposure to drugs of abuse. I. Spontaneous behaviour

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Objectives: Consequences of perinatal exposure to drugs of abuse – morphine and methylenedioxy-methamphetamine (ecstasy, MDMA) – was measured in rats in the early period (3-5 weeks) of development.

Methods: Male and female Wistar rats were mated, the day sperm plugs were detected was designated embryonic day 0. The pregnant female rats were treated daily with either morphine (10 mg/kg sc.) or (+) MDMA (3 mg/kg sc.) from embryonic day 1 until the 21st postpartum day, when the offspring was separated. This day was considered as the termination of drug exposure. Offspring of female rats treated with physiological saline served as control. Spontaneous locomotor activity in novel surroundings, anxiolytic/anxiogenic state and learning performance were checked. Locomotor activity was measured in "CONDUCTA Advances System" (Experimetria Ltd), anxiolytic/anxiogenic behaviour was measured in elevated plus-maze test and learning performance was measured in a shuttle-box apparatus, where the number of intertrial crossings, conditioned avoidance responses and escape failures were recorded. Locomotor activity and the behaviour in elevated plus maze were measured 48 hours after separation, the shuttle-box behaviour was checked two weeks after it.

Results: 1.) Perinatal exposure to morphine enhanced the locomotor activity of both sexes of offspring, the difference compared to control was significant, however, only in males. In the elevated plus-maze test no results indicating anxiolytic or anxiogenic consequences were observed, but the total movement of animals exposed to morphine increased. In the shuttle-box test the number of the conditioned avoidance responses was higher in the drug-exposed females, but not in males. There was no change in the other parameters recorded.

2.) Perinatal exposure to (+) MDMA affected the locomotor activity the same way as morphine exposure did, the activity was higher but the difference was significant only in males. No change in the elevated plus maze behaviour was observed. In the shuttle-box test the number of both the intertrial crossings and the conditioned avoidance responses was higher in the drug-exposed females, and the number of the conditioned avoidance response was higher in males.

Conclusion: Animals exposed to perinatal morphine or (+) MDMA effect possess higher irritability and their ability to adapt to novel surroundings or a challenge suffers.

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Ionotropic glutamate receptors in the spinal dorsal horn

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Glutamate is the main excitatory transmitter in the spinal cord, and is used by primary afferents, many spinal neurons and some descending axons. Synaptically-released glutamate acts on AMPA and NMDA receptors which are concentrated in the post-synaptic membrane. Both receptors have a tetrameric structure. AMPA receptors can be homomeric or heteromeric and are made up from four subunits (GluR1-4, also known as GluRA-D). Subunit composition has important implications for function, for example AMPA receptors that lack GluR2 are Ca2+-permeable. NMDA receptors are heteromeric and contain NR1 together with one or more NR2 subunits (NR2A-D). Spinal AMPA and NMDA receptors both have important roles in pain perception. Spinally-admininstered AMPA receptor antagonists reduce acute pain, allodynia and hyperalgesia, NMDA receptors play a major role in induction of pathological pain states.

Synaptic AMPA and NMDA receptors are difficult to detect with immunocytochemistry due to the protein cross-linking that results from fixation. We have used antigen-unmasking to examine their synaptic distribution in the spinal cord. Following pepsin treatment, punctate labelling was seen with antibodies against each of the AMPA receptor subunits. GluR2-positive puncta were present throughout the spinal cord, and virtually all puncta labelled for any of the other GluR subunits contained GluR2. The other subunits showed a lamina-specific distribution: GluR1 was restricted to the dorsal horn and was concentrated in laminae I-II, while GluR3 and GluR4 were found mainly in deeper laminae. GluR-labelled puncta were associated with glutamatergic boutons and electron microscopy revealed that they were located at post-synaptic sites. Punctate staining for NR1 was present throughout the cord, while NR2B puncta were most numerous in laminae I-II, and NR2A puncta were present in laminae III-IV. For each NMDA subunit, most of the puncta were also labelled with GluR2 antibody.

We have also used this approach to investigate phosphorylation of GluR1 subunits at two serine residues (S831 and S845). Our results suggest that there is a very low basal level of phosphorylation of synaptic GluR1 at S845, but that this increases dramatically in the ipsilateral dorsal horn within 10 minutes of noxious stimulation. In contrast, there seems to be a significant basal level of phosphorylation at S831, and this is not altered by noxious stimulation.

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EEG effect of basal forebrain neuropeptide Y administration in urethane anaesthetized rats

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Neuropeptide Y (NPY) is present both in local neurons as well as in fibers in the basal forebrain(BF), an area that plays an important role in the regulation of cortical activation. In previous studies NPY axons were found to innervate corticopetal cholinergic cells in this area. In addition, identified NPY positive neurons have been shown to be silent during cortical activation, but active during slow EEG waves. However, no in vivo data is available whether or not local release of NPY in the BF can affect EEG. In the present experiments, the EEG effect of injection of NPY (0.5 □l, 300-500 pmol) into the BF of urethane-anaesthetized rats was examined. Fronto-parietal EEG was recorded on both sides and relative EEG power was calculated in the delta (0-3 Hz), theta (3-9 Hz), alpha (9-16 Hz) and beta (16-48 Hz) frequency bands. We found a significant increase in relative delta power and a decrease in the power of all higher frequency bands (theta, alpha, beta) after NPY injection. These results suggest that NPY can inhibit cortical activation acting via the BF.

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Changes in the density of the calretinin-immunoreactive interneurons in control and epileptic human hippocampi

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Calretinin (CR) is a member of the calmodulin superfamily. This calcium-binding protein is exclusively expressed in interneurons in the hippocampus. The human CR-containing interneurons are considerably different from the neurochemically similar cell-type found in rodents. In rats the CR-positive cells with smooth dendrites in the CA1 region are interneuron-specific inhibitory cells, while in humans CR was found in dendritic inhibitory cells as well, forming a functionally heterogenous cell population. The CR-immunopositive cells are morphologically more heterogenous: besides the cells which are present also in rats, there is a group of small neurons in the dentate gyrus (DG), and there is a multipolar cell-type at the border of the stratum radiatum and lacunosum-moleculare. CR-containing cells are particularly sensitive to ischemia and epilepsy in animal models. Therefore we aimed to reveal the fate of this cell type in human epilepsy. We examined the surgically removed hippocampi of drug-resistant temporal lobe epilepsy patients and compared them with control samples with different post mortem delay. The samples were immunostained for CR and the changes in the distribution and density of CR-immunopositive cells were analysed. The epileptic cases were classified and grouped into three types according to the degree of principal cell death. Longer post mortem delay resulted in a reduced number of immunopositive cells. The number of CR-positive cells in the epileptic tissue is considerably decreased in parallel with the severity of principal cell loss. The largest cell loss was found in the hilus and CA3. The number of the cells at the upper border of the stratum moleculare also decreased. However, a moderate number of the multipolar cells in the stratum lacunosum-moleculare and radiatum of the CA1 region are still detectable, but their dendrites are segmented and shortened. Our results suggest that CR-containing interneurons are also sensitive for epilepsy in humans similar to that seen in rat models, thus, interneuron specific inhibition might be decreased in human epileptic hippocampi. Post mortem delays longer than 6 hours of control samples can also cause significant loss of immunostaining, therefore examination of changes of the number and distribution of CR-immunopositive cells can be carried out only in very carefully selected human samples with short post mortem delay and good quality fixation.

Neuronal background of pathological aggression: activational changes in the dorsal raphe

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The serotonergic system is well known for its aggression lowering effects. It was recurrently shown, however, that the serotonergic system is activated during fights, and recent data suggested that it is necessary for the expression of aggressive behaviour. We investigated the interaction between serotonergic activation and aggressive behaviour in Wistar rats by assessing the co-localization of the c-Fos signal (marker of neuronal activation) with tryptophan-hydroxylase activity (marker of serotonin secretion) in the raphe. Control animals were compared with animals exposed to visual and olfactory (but not physical) contacts with opponents (psychosocial stimulation) as well as with animals exposed to aggressive encounters. Fights were accompanied by the activation of the raphe; however, the effect was not aggression-specific, as a similar activation was induced by psychosocial contacts. The lack of behavioural specificity in activation suggests that it was related to arousal rather than to aggressiveness. The activation of serotonergic raphe neurons showed a negative correlation with aggressive behaviour, which is in line with the widespread view that serotonin neurotransmission decreases aggression related behavioural phenomena. The activation of serotonergic neurons did not show a correlation with measures of hypoarousal-driven abnormal aggression, which indicates that factors other than the raphe control this behaviour. The latter finding may explain the low efficacy of serotonergic treatments in conduct and antisocial personality disorders, in which violence correlates with hypoarousal.

Close appositions of CARTP-immunopositive axon terminals on motoneurons in the frog

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Recent light microscopic immunohistochemical studies on the distribution of cocaine- and amphetamine-regulated transcript peptide (CARTp) revealed close appositions between CARTp-immunoreactive (IR) axons and motoneurons both of the cranial nerve nuclei and of the spinal cord. Using immunoelectronmicroscopy, we studied whether these appositions are places of synaptic contacts. Vibrotome sections of the brain and spinal cord were immunocytochemically stained with an anti-CARTp antibody, and blocks containing the motor nucleus of the facial nerve or the ventral horn of the spinal cord were further processed for electron microscopy.

In both locations investigated, CARTp-IR axon terminals contacted motoneuron somata, or (about 2%) the proximal part of their stem dendrite. The axon terminals were heavily packed with small round with a few dense cored synaptic vesicles, and contained several mitochondria. Conventional chemical synapses, however, were rarely found (<10%). Rather, at most places of close appositions, flattened, collapsed part of a subsurface cistern (SSC) with an electrondense substance was attached to the postaxonal plasmamembrane of the motoneuron, mimicking postsynaptic density. The SSCs were frequently continous with a subjacent cistern of rough endoplasmatic reticulum, and closely related to mitochondria. Occasionally, ribosomes were attached to the inner membrane of the SSC. In serial sections, typical synapse of chemical type was found in the near vicinity of these specific contacts.

It is well known that there are local differences in the physiological properties of neuronal plasmamembrane regarding resistance, electric threshold and excitability. The endoplasmic reticulum serves as Ca2+-store in neurons. SSCs are often continous with this reticulum, and functionally coupled to the overlying plasmalemmal microdomains indicating their involvement in the intracellular Ca2+-signalling. Areas of neurilemma associated with SSCs may, therefore, be sites of low transneuronal resistance where increased efficacy of depolarizing the postsynaptic membrane, and, thereby greater impact on the function of the postsynaptic neuron may be achieved. The intercellular junctions observed between CARTp-IR axon terminals and motoneurons likely represent a specific morphological type of synapse in frogs.

The internal structure of the nucleus geniculatus lateralis ventralis in avian brain and its connection with nucleus geniculatus lateralis dorsalis

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The light and electron microscopic structure of the nuclei geniculatus lateralis ventralis (GLv) and dorsalis (GLd) were studied, also the connection between the two nuclei was investigated. Different types of neurons in the GLv of the thalamus of chicks were visualised by Golgi impregnation. The dendritic tree of projection neurons branched in a sphere-like territory in both the ventral and middle areas of the lamina externa. The dendrites of projection neurons in the lamina interna descended into the lamina externa and entered both the ventral and middle dendritic territories. Optic terminals labelled injection of biotinylated dextran amine were found in the dendritic territories garhered into groups in the GLv. They established synapses in synaptic fields with different dendritic profiles also with GABA-positive terminals. No glomerulus-like synaptic complexes were found here. Numerous synapses established by both optic and GABA-positive terminals were found on dendritic stems of the lamina interna projection neurons.

The internal cell layer neurons were labelled with the anterograde tracer. Their axon terminate in the medial part of the dorsal lateral geniculate (GLdm) nucleus. The labelled retinal terminals, however, were found exactly in the lateral part of GLd (GLdl). The labelled terminals in the two parts of the nucleus were also studied with the electron microscope, they showed a different synaptic organisation in the two parts. In the lateral part two kinds of synaptic glomeruli were found: mostly simple glomerulus and complex synaptic unit with several pre and postsynaptic components. No glomeruli were found in the medial part of the nucleus. In the medial part of the GLd nucleus the terminals of internal layer cells axon of GLv establish asymmetrical synapses with dendrites. This connection of GLv with GLdm may responsible for relaying the colour vision into the Wulst.

Histaminergic innervation of cholinergic neurons in the basal forebrain of the rat

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Histamine has a crucial role in regulating arousal, cognition, energy metabolism and neuroendocrine functions. Histaminergic fibers arise from the exclusive source of the tuberomamillary nucleus (TM). While the pharmacology of the histaminergic system has been extensively studied, the synaptic mechanisms of histamine-mediated effects require further exploration. Histamine-immunoreactive (IR) axons heavily innervate the medial septal nucleus, horizontal and vertical limbs of diagonal band of Broca in rats (Panula et al., 1989; Tohyama et al., 1991), regions known to contain cholinergic neurons that modulate hippocampal and cortical activity. The goal of our study was to find out whether or not histaminergic axons contact choline acetyl transferase (CHAT)-IR cells of the basal forebrain in rats and thus part of the behavioral-state control exerted by the histamine synthesizing neuronal population of the TM is mediated via the basal forebrain cholinergic projection system. Double labeling immunocytochemistry at the light microscopic level revealed close appositions between histamine-containing axon varicosities and ChAT-immunopositive cell bodies and dendrites. These results suggest a monosynaptic link between the TM histaminergic system and the cholinergic neuron populations of the rat basal forebrain. Electron microscopic studies are in progress to find out whether histamine-IR axons indeed synapse with cholinergic neurons in the basal forebrain.

Anxiolytics and septo-hippocamapal oscillation: pharmacological and computational analysis of action of GABA-A alpha (1) and alpha (2) receptor allosteric modulators

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Clinically most active anxiolytic drugs are positive allosteric modulators of GABA-A receptors, represented by benzodiazepine compounds. Due to their non-selective profile, however, they actively modulate a number of various suptype specific GABA-A receptors. This non-selective action is presumed to be the liability for their broad-range side effects, including muscle relaxant, sedative, ethanol-potentiating and amnesic effects. Based on observation in genetically altered mice, it has been proposed that anxiolytic action of benzodiazepines is mediated by GABA-A alpha(2) subunit containing receptors [Rudolph and Mohler, 2004]. It is known that the septo-hippocampal activity is greatly affected by anxiolytics, leading to inhibition of its theta oscillation.

In the present study we analyzed the septo-hippocampal action of a preferential GABA-A alpha(1) and an alpha(2) positive allosteric modulator, zolpidem and L-838,417 respectively. We utilized in vivo electrophysiological experiments: medial septum/diagonal band single units and hippocamapal EEG were recorded simultaneously from chloral hydrate anaesthetized rats. Parallel to these experiments, a computational model has been constructed to model pharmacological actions of these compounds on the septo-hippocampal circuitry.

The present results demonstrated that zolpidem inhibited theta oscillation both in the hippocampus and septum, and profoundly inhibited firing activity of septal neurons. L-838,417 also inhibited hippocampal and septal theta oscillation, however, it did not significantly alter firing activity of septal neurons. Our computational model of septal neuronal network showed the capability for autonomous theta-periodic oscillation. However, cessation of periodic firing of hippocampo-septal neurons, representing absence of hippocampal theta activity, disrupted oscillation of septal units, without alteringtheir overall firing activity, similar to changes observed in our in vivo experiments following administration of L-838,417. A substantial decrease in firing activity in the septal network model was observed only after a reduction in constant excitatory input to septal units.

Understanding the correlation between changes in activity of the septo-hippocampal system and anxiolytic action of GABA-A receptor modulators would provide additional advantage in drug design by reducing unwanted side effects of anxiolytic drugs.

Neural microprobes for cortical investigations

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The complexity of the human cerebral cortex requires intense and simultaneous observation of the neural processes to understand its function. A lot of effort has been and being made to expose the spatiotemporal functional properties of the cerebral cortex. Amongst all of these techniques still the electrophysiological approach yields the most, from the functional point of view, mainly because of its high temporal resolution. As far as the elementary neural events are taking place on the synaptic/membrane level, the spatial sensitivity is also a corner stone in the understanding of the cortical functions. While with the existing methods we can easily oversample our data in the time domain, it still needs a great deal of research and development, to do the same in the spatial domain. With the aid of the microelectrode arrays (multielectrodes) one can achieve spatial resolution in the order of ten to hundred microns, which is sufficient to monitor the flow of the synaptic/membrane events. Because of the well known columnar and laminar organization of the human cortex, good approximation can be derived concerning its epileptic, sensory-motor and cognitive functions, by sampling the brain electrical activity from representative points of the columnar volume, and it also minimize the functional damage caused by the manipulation.

Neurosurgical treatment for epilepsy opens a very important window for direct electrophysiological observation of the human cortical functions. During the surgery and accompanying chronic implantation period in the case of epileptic focus localization, various neural probes can be implanted directly into the cerebral cortex.

We examined regions of the human cortex generating epileptic events and event related potentials (ERP) by recording potentials with existing subdural or depth clinical electrodes and with high density multielectrodes. Several types of electrodes were designed to fit the needs of different recording strategies and cause as small additional damage as possible. Since the size of the microelectrode recording sites are in the range of 20-40microns and they are evenly dispersed throughout the depth of the gray matter, they can help to resolve the microscopic neural events taking place in various layers of the cortex. The acquired information are useful in defining the local and long range epileptogenic network interactions, and intracortical generators of epileptic events, cognitive and sensory-motor ERPs.

Our results indicate that short term (20-30min, acute) and long term (chronic), multielectrode implantation is feasible in humans; we were able to record adequate signal quality (clusterable action potentials, clear field potentials and current source density) immediately after and for up to 2 weeks after implantation.

Intracortical Activation Sequence Of Interictal Spike-Wave Complexes

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The defining feature of epileptic brain is its ability to generate seizures (SZ) and interictal spike-wave complexes (SW). SW are clinically important because they are widely used indicators for the location of the epileptogenic zone. Although the origin of the SZ is at issue, SW is much easier to analyze since there are usually thousands of SW to each SZ, and they can be obtained without movement artifacts. Most epileptologists currently believe that surgical treatment requires localization of the SZ discharge itself, but consider SW as providing crucial complementary information. Ictal and interictal activity is variable in animal models, its synaptic basis is poorly understood and questionable whether it can be generalized to the actual human disease.

We examined regions of the human cortex generating SW and SZ by electrical stimulation and recording potentials with subdural clinical electrodes and with high density microelectrode arrays (multielectrodes). The multielectrodes were implanted in cortical areas alongside with the clinical electrodes in intraoperative and chronic settings. We designed a thumbtack like 24 contact linear array multielectrode, intercontact distance: 150micron, contact diameter: 40micron, outer shaft diameter: 350micron. The multielectrodes were inserted in the close vicinity of the subdural electrode in temporal lobe recordings. Electrical stimulation through the clinical electrode (single and train) was used to evoke neuronal responses, spikes and seizure. The tissue containing the electrode penetration site was removed en-bloc and sectioned for recording contact localization.

Spontaneous SW complex started as a sharp surface positivity with middle layer depolarization accompanied by elevated multiple unit activity, suggesting feed-forward excitation. The prolonged surface negativity part of the spike was generated by superficial sinks and less pronounced firing suggesting feed-back excitation. The surface positive part of the wave was accompanied by superficial sources and marked decrease in multiple unit activity suggesting feed-back inhibition, while the final slow surface negative part was marked by middle layer sources and neuronal silence, suggesting disfacilitation or feed-forward inhibition. Low intensity single electrical stimulation evoked similar initial current source density profile as the presumed feed-back excitation, however high intensities were able to evoke the whole SW sequence.

In conclusion, since the intracortical current source density and multiple unit activity distribution during SW were very similar across patients, cortical regions and etiologies, it suggests a common final pathway in epileptic manifestations. These results could help us focus treatment strategies as well as aid EEG/MEG source localization techniques by applying additional spatiotemporal constraints.

The role of prefrontal alpha-2 adrenoceptors on the operant behaviour of rats with different response to novel environment

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The medial prefrontal cortex (mPFC) has been reported to be a component of the motive circuit that is involved in reward-oriented behaviours. The mPFC consists of the infralimbic, prelimbic, and anterior cingulate cortical areas. The mPFC receives a strong noradrenergic innervation from the locus coeruleus, that decreases the basal activity of the neurons, while the locus coeruleus receives a reciprocal excitatory innervation from the mPFC. This circuit may have a particular relevance for attentional regulation. Norepinephrine has higher affinity to alpha-2A receptors, than for alpha-1. An alpha-2 agonists, such as guanfacine, has been shown to improve the performance of PFC tasks.

In the present study we wanted to identify any bias that exists between the operant conditioning performance of HR and LR rats to prelimbically applied alpha-2A agonist, guanfacine and antagonist, yohimbine.

Adult high(HR) and low(LR) responders to novelty are male rats that normally occur in every outbred strain of Wistar rats, but some characteristics remarkably differ between these animals. Differences has been shown in the make-up of the brain, in the neuroendocrinological, immunological systems, in behaviour, and the response to certain pharmacological agents.

In our experiment rats were divided into three groups: HR, MR (middle responder) and LR, according to their locomotor response to a novel open field. Then they learned an operant conditioning task: when six leds were on they had to press a lever in order to get water, but when only one led was on they should not press the bar, or else all light went out as punishment. After acquiring the task, a bilateral guide cannula was implanted in the prelimbic cortex, through which on test days certain doses of guanfacine or yohimbine(0,5 mikroliter) were injected. Task performance and reaction times were recorded and evaluated.

Guanfacine application resulted in a U-shaped dose-response curve by decreasing the reaction time of LR and MR rats, but had no significant effect on HRs. It also impaired the learning rate of the previous groups, but did not influence HR rats. Yohimbine on the other hand improved the performance of HRs, but not LRs. Most doses of the antagonist lengthend the reaction time of the rat groups.

Our data show that rats with different response to a novel environment also vary in an operant behaviour test. The reason to their reaction after the alteration of the adrenergic system is probably due to different receptor distribution of the prelimbic cortex. Our work may provide futher informations about the individual differences to pharmacological products.

Changes in small intracellular proteins and peptides in physiologically and neurochemically controlled epileptic cellular stress in rat cortex

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Epileptic seizures activate extra- and intracellular proteases. Some proteases, as Caspase-9 and 3 are apoptotic signals; others digest the extracellular matrix making space for structural changes. The proteases and their activation have a growing literature but very little if any is known about the primary targets of them. Our hypothesis was that the protease products could give us information about the protease targets detectable in epilepsy. This pilot study aims to set up a method to identify peptides and small proteins from cortex in behaviourally and physiologically monitored epileptic rats. We also measured amino acids in thalamus from microdialysate under seizures to get a neurochemical information about the seizure. Seizures induced by a potassium channel blocker 4-Aminopyridyn (AP4) can be recorded in Ketamine anaesthesia. It makes the experiment easy and the EEG analysis clearly demonstrated that we obtained seizures know in the literature.

The amino acid concentrations in thalamus show changes when AP4 is applied directly into thalamus via microdialysis. When AP4 crystals were applied on the surface of the opposite brain hemisphere no change in the dialysate was observed. Thus, we claim that AP4 induced seizures do not spread from cortex of left hemisphere to the right thalamus.

Using peptide-protein extraction from the cortical tissue samples, we observed differences in the amount of hydrophilic peptides in cortex in epileptic rats comparing to the control. Our first study gave a promising result of changes in putative peptide fragments derive from seizure induced proteolysis. The method needs further development in regard of being able to analyse the more hydrophobic peptides and improving the verification of peptides of high sensitivity mass spectrometry. Our model is ready to use on wide scale of cellular stress including heat-shock, social stress or sleep deprivation stress.

Compartmentalized distribution of the K+ - Cl- cotransporter 2 (KCC2) in the perisomatic region of neocortical pyramidal cells

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The intracellular concentration of chloride and the polarity of GABAergic responses are predominantly determined by chloride extrusion mechanisms. In the mature cerebral cortex, the K+ - Cl- cotransporter 2 (KCC2) is responsible for the generation of an inwardly directed Cl- electrochemical gradient rendering GABA hyperpolarizing. Differential KCC2 expression in distinct types of neurons was shown to influence reversal potentials in various brain regions, but quantification of this cotransporter in different regions of neurons is lacking.

We applied high-resolution immunolocalization in three rats (P25) to determine the subcellular distribution of KCC2 on layer 2/3 pyramidal cells. First, we calculated the non-specific labelling density over the nuclei (0.21 ± 0.05 gold/ìm2), which should not contain KCC2. Then we compared this background with KCC2 immunogold density on somatic and axonal compartments of pyramidal cells. Although dendrites clearly contained KCC2 labelling, the detailed quantification of KCC2 levels on diverse parts of the dendritic tree in relation to GABAergic response polarity requires a separate study following reports that the polarity of dendritic IPSPs could be a function of distance from the soma. Membranes and cytoplasm of somata (n=38) and membranes of axon initial segments (n=11) contained significantly higher density of gold particles relative to background ($p<0.002$ for soma $p<0.02$ for axon, ANOVA with Tukey's correction). Comparison of immunogold densities after the subtraction of nonspecicifc labelling showed a ~19 fold decrease from somatic to axon initial segment membranes (from 28.98 ± 1.05 to 1.53 ± 0.67 gold/ìm2, $p<0.0002$). Although we measured immunogold density on individual axon initial segments up to 40 ìm from the soma, we could not detect a gradient-like distribution of KCC2; density levels dropped at the border between the hillock and the initial segment. Cytoplasmic labelling of KCC2 may represent exchanger molecules being transported to or from the plasma membrane. Relative to the plasma membrane, the cytoplasm contained lower densities of KCC2 immunogold labelling both in the soma and axon initial segment (0.62 ± 0.14 and 0.28 ± 0.26 gold/ìm2, respectively, $p<0.0002$ and 0.05).

Thus, GABAergic inputs arriving from basket cells are surrounded by a high concentration of KCC2 setting the reversal potentials at hyperpolarized values. Low KCC2 density and decreased chloride efflux in the axon initial segments could support higher internal concentrations of chloride leading to depolarizing effects of axo-axonic cells at physiological subthreshold conditions.

Firing pattern analysis of the HCN1 pacemaker ion channel expressing neurons in the medial septum in vivo

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The medial septum has a pivotal role in the generation of hippocampal electrical activity patterns. A large population of medial septal neurons establishes reciprocal connections with the hippocampus. The majority of them exhibit hippocampal theta-related burst firing. Recently in situ and immuncytochemical studies showed hyperpolarization-activated, cyclic nucleotide-gated cation (HCN) channels on soma-dendritic domain of medial septal neurons. These ion channels are involved in pacemaker mechanisms in heart and brain.

Juxtacellular method were used to record and label neurons in the medial septum, and compound electrode consisting of an iontophoretic and a juxtacellular electrode to locally administer drugs, and record the activity changes. The filled neurons were identified to demonstrate the HCN1 and parvalbumin (PV) expression of the cell. Colocalization of the HCN1 PV and ChAT immunopositive neuron populations was investigated by double immunocytochemistry.

The counting of 500 neurons on double stained medial septal sections showed that 20% of the PV neurons colocalized with the 55% of the HCN1 immunorecative cells, and no overlap were found with cholinergic cells. The analysis of the neuron activity showed that the presence of the HCN1 on a cell is a good indicator of regular burst firing behavior, since 7 out of 8 HCN1 immmunoreactive neuron fired theta related rhythmic burst and only one out of 5 HCN1 negative neuron showed similar pattern. Simple and complex (high frequency transients) burst firing neurons were also found among the positive cells. Initial results showed that local iontophoretic application of Cesium – at low concentration a selective HCN blocker – were able to decrease the regularity of cell firing. The neurons expressing HCN1 had a preferential firing around the trough of the hippocampal theta recorded in CA1 pyramidal layer.

Our results support the hypothesis that the HCN1 immunoreactive neurons of the medial septum are good candidates for pace making the hippocampal theta.

Effects of insecticides on neuronal activity

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To evaluate the risk of using toxic chemicals as pesticides, it is important to have more information about their effect on different organisms, including the effects on mammalian nervous system. In our present experiments, the neuronal effects of two insecticides, bensultap and fipronil were examined, using in vitro administration.

Bensultap is an insecticide of the neonicotinoide group. Eaten by the insect, it is metabolically converted to nereistoxin which is an antagonist of nicotinic acetylcholine receptors (nAChRs), both at neuromuscular synapses and in the CNS. But also bensultap can cross the blood-brain barrier and antagonize the effect of ACh.

In the mammalian nervous system, muscarinic acetylcholine receptors (mAChRs) are ubiquitous, while most nAChRs are located presynaptically and they enhance the release of excitatory amino acide transmitters.

Experiments were performed on cortical slices of rat brains, by means of measuring extracellular field potentials. Effects on the excitability were examined, long-term plasticity was characterized with induction of LTP (long-term potenciation) and short-term plasticity with paired-pulse tests. Application of bensultap decreased considerably the efficacy of LTP-induction: in case of control slices, the increase in amplitude of evoked responses was 1,887 (n=5), in treated slices it was 1,333 and 1,253, using 25mg/l (n=8) and 12,5mg/l (n=7) bensultap, respectively. On short-term plasticity, application of bensultap had no effect.

Fipronil is an insecticide acting as an antagonist on ionotropic GABA receptors. It is highly toxic to aquatic invertebrates and it can also bind to mammalian receptors.

Experiments were performed on GABA-sensitive neurons of Lymnaea stagnalis (n=8), the effect of fipronil on the frequency of action potentials was examined. Action potentials were registered with intracellular microelectrodes and the occurrence of different interspike-intervals (ISI) was measured. Fipronil increased the ISIs in a concentration-dependent manner: using 1, 2, 4 and 50 □g/ml, it was 112,4, 116,8, 123,0 and 135,8 % of the control, respectively. This result can be explained by the fact that in gastropodes, GABA is usually an excitatory transmitter with depolarising effect, so GABA-antagonists act as inhibitors.

Insecticides often have some effects on mammals and non-target invertebrates, the knowledge that we have about these effects can be useful to recognize the symptoms of an intoxication or to regulate the use of the chemical.

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Effects of 3'-methylation of 2,3-benzodiazepine derivatives on the inhibitory potency at AMPA / Kainate receptors.

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AMPA / kainate channels (AMPA = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) are known as members of ionotrop type in the family of excitatory amino acid receptors. These receptors play the central role in sensory and motor functions, learning and memory formation. Besides, they are also involved however, in spreading neuronal cell death following an ischemic brain injury. Indeed, as it has been demonstrated by both in vitro and in vivo data, negative allosteric modulators to AMPA / kainate receptors such as the 2,3-benzodiazepine derivative GYKI-52466 and its successors exert potent neuroprotective effects. We studied the effects of several 1-(4'-aminophenyl)-2,3-benzodiazepine derivatives on the in vitro inhibition of AMPA / kainate channels in isolated Purkinje cells (with kainate agonist) by whole-cell patch clamp technics, as well as in hippocampal slice preparations (population spikes) and in chicken retina (spreading depression initiated by AMPA).Inhibitory potency of these benzodiazepine derivatives roughly doubled by ortho-methylation on (3'-methyl substitution of) the 4'-aminophenyl moiety. This effect is illustrated here by the IC50 values of the inhibition observed on kainate response in Purkinje cells (patch clamp experiments, data in microM ± SD): ortho-methylation of GYKI-52466, 53405 and 53655 increased inhibitory ability as shown by the decrease of IC50 values from 22.3 ± 0.7 to 10.8 ± 0.2, from 22.2 ± 0.5 to 10.9 ± 0.2, and from 3.1 ± 0.5 to 1.4 ± 0.2, respectively. Dual type substitution (7,8-dichlorination together with ortho-methylation) produced compounds with even lower IC50 values. These results suggest that both ortho-methylation and 7,8-dichlorination may favor the neuroprotective efficiency, as well.

Heterogeneous population of octopamine receptors for feeding modulation in the buccal network of the pond snail Lymnaea stagnalis

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Octopamine plays a role as both neurotransmitter and neuromodulator in the buccal feeding system of the pond snail Lymnaea stagnalis.

Previous pharmacological experiments on the synaptic connections of the octopamine-containing OC neurons suggested diverse mechanisms for octopame effects. The inhibitory connection between OC-B3 neurons is most likely mediated by ligand-gated receptors, while in the OC-B1 connections we suggested the involvement of cAMPdependent second messenger systems. (Vehovszky et al 2000, Pitt et al 2004).

Octopaminergic regulation of cAMP formation was assayed by mesasuring either by adenylate cyclase activity or the cAMP content of the tissues made from isolated buccal ganglia.

In the presence of 100 µM GTP octopamine (1-100 µM) increased the cAMP content of the tissues in a dose-dependent manner, as well as the adenylate cyclase activator forskolin (50 µM) suggesting stimulation of the adenylate cyclase system by octopamine. In contrast, dopamine (100 µM, an other feeding modulator in Lymnaea buccal system) did no result cAMP elevation.

The presence of the non-hydrolysable analogue of GTP, Gpp(NH)p resulted a further elevation of the cAMP content suggesting the involvement of G-proteins in the octopamine-evoked cAMP increase. A similar cAMP increase was measured in the presence of the octopamine receptor agonist NC-7 (2-cholo-4-methyl-2-phenylimino-imidazolidine) while in the presence of the antagonist mianserin (100 µM) octopamine failed to evoke cAMP increase.

The octopamine receptors involved in our experiments on isolated buccal tissues are most resemble the Lym oa1eceptors cloned from Lymnaea CNS by Gerhardt (1977).

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Lipopolysaccharide-induced changes in blood-brain barrier functions in vitro

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In endotoxemia, which leads to septic shock, multiorgan failure and death in mammals, integrity of the blood-brain barrier (BBB) is impaired. Lipopolysaccharid (LPS)-induced changes in BBB functions has been investigated on an in vitro model of primary rat brain endothelial cells co-cultured with primary rat astroglia cells. Several LPS serotype (Salmonella typhimurium, E. coli O11:B4, O55:B5) has been tested. A dose- and time-dependent decrease in monolayer integrity has been observed in LPS-treated brain endothelial cells. Transendothelial electrical resistance decreased with a maximum at 6 h treatment, although it returned to almost the pretreatment level at 16 h. No change was observed at 24 h and 48 h treatment periods. Immunostaining for ZO1, occludin, claudin-5 and beta-catenin was significantly weaker in LPS-treated endothelial cells. LPS also reduced the intensity and changed the pattern of immunostaining for tight junction proteins in freshly isolated rat brain microvessels. P-glycoprotein activity of brain endothelial cells was also inhibited by LPS. Both reactive oxygen species and nitric oxide production were increased in brain endothelial cells treated with LPS. Pentosan polysulphate, a polyanionic polysaccharide could reduce the deleterious effects of LPS on permeability, P-glycoprotein activity, reactive oxygen species and nitric oxide production.

LPS not only increased permeability of monolayers, but it also affected efflux pump function and stimulated reactive oxygen species and nitric oxide production in brain endothelial cells. The protective effect of pentosan for brain endothelium has been confirmed in LPS-treated cells.

Active vision

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Vision is an active process, through which we, on the one hand, visually characterize our environment and, on the other hand, visually guide our actions. There are two strong arguments that urge us to consider vision as an active process: (i) the neural background of the process of vision is not a passive, "photographic" reflection of a visual scene in our brain, but instead an active processing of this incoming visual information that takes place in specialized neural circuits of the visual system; (ii) it is only a fraction of the visual input that is present in a given moment in our field of view that is fully processed in our brain. The selection of the stimuli, which are to be processed in detail, is an active process, served by the movement of our eyes as well as the movement of our mind's eye, i.e., visual attention.

In this talk, the different types and neural mechanisms of visual attention will be examined, with a special emphasis on the task specific, volitional and stimulus driven mechanisms of attentional selection, as well as the interactions between them. We used psychophysics, eye movement recording and event related potentials (ERP) to uncover the spatiotemporal characteristics of attentional selection and its plasticity. Our results provide evidence that top-down volitional attentional selection consists an explicit and an implicit component: the former affects the processing of the task-relevant visual input, which is in the focus of attention, whereas the later implicit component of selection modulates the processing of the task-irrelevant visual input outside the focus of attention. We will also show that the mechanisms of attentional selection are adaptive and are strongly affected by visual learning. The results on visual attentional selection and eye movement are implemented in our biologically inspired model of active and adaptive vision.

Comparison of the development of the unipolar brush cells in different mammalian species - with emphasis on the human

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The unipolar brush cells (UBCs) are glutamatergic, excitatory interneurons in the granular layer of the mammalian cerebellar cortex, distributed primarily in the vestibulo-cerebellum. Previous studies have shown that UBCs appear in the internal granular layer at a relatively late phase of cerebellar cortical development (at P7-P8 in the mouse and at birth in the cat) and their development continues even beyond the migration of the granule cells (the cytoarchitectonic build-up of the cerebellar cortex). Regarding UBCs in the human, very few data are available. Our aim was to study the temporal and spatial characteristics of the development of UBCs in the human cerebellum.

A series of 20 normal human brains, including 3 adult and 17 fetal or infant brains (from the 24th gestational week till the 11th postnatal month) were used to examine the developmental dynamics of UBCs. In order to visualize UBCs, calretinin (Sigma, developed in rabbit) immunocytochemistry was performed on sections of paraformaldehyde-fixed, paraffin-embedded blocks of the cerebellar vermis.

Our results showed that UBCs were not yet present in the cerebellar vermis at the 24th gestational week. The first UBCs appeared in vermal lobules receiving vestibular input approximately at the 28th gestational week. At birth, UBCs were present in a relatively small number, mostly in the vestibulo-cerebellar lobules. At the 3rd, 5th, 8.5th and 11th postnatal months the number of UBCs gradually increased. As far as the distributional characteristics concern, we found that UBCs could be observed first in the early developing cerebellar lobules, followed by the invasion of the later developing vermal lobules. UBCs appeared to spread in a rostro-caudal direction, „filling up" the lobules from the base towards the apex. Regarding their location within the cerebellar cortex we found that UBCs invade the internal granular layer (IGL) in an outside-in manner, first appearing right below the Purkinje cell layer and only later in the deeper regions of the IGL. Interestingly, we found the same tendency in the mouse, whereas in the cat an inside-out spreading was observed.

Although at the 11th postnatal month UBCs exhibited the same morphological and distributional features as in the adult, their number was still lower than in the adult cerebellum. This implies that the cytoarchitectonical development of the human cerebellum is not completed by about the end of the first postnatal year. In order to determine when the development of UBCs comes to and end, further studies are to be done at later postnatal ages.

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Neurotransmitters of commissural interneurons in the lumbar spinal cord of neonatal rats

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The circuits responsible for generating rhytmic motor activity are located in the ventromedial area of the spinal cord. Commissural interneurons (CINs), which are necessary for correct left-right co-ordination, constitute an essential part of this central pattern generator (CPG). Previous morphological and physiological studies showed that CINs represented a heterogeneous population with respect to their projection patterns and postsynaptic effects on the contralateral motoneurons.

In this study we used morphological approaches to investigate the neurotransmitter properties of CINs. We have injected biotinylated dextran amine (BDA) into the ventromedial gray matter on one side of the lumbar spinal cord in neonatal rats. Anterogradely labeled axons that originated from CINs within the confines of BDA injections crossed the midline in the anterior commissure and arborized intensively in the ventral horn and intermediate gray matter on the contralateral side of the spinal cord. The neuroransmitters used by these BDA labeled boutons were identified by applying antibodies raised against vesicular glutamate transporters 1 and 2 (VGLUT1, VGLUT2), glutamic acid decarboxylase and glycine transporter 2.

With the help of confocal microscopy BDA labeled CIN axon terminals were investigated for inhibitory and excitatory neurotransmitters. Out of 1146 boutons 663 proved to be immunoreactive for inhibitory transmitters; 47% of these terminals contained glycine, 24% were immunostained for GABA and about one third of inhibitory boutons used both transmitters. Vesicular glutamate transporter immunoreactivity was investigated in 410 BDA labeled boutons and 28% of them were found to be immunopositive for VGLUT1 or VGLUT2.

The CINs in the spinal cord of lamprey and Xenopus proved to be inhibitory glycinergic neurons. In contrast to the swimming CPGs, our results suggest that CINs in mammalian spinal cord include both glutamatergic excitatory and GABAergic and glycinergic inhibitory neurons. These data are in agreement with earlier physiological examinations showing that axon terminals of the CINs may excite or inhibit contralateral motoneurons monosynaptically or may also influence the activity of motoneurons through premotor interneurons located in the spinal cord.

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Neocortical c-fos mRNA transcription in repeated, brief, acute seizures: Is c-fos a coincidence detector?

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The expression of the Fos family member protooncogenes is induced in every animal seizures studied so far, indicating that the activation of the c-fos promoter may be an early common pathway in the convulsive state. The effect of acute brief seizures on neocortical c-fos expression was investigated in male Wistar rats with 5 mg/kg 4-aminopyridine (4-AP) administered intraperitoneally.

Electroencephalography (EEG) in freely moving animals with implanted neocortical electrodes detected an average of 2.67 tonic-clonic convulsions within 1 hour following the 4-AP treatment. The first seizure activity appeared on the EEG with an average latency of 20.70±6.48 min. The EEG seizure and the behavioural symptoms did not last after 1 h.

Tissue samples of the somatosensory neocortex were collected for PCR at 30 min, 1 h, 3 h, 5 h and 8 h following the 4-AP treatment. The c-fos mRNA displayed the first significant rise at 1 h; and remained significantly higher through 3 h. After that, c-fos mRNA decreased gradually below the control level by 8 h.

Immunohistochemistry for the localisation of the c-fos gene product protein was performed on frozen, coronal plane rat brain sections. Within the area of interest (S1Tr region of the parietal neocortex), the c-fos immunoreactive cell nuclei were counted. The number of c-fos immunoreactive cells was significantly elevated already at 30 min, peaked at 1 h, and declined by 5 h.

We conclude that in repetitive, brief seizures, the first convulsion does not increase c-fos RNA transcription, whilst the second causes a long-lasting gene expression and a large increase of c-fos protein synthesis. The discrepancy between c-fos mRNA peak and c-fos immunoreactive cell count peak raises the possibility of the seizure-activated translocation of the Fos protein from the cytoplasm to the nucleus. Fos acts as a coincidence detector, potentiating the long-time cellular effects of successive, brief seizures via its transactivating capability. This phenomenon may have implications in the pathogenesis of human and animal epilepsies.

Regulation of interendothelial junctions by serine and tyrosine phosphatases

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Interendothelial junctions are complex structures of interacting transmembrane, membrane-associated and cytoplasmic proteins. Junctional protein function can be affected by phosphorylation which is tightly regulated by protein kinases and phosphatases.

We analyzed the role of serine/threonine and tyrosine phosphatases in the function of junctional proteins in primary cultures of rat brain endothelial cells (RBEC) and immortalized rat brain endothelial cells (GP8).

We found that phenylarsine oxide (PAO), a potent inhibitor of tyrosine phosphatases induced a strong phosphorylation on tyrosine residues of proteins in the range of 50-110 kDa and the phosphorylation was restricted to Triton X-100 insoluble fraction. PAO treatment of RBEC led to a decrease of occludin and cadherin expression and induced a redistribution of ZO-2 and alpha/beta-catenin to the Triton X-100 insoluble fraction. Interestingly we could not detect tyrosine phosphorylation of alpha-, beta-catenin, ZO-1, ZO-2, cadherin or occludin in response to PAO treatment. Immunofluorescence studies have revealed changes in the localization of the junctional proteins. The continuous membrane staining was distrupted (claudin-5) or completely lost (beta-catenin and ZO-2).

Inhibition of the protein phosphatase 2A by okadaic acid also induced a decrease of occludin in the soluble fraction accompanied by a less pronounced redistribution of ZO-2. Accordingly, we found complete loss of occludin staining in the cell membrane, whereas ZO-2 and claudin-5 became discontinuous.

Our results suggest that Ser/Thr and/or Tyr phosphorylation dependent mechanism may regulate directly or indirectly the function of junctional proteins which may have significant effect on the barrier properties of RBEC.

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Multilevel regulatory hormone system in Eisenia fetida

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Occurance and distribution of two peptide hormons (TSH and TGB) and their receptors (TSHR and THR) were studied in the earthworm (Eisenia fetida, Annelida, Oligochatea). Examinations were carried out on paraffin embedded sections of the anterior, medial and caudal segments of the body and smears of coelomocytes were used for immmunostaining. Primary antisera against TSH/TGB and TSHR/THR were visualized with fluorochromes, or with DAB.

In annelids there are no endocrin organs. Various neuron types produce neurohormones. These signal molecules might be released directly into the neurohemal organ or the vascular system. TSH and TGB immunopositive neurons and their receptors are present in all ganglia of the central nervous system. The immunopositive neurons are situated in the cortex of the ganglion. The axonal processes of the neurons might connect to the capillaries running near these cells or to the neurohemal organ. Distribution of the stained neurons is uneven. Most of the TSH/TSHR-positive neurons were found in the cerebral and subesophageal ganglia (e.g. (58 cells out of 1675) and less in ventral ganglia, while TGB and THR occured more frequently in the ventral ganglia. The number of neurons, which contain the above hormones and/or their corresponding receptors was also different, with less receptor-bearing cells.

Stained non-neuronal elements are found in the capsule of the ganglia, coelomocytes in the body cavity and in the connective tissue layer in the wall of the foregut. In the capsule only TSH-positive cells were found, while in the pharynx all the studied hormones and receptors could be detected. None of the coelomocytes showed THR immunopositivity, while large granulocytes were stained for both TSH and its receptor and also for TGB. Many eleocytes showed strong immunostaining with the TSH antiserum, and some of them with TSHR staining also.

In Tunicata a region of the pharynx the endostyle has been considered as a homologue of the vertebrate thyroid gland, while the neurohaemal organ as a hormone storage place. In our experiments the pharynx is the only organ showing strong immunopositivity for all antisera tested. The stained coelomocytes similarly to the mammalian immune cells may conduct immune functions in Eisenia. These hormones might enhance cell metabolism and improve the immune functions of granulocytes and eleocytes.

Origin of cocaine- and amphetamine-regulated transcript (Cart)-containing axons innervating hypophysiotropic corticotropin-releasing hormone (CRH)-synthesizing neurons in the rat

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Cocaine- and amphetamine-regulated transcript (CART), an anorexigenic neuropeptide, has stimulatory effects on the hypothalamic-pituitary-adrenal axis through direct effects on hypophysiotropic CRH neurons. Recently, nerve terminals containing CART have been demonstrated to densely innervate CRH neurons in the PVN. Based on the anatomy of known sources for the innervation of CRH neurons and the CART innervation of the PVN, the origin of these CART-containing fibers could include hypothalamic arcuate nucleus neurons that co-express alpha-melanocyte-stimulating hormone (alpha-MSH) and medullary neurons that produce adrenaline. To determine whether either of these cell groups contribute to the CART innervation of CRH neurons in the PVN, we performed quadruple-labeling immunofluorescence using antisera against CRH, CART, alpha-MSH and phenyl-ethanolamine-N-methyl-transferase (PNMT), the latter as a marker for adrenaline. Consistent with previous observations, PNMT-immunoreactive (IR) and CART-IR axons densely innervated all CRH neurons, whereas the alpha-MSH-IR innervation was sparse. While approximately 60% of CART boutons in juxtaposition to CRH neurons co-contained PNMT, only approximately 16% were positive for alpha-MSH-immunoreactivity. All alpha-MSH-IR boutons and approximately 90% of PNMT-containing varicosities on the surfaces of CRH neurons were labeled for CART. The remaining 26% of CART boutons in contact with CRH neurons contained neither alpha-MSH or PNMT. These results indicate that medullary adrenergic CART-containing neurons are the major source for the CART innervation of CRH neurons in the PVN, whereas the arcuate nucleus has only a minor contribution. The observation that about 1/4 of the CART innervation contains neither alpha-MSH or PNMT, however, suggests that additional source(s) are present that contribute to the CART-IR input of hypophysiotropic CRH neurons.

Genetic approaches to sodium channel function in pain pathways

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Acute, inflammatory and neuropathic pain can all be attenuated or abolished by local treatment with sodium channel blockers such as lidocaine. The peripheral input that drives pain perception thus depends on the presence of functional voltage-gated sodium channels. Remarkably, two voltage-gated sodium channel genes (Nav1.8 and Nav 1.9) are expressed selectively in damage-sensing peripheral neurons, whilst a third channel (Nav 1.7) is found predominantly in sensory and sympathetic neurons. An embryonic channel (Nav 1.3) is also upregulated in damaged peripheral nerves and associated with increased electrical excitability in neuropathic pain states. A combination of antisense and knock-out studies support a specialised role for these sodium channels in pain pathways, and pharmacological studies with conotoxins suggest that isotype-specific antagonists should be feasible. In this presentation I will cover recent work on tissue-specific knock-outs as an approach to understanding the function of broadly expressed genes in nociception

Behaviour related changes in extracellular level of amino-acids in the medial striatum of the domestic chick: An in vivo microdialysis study

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The medial sriatum (MSt) plays a role in the taste aversion learning as well as in reinforcement learning of the domestic chick (Csillag 1999, Izawa et al. 2003). MSt receives dopaminergic and glutamatergic inputs, and excitatory amino acids such as glutamate are necessary for memory consolidation (Rickard et al 1994). Daisley et al. (1998) showed that the glutamate level in the MSt is sensitive to stress. In the present study we used both one trial aversive and serial reinforcement conditioning during in vivo microdialysis to follow the changes of the extracellular level of ten amino acids. Microdialysis probes were stereotaxically implanted to the medial striatum of one-day-old chicks. On the following day, chicks were trained either using one-trial passive avoidance paradigm or by a combination of operant conditioning using water reinforcement, habituation learning and taste aversion learning. As conditional stimuli, coloured beads were presented to the individuals, and the number of pecks was recorded during the experiments. The occasional handling of chicks was also recorded as possible stressor. Microdialysis samples were collected during the experiment using 15 minute time resolution. The samples were analysed by HPLC method. Our results showed that stress highly influenced the level of aspartate and glutamate but not those of other amino acids. This effect increased the variance of the baseline concentration and masked most of the behaviour related transitions. However a slight but significant decrease of aspartate and glutamate occurred after the presentation of the aversive stimulus. In Conclusion it is possible to detect neurochemical correlates of behaviour by in vivo microdialysis in the MSt of freely moving chicks. Excitatory amino acid levels show some behaviour related changes. Stress could be a potent factor affecting the levels of excitatory amino acids and thereby might affect the NMDA and non-NMDA receptor mediated memory consolidation. The slight decrease of glutamate and aspartate after aversive learning might be a result of an enhanced reuptake of excitatory amino acids related to memory formation.

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Altered behaviour of neural stem cells in intact and lesioned brain areas

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Our investigations were focused on the survival of implanted neural stem cells in cortical cold lesion model in mice. Cold lesion is a widely used approach to provoke cortical injury characterised by a necrotic core and penumbra zone. We implanted NE-4C neuroectodermal stem cells to the penumbra zone of the injury one week after the induction of the lesion.

NE-4C stem cell line originates from the fore- and midbrain vesicles of 9-day-old mouse embryos lacking functional p53-gene. It is a pheno- and genotypically identical one-cell-derived cell line. (Schlett, Madarász, 1997). NE-4C stem cells are continuously dividing under normal tissue culture conditions, while they form compact cell aggregates, then differentiate into neurons and astrocytes if induced by all-trans retinoic acid. The induced cells display specific immunocytochemical, electrophysiological and molecular biological characteristics on defined days of retinoic acid treatment (Schlett and Madarász 1997, Herberth, 2002, Jelitai, 2002, Tárnok, 2002). For implantation studies, a sub-clone of NE-4C cells (GFP-4C) expressing constitutively the green fluorescent protein (GFP) reporter-gene, was used. GFP-4C cells preserve the characteristics of NE-4C stem cells, but can be recognized in vivo after implanting into the brains of the animal models. NE-4C cells were shown to integrate into and differentiate inside the embryonic brain, while they formed aggregates, showed a very low rate of differentiation and survived no longer than 4 weeks in the intact adult brain (Demeter, 2004).

In contrast to their fate in the intact brain, GFP-4C cells showed significantly longer survival (>2 months) inside the penumbra zone of the lesion, but cells were barely observed in the necrotic core. At later stages of survival, GFP-4C cells repopulated the past infarct core and formed tumour-like aggregates. The tumour-like inclusions neither expand invasively, nor cause a midline shift in the forebrain. They did not infiltrate neighbouring brain structures, and did not show significant differentiation, either. To find out, whether the intrinsic properties of grafted cells were changed, or only the lesioned brain environment provoked an altered physiological response, the implanted cells were re-cultivated from the lesioned brain region on the 62nd post-operative day. Under normal stem-cell tissue culture conditions, the implanted cells were successfully reselected and maintained.

Morphology, inducibility, immunocytochemic properties and viability of the re-plated GFP-4C cells did not show any difference from the master culture. These data indicate, that the altered proliferation/differentiation of stem cells is due to the effects of some factors present in the lesioned brain environment. The results underline the importance of the host environment in elaboration of any potential cell replacement therapies.

The effect of late intrauterine 5-Bromo-2'-Deoxyuridine administration on the development of GnRH neurons in the mouse

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We have studied the effect of prenatal (late intrauterine) 5-bromo-2'-deoxyuridine (BrdU) administration on the development of the GnRH pathway. BrdU is widely accepted as a tool for studying neurogenesis (pre and postnatal). On the other hand, this thymidine analogue proved to be effective influencing different developmental events too.

Dams of C57Bl6 mice were treated on the last days of pregnancy (between E16, E17, E18, E19 and the time of birth, E20/21 days, respectively). The dams received daily injections of 15 μg/g body weight BrdU subcutaneously, dissolved in physiological saline, between 11 and 12 am.

BrdU treatment during the last 3-4 days of pregnancy resulted in a significant decrease of the number of GnRH immunoreactive (ir.) neuronal cell bodies as well as ir. fibers. This decrease could be detected at all postnatal ages (P0/P1, P3/4, P6/8 and P21/23). The higher cumulative doses of BrdU produced a more significant decrease of the GnRH ir. structures in comparison with the lower ones. The morphological appearance of GnRH ir. neuronal cell bodies proved to be different from that of the controls too: bipolar cells were few in number, and most of the cells were round shaped, smaller in size, and in many cases they seemingly did not exhibit arborisation. As far as the distribution of GnRH ir. cell bodies concerns, it was similar to that of the control animals (most of the cells were found in the preoptic area), however, immunopositive cells were detected in the nasal septum and along their migratory root at later postnatal ages (up to P6/P8 postnatal days) in comparison with the control (up to P3 postnatal day). However, the amount of the "missing" GnRH positive neurons from hypothalamic areas was less, than that we have found along the migratory path: near to the vomeronasal organ, within the nasal septum, passing through the cribriform plate and around the olfactory and accessory olfactory bulbs. Significant differences were seen in the amount of GnRH ir. fibers in BrdU treated animals at all postnatal ages studied, especially at the lateral septal area, in the habenula, the preoptic and more caudal hypothalamic areas next to the arcuate nuclei and also to the mammilary bodies.

As a consequence, late intrauterine BrdU administration influences the development of the GnRH system in a dose-(and time) dependent manner. One of the effects of BrdU is the retardation and diminishment of the migration of GnRH neurons, however, the marked decrease in their number (at hypothalamic territories) could be the reason either of apoptotic cell death or of the decrease of the GnRH synthesis below that level, detectable with immunocytochemical method (or both). Application of the BrdU at low doses during the proper time-window of the pregnancy could be a useful tool in further studies on disturbed neuronal migration and development of the GnRH axis.

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Analogic sensory computers

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When Cellular Neural Network (CNN) paradigm was proposed 15 years ago, it promissed ultra high speed bio-inspired processor arrays. For now, these complex analogic processor arrays, combined with various sensors, are implemented. The largest analogic sensor-processor array is the ACE16k, which contains over 16,000 tiny analog processors. This can capture and process over 10,000 pieces of 128x128 sized images in a second. Based on this chip, a sensor processor computer was designed and built. The presentation will introduce these devices.

The intrinsic organization of the nucleus lentiformis mesencephali magnocellularis: a light- and electron-microscopic examination

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The nucleus lentiformis mesencephali magnocellularis (nLMmc) is an essential part of the accessory optic nuclei and is responsible for stabilization of the horizontal eye movement. The morphology of this nucleus and its intrinsic structural connectivity were studied with Golgi, biotinylated dextran amine anterograde immunotracer and GABA immunostaining methods by light and electron microscopy. In the Golgi preparations neurons of large, medium-large, medium and small sizes were distinguished. The small neurons are GABA-immunopositive local circuit neurons, the others are proposed to be partly projection, partly local circuit neurons. The large and medium-large projection neurons are located in a tight topographical relationships observed in the Golgi preparations. The dendrites of the large and medium-large cells are also observed to be in close proximity with each other, and also with retinal fiber terminals. The morphological arrangement suggests that the retinal fibers make synaptic contacts with dendrites from both types of cell, and this is confirmed by the examination of retinal fiber terminals using electron microscopy. The optic fiber terminals establish synaptic contacts with small dendritic branches, dendritic processes and dendritic spines of large and medium-large neurons in the nLMmc. This arrangement allows the two types of nLMmc neuron access to very similar, if not identical, inputs, which may facilitate some of the different aspects of visual processing. Optic transmission by these cells may be modulated by the GABA-immunopositive terminals from various local circuit neurons, and very probably from GABAergic myelinated fibers as well, which may originate from the controlateral nLMmc and/or the visual Wulst.

Changes in the sensitivity of the hypothalamo-pituitary-adrenal axis in the absence of vasopressin in Brattleboro rats

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The hypothalamo-pituitary-adrenal axis (HPA), a key component of the stress reaction, is under the central regulation of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) both originated in the nucleus paraventricularis hypothalami (PVN). The natural AVP deficient mutant Brattleboro rats seems to be a good tool for studying the role of AVP in the activation of the HPA. Homozygous diabetes insipidus (di/di) rats were compared to heterozygous (di/+) animals. To examine the role of AVP in the maintenance of basal HPA activity the CRH mRNA in the PVN and amygdala and the POMC mRNA in adenohypophysis were measured by semiquantitative in situ hybridization technique. In vivo the CRH sensitivity was examined by serial blood sampling. In vitro the hypophysis CRH and the adrenal gland ACTH reactivity were also studied by static incubation or by superfusion method. The feedback efficacy was tested by dexamethasone supression test. Adrenocorticotrop hormone (ACTH) and corticosterone plasma and medium levels and tissue cAMP content were measured by radioimmunoassay. The basal CRH mRNA level in PVN and in the amygdala were higher in AVP negative than in AVP positive animals. There was a tendency for the POMC mRNA signal in the adenohypophysis and for the unstressed corticosterone plasma level to be higher in AVP deficient rats, too. Both the in vivo and in vitro CRH sensitivity of the adenohypophysis was decreased in the absence of AVP, while the adrenal glands were hyperreactive to ACTH. The AVP deficient rats showed higher sensitivity to dexamethasone supression. Our conclusion is that AVP has a role in the HPA axis regulation also under basal conditions. Although one could hypothesize that the lack of a stimulatory component would lead to decreased activity with higher sensitivity to secretagogues and reduced feedback sensitivity but we could detect opposite changes. However the excessive water turnover in AVP deficient Brattleboro rats may influence our result

Ih controls the resonance properties of hippocampal neurons in a permissive manner

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The hyperpolarization-activated cation current – termed Ih – has been shown to play a key role in cardiac and neuronal pacemaker activity, therefore it could be crucial in controlling the synchronized oscillatory activity of neuronal networks. In this study we investigated the contribution of Ih to the resonance properties of different cell types recorded in the CA1 region of the rat hippocampus.

Horizontal hippocampal slices were prepared from young Wistar rats (p14-p24) and whole-cell patch-clamp recordings were performed. To determine the resonance properties 3 s long sinusoidal currents at fixed frequencies were injected into the cell at different membrane potentials. In the voltage response of the cell to negative current steps of appropriate magnitude a sag can be seen due to the activation of Ih, which can be blocked by the specific HCN-channel blocker, ZD7288. To extract the features of Ih, the properties of this sag were compared between the different cell types.

Pyramidal neurons showed resonance in the theta frequency range (4-8 Hz), especially at hyperpolarized potentials (-70, -80 mV, n=22). The blockade of HCN channels with 10 uM ZD7288 abolished the resonance (n=8), and a significant correlation was found between the amplitude of the sag and the magnitude of the resonance ($R=0.6$; $p<0.05$).

In spite of the presence of the sag in O-LM (n=15) or interneuron-selective interneurons (IS-cells, n=15), the resonance was absent in the majority of these inhibitory cells. In those IS-cells where resonance occurred (n=5), it had a peak at theta frequencies and could be blocked by ZD7288 (n=3). Most of the perisomatic inhibitory cells lacked sag as well as sub-threshold resonance (n=8). In some perisomatic inhibitory cells the sag could be observed but none of these cells showed resonance (n=5).

These results indicate that Ih is necessary but not sufficient for creating resonance.

Subcellular Aspects Of Thyroid Hormone Activation In The Brain

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Type 2 deiodinase (D2) is the key enzyme of thyroid hormone activation in the developing and adult brain. D2 belongs to the deiodinase enzyme family consisting of type 1, 2 and 3 (D1, D2 and D3) deiodinases. These thioredoxin-fold containing selenoenzymes catalyze the conversion of T4 to T3. All deiodinases are integral membrane proteins with a single N-terminal transmembrane domain, but nothing is known about the signals controlling their subcellular localization. While D1 and D3 are in the plasma membrane, D2 is an endoplasmic reticulum (ER)-resident protein.

To find out whether D2 is retained in the ER or is rescued from the Golgi complex, we produced fusion D2 molecules containing signals that direct ER- (NKT; N-linked) or Golgi-specific (YTPPP; O-linked) glycosylation. N-FLAG-hD2Cys-C-terminal-NKT and N-terminal NKT-hD2Cys-C-FLAG proteins were transiently expressed in HEK-293 cells and pulse labeled with 35S-methionine/cysteine. After immunoprecipitation (anti-FLAG antibody) the pellets were exposed to Endoglycosidase H (EndoH) and resolved by SDS-PAGE. Cells transfected with N-terminal NKT-hD2Cys-C-FLAG expressed also an EndoH sensitive glycosylated ~40 kDa D2 form confirming that D2 is a Type 1 integral membrane protein. Cells expressing D2-fusion proteins with the Golgi-specific glycosylation signals, N-FLAG-hD2Cys-C-terminal-YTPPP and N-terminal-YTPPP-hD2Cys-C-FLAG, did not reveal glycosylated bands. We next tested whether D2 could be reassigned to the plasma membrane by fusing a truncated transmembrane-less D2 to the cytosolic FLAG-tagged C-terminus of the sodium-iodide symporter (NIS). Cells transiently expressing NIS-D2 were subjected to cytochemistry and examined with laser confocal microscopy. Specimens were co-stained with anti-FLAG and an ER-specific tracker dye. NIS-D2 showed a peripheral cellular distribution and did not co-localize with the ER-tracker, compatible with a plasma membrane localization of the NIS-D2 fusion protein. On the other hand, a similarly truncated D1 molecule fused to the cytosolic C-terminus of the ER-resident Sec62, co-localized with ER-tracker. We next studied whether the lysine residues in D2 protein can serve as ER retention signals. Intracellular localization of lysine mutant D2 proteins was examined by confocal microscopy. We did not find change in D2 localization indicating that the lysine residues of D2 do not serve as ER retention signals. Our data indicate that D2 is subjected to static retention in the ER but is re-assigned to the plasma membrane when fused to NIS. D1 is re-assigned to the ER after fusion to Sec62.

Electrophysiological correlates of contour integration

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Integration of local features into global shapes has been studied in a contour integration paradigm. We investigated the neural bases of contour integration with the help of event related potentials (ERPs). Observers had to either detect an egg-shaped contour (DET – yes or no), or discriminate between to directions that the egg-shaped contours were pointing at (DISCR – left or right). In both conditions the same stimuli were used: closed contours of Gabor patches on a background of randomly positioned and oriented Gabor patches. Task difficulty was varied by gradually rotating the contour patches from the predeterminated path of the contour, that resulted in six levels of difficulty, and undetectable contours in about half the trials. We repeated both tasks at high and low contrast values for the patches. While subjects performed 360 trials for each condition we obtained ERPs (recorded from 23 channels, positioned according to the standard 10-20 system). DET and DISCR trials were recorded in separate blocks. Difference waves were constructed by subtracting ERPs for undetectable from that of for detectable contours. Contour integration (as reflected in the difference wave) was characterised by a more negative wave between 200 and 300 msec. This difference is generated by a smaller P2 (at around 200-220 msec) and an enhanced N2 (at 260-280 msec) at occipito-temporal electrodes. Increased attention to the shape of the figure in the DISCR condition only slightly increased the magnitude of this effect. Reducing the contrast of the images also led to an increased of the effect between 200 and 300 msec, and extended the difference to the N3 (at 350-380 msec) component of the ERP. The time course of these results is consistent with earlier findings in the monkey cortex (e. g. Zipster et al, 1996, Bauer and Heinze, 2002), suggesting the relevance of a later, „tonic" response phase within the early visual cortex in the integration of orientation information across the visual field.

Reventilation with 21 or 100% oxygen after asphyxia differentially affects cerebral neuropathology in newborn pigs

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Aims- We tested if reventilation with 21% or 100% oxygen (O2) after asphyxia would affect neuronal damage in different brain areas of piglets.

Methods- Anesthetized (Na-thiopenthal (45 mg/kg, i.p.) followed by α-chloralose (40 mg/kg, iv)), artificially ventilated piglets were divided into 3 experimental groups. The first group (n=7) served as time controls. Asphyxia (10 min) was induced by turning the respirator off and occluding the intratracheal tube. Reventilation started with either 21% (A/R 21%, n=17) or 100% (A/R 100%, n=17) O2 for 1h, and was continued with 21% O2 in both groups for 3h. Physiological parameters were regularly monitored and cortical blood flow (CoBF) was continuously measured using laser-Doppler flowmetry throughout the experiments. Animals were killed while anesthetized, and haematoxylin/eosin stained sections from 6 brain areas were prepared for blinded neuropathological examination and scoring.

Results and Discussion- There were similar changes in the monitored physiological variables during the course of asphyxia/reventilation in both groups, except for pO2 in the 1st h of reventilation (80±5 versus 327±69 mmHg). In A/R 21%, by the end of asphyxia MABP decreased from 80±6 to 37±4 mmHg, pulse rate (PR) from 184±11 to 60±8 min-1, and CoBF to 18±3% of baseline values. At the onset of reventilation MABP, PR, and CoBF greatly but transiently rose (108±6 mmHg, 248±10 min-1, and 136±16% of baseline, respectively). Based on the effect of 21% or 100% O2 reventilation on neuropathological scores the brain areas studied could be divided into 3 categories. 1. Both in the frontal and parietal cortices the hypoxic damage was similar in the A/R 21% and A/R 100% groups compared to controls. 2. In the basal ganglia hypoxic damage was less severe in the A/R 100% group. 3. in the hippocampus and the cerebellum very severe hypoxic damage could be observed only in the A/R 100% group. In the pons A/R did not cause significantly detectable hypoxic damage compared to controls. We conclude that the detrimental or beneficial effect of supplementary O2 given during reoxygenation may be different in distinct brain areas/neuronal populations. However, the most chemosensitive structures (hippocampus, cerebellum) appear to be negatively affected by 100% O2.

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Acute effects of 3-nitropropionic acid on neurobehavioral performance in rats

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Mitochondrial dysfunction and oxidative stress are often linked to various neurodegenerative disorders including ischemic stroke and Huntington's disease (HD). Animal models of the latter disease can be created with application of mitochondrial toxins such as 3-nitropropionic acid (3-NP). 3-NP functions as the inhibitor of the complex II of the mitochondrial respiratory chain, and it has been found to effectively induce specific behavioral changes and selective striatal lesions in rats mimicking those in HD. The aim of this study was to investigate how 3-NP, given acutely, influences various quantifiable features of the rats' behavior.

Young adult male Wistar rats (8 weeks old, ca. 200 g) were used. A week before administration of 3-NP, spontaneous activity in the open field, motor performance in rota-rod and climbing test, and the acoustic startle response (ASR) with and without pre-pulse inhibition (PPI) was investigated. Acute treatment was done by intraperitoneal injection of 10 or 20 mg/kg 3-NP to the rats (10 per group). Control animals were left untreated. 90 minutes after the treatment, the behavioral tests mentioned above were repeated.

Before 3-NP administration, there was no noteworthy difference between the performance of treated and control groups. In the open field activity, several parameters were altered in the treated rats. The general trend was decreasing activity. The distance done by running in the first minute and during the 10-minute session was significantly reduced in the high dose group compared to the controls, as was the speed of run, but the decrease in the total distance of movements was not significant. Significantly reduced number of groomings was also observed. The climbing ability of treated rats showed a clear, but not significant, increase. Rota-rod, however, failed to reveal differences in motor performance. ASR and PPI test results also proved to be insignificant.

Following the behavioral tests, the rats underwent electrophysiological recording, whereby certain alterations seemed to be in parallel with the behavioral effects of 3-NP.

Some of the behavioral tests applied proved to be good indicators of alterations in the higher nervous activity induced by acute 3-NP treatment. These can be useful in widening the test battery applied in modelling Huntington's disease and elucidating the mechanisms and structures involved.

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