# Sympsium 1 - Molecular mechanisms of selective neuronal injury in brain ischemia

## ROLE OF CALCIUM IN ISCHEMIC NEURONAL INJURY

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Involvement of calcium ions in mechanisms of ischemic brain injury has been suggested for 20 years. Since massive invasion of $Ca^{2+}$ to ischemic neurons may induce lipo- and proteolysis, mitochondrial dysfunction, damage to membranous structures, and degradation of cytoskeleton, calcium ions were initially recognised mainly as strongly toxic agents directly killing neurons. In parallel to progress in our understanding the role of $Ca^{2+}$ in neurotransmission, in signal transduction, particularly in protein phosphorylation/dephosphorylation and in modulation of gene expression, the calcium hypothesis of neuronal injury evolved continuously. Thus, a role of pathological $Ca^{2+}$ signalling in induction of long lasting processes leading to either necrotic or apoptotic postischemic delayed neuronal death seems to be very probable. Still many questions repeatedly addressed, concerning for instance the nature of main routes of ischemic calcium influx to neurons (VSCCs vs. ROCs and particularly NMDA and non-NMDA receptors, as well as $Na^{+}/Ca^{2+}$ exchange), the role of intracellular $Ca^{2+}$ stores, the exact period of critical increase in intracellular $Ca^{2+}$ concentration (during ischemia or reperfusion), possible subcellular differences in ischemia-evoked calcium imbalance in neurons (soma vs. dendrites) still remain open. Some conclusions concerning destabilization of $Ca^{2+}$ homeostasis in neurons, based on results of in vitro and even in vivo experiments on glutamate excitotoxicity may not apply to in vivo ischemic conditions. This review, apart from emphasising generally proposed mechanisms of calcium transients and toxicity in ischemic neurons, will rise some of these controversial issues.

## INVOLVEMENT OF CALCIUM-DEPENDENT PROTEIN KINASES: PKC* AND CAMKII**, IN POSTISCHEMIC ENHANCEMENT OF GLUTAMATERGIC SIGNALING.

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The paradigm of rapid enzyme activation/translocation with a subsequent inhibition of catalytic activity can be applied to both $Ca^{2+}$-dependent protein kinases: PKC and CaMKII, in their reaction to transient (5') forebrain ischemia in gerbils.
The mechanism(s) participating in inhibition of translocated enzymes would involve: '- irreversible enzyme (auto)phosphorylations
"- limited proteolysis (in the case of PKC)
Inhibition of NMDA receptor by MK801 during ischemia blocks the enzymes translocation/activation in gerbil hippocampus as assayed at 3 h recovery. This treatment results also in a significant attenuation of ischemia-evoked downstream activating responses such as ornithine decarboxylase and transcriptional factor-AP1 induction. In addition to MK801, similar reduction of the above specific responses were achieved by pretreatments of gerbils with PAF*** and NO selective inhibitors (BN52021 and L-NAME respectively) before ischemia.
*In vitro* experiments confirmed further the contribution of these putative retrograde messengers: PAF and NO, to ischemia-evoked enhancement of presynaptic glutamate release by PKC-dependent mechanism. On the other hand CaMKII, which is especially enriched in postsynaptic densities and actively translocated toward membranes during ischemia, may be protected indirectly by these glutamate release inhibitors.
These results indicate that PKC activation may be directly responsible for prolonged enhancement of presynaptic glutamate release while CaMKII would change the sensitivity of postsynaptic signalling system after ischemia.

* calcium, phospholipids dependent protein kinase; ** calcium/calmodulin dependent protein kinase II; *** platelet activating factor.
This project was supported by Med. Res. Ctr. and KBN grant # 4 P05A 026 08.

## Postischemic protein synthesis - regulation and importance for neuronal damage

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Global protein synthesis rate is depressed during the reperfusion period following transient cerebral ischemia. In the vulnerable CA1 region the depression persist until cell death, 2-3 days after the ischemic insult, while in the CA3 region it recovers by 1 day of recovery. In the dentate gyrus cell, protein synthesis recovers by 1h of recovery. The depression of protein synthesis is partly due to a decrease in initiation factor 2 (eIF2) activity as determined by its ternary complex formation with GTP and Met-tRNA. The depression could be either due to and increase in eIF2 phosphorylation or by a decrease in the activation of the guanine exchange factor regulated by among other tyrosine kinases. The depression of global protein synthesis may depress the synthesis of certain populations of proteins, while other proteins such as the immediate early genes (c-jun), the heat shock protein HSP 72 and the tumor suppressor protein p53, are expressed until frank neuronal necrosis occurs. These proteins are functional since both AP1 activity increases and the gene product of p53, WAF 1, are expressed late during reperfusion.

The depression of protein synthesis seems to be due to a persistent stress impinged on the vulnerable neurons and may reflect a deranged intracellular cell signalling damaged by the ischemic insult. In resistant neurons preservation of growth factor coupled signalling may be of importance for recovery.

## Possible role of glutamate and protein synthesis in cell damage after transient cerebral ischemia

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The excitotoxic hypothesis holds that glutamate released during ischemia induces an overactivation of neurons induced by calcium influx through NMDA receptor channels. However, neither the regional intra-ischemic extracellular levels of glutamate nor the glutamate receptor density correlate closely with the vulnerability. In addition, non-NMDA receptor antagonists are neuroprotective even when applied after ischemia. A direct role of glutamate released during ischemia in the development of ischemic cell damage is, therefore, far from being established. Protein synthesis (PS) is severely depressed after ischemia throughout the brain. It recovers in non-vulnerable structures but never in vulnerable ones. This reverse relationship between regional recovery of PS after transient ischemia and vulnerability of the respective region is stable for all experimental conditions studied up to date. In addition, even under "ischemia"-like conditions in vitro the post-"ischemic" inhibition of PS is closely related to the sensitivity to ischemia observed in vivo, as indicated by the observation that after in vitro "ischemia" PS is more pronounced inhibited in gerbil as compared to rat hippocampal slices, independend of the duration of ischemia and temperature of incubation. It is concluded that post-ischemic inhibition of PS as compared to extracellular glutamate during ischemia plays a more prominent role in the manifestation of cell damage after transient ischemia. Whether the post-ischemic disturbance in PS and in glutamate-related neurotransmission (as indicated by the protective effect of post-ischemic applied non-NMDA antagonists) are causally connected has yet to be established.

**NITRIC OXIDE IN CEREBRAL ISCHEMIA. MOLECULAR MECHANISM OF ITS ACTION.**

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Brain ischemia leads to excessive stimulation of glutamatergic receptors. Our studies demonstrated that activation of NMDA receptor induced significant production of cGMP and nitric oxide (NO) via stimulation of NO synthase (NOS). The amount and dynamic of NO/cGMP-release depends on age of animals and the part of the brain. The studies were carried out using hippocampus, brain cortex and cerebellum from 7 days, 4 and 27 months old animals. NOS activity was measured by [$^3$H] cytrulline formation and cGMP level by radioimmunoassay. The type of NOS and it location was determined by mRNA and Northern blot analysis. Brain ischemia in gerbils was induced by ligation of both common carotid arteries for 1, 2.5 and 5 min and then the brain was subjected to the following reperfusion time 15, 30 min, 1, 2, 4 h and 4, 7 days after 5 min ischemia. Brain ischemia increases in time dependent manner of NOS activity. The level of cGMP is significantly enhanced during first 60 sec. During reperfusion time the both NOS activity and cGMP increases biphasically 15 min and 2 h after ischemia. N-nitro L-arginine a specific inhibitor of NOS injected i.p. in a dose of 30 mg/kg b.w. 5 min before ischemia, eliminates the effect of ischemia-reperfusion injury on NOS/cGMP and ameliorates NO-evoked alteration of biochemical processes. Moreover, NNLA protects the neurons in $CA_1$, layer of hippocampus against ischemia. The same effects were observed when animals were treated with specific inhibitor of neuronal form of NOS, 7 Nitroindazole (7-NI), in a dose of 25 mg/kg b.w. 5 min before ischemia. The inhibitor of guanylate cyclase LY-83583 also diminishes NOS stimulation and cGMP elevation. induced by ischemia Hydrocortisone in a dose of 40 mg/kg b.w. injected i.p. 7 days before ischemia has no effect on NOS/cGMP. There was no activation of gene encoding iNOS during reperfusion period. It was found that NO by S-nitrosylation, ADP-rybosylation and free radical mechanism modulated enzymes activity of arachidonic acid metabolism and $Cl^-$ channel properties and function. It seems that NO and NO-activated processes may be responsible for neuronal degeneration during reperfusion. In conclusion our results indicate that ischemia-reperfusion injury activates biphasically neuronal, consitutive form of NOS activity (cNOS). The inhibitor of neuronal form of NOS protects the brain agonist release and action of nitric oxide and offers novel therapeutic strategies for brain ischemia.

GENE EXPRESSION AND INDUCED ISCHEMIC TOLERANCE FOLLOWING BRIEF INSULTS

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The brain is highly responsive to ischemia, with striking changes in the expression of many genes in neurons and other cell types. Even mild insults induce widespread responses, including induction of the 70 kDa heat shock protein, hsp72, as well as other stress proteins, transcription factors, growth factors, enzymes and structural proteins. Recently it as been shown that brief periods of ischemia can induce tolerance to later insults. This presentation will provide an overview of progress in this area and critically examine possible roles of gene expression in tolerance mechanisms. New data will be presented identifying ischemic thresholds for tolerance induction and for expression of ischemia-induced mRNAs in hippocampal neurons.

Anesthetized gerbils were fixed in a stereotaxic frame, and microelectrodes placed in hippocampus to record DC potential during ischemia and recirculation. In preliminary studies 6.5 min depolarization was found to produce maximal CA1 injury, and this was used as a standard test insult. Animals were subjected to brief ischemia (0-4 min depolarization) and tolerance to the standard insult was evaluated two days later, with histological evaluation at 1 week. In situ hybridization was used to determine relative levels of mRNAs encoding hsp72 and several immediate-early genes at various times after the same range of brief depolarizing insults.

Tolerance was maximally induced after 2 min depolarization. Increases in junD and junB mRNAs occurred over a similar range, while hsp72 was detected only after longer depolarizations. These results indicate that hsp72 is not required for ischemic tolerance but leave open the possibility that the induced transcription factors could contribute. More importantly, they establish an optimized model that should be of value in future studies of tolerance mechanisms.

## Symposium 2 - Plasticity of the spinal cord

**RECOVERY OF LOCOMOTION IN CATS AFTER LESIONS OF THE SPINAL CORD.** S. Rossignol, Center for research in neurological sciences, Faculty of Medicine, Université de Montréal, Québec, Canada.

The recovery of locomotion following a total or a subtotal chronic section of the spinal cord at T13 was studied in cats using electromyographic (EMG) and video analyses. In early-spinal cats (<8 days post-lesion), hindlimb locomotion on a treadmill can be evoked by alpha-2 noradrenergic agonists such as clonidine given intraperitoneally or intrathecally. The characteristics of this pattern evolves with time after the lesion and recovery of the pattern can be accelerated with intensive early training. After 2-3 weeks, the EMG pattern on the treadmill resembles that observed before the lesion. Noradrenergic and serotoninergic drugs as well as amino acids can modulate various aspects of this pattern. Thus, noradrenergic agonists increase the duration of the EMG bursts and therefore the overall step length and duration but do not produce noticeable changes in EMG amplitude. There is also a reduction in cutaneous reflex excitability (e.g. fast paw shake is abolished). On the other hand, serotoninergic agonists markedly increase both the EMG amplitude and cutaneous reflex excitability. Spinal locomotion is blocked by the NMDA antagonist AP5 and restored by NMDA. Similarly, the gabaergic agonist baclofen can block locomotion while the antagonist bicuculine can restore it. Preliminary studies with massive partial lesions of the ventral and ventrolateral tracts on both sides show that cats can voluntarily walk with all 4 limbs although there is a significant reduction in weight support and an alteration in interlimb coordination. Drugs are now being assessed for their ability to improve these locomotor deficits and provide a rational basis for a potential locomotor pharmacotherapy in spinal-cord-injured patients. (This work is supported by the Canadian MRC and the Neuroscience Network of Centers of Excellence).

Evidence for spinal stepping generator in paraplegic human

B. Busel, Garches

Not received

DIFFERENT FORMS OF IMPAIRMENT OF THE FORE- HINDLIMB COORDINATION AFTER PARTIAL SPINAL LESIONS IN CATS.

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The fore-hindlimb coordination during overground walking at moderate speed (0.4-1.0 m/s) was analyzed in cats with different partial spinal lesions performed at the low thoracic level. The animals were taught to walk along a stationary walkway covered with cooper wire netting connected to a DC source and wore contact electrodes on the third toe pad of each limb, which allowed to record the stance phases of each limb and to calculate the locomotor speed.The effects of the following lesions were analyzed: 1. section of the dorsal columns, 2. lesions of the ventral quadrants of the spinal cord including, to a different extent, the dorsolateral funiculi and 3. lesions of different extent of the lateral funiculi with or without the damage of the dorsal columns. Lesions of the dorsal columns alone did not affect the fore-hindlimb coordination as compared to intact cats. On the other hand, the remaining two kinds of lesions elicited three different degrees of impairment of the fore-hindlimb coordination, which depended on the extent of lesions: a) a change of locomotion into the direction of pacing, characterized by an increase of the support on homolateral limbs and a reduction or disappearance of support on diagonal limbs, with preservation of the equality of rhythms in the fore- and the hindlimbs; b) a short (lasting usually 3 steps) episodes of fore- and hindlimb rhythm dissociation, characterized by an increase in the hindlimb and a decrease in the forelimb step cycle durations and 3) a permanent difference in the fore- and hindlimb step cycle durations, with forelimb steps being shorter and hindlimb steps being much longer than in intact cats. In general, the extent of spinal lesions eliciting each of these three forms of impairment of the fore-hindlimb coordination was smaller in the group of cats with lateral funicular lesions than in the group of animals with lesions of the ventral quadrants and part of the dorsolateral funiculi. Moreover, in the former group the differences between the fore- and hindlimb step cycle durations in the episodes of and in the permanent rhythm dissociation were greater than in the later group. Comparison of the results obtained in these two groups of operated cats points to the more important role played by the lateral, than the ventral funiculi, in the appearance of the episodes of and the permanent fore- and hindlimb rhythm dissociations, while the destruction of either the ventral or dorsal quadrants of the spinal cord resulted in a preservation of equal fore- and hindlimb rhythms, although the movements of the homolateral limbs became more synchronized.

**THE INNERVATION OF DISTAL AND ELBOW FORELIMB MUSCLES BY BETA-MOTONEURONES**

Illert, M., Kümmel, H., Scott, J.J.A.*

The motor nuclei to the long digit extensors of the cat forelimb have a variety of specific properties in their neuronal connections not found in elbow muscles. Thus they display very specific and restricted Ia connections (Fritz et al., 1989), neither receive nor give rise to recurrent inhibition (Hahne et al., 1988), and are devoid of axon collaterals (Hörner et al., 1991). Their MNs mainly have soma diameters in the range of small $\alpha$-MNs (Illert et al., 1992), although the innervated muscles mainly consist of fast motor units (Fritz et al.,1992).

These characteristics point to a neuronal organization which is specific for finely controlled manipulative movements. We hypothesised that the distal muscles of the cat forelimb may have a high degree of skeletofusimotor innervation.

In 8 long digit extensor muscles (m. extensor digitorum communis and lateralis, EDC and EDL) and 7 elbow muscles (m. anconeous, An, m. triceps lateralis and medialis, TLa and TM) the motor endplates were stained by means of a silver impregnation method, and the number of P1 endplates on the muscle spindle poles analysed.

The distal muscles had an estimated proportion of more than 70% ß innervation of their muscle spindles, the proximal muscles of 41-47%. We suggest that the high degree of ß-innervation in the EDC and EDL could support fast corrections from the intended muscle tension during manipulative movements. Recurrent inhibition of the Ia EPSPs would interfere with and disadvantage this function, whereas small motoneurones and specific Ia projections could support the precision movements.

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Excitatory Ia-pathways in the cat forelimb to the shoulder, elbow, wrist and digit motoneurones.

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In systematic studies we have investigated with intracellular recordings from cat motoneurones the connectivity pattern of the respective motor nuclei. The talk will survey these connections and discuss them in relation with emg- and kinematic data obtained during natural behaviour. It will be shown that the functional synergies supported by the Ia system are different at the various joints. This is also evident in the organization of the recurrent system which, in the proximal forelimb, is more extended than the Ia-system, whereas it is missing in the distal forelimb.

FUNCTIONAL REORGANIZATION OF THE PARTIALLY DENERVATED HINDLIMB EXTENSOR AND FLEXOR MUSCLE ACTIVITY IN RAT

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The effects of partial denervation on EMG activity of the slow *soleus* (SOL) and fast *extensor digitorum longus* (EDL) muscles of rats were studied. Partial denervation of SOL was achieved by section of the L5 ventral ramus (50-70% of SOL's innervation) while EDL was partially denervated by section of the L4 ventral ramus (60-80% of EDL's innervation). The EMG signal of the partially denervated, and contralateral unoperated muscles was examined during exploratory behaviour or regular locomotion along a runway.

The aggregate EMG activity (number of EMG signals crossing a threshold level per min) measured in the partially denervated SOL was lower than in the contralateral unoperated SOL. However, the activity per motor unit was significantly higher than in the unoperated SOL muscle because the operated SOL had only 1/3-1/2 of its normal complement of motor units. The aggregate EMG activity of the partially denervated EDL was increased up to 4 times of that in the contralateral EDL. Moreover, unlike normal EDL muscle, the partially denervated EDL was active during standing, as well as during the stance phase of locomotion. In standing or walking animal, the partially denervated SOL showed the activity pattern similar to that recorded in normal animals. The EMG burst duration of SOL during regular locomotion was correlated to the duration of the step cycle, like in normal rats. However, the burst duration of the partially denervated SOL muscle was significantly shorter than in unoperated SOL. The partial denervation of EDL induced changes in burst activity of the operated EDL; the burst duration was strongly correlated with the step cycle duration, while the burst duration of the unoperated EDL stayed constant for various step cycle duration. In this respect the EMG activity of the contralateral EDL muscle was similar to that of unoperated animals. It appears that SOL can function relatively well even when it is innervated by a reduced number of motoneurones, while the remaining motor units in the denervated EDL develop different pattern of activity.

Summarizing, one can say that partial denervation generally leads to an overall increase of activity of the remaining motor units in both extensor (SOL) and flexor (EDL) muscles, while the temporal pattern of the muscle activity during locomotion was drastically altered in the case of EDL muscle.

VARIETY OF MUSCLE RESPONSES TO TACTILE STIMULI.
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In the postural reaction of contact placing (CP) it was found that depending on the location of tactile stimulus on the dorsal, medial or lateral aspects of the forepaw, the animal used different strategies of the forelimb movement to place the limb on the touched object. One of the differences arose from a various activation of the elbow flexor and extensor muscles in these reactions. When tactile stimulus was applied to the lateral side of the paw, the reaction was initiated by the elbow flexion movement. During dorsal and in some medial CP reactions the elbow flexion was delayed due to a co-contraction of the elbow flexor and extensor muscles which locked the elbow joint. It is postulated that tactile stimuli applied to the lateral or to the dorsal side of the paw activated different subsets of spinal interneurons on their way to the elbow flexor and extensor motoneurons. Depending on the requirements, excitatory pathways both to the flexor and extensor motoneurons or only one of them might be used. Our experiments show that the location of tactile stimulus is an important factor determining which of these interneuronal pathways might be chosen. This raises the question which subpopulation of cutaneous mechanoreceptors is responsible for activation of a respective interneuronal pathway to motoneurons? A monosynaptic Hoffmann (H) reflex technique was used to measure the influence of a cutaneous input on the $\alpha$-motoneurons excitability of the soleus muscle in awake rats. The H-reflex was elicited by direct electrical stimulation of Ia fibers of the tibial nerve with chronically implanted, bipolar, cuff electrodes and recorded with a bipolar electrode implanted into the soleus muscle. Tactile stimulus applied to various skin areas was used to modulate the excitability of the soleus muscle. It was found that the amplitude of the test H-reflex increased over two times when Ia stimulus was preceded by tactile stimuli applied to the tail. This facilitatory effect depends on the type of tactile stimulus indicating that not all the cutaneous mechanoreceptors, located in the same area, influence the excitability of spinal motoneurons in the same way.

## Symposium 3 - Dynamics of interactions between circulatory and respiratory neuronal control systems

Introduction: cardiorespiratory oscillations and instabilities
A. Trzebski, Warsaw

Not received

Mechanisms Responsible for Human Autonomic Periodicities
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In healthy, young, resting humans, electrocardiographic R-R intervals and arterial pressures fluctuate with a strong respiratory periodicity. Although both rhythms result in part from the mechanical effects of breathing, both also are influenced strongly by fluctuations of efferent nerve activity.

Fluctuations of R-R intervals are due almost exclusively to changes of vagal and sympathetic nerve traffic to the heart, and are determined importantly by the kinetics of sinoatrial node responses to acetylcholine and norepinephrine. R-R interval rhythms at all frequencies are nearly abolished by muscarinic cholinergic blockade, and are augmented by $\beta$-adrenergic blockade. Very low frequency R-R interval rhythms ($< 0.03$ Hz) are augmented by angiotensin converting enzyme blockade. Central respiratory activity gates responsiveness of sympathetic and vagal motoneurons, such that both groups of motoneurons respond to stimulation more in expiration than in inspiration. The degree of respiratory gating is not influenced importantly by the level of inspiratory motoneuron activity, but is influenced importantly by the level of autonomic sensory stimulation of vagal and sympathetic motoneurons. Respiratory effects are maximal at usual arterial pressures, and are minimal at low and high arterial pressures. The degree of R-R interval and arterial pressure fluctuations during breathing depends critically on respiratory rate. Rapid breathing nearly abolishes, and slow breathing vastly augments both R-R interval and arterial pressure fluctuations. Slow breathing increases arterial pressure fluctuations by increasing sinus arrhythmia, and by entraining spinal sympathetic motoneurons.

Study of R-R interval and arterial pressure rhythms opens a window onto the human central nervous system. The view through this window reveals a physiology of extraordinary richness and complexity.

PROPERTIES OF PRE-SYMPATHETIC NEURONES IN THE ROSTRAL VENTROLATERAL MEDULLA IN THE RAT: AN INTRACELLULAR STUDY 'IN VIVO' Lipski, J., Kanjhan, R., Kruszewska, B., Rong, W. and Smith, M. Department of Physiology, University of Auckland, Private Bag 92-019, Auckland, New Zealand.

There is now considerable evidence that the activity of vasomotor preganglionic sympathetic neurones largely depends on synaptic excitation from antecedent reticulospinal neurones located in the rostral and ventrolateral part of the medulla oblongata (the rostral ventrolateral medulla, RVLM). These *pre-sympathetic* neurones have been extensively studied with extracellular microelectrodes but so far only a few attempts have been made to examine their intracellular properties 'in vivo'. Using intracellular recording and labelling techniques in anaesthetised rats, we have addressed three questions: (1) Is the activity of these medullary neurones due to synaptic inputs, or is it determined by intrinsic pacemaker properties? (2) Are these neurones of simple 'relay' type or are they also involved in local synaptic interations? (3) What is the relationship between these neurones and adrenergic neurones of the C1 group located in the same area? The following results will be presented and discussed: (a) All neurones displayed a substantial synaptic noise, action potentials were usually preceded by identifiable fast EPSPs, and no evidence was found for the presence of gradual depolarization (autodepolarizations) between individual action potentials. Therefore these results are consistent with the 'network' hypothesis for the generation of sympathetic vasomotor tone. (b) Axons of some pre-sympathetic neurones intracellularly labelled with Neurobiotin or Lucifer Yellow had collaterals arborizing in several distinct medullary regions. Thus these neurones have synaptic inputs not only to preganglionic sympathetic neurones, but also to other, yet unidentified cells in the brainstem. (c) C1 adrenergic cells were revealed by immunofluorescence using an antibody to tyrosine hydroxylase. As some injected neurones were double-labelled, our results are consistent with the hypothesis that a subset of RVLM pre-sympathetic neurones has an adrenergic phenotype.

**INVESTIGATION OF SHORT-TERM FIRING CORRELATIONS BETWEEN SYMPATHETIC PREMOTOR NEURONS**

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**The bursting rhythms that characterize sympathetic whole nerve discharge are believed to originate supraspinally. Most ongoing sympathetic vasomotor drive is attributable to premotor neurons in the brainstem, particularly the rostral ventrolateral medulla (RVLM). One possibility is that sympathetic bursts are generated by cross-connections between, or involving, premotor neurons. Alternatively, premotor neurons may transmit bursts generated by antecedent neurons. Either way, one would expect to see short-term firing correlations between RVLM premotor neurons.**

**We generated cross correlograms from the spontaneous activity of 35 pairs of sympathetic premotor neurons. These were recorded extracellularly from the RVLM of chloralose-anesthetized cats (by single or 2 electrode methods). One or both of each pair were proven to be bulbospinal (collision test); all were inhibited by baroreceptors (carotid blind sac inflation).**

**We found no clear evidence for direct short-term (1/few millisecond) synaptic interactions. Two of the 35 neuron pairs showed strong synchrony within +/-100ms, which was independent of the cardiac cycle, indicating common inputs other than baroreceptors. Five further pairs showed such synchrony very weakly. We conclude that direct crosstalk between these cells is rare, weak or absent, and that only a minority is synchronized by common drives.**

**NH&MRC supported this work by a Block Grant to the Howard Florey Institute and a C.J. Martin Fellowship to A.M.A.**

**THE PRESENCE OF COHERENCE IN SYPATHETIC (SYMP) AND PHRENIC (PHR) ACTIVITIES IN A DEVELOPING MAMMAL.**
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Coherence or entrainment of discharge is a property of biologic rhythms. Such coupling is a reflection of maturation. While synchronization of SYMP motoneurons pools has been well dcoumented in adult mammals over the years (e.g., Gootman & Cohen, Acta Physiol. Polon. 1973, 24: 97), the developmental time course of correlated periodicities has had only limited examination. Utilizing our standard (Gootman et al. Am. J. Physiol. 1991, 261: H1147) model (paralyzed, artificially ventilated Saffan-anesthetized piglets <1 day to 2 months of age) for simultaneous recordings of cervical SYMP, splanchnic (monitors of the SYMP rhythm generating systems) and PHR activity (monitor of the inspiratory (I) pattern generator) along with aortic pressure (AoP), EKG and end-tidal $CO_2$, we examined age-related changes in SYMP activity utilizing partial coherence analysis to remove influences of pulse-sychronous baroreceptor activity (reflected by AoP) and influences of the central circuits renerating I rhythms as revealed by PHR activity. Partialization with AoP increase coherence in the 3-6 Hz frequency band in <3 wk. old piglets. The 8-12 Hz band (present in normal coherence after 21 days) was revealed in piglets <14 days old after PHR rhythmicity was mathematically eliminated; increased coherence was then also observed in the 16-18 Hz band though 3 wk. Thus use of partial power and coherency should begin to disentangle the complex relationships seen in developing SYMP outflows. The results suggest that there is a period of reorganization within the SYMP rhythm generating circuits, which may be essential for normal development. (Supported by NIH grants HD-28931 and HL-20864).

GANGLIONIC NEURONAL MECHANISMS INVOLVED IN CIRCULATORY CONTROL SYSTEM
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Intracellular tonic activity and responses to electrical stimuli applied to preganglionic nerve were recorded from the unidentified neurons of rabbit superior cervical ganglion (SCG) and compared with the responses of the whole pre- and postganglionic nerves to the stimuli applied to single SCG neuron or single preganglionic nerve fibre recorded with the use of averaging technique. It was found that: (1) each ganglion neuron is innervated by two preganglionic inputs, single and multiple, formed by a single nerve fibre whose firing always triggers a postsynaptic spike, and by a few nerve fibres triggering postsynaptic spike only if they fire together, correspondingly; (2) about 100 ganglion neurons discharge synchronously (within 85 ms time interval) during their tonic activity, being driven by only three preganglionic nerve fibres, while about 76% of the neurons innervated by each preganglionic nerve fibre remain silent; (3) single preganglionic input is driven by cardiac rhythm, suggesting the neurons examined were the vasomotor (VM) ones; in contrast, the multiple input either is driven by the single one, or operates irregularly. In addition, intracellular tonic activity was recorded from the neurons of rat SCG innervating the submaxillary gland (SG neurons). In contrast to the VM neurons, the SG neurons are driven by only a single preganglionic input lacking modulation with cardiac rhythm. These results suggest the circulation controlling pathways through the mammalian sympathetic ganglion include much more complex integrative neuronal mechanisms than it has been commonly thought.

**INFLUENCE OF SYMPATHETIC INNERVATION ON CEREBRAL MICROCIRCULATION AND ON THE MORPHOLOGY OF ITS SPONTANEOUS OSCILLATIONS: POSSIBLE FUNCTIONAL IMPLICATIONS.**

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It is generally accepted that, under hypertensive states, activation of the sympathetic supply to the brain vessels exerts a protective action on the integrity of the blood-brain barrier (BBB). We investigated whether, beside the mean level of cerebral perfusion, the morphology of cerebral blood flow (CBF) oscillations could also be a factor conditioning the state of the barrier.

The effect of bilateral stimulation of the cervical sympathetic nerve (CSN) on CBF was studied through laser-Doppler flowmetry in rabbits anaesthetised with a cocktail of urethane, ketamine and xylazine (400, 5 and 1.5 mg/kg i.v. respectively) which preserves a good extent of vasomotion. This study was mainly focused on the low frequency spontaneous oscillations attributed to vasomotion (4-12/min); the large amplitude excursion of the vessel wall related to these oscillations is liable to condition the BBB permeability.

Stimulation of CSNs produced, in 52% of the tested areas, a modest CBF reduction. Transient phases of increase were also observed, more often at low stimulation frequency, in agreement with the findings of Saeki et al. (Japan J Physiol 1990). Sympathetic action on mean CBF was more marked and more often effective in the territories supplied by the carotid than in those supplied by the vertebro-basilar system, which fits with the distribution of sympathetic innervation in such territories. CSN stimulation produces in the majority of the tested areas a marked reduction in amplitude and an increase in frequency of these waves.

The non-uniform sympathetic action on mean CBF should be evaluated at the light of the well known heterogeneous autoregulatory capacity of the various brain structures. It is suggested that the sympathetically-induced decrease in amplitude of pulsatility could constitute a protective factor under conditions in which the BBB is more susceptible to disruption. This may be the case in pathological conditions such as some types of vascular headache in which phenomena of cerebral hyperperfusion associated with signs of sympathetic dysfunction on the side of the pain have been recently shown.

*Supported by MURST and CNR grants.*

## Plenary lectures

The neurobiology of "gnostic" neurons and networks in primate prefrontal cortex - *Jerzy Konorski memorial lecture*

P. Goldman-Rakic, New Haven

Not received

Pathology of brain aging

M.J. Mossakowski, T. Wiśniewski, Warsaw, New York

Not received

# Workshop 1 - Mechanisms of synaptic transmission - electrophysiological *in vitro* studies

## GLUTAMATE RECEPTOR DIVERSITY IN THE CNS

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L-glutamate, the major excitatory neurotransmitter in the brain, exerts its action by activating different cation-selective ion channels that differ in their biophysical and pharmacological properties. The expression of many glutamate receptor subunits is developmentally regulated and leads to an unforeseen receptor diversity in the developing and in the adult brain. One receptor subfamily should serve to illustrate how different genetic mechanisms control the functional properties of the receptors. The fast component of excitatory postsynapic currents (EPSCs) in central neurons is mediated by AMPA receptor channels. These receptors assemble from subsets of four subunits - GluR-A to -D that can self-assemble to form functional homooligomeric channels. When several AMPA receptor subunits are co-expressed, heterooligomeric channels form with properties distinct from those of homooligomeric channels. Posttranscriptional mechanisms - alternative splicing, RNA editing - generate additional molecular forms of these subunits with profound consequences regarding ion conductance and kinetic properties of AMPA receptor channels. Analysis of single neurons in acute brain slices with respect to their electrophysiological and molecular characteristics can reveal the cell-specific use of these genetic mechanisms in the generation of functionally different glutamate receptors.

## $GABA_A$ and $GABA_B$ receptor-mediated inhibition.

U. Misgeld, W. Jarolimek, J. Rohrbacher, S. Honerkamp, H. Brunner and A. Lewen
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GABAergic synaptic transmission in the mammalian CNS is mediated by two entirely different classes of receptors: $GABA_A$ receptors in which the channel is an integral part of the receptor protein and $GABA_B$ receptors which belong to the family of G-protein coupled receptors. We study mechanisms mediated by these receptors in neuronal cell culture. Activation of $GABA_A$ receptors results in a $Cl^-$-conductance increase. The effect of the $Cl^-$-conductance increase on neuronal excitability is determined by the $Cl^-$-homeostasis of the target cell. Pharmacological blockade of $Cl^-$-transporters results in a change of intracellular $Cl^-$-activity and, hence, efficacy of synaptic inhibition. Activation of $GABA_B$ receptors results in $K^+$-conductance increase and $CA^{2+}$-conductance decrease. Both these effect contribute to a presynaptic reduction of transmitter release. In addition, activation of $GABA_B$ receptors takes a direct influence on transmitter release independently from $Ca^{2+}$- and $K^+$-channels.
Supported by grants to the SFB 317/B13 and BMFT 01KI9001(P).

## LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION IN LAYER II/III HORIZONTAL CONNECTIONS OF RAT MOTOR CORTEX

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Horizontal connections of the neocortex have been suggested to play a role in the plasticity of cortical representations. The present studies investigated the conditions for the induction of long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission in local horizontal pathways of layers II/III of adult rat motor cortex (MI) in the *in vitro* slice preparations. Field potentials evoked in the superficial MI horizontal pathways potentiated by 25 - 35% after theta burst stimulation (TBS), but only when the $GABA_A$ receptor antagonist, bicuculline methiodide (Bic) was transiently applied at the recording site immediately before TBS. LTP was input-specific and the amount of potentiation was greater when two converging horizontal inputs were stimulated together. Horizontal LTP could also be induced by co-tetanization of vertical inputs simultaneously with horizontal activation without Bic application. LTD was induced by low-frequency stimulation at 2 Hz for 5 - 10 minutes. Mean decrease of the response amplitude reached 21 - 40%. LTD in horizontal connections was input-specific. Horizontal connections in which LTD was induced retained the capability of increasing synaptic strength as demonstrated by LTP induction in previously depressed pathways. Intracellular recordings confirmed synaptic character of observed effects.

These results demonstrate the potential for bidirectional activity-dependent modifications of the synaptic efficacy within intrinsic horizontal connections in the superficial cortical layers. The strength of synaptic coupling between horizontally connected neurons may depend both on the local circuitry and the capability of modification at individual synapses. These properties are likely to form a substrate and mechanisms for plasticity observed in adult cortical representations.

## MONOAMINE MODULATION OF THE SYNAPTIC INHIBITION IN THE HIPPOCAMPUS

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Dense noradrenergic and serotonergic innervation of the hippocampal dentate gyrus is reportedly directed to GABAergic hilar neurons. These neurons contribute to inhibition of granule cells. We studied the effects of noradrenergic and serotonergic agonists on the hilar neuron activity and granule cell inhibition. Activation of ß-noradrenergic receptors enhanced discharges of hilar neurons and increased the frequency of spontaneous inhibitory postsynaptic potentials (IPSPs) in granule cells. The frequency of tetrodotoxin-resistant IPSPs was also increased, which suggests a direct effect of ß-noradrenergic agonists on the GABA release. Activation of α-noradrenergic receptors decreased the discharge rate of hilar neurons and the frequency of IPSPs in granule cells. 5-HT induced either a hyperpolarization or a slight depolarization of hilar neurons. It also decreased the slow afterhyperpolarization, which was in part due to activation of $5\text{-HT}_4$ receptors. Accordingly, in a number of granule cells the frequency of IPSPs was increased by 5-HT. The $5\text{-HT}_{1A}$ receptor agonist 8-OH-DPAT hyperpolarized 60% of hilar neurons and decreased the frequency of IPSPs in granule cells. Our results suggest that noradrenergic and serotonergic agonists modulate the synaptic inhibition in the dentate gyrus by exerting a direct effect on inhibitory hilar neurons.
Supported by the KBN grant no. 6P20702405.

DEVELOPMENT OF OSCILLATORY ACTIVITY IN THE LIMBIC CORTEX IN VITRO.

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The generation of EEG theta rhythms (3 - 12 Hz, 0.3 - 1.5 mV) in the mammalian limbic cortex is a prime example of rhythmic activity that involves central mechanisms of oscillations and synchronization. In 1986 we demonstrated the first time that bath perfusion of the hippocampal slices with cholinergic agonists resulted in induction of theta-like oscillations which closely resembled the in vivo recorded theta. Since this initial demonstration, we have been carried out a number of experiments in an attempt to answer the general question: what are the similarities between the cholinergic-induced in vitro theta and physiological theta rhythm, which normally occur in the in vivo preparation. Thus far, our in vitro studies provided evidence that the in vitro recorded theta oscillations replicate in many aspects the physiological and pharmacological properties of the in vivo recorded theta rhythm. In addition, our studies validate the in vitro limbic cortex as a model to investigate neural mechanisms of theta activity.

## Workshop 2 - Brain mapping

TOPOGRAPHIC REPRESENTATION OF COHERENCE AND DIRECTIONS OF EEG ACTIVITY FLOW IN BRAINS

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In the investigation of brain functional dynamics mutual relationships between brain regions, their synchronisation and flow of EEG activity between them are of primary importance. A measure of the synchronisation is coherence, the EEG propagation can be estimated by means of directed transfer function (DTF). In the framework of multichannel AR model a method of topographical representation of coherencies and DTFs was elaborated. An important feature of the method is that all 21 channels of EEG are evaluated simultaneously not pair-wise, and EEG signals are treated as realisations of one process. The method was applied to the study of a whole night sleep of normals and depressed. It was found that ordinary coherencies are of a little use, since their values decrease monotonically with the distance between electrodes. On the other hand partial coherencies revealed intrinsic structure of relations between brain regions. By means of DTF the main centers of EEG generation during different sleep stages were identified. A topographical representation of coherencies and DTF patterns was elaborated.

This work was supported by KBN grant 8 8661 9102

BEYOND MAPPING: ESTIMATING COMPLEXITY OF MULTICHANNEL EEG RECORDINGS

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A state-space representation of multichannel EEG signal provides a synthesis of the two traditional approaches to EEG dynamics, the time-oriented and the space-oriented. In this approach, each momentary snapshot (map) of spatio-temporal potential field dynamics is represented by a point in an abstract $K$-dimensional space ($K$ = number of recording channels). A multichannel record of certain time extension can then be represented by trajectory in the state space. Basic properties of trajectories can be assessed by simple quantitative global descriptors like, among others, a suitable descriptor of complexity.

We propose a quantitative measure of complexity of $K$-dimensional signal as

$$\Omega = \exp\left\{-\sum_{i=1}^{K} \lambda_i' \log \lambda_i'\right\},$$

where $\lambda_i' = \lambda_i / \sum_{j=1}^{K} \lambda_j$ are normalized eigenvalues of the $K \times K$ covariance matrix of the signal.

$\Omega$ yields values ranging from 1 (low complexity = maximal synchronization) through $K$ (maximal complexity = no synchronization). The descriptor is by definition independent from voltage and frequency of the signal, so it might bring a novel information combined with global analogs of traditional measures.

To obtain a time-compressed picture of dynamics of long-term recordings, $\Omega$ can be combined with global power $\Sigma^2$ and generalized frequency $\Phi$. Each epoch of several seconds of EEG is thus reduced to a single point in the $\Sigma - \Phi - \Omega$ space. This representation allows to study dynamics of spontaneous or induced state changes on a large time scale.

First experiments with $\Omega$ computations in different physiological states like sleep stages, non-paroxysmal/paroxysmal activity etc. indicate $\Omega$ to be a promising indicator of complexity of multichannel EEG. Unlike various complexity measures introduced by theory of non-linear dynamical systems and used tentatively in EEG studies, $\Omega$ is easy to compute and understand.

## ANALYSIS OF COMPLEXITY OF EEG DURING SLEEP.

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Nocturnal sleep may be considered a cyclic sequence of synchronization and desynchronization of EEG activity. Wackermann (1995) developed a simple quantitative descriptor of the EEG complexity (Ω), which is a measure of synchronization of bioelectrical activity of the brain. The Ω is a single-valued quantity, computed from an epoch of multichannel EEG record. It is based on the analysis of covariances between activities of different scalp locations, ranging from 1 (maximal synchronization) to the value equal to the number of channels (no synchronization).

The aim of our study was to verify a usefulness of Ω in sleep studies. Analysis was performed on polysomnograms of 11 healthy subjects, aged 21 - 53.

Polysomnograms were recorded in 21 standard EEG derivations (10-20 system), referenced to the average electrode, and in the classical polysomnographic EOG and EMG derivations. EEG was amplified in a band 0.16-40 Hz. The sampling frequency was 102.4 Hz. Sleep stages were visually scored in 20 sec epochs, according to the Rechtschaffen and Kales (1966) standard. Artifacts were visually marked in all EEG channels, in 2.5 sec segments. The Ω was calculated from 21 EEG channels in 2.5 sec segments and averaged to 20 sec epochs to enable parallel presentation with the visual sleep scores.

In all subjects the value of Ω was significantly differentiated between sleep stages. In all subjects but one, the lowest value of Ω was obtained in stages 3 and 4, while the highest in stages 1 and REM. The dispersion of results was the highest in REM sleep. The Ω increased in successive sleep cycles in stages 2 and REM, but not in slow wave sleep.

The use of the Ω as a direct descriptor of the of complexity or synchronization of EEG maybe a method of promise in further neurophysiological studies.

Supported by KBN, Grant No.: 4 1106 91 01

## High Sampling Frequency Epileptic Spikes Mapping.

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It is very important to analyze properly the spike discharges that take place between attacks when studying epilepsy. The only possibility to do that exactly is to apply a computer system, that digitizes, registers and analyzes the EEG.

The correct analysis of the very fast spikes requires high sampling frequency. The spike that lasts from 20 to 40 ms. should have at least 20 sampling points to give maps that allow analysis of propagation. This criterion is accomplished with high sampling frequency like 512 Hz or 1024 Hz. These sampling frequencies result in 20 to 40 samples per spike depending on the spike template. This is fast enough to make consistent and continuous image cartooning. The standard 128 Hz sampling frequency, that is good enough for normal EEG is not the best for fast components. The spike gives 3 to 6 samples while sampling at 128 Hz and the maps are very rough and non continuous and give no possibility to analyze the propagation of the discharge.

The authors present examples of undersampled and well processed spikes.

# Workoshop 3 - Melatonin: origin and functions in an organism

## THE ORIGIN AND FUNCTIONS OF MELATONIN IN VERTEBRATES

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Melatonin (*N*-acetyl-5-methoxytryptamine) is mainly secreted by the pineal gland into the blood circulation in most species studied. Experiments carried out during the last decade provided evidence that some extrapineal sites (notably the retina and Harderian gland) are also capable of synthesizing the hormone. The circadian synthesis of melatonin and rhythms of associated enzymes (tryptophan hydroxylase and *N*-acetyltransferase) at least in the pineal gland and retina have long been known to be regulated by the prevailing light-dark environment, with peak values occurring at night (or during the dark phase of any imposed daily light-dark illumination cycle). Although the ability of light to suppress the nighttime melatonin production is intensity- and wavelength-dependent, there is a wide variation among species in their sensitivity to such an inhibitory light action.

Melatonin is a highly lipophilic compound, and once secreted (*e.g.* by the pineal) it can easily enter each cell in the body *via* the blood circulation. However, despite this, most (if not all) described effects of the hormone are mediated through specific membrane receptors (named ML-1 and ML-2), localized in a large number of neural areas and extraneural tissues. Such a widespread distribution of these receptors is in line with the diversity of effects the hormone induces in an organism. The neural melatonin receptor sites that have been most routinely documented include those in the suprachiasmatic nucleus (SCN), pars tuberalis (PT), and other hypothalamic regions. Melatonin receptors in the SCN are believed to regulate the central circadian clock (at least in mammals), whereas those in PT and the median eminence area (ME) likely mediate various melatonin actions on the neuroendocrine system. In addition to hormone-like actions, melatonin works also as a paracrine factor, playing a role of a local neuromodulator (*e.g.* in the retina).

## MELATONIN AS A CHRONOBIOTIC: PROS AND CONS

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The mammalian pineal gland is a major component in the regulation of photoperiodic responses and hence, saisonality. All functions which appear to be controlled by the daylength can be influenced by modifying the output signal of the pineal gland, i.e. melatonin. An important property of the melatonin signal is that the duration of melatonin synthesis and release varies proportionally with the length of the night. Infusion of melatonin into pinealectomized hamsters leads to short photoperiod responses (e.g. gonadal involution) if the duration of the melatonin infusion is more than 8 hrs per 24 hrs; infusion of melatonin for only 6 hrs daily stimulates gonadal growth (long day response). These and similar experiments strongly support the hypothesis that the duration of elevated melatonin is the most important signal conveying the photoperiodic message. Other data, however, suggest that the melatonin-free period is important as well.

In addition to the temporal coordination of saisonal phenomena, melatonin appears to be involved in the control of circadian rhythms in both mammals and birds. Activity-rest cycles of rats but not Syrian hamsters can be entrained by infusion or injection of melatonin at appropriate times of the daily cycle. Rhythms of neuronal activity of the suprachiasmatic nuclei (SCN) of the hypothalamus can be inhibited and phase-shifted by application of melatonin. The firing rate of SCN neurons of rats can be inhibited by iontophoretic application of melatonin. These data are in good agreement with the high density of melatonin receptors found in the SCN of most - but not all - mammalian species. On the other hand, activity-rest cycles appear to be perfectly normal in animals which lack a daily melatonin rhythm.

Several studies indicate that melatonin is involved in changing the animals' sensitivity to light on a daily as well as seasonal basis. In sparrows, for example, the zeitgeber strength of week light/dark cycles can be increased even by chronic melatonin-releasing capsules.

## USE OF MELATONIN IN CIRCADIAN RHYTHM DISORDERS AND FOLLOWING PHASE SHIFTS

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The pineal hormone melatonin is normally secreted at night. Following abrupt phase shifts (real or simulated time zone changes, night shift work) there is desynchronisation between the internal circadian rhythms (including melatonin) and the external environment with consequent disturbances in sleep, mood and performance. Early work showed that suitably timed melatonin administration phase advanced the activity-rest cycle in rodents and prolactin, fatigue and the endogenous melatonin rhythm in humans. Recently melatonin (single evening dose) has been shown to induce a significant suppression of core body temperature and self-rated alertness followed by a phase advance of the onset of endogenous melatonin the following evening. Whether these phase-shifting effects of melatonin are of therapeutic benefit in facilitating adaptation to forced phase shifts and in conditions of circadian rhythm disturbances has been investigated in our laboratory.

In the first placebo controlled jetlag study, melatonin significantly improved self-rated jetlag, daytime alertness, sleep latency and quality, and hastened the rate of resynchronisation of endogenous melatonin and cortisol rhythms. Results from all placebo-controlled and uncontrolled studies (melatonin n=474, placebo n=112) indicate that, suitably timed, melatonin reduces self-rated jetlag by 50 % ($P < 0.001$) in the majority of air travellers (irrespective of age, sex and direction of travel). Preliminary results in shift workers showed that melatonin taken at the desired bedtime improved self-rated sleep quality and duration and night-shift alertness. In simulated phase shift experiments, appropriately timed melatonin improved subjective sleep, alertness and performance and facilitated the readaptation of the melatonin rhythm following a rapid 9 h advance phase shift. Melatonin has also been assessed in conditions of biological rhythm disturbances with disturbed sleep (blind subjects and patients with delayed sleep phase insomnia). Compared with placebo, melatonin significantly improved sleep and synchronized the sleep wake cycle in some blind subjects. Melatonin treatment significantly advanced the sleep onset time in delayed sleep phase insomnia. These findings suggest that melatonin is of benefit in facilitating adaptation to forced phase shifts and in conditions of circadian rhythm disturbances. Optimisation of dose and timing of administration is currently underway.

## MELATONIN IN RETINA: REGULATION OF BIOSYNTHESIS AND FUNCTIONS

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Melatonin is synthesized in photoreceptor cells of vertebrate retina in a light-dependent circadian rhythm. The activity of serotonin N-acetyltransferase (NAT, a key regulatory enzyme in MEL biosynthesis) and MEL level are low and high during the light and dark phase, respectively, of any ligh:dark illumination cycle. The rhythm of MEL production is driven by the photoperiodic environment to which animals are exposed, and is generated by an endogenous circadian clock(s). The nocturnal increase of NAT activity is a cAMP- and $Ca^{2+}$-dependent process that requires protein synthesis. Activation of A2a-like adenosine receptors or $GABA_A$ receptors mimics the effect of darkness and increases retinal NAT activity. On the contrary, light exposure at night dramatically suppresses MEL biosynthesis. In addition, pulses of light produce phase-dependent phase shifts in MEL rhythm. There is fairly good evidence that dopamine (DA), released in response to light stimulation and acting on postsynaptic $D_4$-like (and/or $D_2$) DA receptors, is partially responsible for the light-induced suppression of retinal MEL production. On the other hand, it is less clear whether the DA system is of major importance in the light-evoked process of entrainment of the circadian oscillator generating the MEL rhythm in the retina.

MEL in the vertebrate retina functions as a neuromodulator. It regulates several processes that depend on environmental lighting conditions and circadian clock, including adaptive photomechanic movements of photoreceptors and melanin granules in the retina-retinal epithelium complex, membrane turnover of photoreceptor outer segments, and electrophysiological properties of horizontal cells. In addition, melatonin is also a potent inhibitor of DA release and metabolism in the retina. In line with the local role of MEL within the retina is the demonstration of functional MEL receptors in this tissue.

*Supported by the KBN grant 0540/P2/04/93*

## FUNCTIONAL CONNECTIONS BETWEEN PINEAL GLAND AND IMMUNE SYSTEM

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Involvement of the pineal gland, and in particular its principal hormone, melatonin (MEL), in the modulation of immune system function is already well accepted. In laboratory rodents, an immunostimulatory, anti-stress, and anti-glucocorticoid MEL activity has been demonstrated; it increased several immune parameters in a dose- and time-of-day-dependent manner. MEL effect was also modified by the season and sex, and was blocked by the endogenous opioid receptor antagonists. Additionally, MEL seems to be a potent oncostatic and anti-proliferative agent, used recently in a human oncotherapy. On the contrary, in the chicken we were not able to obtain any of the above-mentioned MEL effects, despite its influence on the circadian rhythm in several immune parameters.

The mechanisms by which MEL exerts its effects within the immune system remains still obscure. Nevertheless, several possibilities can be invisaged: 1) MEL, being a highly lipophilic compound, can attain antigen-primed T-helper lymphocytes and induce herein a synthesis of endogenous opioids, acting as immunostimulatory factors, influencing cytokine synthesis and release (mainly IL-2 and INF-γ) and NK activity; 2) MEL might directly participate in the regulation of cell growth and division, as under *in vitro* conditions it inhibited or increased the proliferation of normal lymphocytes and certain neoplastic lymphoblastoid cell lines; 3) MEL binding sites, as demonstrated recently in the membrane preparations isolated from both mammalian and avian lymphoid organs, could transduce hormonal signal into immune cells. In particular, we have found that MEL binding by chicken thymus membrane preparations was very weak and practically limited to the first week of postnatal development. This may explain, at least partly, why MEL does not stimulate immune response in the chicken; 4) On the other hand, cytokines secreted by activated immune cells can convey their message into pineal gland, modifying thereby MEL synthesis and release. It implies the existence of bidirectional interrelationships between pineal gland and immune system function.

# Oral communications - Neuropharmacology I - Dopaminergic transmission

NITRIC OXIDE (NO) AND CENTRAL DOPAMINE (DA) RECEPTORS REACTIVITY IN RATS.
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NO has been implicated in large number of patologies and in normal physiological function of the brain. The aim of this study was to recognise the effect of N-Nitro-L-Arginine Methyl Ester.HCl (NAME) and L-Arginine Ethyl Ester.HCl (ARGININE) on reactivity of the central DA receptors ($D_1$, $D_2$, $D_3$) to agonists (SKF 38393 and Quinpirole) and antagonists (SCH 23390 and Haloperidol). For this we have been used specific behavioral procedure such: oral activity, yawning and catalepsy. Experiments were perform in adult male Wistar rats treated ICV on 3rd postnatal day with 6-OHDA or saline (control). Other animals were treated daily IP with Qinpirole (0.05 mg/kg) or vehicle for the first 11 days from birth to obtain of the central $D_3$ receptor supersensitivity. NAME and ARGININE in different way influence on DA receptors agonists and antagonists action expressed in change of animals behavior. Theses findings suggest the role of NO in the central DA system function. (Supported by KBN and Fogarty International Center Health Sci. Exchange with Poland-RMK).

SENSITIZATION TO DOPAMINE (DA) AND 5-HYDROXY-TRYPTAMINE (5-HT) AGONISTS IS NOT CORRELATED WITH ALTERATIONS IN DA AND 5-HT CONTENTS OF NEOSTRIATUM AFTER NEONATAL INTRASTRIATAL 6-HYDROXYDOPAMINE INJECTIONS IN RATS. R.M. Kostrzewa, R. Brus, K.W. Perry and R.W. Fuller. Quillen College of Medicine, East Tennessee State Univ., Johnson City, TN, USA (RMK); Silesian Academy of Medicine, 41-808 Zabrze, Poland (RB); Lilly Research Labs, Eli Lilly Co., Indianapolis, IN, USA (KWP, RWF).

Oral activity responses to DA $D_1$ (SKF 38393) and 5-$HT_2$ (*m*-CPP, *m*-chlorophenylpiperazine) agonists are enhanced in rats in adulthood when brain DA neurons are destroyed neonatally by intraventricularly administered 6-hydroxydopamine (6-OHDA). In an attempt to produce a discrete lesion of DA neurons, 6-OHDA was injected directly into the striatum (STR) (4 μg/side) at birth (P0) or 3 days later (P3). The P0 lesion destroys DA inputs to the striosomal or patch compartment only, while the P3 lesion destroys DA inputs to both the patch and matrix compartments of STR (Gerfen et al., J. Neurosci. 7:3935, 1987). We found that there were enhanced responses to SKF 38393 and *m*-CPP in adult rats lesioned at P0, but not at P3. Although the endogenous contents of DA and 5-HT in STR and nucleus accumbens (NAC) were unaltered by 6-OHDA at P0, there was a 15-30% reduction in DA in STR and NAC and 10% elevation in 5-HT in caudal STR after 6-OHDA at P3. The findings in P0- and P3-lesioned rats indicate that behavioral sensitization was not correlated with changes in monoamine content of the STR and NAC. We propose that DA inputs to striatal striosomes, which would have been selectively destroyed by 6-OHDA at P0 but not at P3, underlie DA and 5-HT receptor sensitization. (Supported by NS 29505 and the Fogarty International Center)

## THE IMPACT OF COMPETITIVE AND NON-COMPETITIVE NMDA RECEPTOR ANTAGONISTS ON DOPAMINERGIC NEUROTRANSMISSION IN THE RAT VENTRAL TEGMENTAL AREA AND SUBSTANTIA NIGRA

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The present study compares effects of the competitive and non-competitive NMDA receptor antagonists, CGP 40116 and MK-801 respectively, on the metabolism of dopamine and on the density of dopaminergic receptors of D-1 and D-2 subtypes in the rat ventral tegmental area and substantia nigra. The effects of CGP 40116 on the above parameters were tested in a range of doses which either were devoid of or had locomotor- or stereotypy-stimulating effects. It was found that (1) CGP 40116 given in a dose of 5 mg/kg enhanced the locomotor activity of rats and evoked a stereotypy-like activity. A dose of 1.25 mg/kg was devoid of such effects; (2) CGP 40116 in a dose of 5 mg/kg enhanced the concentrations of dopamine, DOPAC and HVA in the ventral tegmental area; the lowest dose, 1.25 mg/kg had no effect; the only effect of CGP 40116 (5 mg/kg) observed in the substantia nigra, was an increase in dopamine concentration. (3) MK-801 in doses of 0.2 and 0.4 mg/kg enhanced the concentrations of dopamine, DOPAC and HVA in the rat ventral tegmental area and substantia nigra. The effects of MK-801 in the substantia nigra were quantitatively weaker than those observed in the ventral tegmental area. (4) Both CGP 40116 (5 mg/kg) and MK-801 (0.4 mg/kg) evoked alterations in the density of dopaminergic receptors. D-2 receptors, labelled with [$^3$H]spiperone, were upregulated by MK-801 in the ventral tegmental area and subregions of the substantia nigra, i.e. pars compacta and reticulata, whereas CGP 40116 evoked similar effects in the ventral tegmental area only. That effect was seen at 4 or 24 hours after administration of the respective drug. D-1 receptors, labelled with [$^3$H]SCH 23390 in pars compacta and pars reticulata of the substantia nigra, were consistently downregulated at 4 and 24 hours after administration of either drug.It is concluded that competitive NMDA receptor antagonists, such as CGP 40116, given to rats in doses which evoke hyperlocomotion and stereotypy-like activity, may have a substantial impact on the dopaminergic neurotransmission in the rat ventral tegmental area and substantia nigra, similar to that described for MK-801, a non-competitive NMDA receptor antagonist. The obtained results may suggest that CGP 40116 and, possibly, other competitive NMDA antagonists may have dopaminomimetic properties, and that their clinical potentials may be limited by the risk of evoking dopamine-dependent psychotomimetic and abusing effects, similar to those described for MK-801.The obtained results may suggest that CGP 40116 and, possibly, other competitive NMDA antagonists may have dopaminomimetic properties, and that their clinical potentials may be limited by the risk of evoking dopamine-dependent psychotomimetic and abusing effects, similar to those described for MK-801.

## ADAPTIVE CHANGES IN DOPAMINERGIC TRANSMISSION UPON PROLONGED ADMINISTRATION OF LITHIUM

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Lithium salts are among the most effective drugs in the prevention and therapy of manic-depressive episodes; however, the role of dopamine (DA) in the therapeutic effects of lithium remains unclear. The present study was designed to find out whether prolonged administration of LiCl induced any changes in the level of DA and its metabolites in the rat nucleus caudatus (NC) and nucleus accumbens septi (NAS). Additionally, using an *in situ* hybridization technique, the level of mRNA coding for the dopaminergic D-2 receptor in those brain areas, as well as the binding of [3H]spiperone to D-2 receptor following prolonged lithium administration were measured. The results obtained in the present study allow a conclusion that upon prolonged administration of lithium the DA transmission is attenuated, that effect being stronger in the NAS than in the NC. The assay of DA metabolites indicate that inhibitory effects of lithium appear at the level of DA release from terminals rather than at the level of DA synthesis and storage. There were no changes in the parameters characteristic for the binding of [3H]spiperone in either the NC or limbic forebrain (an area containing NAS); however, a statistically significant increase in the level of mRNA coding for D-2 receptor was observed, which points to stimulation of the biosynthesis of receptor protein upon prolonged lithium administration.

**BEHAVIORAL AND BIOCHEMICAL WITHDRAWAL EFFECTS AFTER CHRONIC TREATMENT WITH HALOPERIDOL AND THEIR MODIFICATION BY NIFEDIPINE AND VERAPAMIL**

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Chronic treatment with neuroleptics leads to development of tolerance and a few days later after the last dose withdrawal effects connected with supersensitivity of dopamine system. Our earlier experiments have shown that the effects of chronic treatment with psycho or neurotropic drugs may be altered if the drug is administered during blockade of calcium channels. The aim of this study was to investigate the behavioral and biochemical effects of withdrawal from haloperidol, and to find out whether calcium channel blockade during neuroleptic administration affects the results. Experiments were done on male Wistar rats. The biochemical investigations included: calcium channel receptors ([$^3$H]nitrendipine in the cortex), noradrenergic alpha1 receptors ([$^3$H]prazosin in the cortex) and dopamine D1 receptors ([$^3$H]SCH-23390 in the limbic structures) were carried out 24 h after the end of the treatment. In behavioral studies we investigated motor and stereotypy responses to dopamine agonists (apomorphine, amphetamine). Haloperidol, 1 mg/kg ip was administered daily for 15 days and withdrawal effects were investigated 24 h and 9 days after the last dose. Nifedipine, 5 mg/kg ip and verapamil 10 mg/kg ip were given always 15 min before the neuroleptic. The motor and stereotypy responses to apomorphine (1 mg/kg ip) and amphetamine (0.7 mg/kg ip) were significantly augmented in haloperidol treated group in comparison to apomorphine or amphetamine control groups. Nifedipine, calcium channel antagonist from dihydropyridines prevented the higher response to apomorphine, but strongly potentiated the effect of amphetamine. Verapamil, calcium channel antagonist from phenylalkylamines contrary to nifedipine did not change higher response to apomorphine, and significantly reduced the effect of amphetamine. The binding studies have shown, that chronic treatment with haloperidol significantly increased the density of calcium channels (by approx.40%) in the cortex and dopamine D1 receptors (by approx. 35%) in the limbic structures and did not change the density of alpha1 noradrenergic receptors. Co-administration of nifedipine antagonized the augmenting effect of haloperidol on [$^3$H]nitrendipine and [$^3$H]SCH-23390 binding sites and significantly increased the density of alpha1 receptors in the cortex. The results indicate the important role of voltage-dependent calcium channel in neuroleptics mechanism of action.

RECIPROCAL CONNECTIONS BETWEEN THE VESTIBULAR NUCLEI AND THE RETICULAR FORMATION OF THE LOWER BRAINSTEM: RETROGRADE TRACING STUDY IN THE RABBIT

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**The organization of the secondary vestibular projections onto the reticular formation of the lower brainstem /RF/ in reference to the reticulovestibular connections indicated previously /Neurosci.Res.,12, 1991,185-220/ was investigated by the retrograde horseradish /HRP, WGA-HRP/ technique in the rabbit. After single iontophoretic injections of the tracer into RF that involved mainly the gigantocellular nucleus and the caudal pole of the caudal pontine nucleus, distribution of retrogradely labelled neurones in the vestibular nuclear complex /VNC/ was analyzed. The findings indicate that the projections are rather abundant and originate bilaterally in all main VNC nuclei. Vestibular neurones projecting onto RF were scattered over the length of the superior vestibular nucleus. Those in the lateral vestibular nucleus were present mainly in the ventral and ventromedial regions of its central aspect; some of them were of giant size. Within the medial and inferior vestibular nuclei neurones projecting to RF were found to arise especially near their mutual border. The present data on the vestibuloreticular projection compared with those on the reticulovestibular projection show that some reciprocity exists between VNC nuclei and RF in the rabbit.**

# Poster sessions - Motor control

## Interlimb coordination in intact rats

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In 7 intact rats freely moving with speeds of 0.23–0.59 m/s the support pattern and the interlimb coordination were investigated to be further compared with those obtained in rats subjected to partial spinal lesions in order to identify the role of spinal structures in locomotion control. The animals were taught to locomote on 2 m long and 12 cm wide platform placed 1.5 m above the ground. The platform was covered with conductive material and the animals wore on palmar surface of each limb small cooper wire contact electrodes placed between the distal pads. The locomotion velocity was measured every 25 cm using photocells. In the four legged step cycle both phases of support on the diagonal limbs occupied up to 80% of the step cycle and their durations were positively correlated with the step cycle duration. Phases of support on other limbs were also positively correlated with the step cycle duration and among them supports on three limbs or on one limb predominated. The coordination between homologous limbs was characterized by mean phase shifts ranging between 0.42 ±0.04 and 0.55 ±0.06. The coordination between the fore- and hindlimbs was characterized by mean diagonal phase shifts ranging between –0.01± 0.05 and 0.20± 0.03. All these phase shifts were not correlated with the step cycle duration. The diagonal phase shifts in intact animals were negatively correlated with the relative swing duration only in the hindlimbs. Moreover, in these animals the homologous phase shifts were positively correlated with the diagonal phase shifts. These features of interlimb coordination appeared especially useful in identification of the fore- hindlimb coordination after partial spinal lesions.

MOVEMENT TESTS APPLIED TO EVALUATION POSTURAL STABILITY IN MAN

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Postural destabilizations in response to cyclic pull-and-push arm movements were compared in young and elderly subjects, with the goals of determining how age-related declines in stability influence cyclic arm movements made at different speeds, against different loads and while standing on support surfaces of different compliances. The characteristics of postural control have been studied using sinewave input produced by the subjects themselves. The results confirmed a decline with subject age of the preferred speed. The elderly subjects performed the experimental task more slowly with a lower mean movement frequency and a smaller amplitude. The older adults exhibited lower damping of the disturbing torques and center of mass excursions produced by the arm movements as evidenced by a higher amplitude of the center of foot pressure excursions in comparison to the young adults in response to the same rate of the voluntary arm movement. The results illustrate the age-related changes in postural stability and document reciprocal motor and posture interaction.

## THE INFLUENCE OF PATTERN OF STIMULI ON CHANGES IN TENSION OF UNFUSED TETANI OF MOTOR UNITS IN RAT'S MEDIAL GASTROCNEMIUS MUSCLE

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The influence of changes in regular pattern of stimuli on tension of unfused tetani was tested on motor units of rat's medial gastrocnemius muscle. This influence was investigated in tetani of different fusion for each motor unit. Shortening or lengthening of interpulse time break was used. Lengthening of interpulse distance evoked fall in tetanic tension which was the stronger the bigger lengthening was introduced. The decrease in tension tended to be higher in more fused tetani. On the other hand, shortening of interpulse distance evoked increase in tension which was the stronger the more this distance was shortened. Higher increase in tension was observed in less fused tetani. An effort was made to find out the degree of tetanic fusion when shortening as well as lengthening of interpulse time break evoke similar changes in tension of tetanus. Preliminary analysis shows that the two opposite effects are nearly equal when fusion index is high. The influence of very strong shortening of interpulse distance (up to 5 ms) on tension of further part of tetanus was also investigated. The tension of tetani evoked by pattern of stimuli starting with two pulses in 5 ms break ("doublet" tetani) was higher but less sensitive to shortening of interpulse distance in post-doublet part of tetanus and more sensitive to lengthening, when compared to "non-doublet tetani".

## ACOUSTIC SIGNALS GENERATED DURING ACTIVITY OF SINGLE MOTOR UNITS IN RAT'S MEDIAL GASTROCNEMIUS MUSCLE

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Acoustic signals generated during evoked isometric contractions of motor units of rat's medial gastrocnemius muscle were recorded using piezoelectric sound sensor placed in a paraffin oil bath. The motor unit tension and action potentials were recorded paralelly. Using this sound sensor we were able to record acoustic phenomenas during activity of most of studied motor units. The acoustic signals accompanied single twitches, unfused and fused tetani. An acoustic signal recorded during twitch of motor unit was a series of waves of amplitude decreasing in time. A peak to peak amplitude of this record was measured. The amplitude of acoustic wave depended on the tension of motor unit twitch. In unfused tetani the time relationships between components of a tetanus and of an acoustic signal were analysed. In fused tetani the highest amplitude was usually observed in the first phase of the tetanus when the tension of the tetanus increased and during relaxation following the end of stimulation. However, when the maximal tension of fused tetani was kept on constant level, acoustic phenomenas were almost absent.

## THE SPINORETICULAR NEURONES IN THE SECOND SACRAL SEGMENT OF THE CAT'S SPINAL CORD.

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The location of neurones of origin of spinoreticular tracts in sacral spinal segments of the cat was not investigated in details. In order to evaluate their topography in S2 segment and projections to the reticular gigantocellular nucleus, both the method of intracelullar staining with HRP and the antidromic excitation of axonal terminals have been used in α-chloralose anaesthetized animals. Following electrical stimulation (with pulses of about 120 μA) via the tungsten electrode stereotactically placed in the contralateral brainstem, both extra- and intracelullar recordings were performed from 37 neurones located in the medial lamina VII and the area adjacent to the central canal (n=13), the medial lamina VIII (n=12), medial laminae V and VI (n=10) and in laminae II and III (n=2). Axons of those cells might be additionally invaded from the dorsolateral funiculus of contralateral (n=37) and ipsilateral (n=31) sides at the Th13 level. The latencies of antidromic excitation from the brainstem to S2 segment ranged from 3.2 to 11.8 ms (mean of 5.9 ms) whereas the corresponding conduction velocities were between 130 and 44.2 m/s (69.8 m/s). Three medium-size, triangular-shape neurones have been visualized in medial laminae VII and VIII following the intracellular staining.

## X-RAY KINEMATIC ANALYSIS OF CAT TARGET-REACHING AND FOOD-TAKING MOVEMENTS.

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To investigate the neuronal organisation of goal-directed and manipulatory movements of the cat forelimb, Gorska and Sybirska (Acta Neurobiol. Exp., 1980) introduced a paradigm known as target-reaching (TR) and food-taking (FT). Lesion studies indicate that the command for TR is transmitted via C3/4 propriospinal interneurones whereas FT depends largely on interneurones at the segmental level. The kinematic components of this complex behaviour in intact and lesioned cats are, however, to a great extent unknown, because relevant proximal and distal structures are not accessible for conventional movement recordings. Here, we report three-dimensional x-ray recordings (time resolution 20ms, spatial resolution ~ 1mm) during TR and FT in cats before and after a lesion dorso-lateral C5, thus interrupting descending input to segmental interneurones.
The most striking deficit after the lesion is the inability to grasp the food correctly, whereas TR seems unaffected. In intact cats the grip is formed ~100 ms prior to food contact by a meta-carpo-phalangeal (MCP) and/or a proximal-interphalangeal (PIP) extension. The cat then gets hold of the object by a PIP flexion. After the lesion the amplitude of precontact MCP and PIP extension is greatly reduced. The relative timing, however, remains fairly unchanged. PIP flexion after grasping decreased and slowed down. Discrete effectswere observed also in more proximal joints. Whether the changes in humeral adduction and rotation and of radio-ulnar supination are due to a lesion effect or are the sign of a compensatory strategy remains to be determined.

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## OSCILLATIONS WITHIN PREMOTOR NETWORK AS A COMPENSATORY RESPONSE FOLLOWING CORTICAL HEMICEREBELLECTOMY

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We studied the effect of cortical hemicerebellectomy on premotor network. In particular the changes observable after a significant compensation of motor deficits occured.
Under semi-sterile conditions our cats had removed cerebellar cortices of one hemisphere via suction lesion. Their functional recovery was assessed neurologically for a variable period of time (between 2-6 weeks). When a significant improvement in motor functioning could be seen e.g. cats would climb steps independently, animals were reoperated. Second operation involed exposure of brain for passage of recording microelectrodes placed under stereotactic control in cerebral cortices and red nuclei. Spontaneous firing and following peripheral stimulation of forelimbs were recorded. Histological documentation of recording sites and extent of lesions completed experimental protocol.
Cross-correlograms(CC) of trains of spikes recorded intra- and extracellularily exhibited following features. CC within red nucleus contralateral to lesion showed oscillations of (26)Hz frequency (n=36). CC between red nuclei displayed no oscillations. CC of red nucleus ipsilateral to lesion and contralateral cortex showed oscillations of (33)Hz frequency (n= 25). We also noted an intriguing absence of oscillations in CC between contralateral (with regard to lesion) cortex and red nucleus
We postulate that these oscillations, could be a part of mechanism responsible for functional recovery following first operation, and we shall study that phenomenon in greater detail in our next experiments.

## ESTIMATION OF THE DISTRIBUTION OF THE EMG SIGNAL AMPLITUDE

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In many experiments, in which the electromyographic activity (EMG) is analyzed, one needs the method of assessment of this activity. The EMG signal depends on many factors related both to the nature of the signal, and the method of recording and the equipment. One of possible ways of analysis is the quantitative evaluation of EMG activity by counting number of threshold level crossings of the signal (called an aggregate activity). Crossings of the threshold level can be detected, e.g. with the use of a spike trigger connected to computer which counts the triggered pulses, monitored simultaneously with the analyzed raw signal on an oscilloscope. The threshold level should be set up just above the noise level. Such assessment is not, however, quite objective, and we propose another solution.

Our method should allow an estimation of the distribution of the EMG signal amplitude. The estimation is based on the detection in the analyzed signal of crossings of threshold levels. First the EMG activity is digitized using an A/D converter, and the objective parameters such as the mean value (MEAN) of the signal and the standard deviation (SD) are calculated for each analyzed channel during the chosen time interval. On the basis of these parameters 64 threshold levels are determined: the lowest level is equal MEAN-4*SD, and the highest one equals MEAN+4*SD. Typically the distance between two successive levels is equal 0.125*SD, but it can be easily changed, if necessary. The standard definition of levels makes the number of crossings of a particular level to be independent from the amplification of the signal. Written by us program enables calculation of the threshold level values, and number of crossings for each level. This allows comparison of the results for different records.

The application of the method described above will be shown on two examples. One example is the presentation of the comparison of activity of a muscle before and after its partial denervation. The other example would show the analysis of muscle activity in matured animal with early partial denervation of one hindlimb. In this case comparison of the muscle activity before and after partial denervation is not possible, thus the EMG activity of the contralateral muscle, simultaneously recorded with the activity of the affected muscle, is used as a control and the reference.

Detection and recognition of single motor unit potential is a difficult task and it is only possible in case of poor muscle activity. In our method, on the basis of the distribution of high, medium or low amplitudes of analyzed EMG signal it might be possible to assess the changes in amplitude of active motor unit potentials, induced by various experimental manipulations.

## LOCOMOTOR RESPONSE TO UNILATERAL VTA STIMULATION IS FACILITATED BY CONTRALATERAL VTA LESION

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Unilateral electrolytic lesions of the ventral tegmental area /VTA/ facilitate feeding response to contralateral VTA stimulation . This effect which was found in our previous works we call "contralateral facilitation" of function. It may constitute an important, not yet explored, mechanism of recovery of function after acute brain injury. In the present work we studied whereas contralateral facilitation can be also replicated on other functional models.We choose forward locomotion, the most commonly observed response to stimulation of VTA.

Twelve male Wistar rats were implanted with chronic bilateral VTA electrodes serving for electrical stimulation and electrolytic lesions. Latency to initiate locomotion in response to VTA stimulation was measured as a function of stimulation frequency. Then, unilateral electrocoagulation of VTA /n=5/ or the tissue above VTA /n=7/ was performed in the hemisphere contralateral to the stimulating electrode, and latency-frequency functions were determined daily for 5-14 days postlesion. It was found that lesions facilitated locomotor response to VTA stimulation which manifested as an immediate and long-lasting decrease of frequency threshold /up to 43% of the prelesion baseline/, and a leftward shift of the latency-frequency function. No such effect was found when control lesions were localised above VTA in the hypothalamus and the thalamus. Instead profound impairment of locomotor response was observed. The results provide further evidence for anatomically specific facilitation of function of the intact hemisphere after acute unilateral brain injury.

## GABA-ergic MECHANISM OF FACILITATION OF VTA STIMULATION-INDUCED LOCOMOTOR RESPONSE AFTER CONTRALATERAL VTA LESION

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As we found previously unilateral electrolytic lesions of the ventral tegmental area /VTA/ facilitated feeding and locomotor response elicited by stimulation of VTA in the other, intact hemisphere. In the present experiments we tested a hypothesis that unilateral VTA lesions exert their facilitatory effect by destroying interhemispheric GABA-ergic projections which normally are responsible for reciprocal inhibition of VTA functions. If so, unilateral blockade of GABA-ergic transmission should mimick facilitatory effect of electrolytic lesions.

The behavioural model used was locomotor response to electrical VTA stimulation. In male Wistar rats implanted with VTA cannula and contralateral VTA electrode a latency to initiate locomotion in response to VTA stimulation was measured as a function of stimulation frequency. After establishing a baseline responding, contralateral VTA injections of a $GABA_A$ receptor blocker bicuculline in a dose of 0.5 or 5.0 ng were performed, and latency-frequency functions were determined postinjection. 0.5 ng of bicuculline evoked a clear facilitation of locomotor response which manifested as a decrease of the reaction threshold by mean 25.5% of the baseline and a leftward shift of the latency-frequency function. This mimicked the effect of electrolytic lesions studied in our previous experiments. Bicuculline in a dose of 5.0 ng was less effective causing a decrease of threshold by mean 5.2%. The results suggest that contralateral facilitation of VTA function after unilateral VTA lesion is, at least in part, dependent on destruction of interhemispheric GABA-ergic projections.

## THE INFLUENCE OF A BILATERAL 6-OHDA LESION OF THE NIGROSTRIATAL PATHWAY ON THE MUSCLE TONE IN RATS

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The aim of the present study was to find out whether a lesion of the nigrostriatal pathway with 6-OHDA induces muscle rigidity in rats, similar to that seen in parkinsonism. 6-OHDA was administered in a dose of 6.5 μg/ 1μl bilaterally into the pars compacta of the substantia nigra. The muscle tone was estimated as resistance of the hind foot to passive bending and stretching in the ankle joint. Simultaneous recording of the electromyographic (EMG) activity of the gastrocnemius soleus and tibialis anterior muscles was carried out. The magnitude of the lesion was checked histologically by a tyrosine hydroxylase immunohistochemistry. It was found that the lesion that involved more than 90% of dopaminergic neurons induced an increase in the muscle resistance, measured 2 weeks after the lesion, and in the stretch-induced short-latency (spinal) and long-latency (supraspinal) EMG components in the gastrocnemius soleus muscle 1 and 2 weeks after the surgery. The results suggest that a lesion of the dopaminergic nigrostriatal pathway with 6-OHDA induces muscle rigidity which is somewhat similar to that seen in Parkinson's disease.

## DOES HALOPERIDOL INDUCE PARKINSONIAN RIGIDITY ?

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The aim of the study was to find out whether the haloperidol-induced rigidity was resembled that seen in parkinsonism. Simultaneous measurements of the muscle resistance (MMG) of the hind foot to passive bending and stretching in the ankle joint, as well as those of the electromyographic (EMG) activity of the gastrocnemius and tibialis anterior muscles of rats were carried out. Haloperidol was injected in doses of 0.5-10 mg/kg 1 h before the start of measurements which lasted 2 h. Haloperidol increased in a dose-dependent manner the muscle resistance of the rat's hind leg to passive movements. The muscle rigidity was accompanied with an increase in the resting, as well as stretch-induced long-latency EMG activity (influenced probably by supraspinal mechanisms) in the gastrocnemius muscle, whereas the short-latency EMG activity (first, 0-20 ms large bursts of the EMG activity, probably of a spinal origin) was significantly decreased. The obtained results suggest that the haloperidol-increased MMG/EMG activity seems to be a good model of parkinsonian rigidity.

## THE EFFECT OF LOW EXTERNAL CAESIUM ON MUSCLE RESTING POTENTIAL IN MEALWORM LARVA

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It is known that potassium current, mainly inward rectifier, plays an important role in determining the resting membrane potential (RMP) in many cells. There is a considerable evidence that external $Cs^+$, even at low concentration, blocks inward $K^+$ current. The influence of extracellular caesium on the membrane potential (MP) in ventral longitudinal muscles of mealworm larvae was studied by using conventional microelectrode technique. The ionic content of standard physiological saline was (in mmol/l): NaCl 80, KCl 40, $CaCl_2$ 5, $MgCl_2$ 10, glucose 435. $Cs^+$ was used as a substitute for $K^+$ and thereby 5 or 1 mmol/l of the KCl was replaced with equimolar CsCl in the test solutions. The average value (±SEM) of muscle resting potential was -47.4±0.5 mV in standard physiological saline which did not change significantly with time (90 min). Exposing studied muscle fibres to the saline containing 5 mmol/l CsCl caused immediately a significant hyperpolarization of MP (by 8.9-20.5 mV, depending upon the measuring time) which gradually increased up to 30 min. After 1h the level of MP slightly decreased being still significantly higher than that observed in the control. In the case of application the saline containing 1mmol/l Cs ions, their influence on the MP was less distinct. However, the muscle MP usually hyperpolarized slightly. The effect of $Cs^+$ was fully reversible upon washing. The suppressing effect of $Cs^+$ on the $K^+$ inward current depends not only on the $[Cs^+]_o$ but also on $[K^+]_o$. It is known that K ions have dual effects on channel blocking, facilitatory or inhibitory. It seems that in the case of 5 mmol/l $[Cs^+]_o$, K ions exert the facilitatory effect. However, the influence of higher $[K^+]_o$, present in the saline containing only 1 mmol/l CsCl, is not facilitatory anymore, and becomes even slightly inhibitory. The influence of Cs ions on the MP, shown in the present study, might be explained by a preferentially suppressing or blocking effect on the inward $K^+$ current rather than on outward currents. Thereby it seems reasonable to assume that during Cs-treatment the outward $K^+$ current might prevail over the inward one resulting in the hyperpolarization of MP in studied muscles. The present results confirm that inward $K^+$ current might have some contribution to the RMP electrogenesis in mealworm larva muscles.

## THE SYNCHRONIC EFFEC OF HYPOXIA AND OUABAIN ON THE MUSCLE RESTING POTENT L OF PERIPLANETA AMERICANA L.

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Many investigators have shown that the decrease of external oxygen content causes a membrane depolarization. Ouabain - the selective inhibitor of Na-K-ATPase has the similar effect on the membrane potential. Our studies on the influence of hypoxia and ouabain on the muscle resting potential (RP) of Periplaneta americana L. also confirm these data. The aim of our studies was to explore what is the synchronic effect of these two factors. The experiments were done in four independent groups of insects: the first group - control - reared in normal content of oxygen (21%), and RP was measured in standard physiological saline, the second group - RP after 2 h rearing in hypoxic conditions ,the third group - reared in normal content of oxygen - treated with ouabain, and the fourth group - kept for 2 hours in hypoxic condition and then treated with ouabain. The resting potential was studied for 90 min using the conventional microelectrode methods. The resting potential of the control group just after the equilibration time was 66.8±0.3 mV and did not change significantly within 90 min of experiment duration. Two hours of keeping animals in hypoxic conditions caused a decrease of RP to 55.2± 0.6 mV. In the third and fourth experimental groups two concentrations of ouabain were used: $10^{-5}$ and $5x10^{-5}$ . Ouabain in the third group of insects caused a decrease of RP from 68.4±0.4 mV and 69.8±0.6 mV to 58.5±0.6 mV (ouabain $10^{-5}$ ) and 54.5±0.6 mV (ouabain 5 x $10^{-5}$ ) after 90 min of experiment respectively. After 2 hours of hypoxia and application $10^{-5}$ and $5x10^{-5}$ M of ouabain the RP decreased to 38.3±0.5 mV and 35.5±0.5 mV respectively. The decrease of RP obtained in insects where ouabain was applied, point out the role of Na-K-ATPase in producing of the RP in P.a.. Under hypoxia the rate of oxygen transfered to the cells is not sufficient to meet the ATP requirements. In such conditions the ionic homeostasis is disturbed. Ouabain, influencing Na-K-ATPase, intensifies the effect of hypoxia.

SACRAL PROJECTIONS OF PROPRIOSPINAL NEURONES OF THE SIXTH CERVICAL SEGMENT OF THE SPINAL CORD IN THE CAT

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The sacral projections of cervical propriospinal neurones were investigated in the cat. An electrophysiological method of intracellular and extracellular recording from neurones located in the sixth cervical segment was used. The cells were identified following antidromic activation from the spinal grey of the second sacral segment and they were found predominantly in the ventral horn of C6 segment. Their axons descended in the dorsal part of lateral funiculi on the ipsilateral and/or contralateral side and they terminated in the ipsi- or both ipsi- and contralateral grey matter of the S2 segment. These results indicate that there are direct interconnections between cervical and sacral part of the spinal cord, suggesting the possible role in coordination of movement of the limbs.

CERVICAL PROJECTIONS OF NEURONES LOCATED IN THE SECOND SACRAL SEGMENT OF CATS SPINAL CORD

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The cervical projections of neurones located in the second sacral segments of the cats spinal cord were investigated using an electrophysiological method of both intracellular and extracellular recording. Neurones, identified by antidromic activation from the spinal grey matter of the sixth cervical segment as well as thoracic and lumbar levels of the spinal cord, were found predominantly in lamina IV, V, VI of the S2 segment. Their axons ascended in the dorsal part of lateral funiculi on both the ipsilateral and contralateral side but their cervical terminations were found in the ipsilateral only or both ipsi- and contralateral spinal grey of C6. The findings suggest that these connections may by of functional importance in movement coordination.

## Poster sessions - Sensory processing

DIFFERENTIAL EFFECTS OF SHORT-LASTING APPETITIVE AND AVERSIVE CLASSICAL CONDITIONING UPON CORTICAL BODY MAPS IN THE BARREL FIELD OF ADULT MICE, A 2DG STUDY.

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Changes in topographical maps in the first somatosensory cortex were visualized with 2-deoxyglucose (2DG) autoradiography after appetitive or aversive sensory conditioning training. In this study we used paradigms of classical conditioning involving stimulation of mystacial vibrissae row B. Row B of vibrissae on one side of the snout was stroked immediately before delivery of sweet water to the mouth or delivery of a mild tail shock. The pairings were repeated 4/min for 10 min. Training lasted 3 days. 2DG experiments were done after the last training session. Following injections of [$^{14}$C]2DG rows B of vibrissae were stimulated on both sides of the snout. Stimulation of "trained" row resulted in labeling of a larger area of the barrel field than stimulation of the "untrained" row on the other side of the snout. In the case of appetitive conditioning, enlargement of row B representation was visible in layers II/III (27%) and layer V (28%). In the case of aversive conditioning, the enlargement of row B representation was found in layer IV and IIIB. The labeling was centered on row B of barrels and overlapped neighboring rows. The results demonstrate learning induced changes in cortico-cortical and thalamo-cortical connections.

CLASSICAL CONDITIONING INDUCES SHORT-LASTING CHANGES IN SOMATOSENSORY EVOKED RESPONSES IN THE RAT'S BARREL CORTEX

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Five hooded male rats were used for experiments. Under chloral hydrate anesthesia four bipolar electrodes were implanted at the depth of the layer IV of the barrel cortex and reference electrode to the frontal part of the skull. For chronic experiments rats were accustomed to lie in plexiglass tubes with heads restrained to metal holder. The piezoelectric device stimulated precisely rat's vibrissae to produce the evoked responses. The whole experiment consisted of a few (5-10) sessions, one session per day. During one session, lasting for about an hour, every rat received 100 stimuli to one of the vibrissae with intervals randomly scattered from 30 to 45 seconds. First 2-3 sessions allowed the habituation of the evoked responses for the stimulation of the chosen vibrissa. During following conditioning sessions, we accompanied the stimulation of the vibrissa with an electric shock applied to the ipsilateral ear. During the first conditioning session the evoked responses changed significantly. Immediately following the first shock, we observed the increase in the slope of first negative (N1) and amplitude of the second positive (P2) components of the evoked responses. These changes lasted up to 10 minutes and ceased at the end of the session.

We conclude that classical conditioning evokes the plastic changes in both thalamic relay and cortical networks during first minutes of the process.

## THE INFLUENCE OF ATROPINE ON INFORMATION PROCESSING IN NEIGHBORING COLUMNS OF THE RAT'S BARREL CORTEX

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Four hooded male rats were used in the experiments. Under urethan anaesthesia the scull was opened to expose barrel field and the dura matter was removed. Bipolar electrode was dipped in the cortex at the depth of the granular layer. By stroking each vibrissa with a piezoelectric device we recorded evoked potentials (EPs) and produced a map of its receptive field (map of responses) at the particular electrode location. The principal whisker (PW) - corresponding to biggest EP - was then continuously stimulated with a frequency of 0.2 Hz. In the mean time a drop of Atropine sulphate (20mM) was applied to the surface of the cortex for about 5-7min and subsequently washed out. The evoked potentials were observed on line and recorded on the analog tape. Response maps were investigated at approximately 10, 30, 45 min after applying the drug. The amplitudes of EPs were enhanced slightly after 1-3 min from atropine application and then ceased for subsequent 3-5 min There was no response to whisker stimulation for the following 15-20 min and the responses recovered after additional 10-25 min with the amplitudes of the main components (N1, P2) bigger then at the beginning of the experiment. These changes were different for the responses to the whisker undergoing continuous stimulation as compared to surrounding ones, The PW responses ceased earlier, were blocked for a longer time and the rebound effect was also more striking. Our results strongly support the notion of the ACh involvement within the sensory processing in the barrel cortex.

## RETICULAR FACILITATION OF RAT VISUAL CORTICAL RESPONSES IS BLOCKED BY SYSTEMIC ADMINISTRATION OF A MUSCARINIC AND NICOTINIC ACh - ANTAGONIST

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Our previous results indicated that intravenous administration of the muscarinic antagonist atropine and scopolamine and the nicotinic antagonist mecamylamine individually failed to block the effect of reticular facilitation. The aim of the present study was to explain whether simultaneous administration of both antagonists fully eliminated the facilitation of cortical visual responses in rat. Therefore we investigated the effect of intravenously applied antagonist of muscarinic (atropine) and nicotinic (mecamylamine) cholinergic receptors at doses 2 and 4mg/kg on reticular facilitation of evoked potentials in the visual cortex of adult rats. The experiments were conducted in anaesthetised rats. Cortical responses were evoked by electrical stimulation of the dorsal lateral geniculate nucleus (dLGN) via bipolar concentric stimulation electrodes and recorded with silver ball electrodes positioned on the dura mater overlying visual cortex. In addition, stimulation electrodes were inserted into the lateral dorsal tegmental nucleus (LDTg) to induce reticular facilitation. We found that only combined administration of muscarinic and nicotinic antagonists eliminated or significantly reduced the effects of reticular stimulation. However, the transient suppression of the facilitatory effect of LDTg stimulation might indicate that other neuronal substrates are involved in this mechanism. Particularly that the interaction of cholinergic, noradrenergic, and serotonergic neurones in the brainstem has long been suggested to be a key factor in behavioural state control. Additional experiments are needed to make sure that in reticular facilitation of rat visual cortex responses, other neurotransmitters are involved.

## THE DYNAMIC STRUCTURE OF VISUAL RECEPTIVE FIELDS IN THE CAT'S SUPERIOR COLLICULUS

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The visual receptive field structure of superior colliculus cells was examined using single unit extracellular recording. Twelve cells were tested with a light bar moving along the horizontal axis of their receptive fields. The stimulus moved in both directions covering a limited range of the receptive field, 0.5° to 10°, the position of this movement varied. The intensity of cell responses and their directional sensitivity varied depending on the location of the tested region within the receptive field. Most collicular cells (9) showed structural heterogeneity, six of them had opposite types of responses to stimulus movement within the test-zones when compared to the clear-cut direction sensitive response of a cell for movement throughout the whole receptive field. Three collicular cells, directionally nonsensitive when tested with full length movement along all of the horizontal axis, showed directional responses to small amplitude movements in different test-zones of the receptive field. The receptive fields of three other collicular cells with nondirectional characteristics were homogenous.

These results suggest that receptive fields of most collicular cells are composed of elements with different characteristics which interact to form a global response.

## THE INFLUENCE OF THE HEMICEREBELLECTOMY ON THE CORTICAL SOMATOSENSORY EVOKED POTENTIALS IN THE CAT

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It is well known that the cerebellum plays an important role in determining the excitability of the cerebral cortex network. However the mechanism of this modulation activated by cerebello-thalamo-cerebral projection system is yet unclear.

Animals were immobilized by gallamine under chloralose or nembutal anesthesia. Brain potentials (BP) from sensorimotor cortex of both hemispheres evoked by electrical stimulation of limb nerves were recorded before and after acute or chronic hemicerebellectomy. Operation was always confined to the right side of the cerebellum.

We did not observe any significant differences in amplitude and latency of the BP collected from either side before acute lesion. After hemicerebellectomy results were strongly depended on extension of the impairment and kind of anesthesia. BP amplitudes were unchanged or reduced comparing to the preoperative period when the lesion performed under chloralose anesthesia was complete and involved as well cortex as deep nuclei. The time duration of the potentials increased after operation. Amplitudes of the signals were distinctly bigger at the left side during right limb stimulation - according to the main crossed cerebello-thalamo-cerebral projection - in the case of lesion was narrow and spared cerebellar nuclei. The first component was about the same while amplitude of the long latency components became unchanged or attenuated at another side. The time duration increased in all above situations.

There were no BP amplitude changes of the type after chronic surgery.

We did not noted any crucial differences in latency before and after acute cerebellectomy in cats under nembutal anesthesia. Amplitude of the potentials was the same or reduced after operation.

During experiments we were using PC computer programs designed in our laboratory for recording, reviewing and processing obtained data. The set of currently available analytical procedures comprises FFT, correlation functions and Brain Electrical Activity Mapping (BEAM) technique.

CHANGES IN THE PATTERN OF 20 HZ SYNCHRONIZATION WITHIN CAT'S VISUAL CORTICO-THALAMIC SYSTEM AS A FUNCTION OF ATTENTIVE PERCEPTION

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The Pearson Product-Moment Correlation Coefficients (normalized cross-correlation coefficients with zero lag) were calculated for pairs of recorded EEG signals from the lateral geniculate nucleus (LGN) and primary visual cortex (VCx) in cats performing visual or acoustic task requiring attention. For computation band-passed filtered signals were used in the range 16-24 Hz. The spectrum within this range is characterized by increased power during attention to visual stimuli (Bekisz and Wróbel, Acta Neurobiol. Exp. (1993), 53: 175-182).

The analysis revealed that during nonvisual situations the recordings from most electrode sites within the visual system show positive synchronization indicating oscillatory rhythm of a general nature. In periods requiring visual attention the synchronization changes toward negative values of the Pearson coefficient, with the exception of a few recording sites from which especially high levels of complaince between signals were observed.

We suggest that such a specific pattern of synchronization for 20 Hz beta activity mirrors the functional connections temporarily appearing in the visual system during attentive seeing.

INFLUENCE OF MONOCHROMATIC AND POLYCHROMATIC BACKGROUNDS ON THE ACTIVITY OF CAT VISUAL NEURONS

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Colour sensitivity of night active cats does not seem to play an important role for orientation. Cats have been generally classified as dichromats with a poor ability of colour discrimination. Although this capacity has not been fully understood, the stimulus size may be the decisive factor. To investigate the colour sensitivity in feline cortical neurons in areas 17/18 we stimulated with white stationary (on/off) and moving (forward/backward) light bars. Mono- and polychromatic large visual noise processes (50*50°) of identical luminance were added. Backgrounds were moved at different speeds either inphase or antiphase to the moving bars. Responses of single units were analysed off-line on the basis of PSTHs including maximum amplitude, direction index (DI), receptive field (RF) width and latency.

In general, the amplitudes of 20% (forward) or 60% (backward) of all neurons were modulated by the visual noise process. The most sensitive characteristic altered by composition of the visual noise was the DI. Up to 90 % of the tested cells showed either an increase or decrease of the DI, depending on the stimulus-background constellation. On the contrary, visual latencies and RF-widths were not influenced by the spectral composition of the background.

In conclusion, a large proportion of cat visual neurons was sensitive to the spectal composition of the background. However, we found no general preference of either the mono- or polychromatic noise process.

## Poster sessions - Learning, memory and cognitive functions

NMDA RECEPTOR ANTAGONISTS ENHANCE LEARNING IN ANIMAL MODELS OF COGNITIVE DEFICIT

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NMDA receptor antagonists are neuroprotective against ischaemia, hypoxia and hypoglycaemia. NMDA receptor antagonists are usually tested for their neuroprotective potential against increased levels of activation of glutamatergic receptors which also suggested to occur in early stages of Alzheimer's disease. However, inhibition of NMDA receptors may also produce negative effects on cognitive functions. Memantine (MEM), an uncompetitive NMDA receptor antagonist showing a symptomatological effect in Alzheimer's disease was compared in animal models of cognitive deficit with the classical NMDA channel blocker MK-801 and d-cycloserine (DCS), a partial agonist of the glycine site coupled to the NMDA receptor, recently tested in the treatment of dementia. In the present study, quinolinic acid-induced entorhinal cortex (EC) lesions produced negative effects on cognitive functions in radial maze. MEM and MK-801 were infused with Alzet osmotic minipumps to assure steady-state plasma drug levels. MK-801 (0.312 mg/kg/day) enhanced cognitive deficits induced by EC lesions, while MEM (20 mg/kg/day) reversed this deficit. DCS (6 mg/kg) injected before each daily session had no effect. In the dark avoidance paradigm, a learning deficit induced by administration of NMDA (25 mg/kg) was reversed by both MK-801 (0.05 mg/kg) and MEM (2.5 mg/kg). DCS (3 mg/kg) reversed the learning deficit induced by MK-801 (0.2 mg/kg). In summary, MEM but not MK-801, attenuates cognitive deficits produced by brain damage such as lesions of the EC, a structure affected early in Alzheimer' disease. Moreover, both drugs reversed the learning deficit induced by stimulation of NMDA receptors, suggesting their potential usefulness in the therapy of Alzheimer's disease, if amnesia resulting from NMDA receptor over-activation takes place in this disorder. The cognitive enhancing potential of DCS may be limited to acute models.

**TRANSIENT AMNESIA FOR REACTIVATED MEMORY AFTER NMDA RECEPTOR BLOCKADE.** Jean Przybyslawski & Susan J. Sara, Institut des Neurosciences, Université P & M Curie, Paris, France.

Rats were trained in a radial maze to choose 3 fixed arms/8, a task requiring integration of distal spatial information contained in cues strategically placed around the maze. Pretrial injection of the noncompetitive NMDA receptor antagonist, MK-801, at a dose which had no effect on overt behavior, markedly disrupted the well-trained performance of the task. Surprisingly, the behavioral deficit persisted on subsequent drug-free trials, 24h and even 48h later. Posttrial injections produced the same proactive effects on performance on one or two subsequent daily trials. There was a temporal gradient of efficacy of drug treatment: delay of injection up to 2h post trial induced significant amnesia, when the rats were tested 24h later. A more difficult version of the task yielded a still longer temporal gradient. In this experiment rats were submitted to several trials within a single 5 min session and performance was reinstated within the session, indicating that the memory deficit was only partial and transient. Optimal performance could be rapidly reinstated with a few massed trials. Thus it appears that activation of a well-established memory circuit triggers cellular events which depend upon NMDA receptors for more than 2h. To what extent the entire postacquisition cascade of intracellular events associated with long term memory consolidation is recapitulated each time a memory is activated and reorganised is probably a function of the age and complexity of the memory and the amount of new information to be integrated into the circuit. These results provide physiological evidence that memory, as a dynamic process, undergoes continual reorganisation as a function of the ongoing experience of the organism.

Supported by the CNRS Programme Cognisciences and by the ESF Programme in Neuroscience.

## LATERALIZATION OF SEROTONINERGIC MODULATION OF LEARNING AND MEMORY IN RAT HIPPOCAMPUS

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The modulation of learning and memory after left or right microinjections of the selective 5-HT1A receptor agonist 8-OH-DPAT and of the 5-HT1A receptor antogonist NAN190 into the hippocampal CA1 area of male Wistar rats was studied. Microinjections of 8-OH-DPAT (1 μg) into the right or left CA1 hippocampal areas produced a significant decrease in the number of avoidances in shuttle box. The impairing effect of 8-OH-DPAT was more pronounced when injected into the right hippocampus compared to the left one. Microinjections of NAN190 (1 μg) into the right or left CA1 hippocampal areas produced a significant increase in the number of avoidances in shuttle box. Right microinjections of NAN190 tended to increase the number of avoidances compared to left injections. These opposite effects on learning and memory were more pronounced after injection of either of serotonergic agents into the right CA1 hippocampal area compared to the left. The stronger memory-modulating effect after injection of 8-OH-DPAT or NAN190 into the right CA1 hippocampal area suggests right-prefered asymmetry in the rat. The binding parameters of [$^3$H]8-OH-DPAT to 5-HT1A receptors in the left or right hippocampal membranes were compared. A tendency for a higher affinity (lower $K_d$) and lower $B_{max}$ of 5-HT1A receptors was observed in the right compared to the left hippocampus.

## CHANGES OF THE ACOUSTIC STARTLE REFLEX IN ONTOGENESIS

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The mammalian startle reflex is activated by a sudden intense stimulus and consists of a strong muscle contraction of the whole body. Changes in the acoustic startle reflex (ASR) have been studied in 12 Wistar albino and 15 hooded rats (male and female) for ten weeks starting from the sixth week of their life. The ASR was produced by a pair of brief acoustic pulses (duration 2 ms, amplitude 124 db) with interstimulus intervals range 2 to 12 ms. Changes in the ASR amplitude and latency during ontogenetic development were analyzed. Statistical analysis (ANOVA mixed design) revealed significant differences of the ASR characteristics between male and female rats. Generally, male subjects responded with a greater ASR amplitude even when normalized for body weight. In contrast no differences were found between Wistar and hooded rats. Both ASR parameters were strongly dependent upon the interstimulus intervals with the maximal response for the 2 ms interval. The differences in the ASR may be attributed to a development of the muscular system in both sexes of the rats.

## PHARMACOLOGICAL CORRECTION OF DISTURBED MEMORY BY NOOTROPIC DRUGS

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The influence of nootropic drugs of different chemical structure and mechanism of action (piracetam 200 mg/kg, meclofenoxate 50-100 mg/kg, nicergoline 1-4 mg/kg) on mnestic disturbances induced with electroconvulsive shock (ECS), scopolamine (2,5 mg/kg) and phenazepam (2,0 mg/kg) was studied in mice using passive avoidance test. All the investigated drugs possess distinct protective properties concerning different models of amnesia. In ECS-induced amnesia meclofenoxate was most effective, in scopolamine-induced amnesia - meclofenoxate and nicergoline, in phenazepam-induced amnesia all drugs were equally potent in profundity. It is supposed that neurophysiological mechanism of action of nootropic drugs is connected with directed activation of weakened memory trace of different etiology and its transmission into form receivable for transcription.

## ODD EFFECT AND SEX RELATED DIFFERENCES IN COGNITIVE ABILITIES.

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Hines (Hines et al., 1982; Hines, 1990) reported that even digits are processed faster or more accurately than odd digits. As in English the word "odd" has two meanings: that of not being divisible evenly by two and that of strange or bizarre, it is difficult to reconcile which of them is crucial for the observed effect. In the present experiment we examined whether the "odd effect" can be also observed in Polish language in which adjectives "odd" and "even" have no other than mathematical connotation. Twenty subjects (8 female and 12 male) were presented with three types of material: arabic numerals, words and dots arranged in die patterns. The stimuli (denoting digits from 1 to 8) were exposed centrally on a computer screen and remained visible until the subject made a response. The subjects were asked to judge whether digits were odd or even by pressing one of two buttons on the computer keyboard. The results showed significant "odd effect" (shorter reaction time for even stimuli). This effect, however, was sex and material dependent: in female subjects significant effect was found for words and in male subjects for dice. The results suggest that differences in oddness/evenness processing are not language specific. They are, however, related to different cognitive abilities of the two sexes.

Hines, T. et al. (1982) Communication presented at the 90th Annual Meeting of the American Psychological Association, Washington, DC.
Hines, T. (1990) Memory and Cognition, 18 (1), 40-46.

SPONTANEOUS RUNS THROUGH THE MAZE TO A SEXUALLY ACTIVE FEMALE IN MALE RATS

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Eight male rats were trained to run through the maze from the start compartment (SC) to the goal compartment (GC) where the incentive estrous female was tethered. The distal corridor of the maze and the GC as well as the GC and the SC were connected by one-way door enabling the male to run only in one direction. The contact with an incentive female lasted up to the end of mount bout (it is a clouster of 1 - 5 copulatory events separated only by the genital grooming or the female directed behaviour). After 3 - 4 experimental sessions the male was trained to open the door between the GC and the SC. Then, after the contact with female, he spontaneously passed from the GC to the SC and the new run started. In such a way the male performed the runs through the maze without any intervention of the experimenter. This phenomenon was observed not only after the end of the mount bout, but also after ejaculations as well as after the contacts when no copulatory behaviour was diplayed.

ROLE OF SHOCK AND CS RELATIONS IN FEAR AND SAFETY CONDITIONING IN RATS

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The Estes-Skinner /1941/ conditioned suppression method was used for testing influence of the time arrangements of the same pair of stimuli: an external CS and a grid shock, in three groups of rats. The shock presented concurrently with the CS termination /Forward Group/ or given in the middle of the CS action /Embedded Group/ resulted in suppression of bar presses for food indicating acquisition of fear to the CS. The same shock presented together with the CS onset /Backward Group/ caused enhancement of bar presses indicating acquisition of the opposite motivation: the state of safety during CS action. Similar enhancement of bar presses was also observed after termination of the forward CS . The acquired safety motivational state was maintained during test sessions when non-signaled shocks were presented in experimental context. This experiment indicate that contingency imposed by the time arrangements of the pair of stimuli /CS and shock/ determined classically conditioned motivation underlying the shape of the overt responding.

LESIONS TO AMYGDALA AND HIPPOCAMPUS ATTENUATE MEMORY ENHANCING EFFECT OF THE 3-7 FRAGMENT OF ANGIOTENSIN II

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We have previously shown that 6-OHDA lesion to the central amygdala (CA) abolishes, and to the hippocampus (HI) diminishes the facilitating effect of angiotensin II (AII) the retrieval of memory, whereas lesions to the other mesolimbic structures do not. The present investigation was aimed at the answering the question whether the dopaminergic projections from ventral tegmental area (VTA) and substantia nigra to CA and HI are responsible for the improving effect of the 3-7 fragment of AII [AII(3-7)] on memory retrieval. The bilateral 6-OHDA lesions to CA were made in 18 and to CA4 field of HI in 16 male rats before behavioural testing of the influence of intracerebroventricular AII(3-7) injection on recall in a passive avoidance situation. 32 additional rats served as sham-operated controls. Bilateral lesions to the CA totally abolished, while to HI significantly diminished facilitating effect of AII(3-7) on recall. Some increase of locomotor exploratory activity in CA lesioned animals as well as the decrease in HI lesioned rats were unlikely to interfere with the cognitive effects of AII(3-7). We therefore hypothesize that the anatomical substrate of facilitating retrieval of information activity of AII(3-7) is closely related to the dopaminergic projection from VTA and substantia nigra to CA and HI.

EFFECT OF INTRAHIPPOCAMPAL INJECTIONS OF COOPER SALTS ON RAT SPACE MEMORY

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It was previously found that copper salts injected into the lateral brain ventricle impair space memory of rats. In the present study a presumable neurotoxic effects of copper salts injected bilaterally into hippocampus were investigated on Wistar rats. Two copper salts: $CuSO_4.5H_2O$ and $Cu(CH_3COO)_2.H_2O$ were injected bilaterally into hippocampus in doses of 1-100 nmols at each side and space memory was determined using a water mze test. A significant impairment of the test performance was showed.

These results indicate that memory deficit induced in rats by icv injections of copper salts is at least in part due to their effect on hippocampus.

Supported by: Silesian Medical Academy grant NN-4-132/94

**Study on laterality effects in patients with focal lesions to the hippocampus and amygdala.**

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Numerous studies demonstrate that function of the medial temporal lobe structures is lateralized. It has been shown that patients with damage to the left hemisphere suffer from verbal memory deficits, whereas those with lesions to the right hemisphere from visuo-spatial impairment. It is not clear whether these laterality effects result from functional differentiation of temporal cortex or of hippocampal formation *per se*, since in most of these studies lobectomies involved large portion of both those structures. Seven patients with unilateral stereotaxic damage to the anterior hippocampus or/and medial amygdala and 11 control subjects participated in this study. Four experiments were run in which subject's task was to recall verbal or visuo-spatial material presented in a simultaneous or sequential manner. The data demonstrated that (1) patients performed significantly worse than controls in both visuo-spatial tasks but they did not differ from controls in verbal tasks, (2) the impairment did not depend on the side of the hippocampal lesion. The results suggest that memory function subserved by the hippocampus is not lateralized. Differential effects of left and right lobectomies found in previous study were, thus, probably 3due to the damage to temporal cortex.

**TEMPORAL MECHANISMS OF CONSCIOUSLY CONTROLLED ACTIONS: EVIDENCE FOR HEMISPHERIC DOMINANCE**

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Neuropsychological and electrophysiological evidence suggests that a substantial amount of time of presumably cerebral activity is required before-after stimulus occurrence-conscious experience sets in. This means that physical events and mental events do not co-concur, but that there is a time delay between the two; consciousness is always late. The aim of the present study was to look deeper into this relationship and to study potential hemispheric differences in temporal mechanisms that control consciously mediated actions. Fourteen right-handed students were presented binaurally with a computer - generated pure tone. The subject's task was to react as fast as possible or with a defined time delay to the stimulus by pressing a response-key by the index finger. Responses had to be given with the left index finger *(predominantly addressing the right hemisphere)* or with the right index finger *(left hemisphere)*. Requested delays varied between 200 and 750 ms in steps of 50 ms. (In young adults simple auditory reaction time is well below 200 ms). Subjects got feedback after each individual trial; by doing so they attempted to get as close as possible to the requested delays. For each subject and each delay time arithmetic mean and transformed standard deviations were calculated. The surprising result was that for the short delays (requested response times 350 ms) variability was much larger then for longer delays (above 350 ms). Interestingly, variability was considerably higher for the responses with the right index finger. Thus, precise temporal control on consciously mediated actions sets only in after a rather long delay (in some cases after half a second). Neuronal mechanisms underlining conscious temporal control of actions appear to be different in the two hemispheres, the left hemisphere showing surprisingly higher temporal variability. These observations are discussed with respect to speech perception.

SEMANTIC MEMORY IN THE EARLY STAGE OF ALZHEIMER´S DISEASE

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Semantic memory comprises knowledge of concepts, words and rules governing their usage. Semantic memory is susceptible to a little degree to changes occuring in the process of aging. On the other hand, it is very "sensitive" to brain diseases of degenerative type. One of them is Alzheimer´s disease. Its distinctive feature is an impairment in retrieving once encoded information apart from an impairment in acquiring new information.

The goal of our study was the evaluation of abilities to retrieve words of the specific categories aut of memory at different stages of Alzheimer´s disease.

200 patients were examinad. The comparative results of 48 patients obtained in the verbal fluency test were analyzed.

It was found that persons with Alzheimer´s disease demonstrated significantly lower level of preformance particularly in semantic categories.

Furthermore, it was concluded that both the number of committed errors and also their type describe the level of semantic memory impairment.

Detailed analysis of the results indicates that the memory impairment signs found in our study may have a diagnostic importance in defining the impairment region at different stages of Alzheimer´s disease.

**TEMPORAL LOBE STRUCTURES ARE ESSENTIAL FOR TIME LIMITED STORAGE OF SENSORY INFORMATION**

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Studies on human subjects who have undergone unilateral temporal lobectomies often show that the left-side lesions result in verbal memory deficit whereas right-side lesions impair memory of visual-spatial material. These effects were observed in a condition of relatively long (several minutes) delay between the stimulus onset and memory test. Little in known as to the effect of temporal lobe lesions on a time limited storage of sensory information. Our study addressed this question. Twenty patients who had undergone a unilateral temporal lobectomy for the relief of intractable epilepsy (10 subjects - left hemisphere damage, 10 subjects - right hemisphere damage) and 11 normal control subjects with no brain damage were tested. The subjects were presented with geometrical Vanderplas type figures which were exposed in pairs, each for 100 ms, one after another, with three different interstimulus intervals (ISI): 50 ms, 500 ms, and 3,000 ms. The subjects judged, whether the second stimulus was the same as, smaller or bigger than the first one. The first stimulus in each pair was exposed unilaterally, randomly in the left (LVF) or right (RVF) visual field, and the second one in the centre of the screen. In shorter ISI conditions (50 ms and 500 ms) the RH-damaged group performed worse than both the control group and the LH-damaged group, whereas the LH damaged group did not differ significantly from controls. The differences were mainly due to lower scores of the RH damaged group in the LVF presentation condition. In the control group the LVF presentations resulted in higher performance scores than the RVF presentations. In 3,000 ms ISI condition neither the effect of group nor the effect of visual field were statistically significant. Our results show that temporal lobe structures are essential for time limited storage of sensory information which is lateralized to the right hemisphere.

## Poster sessions - Electroencephalography

### GABAERGIC / CHOLINERGIC INTERACTION IN PRODUCTION EEG THETA-LIKE OSCILLATIONS IN THE LIMBIC CORTEX IN VITRO.

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Since our first demonstration of cholinergic-induced theta rhythm in the hippocampal formation slices, this in vitro experimental model has been successfully used in a number of investigations concerning physiology and pharmacology of rhythmic slow activity. In the present study we emphasize the role of GABA-ergic receptors in inducing in vitro theta-like slow waves. Specifically, we provided evidence for GABA-A-ergic/M1 muscarinic interaction in limbic cortical network (hippocampal formation and entorhinal cortex) responsible for theta production: bicuculine - GABA-A antagonist facilitated the effect of cholinergic agonist - carbachol in inducing in vitro theta both in the hippocampal and entorhinal slice preparations. This bicuculine-carbachol induced theta rhythm was antagonized by M1 muscarinic antagonist - pirenzepine and GABA-A agonist - muscimol. Galamine, M2 receptor subtype antagonist was found to be completely ineffective in blocking bicuculine-carbachol induced theta.

### THE RELATIONSHIP BETWEEN THE SPONTANEOUS RSA RECORDED FROM THE HIPPOCAMPAL FORMATION AND ENTORHINAL CORTEX IN CATS.

M. Błaszczyk, R. Grabowski, B. Eckersdorf,
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Theta rhythm is the most synchronized sinusoidal-like EEG waveform that can be recorded from the mammalian brain. Generally two regions of the mammalian limbic cortex are considered to produce the most pronounced theta rhythm: hippocampal formation (Hipp) and entorhinal cortex (EC). Most of the studies concerning the physiology and pharmacology of theta rhythm were performed on rodents, including rats, rabbits and guinea pigs.

The aim of the present study was to characterize the physiological features (amplitude, frequency, power, and the waveform correlation) of EC spontaneous rhythmic activity in freely moving cats. The second aim was to compare EC rhythm to the theta rhythm recorded simultaneously from the Hipp.

We found the spontaneous EC theta rhythm to be of similar frequency and amplitude, but not closely corelated to the theta oscillation recorded from Hipp. The origin of both Hipp and EC rhythms is discussed.

### THE SPONTANEOUS THETA ACTIVITY RECORDED FROM HYPOTHALAMUS POSTERIOR IN THE CAT *IN VIVO*.

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Theta rhythm is one of the most synchronous slow wave EEG patterns generated in the mammalian limbic cortex. The major generators of these waveforms are located in the hippocampus and entorhinal cortex. The latest studies have demonstrated, that hypothalamus is involved in modulation of hippocampal theta in the rat. However, the role of hypothalamus posterior (Hpt) in generating of theta oscillations in the cat has never been examined.

The aim of our studies was to record the spontaneous theta rhythm from Hpt region in freely moving cats. On the basis of the EEG spectral analysis, we found the area of posterior hypothalamus to produce very well synchronized, high amplitude theta activity. In the second step we recorded theta oscillations from hypothalamus and hippocampal formation simultaneously. The obtained data were subjected to computer Fast Fourier Transform (FFT) and waveform correlation analyses. It lets us estimate parameters of hypothalamic theta rhythm and compare these with oscillatory activity recorded from hippocampal formation.

### STUDY ON THE CHANGES IN THE EEG POWER SPECTRA OF THE θ-1 (7-12 Hz) AND θ-2 (4-7 Hz) ACTIVITIES IN HIPPOCAPUS AND NUCL. POSTERIOR HYPOTHALAMI FOLLOWING CHRONIC STRESS IN RATS

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The θ-2 (4-7 Hz) and θ-1 (7-12 Hz) are the most prominent rhythmic activities in the rats hippocampus [HC], which is a crucial structure in the regulation of the stress response. Their spectral characteristics depend on the general level of arousal and motivation. On the other hand the dorsomedial posterior hypothalamic region [dmPH] is known to be a triggering zone for the hippocampal θ-activities and an "intervening part" of the reticulo-septo-hippocampal θ synchronizating system. That's why using an experimental procedure for chronic stress in rats based on 7-day REM sleep deprivation we studied the changes in the power spectra of θ-1 and θ-2 activities in freely mooving rats with chronically implanted electrodes in HC and dmPH. The data obtained from controls, 1st, 4th and 7th days of stress challenge, which correspond to the three general phases of the stress reaction were analysed. The results show a moderate decrease in the EEG power of θ-2 in HC at the 1st day of the challenge (alarm reaction), followed by a persistence at the same power level in the 4th and 7th day (phases of resistance and exhaustion). The time course of the changes of θ- 1 is similar, except of the moderate increase of the EEG power at the 7th day. In dmPH there is a decrease of θ-2 EEG power during all phases of the stress reaction. The changes in θ-1 are alike, but the decrease of the EEG power during all three phases of the stress reaction are more pronounced. In conclusion, the present data suggest that independently of the different neurotransmitter nature and physiological roles of the θ-1 and θ-2 activities, they react similar under conditions of chronic stress.

**C57 Black/6 J inbred mice as a "natural model" for studying anxiolytic effects**

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It is known that a marker of anxiolytic effect of drugs in the EEG is the slowing of theta-activity in animal brain. In our experiments it is shown that C57 Black/6 J Sto inbred mice possess an extremely slow basal EEG activity (3.58±0.15 Hz) as compared to other rodents which is within the range of delta-waves but not the theta-band. At the same time these mice are characterised by active type behaviour under conditions of "open field" and "conflict situation". Diazepam (1 & 5 mg/kg) evoked an increase but not decrease in the frequency of basal rhythmical activity in the mice and induced anxiogenic effect at the behaviour level. It is possible also that very slow basal EEG activity is a marker of very low sensitivity of C57 Black/6 J mice to convulsive influence.

**CHANGES IN THE EEG POWER SPECTRA OF THE CORTICAL DELTA (0.5-3.5 Hz) AND BETA 2 (24-36 Hz) ACTIVITIES UNDER CONDITIONS OF CHRONIC STRESS IN RATS**

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The cortical beta 2 (24-36 Hz) activity, which is connected with the cortical activation, is thought to be generated by the brain cholinergic mechanisms, which also participate in the stress reactions. On the other hand it is known that delta (0.5-3.5 Hz) waves, which are connected with "drowsy" behaviour, are produced by cholinolytic drugs. That's why using an experimental procedure for chronic stress, based on 7 day REM-sleep deprivation, which might provide an useful tool for investigation on stress resistance and stress-related disorders, we studied the changes in the power spectra and in the bandwidth of the beta 2 and delta activities in motor cortex (MC) in freely mooving rats. The data obtained from controls, 1st, 4th, and 7th days - which correspond to the three general phases of stress reaction were analyzed. The results show that in the 1st and 4th day of the challenge (alarm reaction and phase of resistence) there is a moderate increase in the beta 2 EEG power, which however is connected with well expressed decrease of the bandwidth. At the 7th day (phase of exhaustion) there is pronounced decrease of both EEG power and bandwidth. On the contrary there is pronounced increase in the delta EEG power since the first day of the stress challenge, which is most prominent at the 7th day. This results suggest exhaustion of the cortical mechanisms activation and probably of the cholinergic neurotransmission connected with them following chronic stress.

This study and its companion (Maslarov D. Totev A. et al) were sponsored by the National Found "Scientific Research" and the National Centre for Interdisciplinar Human Studies.

**POWER SPECTRAL ANALYSIS OF EEG IN LATERAL HYPOTHALAMIC INSOMNIAC RATS.**

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As we found in our previous experiments, electrolytic lesions of the lateral hypothalamus (LH) increased EEG waking with simultaneous reduction of the amount of slow wave sleep and paradoxical sleep (insomnia). After the lesion EEG pattern showed several abnormalities difficult to assess by standard visual inspection of the EEG records. Therefore in the present study a power spectral analysis of EEG was applied to diagnose EEG changes after LH damage.

In male Wistar rats bilateral electrolytic LH lesions were performed and hippocampal and cortical EEG was recorded before and after the lesions. One-hour samples of EEG taken from the light part of the day were fed to a computer and power spectral density was calculated off line by Fast Fourier Transform routine at delta (0.4-4.0 Hz), theta (4.1-8.0 Hz) alpha (8.1-12.0 Hz), and beta (12.1-25.0 Hz) bands as well as at 1 Hz bands from 0.4 to 25.0 Hz (the whole registered spectrum).

The following power density distribution was found after the lesion: 55.8% of the whole power density for delta, 33.5% for theta, 6.9% for alpha and 3.8% for beta frequency. Comparison with the prelesion baseline values revealed an increase of a power density in 4.0-5.0 Hz and 5.0-6.0 Hz (theta frequency) and also in bands exceeding 12 Hz. These results correspond with an increase of waking time, and an increase of theta related motor activity found previously after LH damage.

## Cyclic changes in human sleep structure[1)]

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In this study cyclic changes of human sleep structure were examined. For whole night polysomnograms of 35 healthy volunteers of both sexes (23 females, 12 males), aged 19-26 (mean 23.1) manual hypnograms were created (1) and divided into NREM-REM cycles. EEG signals from C3-A2 derivation were analyzed by computer. The Fast Fourier Transform (FFT) method was used in 0.4-25.0 Hz frequency band. For consecutive NREM-REM cycles, individual sleep stages and averaged polysomnogram parameters were analyzed. These parameters were as follows: EEG voltage, EEG power density frequency band width and EEG power density contents for delta, theta, alpha, sigma and beta waves.

For consecutive sleep cycles, clear decrease in NREM sleep duration, specially slow wave sleep (stages 3 and 4) duration was obtained. Also a decrease in EEG voltage ($p < 0.001$) and delta waves power density ($p < 0.001$) was obtained. For consecutive sleep cycles, an increases in: REM sleep duration, EEG power density frequency band width ($p < 0.001$) and theta and alpha waves power density contents ($p < 0.001$) were obtained. In consecutive sleep cycles high amplitude delta slow waves are replaced by higher frequency and lower amplitude waves. Thus stages of NREM sleep are replaced by stage REM. The above results prove that sleep gets shallow in consecutive NREM-REM cycles.

Reference:
1. Rechtschaffen A. and Kales A. (Eds.), U.S. Government Printing Office, Washington, 1968.

[1)] This work was supported by a KBN grant nr 8 T11E 005 08 in 1995.

THE INFLUENCE OF AMITRYPTILINE ON THE PATTERN OF REM SLEEP IN DEPRESSION

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Parameters of REM sleep //latency of REM stage /LR/, REM time/RT/, REM density/RD/and latency of eye movement/LEM/, and mean latency of eye movement/M-LEM/ //were measured in the polysomnograms of 12 depressive patients under 2 conditions a. after 2 weeks wash out period, b. in 7th day of treatment with optimal dosis of amitryptiline /125-300 mg/.
The significant prolongation of LR /p 0.02/, LEM1 /p 0.025/, and M-LEM/p 0.018/, increase of RD/p 0.04/and decrease of RT/p 0.04/during amitryptiline treatment were found.
These results and previous data concerning the changes of the pattern of human REM stage under different conditions were discussed. All results suggest, that the REM sleep pattern may be a sensitive indicator in diagnosing of the Alzheimer disease.
We are planing to do such study.

## Computer paperless sleep analyzing system[1)]

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The computer, paperless system for continuous monitoring and analyzing of human polysomnogram was elaborated and carried out. It consists of the polygraphic amplifier connected through the 12-bit analog-digital converter with a standard IBM/PC computer and the software for monitoring and analyzing of the polysomnogram. From derivations for 2 EEG channels, 2 EOG channels, EMG, ECG, respirogram and motor activity channels data are converted into digital value with the sample frequency of 102.4 Hz. Then they are analyzed on-line using the amplitude-frequency classical analysis and spectral analysis by Fast Fourier Transform (FFT) method. Results of these two analyses and polysomnogram digital data and computer generated hypnogram are recorded on the hard disc. For the purposes of further archive storage the magneto-optical disc was applied. This system can be freely reproduced and all polysomnographic data analyzed.

32 all-night, correct human polysomnograms were examined using visual analysis (1) and computer paperless sleep analyzing system. An agreement of 85.7% was obtained for both these analyses.

Reference:
1. Rechtschaffen A. and Kales A. (Eds.), U.S. Government Printing Office, Washington, 1968.

1) This work was supported by a KBN grant nr 8 T11E 005 08 in 1995.

# Poster sessions - Glia

INTRACELLULAR pH ($pH_i$) REGULATION IN CULTURED MICROGLIAL CELLS FROM MOUSE BRAIN

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Microglia, the resident macrophages of the CNS are activated by any injury or pathologic event. The signal cascade controlling the multi-step activation from the resting to the fully activated form are at present unknown. pH could interfere with such as cascade twofold: firstly, extracellular pH changes are observed in many pathologic events including ischemic insults; secondly, intracellular pH changes might be part of a signal cascade.We therefore set out to study basic mechanisms by which intracellular pH is controlled or affected in microglial cells from embryonic mouse brain using the pH-sensitive fluorescent dye BCECF-AM. We used a classical approach to acidify cells by a pulse of $NH_4^+$ ( 4-5 min; 20 mM) and studied the subsequent $pH_i$ recovery. In $HCO_3^-$-free saline, pH regulation was dependent on extracellular $[Na^+]$ and sensitive to amiloride indicating the involvement of the $Na^+/H^+$ exchanger. In $HCO_3^-$ - containing solution amiloride slightly slowed but did not block $pH_i$ recovery; in $Na^+$-free saline, however, the recovery was completely blocked. The involvement of a $Na^+$ -dependent $Cl^-/ HCO_3^-$ exchanger was infered from the observation that removal of $Cl^-$ or application of 1 mM furosemide decreased the recovery rate. The recovery (in the presence of $HCO_3^-$) was completly blocked in the presence of 1 mM DIDS suggesting the additional presence of a $Na^+/ HCO_3^-$ exchanger. We conclude that microglial cells express a distinct set of pH regulatory carriers which act in concert for $pH_i$ homeostasis.

THE LESION-INDUCED AND AGING-INDUCED ASTROGLIOSIS DIFFER IN THEIR SUSCEPTIBILITY TO PHARMACOLOGICAL INTERVENTION

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We have previously shown that the lesion-induced astrogliosis, demonstrated by the increase in GFAP content, staining and the number of GFAP (+) cells is a differentiated phenomenon, as the intensity of changes of the particular marker of astrocytic activation is not uniform and depends on the type of stimulus and the brain structure investigated (Jegliński et al. J Neurosci Res 40, 1995). We have also reported a structure-differential susceptibility of lesion-activated astrocytes to pharmacological treatment (GM1-ganglioside, previously demonstrated to exert beneficial effects on post-lesion neuronal changes - Oderfeld-Nowak et al. J Neurochem 61, 1993). Now we have compared the two forms of gliosis: lesion-induced (7 days after lateral fimbria transection) and aging-associated (24-month-old rats) in relation to their responsiveness to phosphatidylserine (PS), an agent that was shown to diminish some aging-induced neuronal impairments. Using immunoblot technique we have measured the GFAP content in the septum (sp) and hippocampus (hp) - structures undergoing intense astrogliosis after fimbria transection, and in striatum, corpus callosum, hp, sp from aged brains. Following groups of animals were investigated: - 3-month old (mo), saline-treated; - 3mo PS-treated; - 24-mo saline-treated; - 24-mo PS-treated; - 3-mo FF saline-treated; - 3-mo FF PS-treated. The dose of PS was 15mg/kg (*ip*) daily for 8 days. The results show that spontaneusly elevated GFAP in the aged rat brain is responsive, its content being further enhanced, to PS treatment, while the lesion-activated astrocytes of the adult rat brain does not show such a susceptibility. This distinct differences in the response to pharmacological treatment between lesion-induced and aging-induced astrogliosis further strengthen the idea that gliosis is not a stereotypic phenomenon and may depend on the astrocytic heterogeneity and different character of astrocytic activation

TRIMETHYLTIN-INDUCED NEUROTROPHIC ACTIVITY IN ASTROCYTES OF THE RAT BRAIN

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Astrocytes demonstrate a multitude of responses to brain insults, including hypertrophy and increased glial fibrillary acidic protein (GFAP). A view that astrocytes in response to brain injury may produce neurotrophic factors is recently gaining credence. The hypothesis that reactive astrocytes in trimethyltin (TMT) intoxicated rats may have the capacity to express neurotrophic, specifically nerve growth factor-like (NGF-like) activity was examined in this study. TMT is a neurotoxin of industrial and environmental importance. It destroys specific subfields of the hippocampus in the rat causing degeneration of pyramidal cells. Adult, male Wistar rats were injected with a single dose of 8mg/kg i.p of TMT and examined at 7 and 21 days after injection. We have found that NGF-like immunoreactivity (NGF-LIR) in pyramidal neurons was reduced which was better visible at 21 day. At the latter time point many reactive astrocytes, especially in CA1 subfield and in molecular layer of the dentate gyrus became NGF-immunoreactive. Gliosis, as evidenced by GFAP immunoreactivity and immunoblotting, was seen all over the hippocampus. ELISA assay showed a slight decrease of NGF content in the hippocampus as compared with the control after 7 days, while after 21 days a highly increased content of NGF (over 150% of control) was found. Of particular interest is the early induction in reactive astrocytes of high affinity NGF receptor (trkA) immunoreactivity in the same gliotic areas as the subsequent induction of NGF-LIR. After 21 days much more astrocytes became trkA immunopositive. Interestingly, an induction in microglia of interleukin-1beta, known to stimulate NGF synthesis, has been observed in the same gliotic areas already after 7 days with a noticeable increase after 21 days. The experiments are in progress to verify whether the induced neurotrophic activity in reactive astrocytes represents a transient phenomenon and vanishes with gliosis at later postinjection periods. The trophic activity of astrocytes may contribute to survival of some pyramidal cells as well as may play a role in plastic rearrangements reported to occur in dendrites and nerve terminals in dentate gyrus few weeks after TMT intoxication.

**Unusual interaction of astrocytes and oligodendroglia in human and experimental Creutzfeldt-Jakob disease and scrapie**

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Transmissible spongiform encephalopathies or prion diseases are regarded as disorders characterized by a paradigmatic absence of any immunological reaction toward the infectious agent. We report here an unusual interaction between reactive astrocytes and oligo- and microglial cells in a brain biopsy of human Creutzfeldt-Jakob disease (CJD) and in experimental CJD in mice and in scrapie in hamsters. Such an interaction has been reported so far only in early lesions of multiple sclerosis and their presence in both naturally occurring and experimentally induced TSE may suggest some abbortive response toward the agent. All brain biopsy samples available for ultrastructural study were optimally fixed. Reactive astrocytes were easily identified because of innumerable glial filaments and oligodendrocytes were identified as such because of electron-dark cytoplasm, no intermediate filaments but numerous microtubules. GFAP-immunohistochemistry and low power electron microscopy revealed numerous examples of astrocytes and oligodendroglial cells in a close apposition with cellular membranes of one cell modelled on those of another. At higher magnification, both types of cells were connected by adhesive plaque junctions. More complex structures were also seen. Astrocytic processes penetrated between two oligodendroglial cells or such a cell was observed to be completely surrounded by astrocytic cytoplasm. Analogous phenomenon was identified in mice infected with the Fujisaki strain of CJD agent and hamsters infected with 263K or 22CH strain of scrapie agent. In the latters, the astrocytic cytoplasm was clearly penetrated by oligodendroglial processes.

Liberski PP. The enigma of slow viruses: facts and artefacts. *Arch.Virol.* 1993 (suppl 6): pp 267

**Supported by the KBN grant and a part of EC Biomed-1 project "Prion disorders"**

**GFAP EXPRESSION IN ASTROCYTES PROLIFERATING IN THE RAT BRAIN INJURED AT DIFFERENT AGE.**

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In brains of newborn, 6, 14 and 30-day-old rats a lesion was made. Following the lesion $^3$H-thymidine was injected and the animals survived four hours after the injection. Brain sections were immunostained for glial fibrillary acidic protein (GFAP) and subjected to autoradiography. Thereafter, changes in the distribution of GFAP-immunopositive (GFAP+) and autoradiographically labeled astrocytes were recorded.

In rats injured neonatally, no proliferation of GFAP+ astrocyte was found. In the group of 6-day-old rats only occasional proliferating GFAP+ astrocytes were recorded. In 14-day-old rats, the spatio-temporal pattern of reactive proliferation of GFAP+ astrocytes was similar to that seen in 30-day-old rats.

The study provides evidence that the reactive astrocytes proliferating in the rat brain do not express GFAP during the first postnatal week.

*Supported by the State Committee for Scientific Research, DS/IZ/94/N*

# Poster sessions - Neuropathology

ACCUMULATION OF ALZHEIMER'S ß-AMYLOID PEPTIDE IN CULTURED SMOOTH MUSCLE CELLS IS REGULATED BY ß-PEPTIDE CARRIER PROTEINS. Bozena Mazur-Kolecka*, Janusz Frackowiak*, Henryk M. Wisniewski*, Mark R. Emmerling**.*NYS Institute for Basic Research, 1050 Forest Hill Rd, Staten Island, NY 10314, Parke-Davis, 2800 Plymouth Road, Ann Arbor, MI 48105.

Smooth muscle cells (SMCs) are engaged in deposition of vascular ß-amyloid in Alzheimer's disease (AD), Down's syndrome and in aged dogs. Vascular SMCs isolated from brain of AD patients and aged dogs were found to accumulate intracytoplasmic deposits immunoreactive for ß-peptide and apolipoprotein E (apoE). The accumulated ß-peptide was identified by immunocytochemistry as comprising whole N-terminal sequence. Secretion and accumulation of ß-peptide in vitro could be influenced by factors present in body fluids. The accumulation of ß-peptide was enhanced in the presence of whole serum from amyloid-angiopathy affected dogs and reduced in the presence of cerebrospinal fluid (CSF). ApoE3 and E4, at physiological concentrations induced ß-peptide accumulation in SMCs in a dose dependent manner. The intracytoplasmic granules accumulated in SMCs treated with apoE3 or E4 were also immunoreactive for apoE. This finding together with the increased intracellular content of apoE suggests that the mechanism of induction of ß-peptide accumulation may be mediated by uptake of apoE complexed with ß-peptide. The cellular content of ß-amyloid precursor protein was not affected. Transthyretin at physiological concentrations blocked the ß-peptide accumulation induced by apoE3 and E4 and reduced the spontaneous and serum-mediated accumulation. It is suggested that certain ß-peptide carriers present in serum and CSF can influence the fate of ß-peptide by either keeping it in the soluble form or by facilitation of its internalization and accumulation in SMCs. The observed effects of carrier proteins on cultured cells suggest that these proteins may also participate in development of the ß-amyloidosis in vivo.

INTENSITY OF PATHOLOGICAL CHANGES AND GLIA RESPONSE IN THE HIPPOCAMPAL FORMATION IN ALZHEIMER'S DISEASE

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The hippocampal formation was studied in 11 brains of patients with Alzheimer's disease (AD) aged from 65 to 91 years and in six brains of elderly subjects without signs of dementia aged from 61 to 89 years. The hippocampal formation was cut frontally into 8-µm-thick sections and stained either with cresyl violet or with immunocytochemical methods using antibodies: 4G8 for β-amyloid, Tau-1 for neurofibrillary tangles, anti-ferritin for microglial cells and anti-GFAP for astrocytes. The density of neurons, neurofibrillary tangles, senile plaques as well as microglial cells and astrocytes was estimated in various regions of the hippocampal formation using the morphometric methods.

The most massive neuronal loss was observed in layer II of the entorhinal cortex (55.9%), parasubiculum (48.9%), dentate gyrus (32.5%) and sector CA1 of the hippocampus (28.9%). The pattern of neurofibrillary pathology was similar. In layer II of the entorhinal cortex 62.8% of neurons revealed neurofibrillary tangles, in the sector CA1 - 32.3% whereas in the subiculum - 17.7% . Contrary to that, the largest number of β-amyloid plaques was found in the presubiculum ($32.5 \pm 6.3/mm^2$), layers III and IV of the entorhinal cortex ($28.8 \pm 3.9/mm^2$), and molecular layer of the dentate gyrus ($23.6 \pm 3.2/mm^2$).

A significant increase in the density of astrocytes was observed in the whole hippocampal formation. Most of the astrocytes in AD affected brains were hypertrophied and showed much stronger GFAP immunoreactivity than normal ones. Contrary to that, a significant loss of microglial cells was noted especially in structures in which a large number of senile plaques was observed e.g. the dentate gyrus. In areas characterized by the presence of a large number of senile plaques and neurofibrillary tangles the loss was moderate, whereas in layer II of the entorhinal cortex a significant increase was noted.

WHITE MATTER CHANGES IN ALZHEIMER'S DISEASE BRAINS

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White matter changes (other then leucoaraiosis LA) within Alzheimer's (AD) brains shown by MRI remain unexplained. Vascular alterations may account only for some white matter pathology in AD brain. Other may be due to the presence of amyloid or cytosceletal pathology of neurons.
The aim of our study was to elucidate pathogenesis of myelin alterations in AD. Five - AD, 5 - vascular dementia (VD) cases, and 5 - cases of mixed pathology (AD and VD) were compared with 5 non demented, all matched in age. Frontal cortex white matter was chosen for assessing myelin pathology. All fragments were processed routinely in paraffin and examined in Klüver-Barrera, HE staining and immunohistochemical labelling against MBP, β-amyloid, tau-1, to diagnose myelin, vascular, and Alzheimer's type of degeneration. Myelin palloring and focal patchy changes were analyzed. Vascular congophilic and arteriosclerotic changes were diagnosed. The presence of amyloid deposits - focal and diffuse, with or without cytosceletal pathology - were described.
Myelin focal changes are related to age and neurofibrillar pathology, but surprisingly - not to amyloid. Wallerian degeneration seems at this stage on hypothesis worth pursuing.

**NMDA-INDUCED PROCESSING OF β-APP IN HIPPOCAMPUS IN VIVO AND IN VITRO**

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Accumulation of β amyloid protein (βA) and β-amyloid precursor protein (β-APP) in brain has been found in Alzheimer disease and in several neurodegenerative diseases such as neuronal ceroid lipofuscinosis, Down disease, mukopolisacharidosis and brain ischemia. Abnormal proteolytic degradation of β-APP may result in accumulation of potentially neurotoxic βA. The role of various receptors in regulation of β-APP processing has been suggested. The aim of this study was to establish early changes in the expression of some domains of β-APP in the rabbit hippocampus after 20 min application of 1 mM NMDA via transhippocampal microdialysis probe, and to determine if NMDA receptors and $Ca^{2+}$ ions regulate proteolysis of β-APP in superfused rat hippocampal slices in vitro. Separation of proteins from the hippocampal tissues and superfusates by electrophoresis was followed by their Western blot analysis using antibodies against some domains of β-APP. Application of NMDA to the rabbit hippocampus induced significant changes in the pattern of β-APP fragments in the tissue indicating enhanced processing of this protein. To detect the role of NMDA receptors in modulation of β-APP processing, adult rat hippocampal slices were superfused with NMDA containing media, and immunoreactivity of soluble β-APP derivatives was measured in superfusates. 100 µM and 250 µM NMDA induced a release of amino-terminal β-APP derivatives and a fragment of βA, which was dose-dependent, sensitive to 1 µM MK-801 and 100 µM CPP, and to $Ca^{2+}$ presence. Release of carboxy-terminal fragments of β-APP was not detected. These data indicate that stimulation of NMDA receptors induces cleavage of β-APP. Mechanisms of these effects will be discussed.
*Supported by the KBN grant # 4.P05A.059.08.*

**The majority of dystrophic neurites in Alzheimer disease (AD) and all neurites in Gerstmann-Straussler-Scheinker disease (GSS) do not contain paired helical filaments and do not express MAP tau (τ)**

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Dystrophic neurites (DN) and amyloid plaques comprize the major neuropathological feature of both transmissible (GSS) and non-transmissible (AD) cerebral amyloidoses. The ultrastructural correlate of neurofibrillary tangles of AD are paired helical filaments (PHF) composed predominantly of MAP tau (τ). Besides NFT, PHF are regarded to be a major constituent of neuropil threads and DN. Because τ-immunohistochemistry is so reliable method to detect PHF, and because electron microscopy is no longer that "fashionable", there is a trend to identify τ-immunopositive neurites with those DN defined on a basis of earlier ultrastructural studies. Using quantitative thin-section transmission electron microscopy, we report here that, unexpectedly, majority of DN in AD do not contain PHF and thus they resemble DN of GSS, a transmissible prion disease in which τ-immunoreactivity is absent, except for Indiana and Swedish families. As a result, these DN, albeit widespread, are hidden from τ-immunohistochemistry and are neglected by current neuropathological resaerch. By thin-section transmission electron microscopy, we studied DN for the presence of PHF and lysosomal electron-dense bodies (LEDB). In AD, majority of DN (285 of 332) contained LEDB while only 91 contained PHF; 56 contained both structures. Of 141 DN associated with plaques, 126 contained LEDB, 42 contained PHF and only 27 contained both. Of 191 DN not associated with plaques, 129 contained LEDB, 20 contained PHF and 42 contained both structures. In CJD and GSS, all DN irrespective whether associated with plaques, or not contained LEDB. These quantitative EM studies indicated that majority of DN in AD and all DN in CJD and GSS contain LEDB and not PHF.

**Supported by the KBN grant and a part of EC Biomed-1 project "Prion disorders"**

**AMYLOID β-PROTEIN PRECURSOR IN BRAIN AFTER COMPLETE CEREBRAL ISCHEMIA**

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The distribution of amyloid β-protein precursor (APP) was investigated immunocytochemically in rats subjected to 10 min complete cerebral ischemia (CCI) induced by *cardiac arrest*. APP immunostaining was found extracellularly and intracellularly. Multiple extracellular APP immunoreactive deposits around and close to the vessels appeared as soon as 3h after CCI. Extracellular accumulation of APP occurred frequently in the hippocampus, cerebral and cerebellar cortex, basal ganglia and thalamus and rarely in the brain stem. These deposits were labelled with antibodies against the N-terminal, β-amyloid peptide, and C-terminal domains of APP. Our data suggests that either proteolytically cleaved fragments of the full-length APP or the entire APP molecule accumulates extracellularly after CCI. This finding may not only implicate the participation of APP in postischemic tissue damage but also suggest the involvement of pathomechanisms operating in ischemia in Alzheimer's disease pathology.

**EXTRAPYRAMIDAL SIGNS IN ALZHEIMER'S DISEASE**

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Extrapyramidal signs were assessed in 112 patients satisfying NINCDS/ADRDA criteria for probable Alzheimer's disease. They were present in 50% of the patients. The most common features of them were bradykinesia and rigidity. Resting tremor was rarely encountered. There were no significant differences in age of onset, symptom duration, presnce of primitive reflexes and psychotic symptoms between cases with and without extrapyramidal signs. Whereas presnce of extrapyramidal signs was associated with statistically significant greater dementia severity.

**DETECTION OF OLIGOCLONAL BANDS IN THE CEREBROSPINAL FLUID BY ISOELECTRIC FOCUSING WITH THE PHAST- STYSTEM IN NEUROLOGICAL DISEASES.**

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The demonstration of the presence of intrathecal synthesis of IgG as oligoclonal bands (OB) in the cerebrospinal fluid (CSF) is an important indicator of inflamatory process in nervous sytem, esspecially in cases of multiple sclerosis (MS). The Phast System equipment was used for detection of OB in CSF. Separation was performed on poliacrylamide gels pH 3-9.7 by isoelectric focusing and subsequent silver staining according to manufacter's instruction. One microliter of serum and one microliter of cencentrated (15mg/l) CSF was found optimal. We tested the CSF of 237 patients with neurological diseases : multiple sclerosis n=107, inflamatory diseases (ID) of CNS n=55, other noninflamatory diseases (OND) n=74.
Oligoclonal IgG were found in 100 (93,5%) MS, in 31 (56.4%) cases of ID and only in 2 cases of OND. We compared the latter with mathematical formulas fidings indicating intrathecal synthesis of IgG. In some cases in which OB were absent in isocletrofocusing we used for detection microimmunobloting technique by Phast Transfer equipment. We can conclude that isoelectrofocusing has highest sensitivity of detection of intrathecal synthesis of IgG, but in some cases we need used microimmunobloting for higher sensitivity. Detection of IgG with Phast System equipment is easily performed in the laboratory and reveals good reproduccibility. This method is relatively quick.

## INTERLEUKIN-6 SECRETION BY GLIAL CELLS IN PARKINSON'S DISEASE MOUSE MODEL

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Microglial and astroglial cells could play an important role in the degeneration and regeneration processes in the nervous system in several neuropathological conditions. Loss in the number of dopaminergic cells in substantia nigra (SN), decrease of dopamine content in the striatum and activation of microglial cells were found in brains of patients with Parkinson's disease (PD). Similar changes were observed in mouse model of PD induced by intoxication with MPTP. In the present investigation we have studied microglial and astroglial response (observing the amount and morphology of cells and secretion of cytokines) following MPTP treatment in one group and after pretreatment with pargyline before every MPTP injection in the second group of mice. Microglial cells were stained by lectin derived from Griffonia simplicifolia seeds, astroglial cells by anti-glial fibrillary acidic protein antibody. The secretion of interleukin-6 (Il-6) was estimated by anti-Il-6 antibody. The activation of microglia in SN and striatum was observed from the 1st day following MPTP treatmnet. From the 2nd day the reaction of astroglia was seen. These effects were blocked by pargyline pretreatment. Staining for Il-6 on the microglial cells was positive on the 1st and 2nd day following MPTP treatment, from the 3rd day the astroglia started to produce this cytokine. Our results suggest that microglial and astroglial cells secreting inflammatory cytokines are involved in the pathological process following MPTP intoxication which leads to the damage of dopaminergic neurons.

## COGNITIVE SYMPTOMATOLOGY IN THE NORMAL PRESSURE HYDROCEPHALUS

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Hakim introduced a symptomatic syndrome associated with the normal pressure hydrocephalus. He described the triad of symptoms: gait disturbances, vrinary incontinence and mental disturbances. While studying the cognitive processes he paid special attention to: psychomotor deterioration, difficulties in memory , decrease in concentration preformance, akinetic mutism. In consideration of a significant divergence of results, lack of precise methods, methodological and terminological doubts we performed very thorough neuropsychological tests in our study. They were aimed at the description of cognitive activities and the attempt to determine kinds of disturbances specific to this disease. We examined a group of 60 patients that were treated operatively in the Department of Neurosurgery. This group was found to be heterogenous. There were persons with severe disturbances of their conciousness state, loss of orientation in time, space and situation.

On the other hand, there were persons with intact conciousness that did not exhibit any signs of mental deterioration. The results in this second group of patients were subject of the thorough analysis. It suggests that the disturbances are of selective character. Most of all they affect learning new information and visual-spatial functions.

## Different pathomechanisms of nerve fibre degeneration in the brain of rabbits treated with vincristine

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Vincristine (VCR) is a potent anti-cancer drug used also in the treatment of various chronic inflammatory diseases. Neurotoxic side-effect of VCR (peripheral neuropathy) limits the dosage and duration of the therapy. VCR penetrates the blood-brain barrier poorly, and pathomechanism of neurotoxic changes in the brain parenchyma remains not clear. In the present study ultrastructure of the brain nerve fibres was examined following intraperitoneal single injection of the drug.

Three different pathomechanisms of nerve fibre degenerations have been found.
The first, includes degenerating axons of "dark neurons". In the second, axons were compressed by the large vacuoles, formed between adaxonal lamellae of myelin. Compressed axons underwent atrophy and abaxonal part of myelin sheath remained unchanged. In the third pathomechanism, the myelin sheath was affected by apoptosis of oligodendroglial cells. Axons of such nerve fibres were always well preserved.

Our results provided evidence that apoptosis of glia cells and degeneration of "dark neurons" may play an important role in pathomechanism of vincristine neurotoxicity.

## 1H MAGNETIC RESONANCE STUDY OF THE BRAIN AND SPINAL CORD DURING ISCHEMIC-REPERFUSION INJURY.

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Investigation of changes in the CNS due to ischemic and reperfusion injury using in vivo 1H nuclear magnetic resonance was the aim of this study. Brain ischemia was induced in both Mongolian gerbils and rats by occluding one main carotid artery or by the four vessel occlusion model. Spinal cord ischemia was induced in rabbits by occluding the abdominal aorta below the left renal artery. The 4,7T SISCO imaging spectrometer was used for measurements. The time dependence of the ischemic lesion was determined by T2 weighted and diffusion weighted MR image. Diffusion weighted MR images proved more sensitive than did T2 weighted MR images in detecting lesions due to cell edema produced during the ischemia. The metabolic changes in the rat brain during brain ischemia and reperfusion injury were measured by in vivo localized 1H MR spectroscopy using steam pulse sequence. In the volume of interest 150 ul it was possible to detect several metabolites with low molecular weight. The most interesting finding was a time dependent increased level of lactate during brain ischemia.

***γδ⁺ T Cells in Wilson's Disease***

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*The proportion of γδ⁺ T cells out of all T cells has previously been found increased in cerebrospinal fluid (CSF) of patients with multiple sclerosis, aseptic meningitis, and Parkinson's disease. Wilson's disease (WD) is an autosomal recessive disorder of copper transport. We studied 21 patients with WD. All patients had neurological symptoms of extra-pyramiadal disorders. Group of control patients consisted of age-matched 15 patients with other non-inflammatory neurological diseases (OND). Flow cytometric analysis was performed by FACSort (Becton Dickinson). The following stainings were performed:*
*δTCS1-FITC*/TCRδ1**-PE/CD3-biotin-tricolor*
*CD25-FITC/TCRδ1**-PE/CD3-biotin-tricolor*
**specific for Vδ1Jδ1 or Vδ1Jδ2 (the majority of the Vδ1),*
***"pan"-γδ monoclonal antibody (anti-TCR-γ/δ-1).*

*The mean percentage of γδ⁺ T cells in peripheral blood was 4.6+/-4.4% in patients with WD compared to 2.4+/-1.5% in OND. The level was higher in WD compared to OND. In CSF, the mean percetage of γδ⁺ T cells was 7.1 +/-6.0% in patients with WD, compared to 1.7+/-1.4% in OND. Numbers were higher in WD compared to OND. Nine of 18 WD patients, 5 of 15 OND patients had CD25+γδ⁺ T cells in CSF, they were also found in blood, but less frequently and at lower levels than in CSF patients with WD. The percentages of Vδ1/γδ T cells ranged between 20-50% in all patient groups.*

*Little is currently known of the role of γδ⁺ T cells in neuronal damage in chronic neurodegenerative conditions. The antigen reactivity of γδ⁺ T cells and the possible role of this T cell subpopulation in the immune response are the subjects of intensive studies.*

## Poster sessions - Development and aging

EXOGENOUS NERVE GROWTH FACTOR AFFECTS ENDOGENOUS NGF IN NEURONS OF ADULT AND AGED RAT HIPPOCAMPUS.

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Exogenous NGF ameliorate the degenerative changes in axotomized forebrain cholinergic neurons as well as attenuate aging-induced impairments of cholinergic neurons. NGF also enhances cholinergic parameters in adult intact brain. Recently a great deal of attention has been focused on endogenous NGF regulatory mechanisms in target structures of the brain cholinergic system. The present study summarizes our results obtained in adult and aged rats on regulation of NGF in the hippocampus upon continuous administration of exogenous NGF. 2.5S NGF in saline was infused from Alzet mini- osmotic pump (model 2001, flow rate 1ul/hr) into the lateral ventricle of uninjured adult and aged rats. The animals were infused for 7 days with NGF (25 ug). 4 days after the end of the treatment NGF-like immunoreactivity (NGF-LIR) was measured in the hippocampus using computer image analysis system. Affinity purified polyclonal anti-2.5S NGF antibodies were used in the study and 25um brain cryostat sections were developed for NGF-LIR in the same conditions simultaneously for all investigated groups. As controls saline infused and naive rats were used. The results were evaluated by multivariate analysis of variance (Anova VI) and the differences between groups were assessed using multiple-range Neuman-Keuls test. NGF-LIR in investigated pyramidal and granular layers in hippocampi of adult naive and aged naive animals did not differ significantly. The continuous infusion of NGF resulted in a marked decrease of NGF-LIR in the hippocampal pyramidal and granular layers in comparison to NGF-LIR in saline treated adult and aged rats. In both age groups the level of NGF-LIR in the investigated hippocampal layers of saline treated rats exceeded significantly the level observed in naive rats, while NGF-LIR of NGF treated rats was not statistically different from naive controls. Thus the action of exogenous NGF may reverse endogenous neuronal NGF responses to noxious conditions. Regulation of neuronal NGF-LIR by exogenous NGF suggests that hippocampal cells synthesizing NGF are responsive to external stimuli like trophic factor availability and may adjust their own synthesis. The results obtained suggest that the similar mechanisms of NGF regulation operate in adult and in aged brain.

DISTRIBUTION OF ZINC IN THE MOUSE SI BARREL CORTEX OF MICE DURING DEVELOPMENT

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There are indications that zinc may act as a modulator of neurotransmission in CNS. It could be released from nerve terminals following stimulation of afferent nerve fibre pathways and might mudulate inhibitory and excitatory amino acid receptors (Smart et al., Prog. Neurobiol., 1994, 42:393-441).

We used the selenium method of Danscher (Histochemistry, 1982, 76:281-293) to localize zinc in the cortical representation of mystacial vibrissae (barrel cortex) of mice at different postnatal ages (5, 12, 21, 28, 70 days). For this purpose some modifications of the method were introduced - different survival time after sodium selenite administration, and different time of development of reaction. The distribution of selenite precipitate was compared with cytoarchitectonic pattern on Nissl-stained adjacent sections. In adult mice in sections cut tangentially to the barrel field zinc distribution in layer IV mimicks the pattern of morphologically identified barrel field. The darkest staining was found in the centers of barrels.

Alterations in the pattern of zinc distribution in the barrel cortex may suggest the involvment of zinc in maturation processes and may reflect differences in excitatory input during ontogenesis.

Supported by grant 6/P04C/001/08 from the State Committee for Scientific Research.

## DEVELOPMENT AND MATURATION OF INTERCALATED CELL MASSES OF THE AMYGDALOID BODY

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Intercalated cell masses are clumps of densely packed and darkly stained small cells which are mainly GABA-ergic. Their development is rather ambiguous.

The study was performed with morphometric methods on 33 rat brains from 15th embryonic day (E-15) to the 360th postnatal day (P-360). All the animals were decapitated under general anesthesia; the brains were removed, fixed in formalin solution, and embedded in paraffin. 10-μm-thick sections were stained with cresyl violet and studied using the morphometric program Sigma-Scan (Jandel Scientific Corp., USA).

Intercalated cell masses are first visible at stage E-17, in which they form a compact structure surrounding the basolateral complex mainly on its lateral and inferior side. At this stage we observed the continuity of intercalated cell masses with so-called claustro-cortical reservoir. From stage E-17 to stage P-21 there is an increase in the total number of neurons and in the mean cross-sectional area of both cell bodies and nuclei. After stage P-21 the changes in these parameters are statistically non-significant.

The development of intercalated cell masses differs from that of other amygdaloid nuclei mainly in two respects: 1. the changes of morphometric parameters during the development are less expressed, 2. the initially single cell masse breaks up into several neuronal groups.

These differences between the intercalated masses and other amygdaloid nuclei may suggest the different origin of their neurons.

## ULTRASTRUCTURAL STUDY OF THE DEVELOPMENT OF SYNAPSES IN THE HUMAN EMBRYONIC SPINAL CORD

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Spinal cords of human embryos of developmental stages 16-23 /37-56 postovulatory days/ were fixed in 2% glutaraldehyde and postfixed in 1% osmium tetroxide. Ultrathin sections were inspected in JEM-7 and Philips electron microscopes.
Early in the 6th week the spinal cord is divided into basal plate and alar lamina and the nuclear groups in the basal plate are in the process of formation.
The first synaptic connections were found in the basal plate of the cervical region of the spinal cord at stage 16 /37 days/. These connections are classified as the primitive synapses /protosynapses/ and they are characterized by the following features :
a/ asymmetrical densities of the synaptic membranes,
b/ spherical synaptic vesicles,
c/ differences in thickness of synaptic membranes, the presynaptic being thicker.
Mature synapses were found in the basal plate in embryos at stage 18 /44 days/. The formation of synapses proceeds into cranial and caudal ends of the spinal cord. First protosynapses within the alar lamina were noted in stages 18 and 19 and they also proceeded caudally. The first synypses during development were axodendritic ones with spherical synaptic vesicles.

## DEVELOPMENT OF THE CENTRAL NUCLEUS OF THE RAT AMYGDALOID BODY - A MORPHOMETRIC STUDY

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The central amygdaloid nucleus located in the dorsal, adjacent to the corpus striatum, portion of amygdala is composed of three parts: lateral, medial and capsular. They participate in numerous adaptive behaviors, including arousal, feeding, flight, avoidance and defence reaction and produces the visceral responses like changes in the heart rate, blood pressure, respiration and gastrointestinal functions.

30 rat brains of various age (from 15th embryonic to 90th postnatal day) were studied. The animals were decapitated under ether anaesthesia; their brains were removed and placed in formalin solution. 10-μm-thick paraffin serial sections were cut and stained with cresyl violet and studied using morphometric methods.

The central amygdaloid nucleus and its parts are visible in E-17. From this stage to stage P-14 a rapid decrease in neuronal density and an increase in neuronal size were observed.The neuronal population increases to stage P-7, while the volume of the whole nucleus increases - up to stage P-21.

## NITRIC OXIDE SYNTHASE AND cGMP LEVEL IN DIFFERENT PARTS OF THE BRAIN DURING AGING.

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Nitric oxide is an important messenger molecule that mediates a wide variety of physiological processes and implicates neurotoxicity. Nitric oxide synthase (NOS) in brain occurs in vascular endothelium, nerve fibres of cerebral arteries, in neurons and astrocytes. In central neurons NO is produced in postsynaptic structures in response to the activation of exitatory amino acid (EAA) receptor. A major role of NO is to activate of guanylate cyclase and to elevate cGMP in the target cells. One of the important hypothesis of neuronal death and degeneration during aging is excitotoxicity, unregulation of excitatory neurotransmitter system. In this study NMDA receptor mediated NO-dependent cGMP formation and nitric oxide synthase activity in different parts of aged brain was evaluated. The study was performed using adult 4 months old and aged 27 months old rats. The type of NOS was determined by mRNA and Northern blot analysis. Moreover animals were treated with hydrocortisone in a dose of 40 mg/kg b.w. for 7 and 20 days before decapitation. It was observed that NOS activity was significantly elevated in all investigated parts of aged brain: in hippocampus, cerebellum and cerebral cortex by about 60-80% comparing to enzyme activity in adult brain. There was no activation of gene encoding iNOS during aging. Glucocorticoid has no effect on NOS activity. The constitutive form of NOS was exclusively activated. In spite of higher NO production the NMDA receptor-mediated NO-dependent cGMP formation significantly decreases in aged hippocampus and cerebellum. These changes are probably due to disturbances in $Ca^{2+}$ ions redistribution observed by us in aged brain. In conclusion our results indicate that aging differently affects cNOS activity and cGMP production in brain. The excessive nitric oxide formation in aged brain may be involved in neuronal degeneration.

## AGE-RELATED CHANGES IN GLUTAMATE RECEPTORS: AN AUTORADIOGRAPHIC ANALYSIS.

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The aim of the present study was to examine the influence of aging on the density of glutamate receptors in rats. The experiment was performed on young (Y; 3-month old), middle-aged (M; 12-month old) and old (O; 34-36-month old) female Wistar rats. The distribution and density of NMDA receptors were investigated autoradiographically using both non-competitive ([3H]-MK-801) and competitive ([3H]-CGP 39653) antagonists of those receptors. AMPA receptors were labelled with [3H]-AMPA, and dopamine D2 receptors with [3H]-spiperone. In young rats, the highest density of NMDA and AMPA receptors was found in the hippocampal formation and cerebral cortex. Moderate levels were detected in the striatum, nucleus accumbens and septum. There were no differences in the density of NMDA receptors in M rats compared to Y ones. In old rats, the most pronounced decrease was observed in different parts of the cortex (frontal, insular, parietal), striatum, nucleus accumbens, and the CA3 region of the hippocampus. In old rats, the [3H]AMPA binding level was decreased only in the dentate gyrus and CA3 region of the hippocampus, and in the dorsal striatum. The present results suggest that aging processes lead to changes in the density of glutamate receptors, which may reflect motor and memory disturbances in old rats.

## BINDING OF DIZOCILPINE (MK-801) IN THE BRAINS OF DEVELOPING AND AGEING MICE

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The regulation of N-methyl-D-aspartate (NMDA) receptor-coupled ion channel was studied by measuring the binding of the modulatory ligand, [$^3$H]dizocilpine (MK-801) to cerebral cortical membranes isolated from developing and ageing mice (from 7 days to 22 months of age). The binding was saturable, consisting of only one component at every age studied. The number of binding sites ($B_{max}$) was maximal at the age of two weeks, declining thereafter until 18 months of age. The affinity of binding was lower in immature and aged mice than in adult, 3-month-olds. Glycine and glutamate potentiated concentration-dependently dizocilpine binding, the magnitude of stimulation depending on age, however. The potentiation by glycine or glutamate was smaller in developing than in adult animals. The results confirm the overexpression of glutamate receptors of the NMDA type during early development. The alterations in the binding characteristics during development and ageing could be of importance in the regulation of NMDA receptors, which have been implicated in the synaptic potentiation, developmental processes and various pathological conditions.

Supported by the Academy of Finland and the Research Fund of Tampere University Hospital.

## ALTERATION BY AGING OF PHOSPHOINOSITIDES-DEPENDENT CALCIUM SIGNALLING IN BRAIN CORTEX

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In this study, the cholinergic and serotonergic receptor-dependent inositol phosphates (IPs) formation, through the activation of phosphoinpsitide-specific phospholipase C, in brain cortex synaptoneurosomes from adult (4 month-old) and aged (27month-old) rats was investigated. Moreover, these agonists-evoked intracellular calcium ($[Ca]_i$) mobilization was determined. The release of IPs was measured after prelabeling of synaptoneurosomal lipids with myo-[$^3$H]inositol. Changes in $[Ca]_i$ were monitored by using fura-2 fluorescent indicator. It was observed that in adult brain, nonhydrolysable analog of acetylcholine,1mM carbachol (CCh), and 10μM serotonin (5-HT) together with 10μM pargyline, in the presence of 5mM lithium, stimulated IPs production in a $Ca^{2+}$-dependent manner by about 150% and 30% over $Ca^{2+}$-mediated release during 60 min of incubation. In aged brain, CCh and also 5-HT, more potently than in adult, activated IPs production, increasing it by 300% and 100%, respectively. In both age groups, 5-HT diminished pronouncly CCh response. Concomitantly, in adult brain, activation of cholinergic receptor increased $[Ca]_i$ by about 30% over its resting level (180nM). The maximal effect was seen 5min after CCh addition. This $[Ca]_i$ elevation was completely bloked by TMB-8, an inhibitor of $IP_3$ receptor, located on endoplasmic reticulum. Serotonin significantly reduced by about 50% cholinergic receptor-mediated increase of $[Ca]_i$. This effect was more significant at the early phase of CCh response. In aged brain, in spite of enhanced IPs formation by cholinergic receptor stimulation, there was no detectable $Ca^{2+}$ mobilization from intracellular endogenous stores. These results show the disturbances in cholinergic neurotransmission in aged brain, involving $IP_3$ pathway.

# Poster sessions - Cardio-respiratory regulation

## RAPID CHANGES OF ARTERIAL PRESSURE DISTURB THE BALANCE BETWEEN HYPOGLOSSAL AND PHRENIC NERVE ACTIVITY.

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Rapid fluctuations of arterial blood pressure (AP) are often observed during REM sleep in normal subjects, however they have much greater amplitude in the sleep apnoea (OSA) patients. **Our aim** was to compare changes of hypoglossal nerve (n.XII) activity with phrenic nerve (n.Ph) activity and to measure upper airway (UA) resistance during changes of AP. Rapid rise of AP was induced either mechanically or by i.v. administration of noradrenaline (25 $\mu$g/kg). Rapid fall in AP was induced by a bolus of sodium nitroprusside (0.12 mg/kg i.v.) or nitroglycerine (0.13mg/kg i.v.). We examined 15 anaesthetized rabbits. Both vagus nerves and sympathetic trunks were cut. Respiratory chemical drive was kept constant with a servo-respirator. To inactivate the chemoreceptors the animals were ventilated with 100% oxygen. Electrical activities of the n.XII, and n.Ph were recorded. UA resistance was measured as a pressure to flow ratio. **We found that** administration of a bolus of noradrenaline within 3 seconds rose arterial pressure by 52.6 ± 12%. The maximum level was attained in 32.8 ± 12 sec. AP remained elevated for ca. 3 min. Rise in AP induced either mechanically or pharmacologically, disproportionately more suppressed n.XII ( by 27.6 ± 12%) then n.Ph activity ( by 7.2 ± 6% ). Inspiratory upper airway resistance increased two fold. After administration of vasodilators mean AP dropped by 34.4 ± 12%. The effect lasted 75.3 ± 17 sec followed by slow increase in AP to control value. Decrease of AP almost simultaneously activated n.XII and n.Ph by 42.3 ± 9% vs. 6.3 ±4.2 % respectively. UA resistance (cranial to the larynx) decreased. **We conclude** that changes in AP may disturb the balance between upper airway muscles and diaphragm activity. Rapid rise in AP promotes UA obstruction.

## RESPIRATORY EFFECTS OF SEROTONIN CHALLENGE TO LARYNGEAL ARTERY IN CATS

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It is generally held that serotonin (5HT) challenge to the pulmonary circulation elicits in cats apnoea, bradycardia and hypotension, followed by shallow tachypnoea.The object of this investigation was to examine the range of the respiratory effects of 5HT injection into laryngeal artery in comparison with pulmonary circulation challenge. In addition, we looked at the contribution of laryngeal afferents to this chemoreflex.

Experiments were conducted on 11 anaesthetized, spontaneously breathing cats. Pulmonary airflow was measured from the trachea. Serotonin was administered to the laryngeal artery (0.05 mg/kg of body weight) in the intact animals, following midcervical vagotomy and after subsequent division of the superior laryngeal nerves (SLNs). Intralaryngeal artery injection of 5HT caused expiratory apnoea in five of eleven cats, which was abolished by midcervical vagotomy. During the late phase of stimulated breathing the peak respiratory airflows tended to increase but did not achieve statistical significance in any neural state of the cats. The significant rise in the respiratory rate ($P<0.01$) was present in the intact cats only.

These data demonstrate that injection of 5HT into the laryngeal site induces confined respiratory chemoreflex depending to large extent on the infranodose vagal pathway.

## CHANGES IN TRANSIENT POTENTIATION OF HYPOGLOSSAL NERVE ACTIVITY BY MODULATORS OF NITRIC OXIDE PRODUCTION

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Stimulation of the superior laryngeal nerve (SLN) produces a transient potentiation of the respiratory-related activity of the hypoglossal nerve. The mechanism of this phenomenon is uncertain. We studied a possible role of nitric oxide (NO) in modulation of the hypoglossal potentiation since it is known that NO contributes to long term potentiation in other then the respiratory system. Experiments were performed on anaesthetized, vagotomized, paralysed and artificially ventilated rabbits. The integrated phrenic and hypoglossal activities were recorded. Electrical stimulation of SLN with frequency 50 Hz, duration 0.5 ms and intensity 30-200 $\mu$A was applied during 20 s. Changes in NO production were evoked first by i.v. injection of 300 mg/kg of L-Arginine, a substrate for NO synthesis and after 30 min by 30 mg/kg of N-ώ -Nitro-L-Arginine (L-NNA), an inhibitor of NO synthase. Effects of SLN stimulation were studied in the control and a few minutes after each pharmacological treatment. SLN stimulation caused a transient potentiation of the hypoglossal activity that persisted after cessation of the stimulus from 50 s to 15 min. After the injection of L-Arginine the duration of potentiation was reduced to 0-4 min. L-NNA restored partially this effect. We conclude that NO modifies transient potentiation of the hypoglossal nerve activity.

## THE CAROTID BODY IN THE MOTONEURON RESPONSE TO LARGE INSPIRATION

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Large inspiration has been shown to preferentially attenuate the upper airway hypoglossal (HYP) inspiratory motoneurons relative to phrenic (PHR) activity. In this study we hypothesized that the carotid body might be involved in the disproportional changes of the two pools of motoneurons. We addressed this problem by comparing the integrated electroneurogram activities of the HYP and PHR nerves in response to a stepwise doubling of the respiratory volume on the background of normoxia ($F_IO_2$≈21%), hypoxia ($F_IO_2$≈12%), and hyperoxia ($F_IO_2$≈36%). The study was carried out in the anesthetized, paralyzed, vagotomized, and ventilated rabbits. The arterial blood pressure, end-tidal $CO_2$, and gas content were monitored. The respiratory volume was increased from 15 to 30 mL by adjustment of the respirator cylinder's stroke at a constant frequency of 30/min. We found that the volume doubling decreased markedly the HYP activity with a little change of the PHR activity in each gas condition. When compared with normoxia, however, the disproportion of the HYP-PHR decrease was augmented 2-fold on hypoxia and attenuated 2.5-fold on hyperoxia. We conclude that the carotid body is likely responsible for the heightened HYP decrease relative to the PHR nerve in response to large inspiration, which may promote the upper airway collapse.