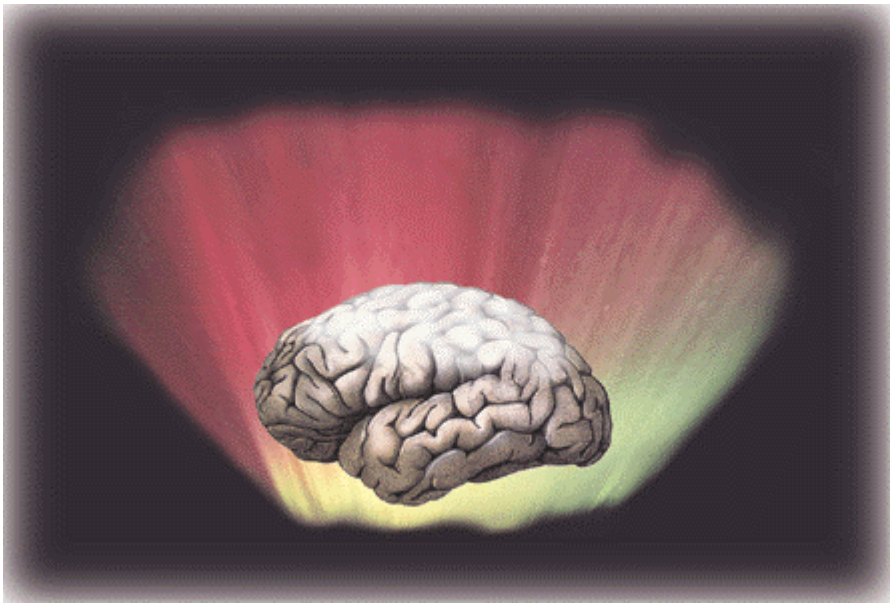
PROCEEDINGS OF THE

ARCTIC SYMPOSIUM ON

MECHANISMS OF MEMORY AND

MEMORY DISORDERS

March 17-18, 2001, Saariselkä, Finland



Satellite of The Second Kuopio Alzheimer Symposium, March 13-15, 2001, Kuopio, Finland

Combined with

NEUROSCIENCE 2001 FINLAND Meeting, March 15-16, 2001, Saariselkä, Finland

The goal of this meeting is to provide a multidisciplinary discussion for leading scientists working on the mechanisms of memory with participation of young European scientists working in different fields of neuroscience. The main aim is to facilitate multidisciplinary co-operation and encourage collaboration between neuroscientists, neurologists and psychiatrists.

The organizers of the meeting hope that participants and lecturers will spend one arctic weekend with other neuroscientists from different disciplines concentrating on the problems we all face: the basic mechanisms of memory and memory disorders. We trust that novel ideas, approaches and fruitful collaborations will spring up from this meeting.

The Organizing Committee:

György Buzsaki, USA (Chairman of the Program Committee, buzsaki@axon.rutgers.edu)

Aarne Ylinen, Finland (Co-Chairman of the Program Committee, Chairman of the Congress Organizing Committee, aarne.ylinen@uku.fi)

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Invited Speakers

Yehezkel Y Ben-Ari France
György Buzsaki USA
Larry Cahill USA
Howard B Eichenbaum USA
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Michael Hausser Great Britain
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Alex Thomson England
Wim Van Paesschen Belgium
Aarne Ylinen Finland

Oral 1

EXPRESSION AND LOCALISATION OF MUTANT AND RECOMBINAT AMPA RECEPTORS

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Background

The alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtypes of the ionotropic glutamate receptors are multimeric proteins of 4 or 5 subunits. AMPA receptor subunits GluRA–D, can be subdivided into distinct functional areas; the extracellular N-terminal X domain of ~400 residues; the S1 and S2 extracellular ligand binding domains; the membrane regions, the pore forming re-entrant loop M2 and transmembrane sections M1, M3, M4; the cytoplasmic C terminal region. This latter region appears to be important for correct localisation of the receptor. A number of intracellular proteins have been identified which interact with this domain of the GluRA, B and C subunits. However, no proteins have yet been identified which interact directly with the GluRD subunit.

Methods

A series of deletion and point mutants of the GluRD subunit were created and expressed in HEK 293 cells. The effect of the mutations on the sub-cellular localisation of the receptor/subunit was studied by immunofluoresence labelling.

Results

Removal of the entire C-terminal domain prevented detectable surface expression by immunofluoresence. Refinement of the region deleted allowed the identification of a region essential for correct targeting. This domain was further investigated by multiple and single point mutations.

Conclusions

The GluRD intracellular tail contains a region which modulates the expression and localisation of the receptor, possibly by interaction with a cytoplasmic protein.

Oral 2

Use-dependent shift from inhibitory to excitatory $GABA_A$ action in interneurons induces beta-gamma oscillations in the hippocampus

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Background:

Glutamatergic excitation has a critical role in the generation and maintenance of fast network oscillations in the hippocampus. In addition to glutamatergic input, excitatory drive to hippocampal neurons can also be provided by synaptic activation of $GABA_A$ receptors ($GABA_ARs$). However, the physiological relevance and the generation mechanisms of this "paradoxical" $GABA_A$ action in different cell types are largely unknown.

Results:

We now show that in the absence of glutamatergic transmission a fast switch from inhibitory to excitatory $GABA_A$ action induces bursts of synchronous beta (15-30 Hz) and low gamma frequency (30-40 Hz) oscillations in the CA3 interneuronal network. The $GABA_AR$-mediated postsynaptic potentials (PSPs) in the interneurons were typically hyperpolarizing, but by an increase in the $GABA_AR$-mediated conductance the PSPs became depolarizing and excitatory. During mutual excitation of interneurons, $GABA_AR$-mediated currents displayed beta-gamma oscillations. A shift of $GABA_AR$-mediated PSPs to depolarizing by the $GABA_AR$ permeant weak acid anion formate (20-30 mM) or by up-modulation of the $GABA_A$ conductance with pentobarbital (100 μM) provoked interneuronal population bursting. The synchronous beta-gamma rhytmicity, however, was dependent on electrotonic coupling, since it was abolished by gap-junction blockers, although the population discharges were preserved.

Conclusions:

We conclude that the use-dependent switch from inhibitory to excitatory $GABA_A$ action provides a mechanism for the generation of synchronous ß-? bursts in the interneuron network. Further, it provides an alternative mechanism for short-term plasticity in excitatory synapses in interneurons.

Oral 3

EPILEPTOGENESIS RELEATED CHANGES IN GENE EXPRESSION REVEALED BY cDNA ARRAYS IN THE RAT MODEL OF TEMPORAL LOBE EPILEPSY.

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Background: Epilepsy frequently develops as a result of brain insult and the epileptic process can be divided into three phases: 1) initial insult, 2) latency period (epileptogenesis) and 3) recurrent seizures (epilepsy). In the present study, we aimed at identification of genes that change their expression during the epileptogenesis.

Methods: We used an amygdala stimulation model of temporal lobe epilepsy. SE was evoked by stimulation of the lateral nucleus of the amygdala. Rats were monitored with video-EEG during SE, and thereafter, until the end of experiment to detect the appearance of spontaneous seizures. Only the animals that had SE but did not experience spontaneous seizures were used for the experiment. Hippocampal RNA was isolated 14 days after induction of SE and was used for hybridization to cDNA arrays. RT-PCR and immunohistotochemistry was used to confirme changes in the expression selected genes.

Results: cDNA arrays analysis revealed about 2 fold increase in expression for 88 genes, and decrease for 32 genes. Increase in immunoreactivity of one of candidate genes, cystatin C and was observed in glial cells.

Conclusions: Changes in gene expression are present in the hippocampus during epileptogenesis, before appearance of first spontaneous seizures. Cystatin C has a novel, unknown function that could be related to recovery from SE induced damage and/or plasticity.

Oral 4

Selegiline can diminish N-methyl-D-aspartic acid (NMDA)-induced perturbation of neuronal transmission, but it does not interfere with NMDA receptor dependent long-term potentiation (LTP) in the hippocampus in vitro

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Background: Selegiline has been shown to exhibit both neuroprotective and antiapoptotic effects in a variety of model systems for human neurodegenerative diseases. Recently, selegiline was shown to rescue dopaminergic neurons from NMDA toxicity (Shimazu et al., EJP, 377,29,1999). The present experiments were undertaken to test the hypothesis that selegiline can be neuroprotective while not interfering with the normal function of NMDA receptors underlying learning and memory.

Methods: Hippocampal slice preparation was used as a model system (Niittykoski et al. Neurosci. Lett., 281,95, 2000). The effects of selegiline, SEL, (given as a continuous bath perfusion at the concentration of 10 nM or 1 μM) on (i) the disappearance, induced by a short exposure of NMDA (100 μM), of field excitatory postsynaptic potentials (fEPSPs) and their gradual recovery as well as (ii) the induction and early maintenance of LTP induced by theta burst stimulation (TBS) were investigated in the field CA1 of rat hippocampal slices.

Results: SEL treatment did not affect the onset time for the action of NMDA to perturb neuronal transmission, but it facilitated the recovery of fEPSPs seen 90 minutes after NMDA exposure (~59% under the control conditions, ~65% and ~71% in the presence of 10 nM and 1μM SEL, respectively). On the other hand, SEL treatment (1 μM) did not influence the amount of LTP (~50%) seen 45 minutes after TBS (10 bursts at 5 Hz).

Conclusions: The present results support the content that selegiline is a safe neuroprotective agent.

Oral 5

Morphine withdrawal selectively increases $GABA_A$ receptor ε subunit mRNA expression in rat locus coeruleus

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Background

The increase in activity of locus coeruleus (LC) neurons has been hypothesised to be one of the major factors behind opiate withdrawal. Current data indicates that neurons of the LC are under inhibition of γ-aminobutyric acid (GABA). It has been suggested that LC has unique and enriched expression pattern of $GABA_A$ receptors containing epsilon (ε) and theta (θ) subunits. The aim of this study was to determine the contribution of $GABA_A$ receptor ε and θ subunits in rat LC under naloxone precipitated morphine withdrawal by *in situ* hybridization.

Methods Opiate dependence was induced by i.p. injection of escalating doses (10-100mg/kg) of morphine during 5 days. Animals were divided in three treatment groups: *Control group* received saline injections for 5 days and naloxone injection on 6th day. *Chronic morphine group* received morphine injections for 5 days. *Morphine withdrawal group* was treated with morphine injections for 5 days and with naloxone injection on 6th day. Rats were then decapitated and brains used for in situ hybridization.

Results Naloxone treated morphine withdrawal group animals presented many clear physical signs of opiate withdrawal. Tyrosine hydroxylase mRNA expression clearly increased in withdrawal group LC and we also detected a significant increase in expression of epsilon ($p<0.0001$) and a marked increase in theta ($p=0.3$) subunit mRNA levels.

Conclusions Our results indicate that expression of these recently cloned $GABA_A$ receptor subunits is modulated in naloxone precipitated opiate withdrawal syndrome.

Oral 6

Dynamics of face perception in the occipito-temporal cortex.

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Background

We have earlier used magnetoencephalography (MEG) to characterize letter-string processing in the occipitotemporal cortex [1]. Now we applied the same tchnique to map the early cortical processes related to face processing.

Methods

We recorded brain activity of 10 healthy Finnish adults with whole-head MEG (VectorviewTM) while they viewd stimuli consisting of photographs and drawn images of faces and common objects. These same sybjects had also participated in the study of letter-string reading. [a].

Results

At 100 ms after image onset the subjects showed occipital activity similar to that in our earlier study [1]. Activation specific for faces occurred at about 150 ms after stimulus onset bilaterally in inferior occipito-temporal corteces.

Conclusions

Activation seen at 100 ms was likely realated to basic visual feature analysis. It did not differ between reading and face processing. The timing of face-specific activation at 150 ms coincided with that of early letter-string specific source areas [1]. While the active areas in the inferior occipito-temporal cortex were very similar for face and letter-string reading, the hemispheric distribution differed. Letter-string processing utilized mainly a left-hemishpheric cortical network, whereas early face processing relied more heavily on bilateral neuronal systems.

[1] Tarkiainen A., Helenius P., Hansen P.C., Cornelissen P.L. & Salmelin R. Brain 122,2119-2131 (1999).

P1 Hyperinnervation of the axon initial segments of granule cells in the epileptic human dentate gyrus

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The majority of granule cells, the principal cells of the dentate gyrus are found to be resistant to damage in epilepsy, and may serve as generators of seizures if their inhibition is impaired. Therefore, the parvalbumin-containing perisomatic inhibitory interneurons (basket and axo-axonic cells) were examined in human control and epileptic dentate gyrus. The number of parvalbumin-containing cells decreased in the epileptic samples, although in some patches of the granule cell layer parvalbumin-positive terminals that form vertical clusters characteristic of axo-axonic cells were more numerous than in controls. Postsynaptic target elements of parvalbumin-positive axon terminals showed that they form symmetric synapses with somata, dendrites, axon initial segments and spines as in the control, but the ratio of axon initial segment synapses was increased in the epileptic tissue. Furthermore, the synaptic coverage of granule cell axon initial segments increased more than three times in the epileptic samples, whereas the amount of somatic symmetric synapses did not change significantly. Basket and axo-axonic cell terminals – whether positive or negative for parvalbumin – are present, moreover, the axon collaterals targeting axon initial segments sprout in the epileptic dentate gyrus. Thus, part of the perisomatic inhibitory interneurons may survive in epilepsy, but their somadendritic compartment and partly the axon looses parvalbumin or immunoreactivity for parvalbumin. The hyperinnervation of axon initial segments could contribute to the generation or amplification of epileptic seizures via the enhancement of the synchronization of granule cell firing, or might be part of a compensatory mechanism designed to balance increased excitation in the area.

P2 PROJECTION FROM THE AMYGDALOID COMPLEX TO THE CLAUSTRUM IN RAT

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Background: Recent studies have implicated that the claustrum is one of the relay stations that spreads epileptiform activity from the temporal lobe, including the amygdala to the other brain areas. There are, however, almost no data available about the amygdaloid complex projections to the claustrum.

Methods: To investigate the distribution, density, and topography of the projections from the amygdala to the claustrum, we iontophoresed the anterograde tracer, *Phaseollus vulgaris*-leucoagglutinin (PHA-L) into various amygdaloid nuclei in 101 rats. After 10-d survival period, brains were processed for immunohistochemistry to detect immunolabeled terminals.

Results: The magnocelullar division of basal nucleus projected moderately to the dorsal aspect of the rostral two-thirds of the claustrum. The parvicelullar and intermediate divisions provided a light projection to the same claustral zone. Parvicelullar division of accessory basal nucleus and the lateral division of amygdalohippocampal area projected lightly to the most caudal aspect of the claustrum. Only single fibres with varicosities were observed in the caudal two-thirds of the claustrum after injections located in different divisions of the lateral nucleus. The anterior cortical nucleus, the periamygdaloid cortex, the posterior cortical nucleus, or the central nucleus of the amygdala did not project to the claustrum.

Conclusions: The amygdaloid complex sends sparse projections to the claustrum which mainly originate in the deep amygdaloid nuclei, particularly, the basal nucleus. These projections target the portion of the claustrum that has bilateral reciprocal connections with the motor and somatosensory cortices. These data provides an anatomic background for the observations suggesting that the claustrum is a candidate brain area via which the seizures of amygdaloid origin may become secondarily generalized.

Supported by the Vaajasalo Foundation, The Academy of Finland and the Sigrid Juselius foundation.

P3 REORGANIZATION OF PARVALBUMIN IMMUNOREACTIVE CIRCUITRIES IN THE LATERAL AND BASAL NUCLEUS OF THE AMYGDALA IN EXPERIMENTAL TEMPORAL LOBE EPILEPSY.

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Background: Status epilepticus (SE) induces substantial neuronal damage in selective nuclei of the amygdala. Little is known, however, about the extent of damage to inhibitory interneurons.

Methods: Parvalbumin labels a subset of GABAergic neurons in the rat amygdala. Here, the reorganization of amygdaloid inhibitory circuitry was investigated by assessing the distribution of parvalbumin immunoreactive (ir) cells and terminals in chronically epileptic aminals. Rats (n=8) were treated with kainic acid (i.p.) to induce SE or with saline to be used as controls (n=5). Occurrence of spontaneous seizures was assessed 2 months later. The number of parvalbumin-ir neurons was counted stereologically, size of neurons was measured, and neurons were classified according to their morphology. The density of basket-like plexus and cartridges was assessed.

Results: In epileptic animals compared to controls: 1) the number of parvalbumin-ir cells was decreased (33 to 85 %) in the basal and lateral nucleus except in the medial division of the lateral nucleus, 2) the size of large multipolar neurons was increased (from 139 ± 51 to 177 ± 81 μm^2), however, different cell types were equally affected, 3) the density of basket-like plexus was decreased in the basal and lateral nucleus except in the ventrolateral division of the lateral nucleus, and 4) the density of cartridges was increased in the basal nucleus and the ventrolateral division of the lateral nucleus.

Conclusions: Our data suggest that the reorganization of the parvalbumin-ir circuitries in the amygdala may underlie hyperexcitability and synchronization of principal cells leading to propagation of epileptic seizures.

P4 STATUS EPILEPTICUS-INDUCED DAMAGE IN DEVELOPING THALAMUS OF THE RAT.

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RATIONALE: To characterize the pattern of status epilepticus (SE) - induced damage in the thalamus of developing rats.

METHODS: LiCl-pilocarpine SE was induced in 54 twelve-day-old rats. Animals were sacrificed 12, 24, 48 hours or one week later and distribution of degenerating neurons was characterized using Gallyas silver impregnation and/or Fluoro Jade B staining. Mechanism of SE-induced neuronal damage was studied using TUNEL, immunohistochemistry for caspase-3, cytochrom c, and OX-42 (microglia). In addition, to provide evidence of the type of neuronal death electron microscopy was performed.

RESULTS: Distribution of silver-positive and Fluoro Jade B – positive neurons exhibited similar pattern in all intervals used. Degenerating neurons were found already 12 h after SE in 6 thalamic regions and they were still present in 5 regions one week after SE. No signs of neuronal damage occurred in controls. The highest density of degenerating neurons was found bilaterally in the mediodorsal nucleus of the thalamus. In the same region, OX-42 positivity was demonstrated. In contrast, no TUNEL-positive neurons or caspase-3 and cytochrom c immunopositive cells were found in any thalamic nucleus at any interval studied. Ultrastructural analysis revealed a condensed or lysed cytoplasm and disintegration of the cellular components of damaged neurons.

CONCLUSION: Our data suggest that status epilepticus induces neuronal degeneration in the mediodorsal thalamic nucleus at postnatal day 12 and that the mechanism of neuronal damage is necrosis rather than apoptosis.

Supported by the Exchange Visitor Program between the Academy of Sciences of the Czech Republic and the Academy of Finland (to H.K. and A.P.)

P5 Changes in the $GABA_A$ receptor subunits induced by chronic temporal lobe epilepsy in the rat brain

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Background

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain. The fast effects of GABA on neurons are mainly mediated via the $GABA_A$ receptor, which is a pentameric assembly composed of 16 possible subunits grouped into seven families (α1-α6, β1-β3, γ1-γ3, δ, ε, θ and π). Decreased GABA receptor function and/or expression is suggested to contribute to some forms of epilepsy.

Methods

We used a new animal model of human temporal lobe epilepsy, in which epileptogenesis was induced by electrically stimulating the amygdala of rats (Nissinen et al., Epilepsy Res. 38, 177, 2000). This resulted in spontaneous seizures. The changes in different $GABA_A$ receptor subunits were studied in hippocampus with *in situ* hybridization using rat subunit-specific oligonucleotide probes for α1, α2, α4, β2, β3 and γ2 subunits.

Results

Our results indicate changes in the $GABA_A$ receptor subunit expression in the hippocampus of epileptic rats compared to normal control rats. At least α2, α4 and β2 showed a decrease in the CA1 and CA3 regions of epileptic rats. The expression of some subunits, e.g., β2 and β3, increased in the dentate gyrus (DG) region of this group.

Conclusions

These results suggest that decreased expression of the $GABA_A$ receptor subunits in the CA1-CA3 region during epileptogenesis may be associated with reduced inhibition in epileptic rats. The significance of increased expression in the DG region remains to be established.

Supported by: The Academy of Finland, Arvo and Lea Ylppö Foundation, Sigrid Juselius Foundation

P6 Enhancement of depolarizing $GABA_A$ responses by pentobarbital or by direct shift in E_{GABA-A} has differential effects on synchronous epileptiform oscillation in hippocampus

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Background:

Tonic $GABA_A$R-mediated excitation is sufficient to drive fast synchronous oscillations in interneuronal network in the absence of ionotropic or metabotropic glutamatergic excitation in rat hippocampal slices. Here we examined the role of $GABA_A$R-mediated depolarization in the function of pyramidal cell population.

Results:

In Mg^{2+}-free solution single pulses applied to Schaffer collaterals induced 100-200 ms long gamma- (60-100 Hz) and high frequency (>100 Hz) oscillation in CA1 field potential recording. Exposure to $GABA_A$R-permeant weak-acid anion formate, which induces a positive shift in E_{GABA-A} and an increase in the mutual interneuronal excitation, induced 2-3-fold lengthening in the oscillation. However, $GABA_A$R up-modulator pentobarbital, which accentuates interneuronal oscillations completely blocked the pyramidal cell oscillations. The carbonic anhydrase inhibitor ethoxyzolamide (EZA), which curtails the $GABA_A$R-mediated excitation by inhibiting the production of the channel-permeant anion HCO_3^- attenuated the pyramidal cell oscillations. Interestingly, the oscillations were augmented by formate thus speaking for the critical role of depolarizing $GABA_A$ responses. Formate-induced oscillations were blocked by the $GABA_A$R-antagonist picrotoxin, but not by EZA.

Conclusions:

Thus, positive shift in E_{GABA-A} promotes network oscillations in both pyramidal cell and interneuronal network. However, if shift is accompanied by increased $GABA_A$ conductance (e.g. induced by pentobarbital) only interneuronal oscillations are likely to ensue.

P7 INHIBITION OF SPONTANEOUS ACTIVITY IN CA1 AREA DURING EARLY POSTNATAL DEVELOPMENT: EFFECT ON EXCITATORY AND INHIBITORY TRANSMISSIONS.

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Background: It has been hypothesized that the maturation of excitatory synapses requires the activity-dependant incorporation of AMPA receptors at pure NMDA synapses. In the neonatal hippocampus, this maturation maybe driven by a spontaneous oscillating neural activity, mediated by both GABA and glutamate transmissions. Here, we test, *in vivo*, this hypothesis by inhibiting the spontaneous activity in the rat CA1 area using a single injection of tetanus toxin at postnatal day (P) 1.

Methods: Spontaneous EPSCs, mediated by NMDA, AMPA and $GABA_A$ receptors, were recorded from CA1 pyramidal neurons, in control and in tetanus-injected rats over the first postnatal week.

Results: Surprisingly, in controls, AMPA/NMDA ratios for frequency and amplitude were stable over the first postnatal week, 0.97 ± 0.1 and 0.77 ± 0.07 (P1-8), respectively. Tetanus toxin injection initially blocked about 80% of the spontaneous glutamatergic and GABAergic synaptic events. Spontaneous $GABA_A$ IPSC amplitudes were significantly reduced after tetanus injection. However, tetanus injection didn't change 1) AMPA and NMDA amplitudes, CVs, or kinetics, or 2) AMPA/NMDA frequency ratio over the first postnatal week, suggesting that the spontaneous activity is not influencing these postsynaptic aspects of glutamatergic synaptic signalling.

Conclusions: Together, these results do not support the hypothesis of a sequential activity-dependant maturation of NMDA and AMPA signalling in CA1 pyramidal cells over the first postnatal week.

P8 VESICLE RELEASE PROBABILITY AND PRE-PRIMED POOL AT GLUTAMATERGIC RELEASE SITES IN AREA CA1 OF THE NEONATAL HIPPOCAMPUS.

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BACKGROUND: It is assumed that release probability at a release site is decided by the number of immediately releasable vesicles (the pre-primed pool) and the probability of releasing any of those vesicles (P_{ves}). Here we have determined these two factors for glutamatergic synapses in the neonatal hippocampus and explored their influence on the heterogeneity in release probability and short-term facilitation/depression.

METHODS: Putative single glutamatergic synapses, containing a single release site, were stimulated using "minimal stimulation" (10-20 impulses @ 50 Hz) and synaptic responses were recorded using whole cell patch-clamp recordings from pyramidal neurones in the CA1 region of hippocampal slices from 1-7 days old rats.

RESULTS AND CONCLUSIONS: Initial release probability (P_1) varied among the synapses from 0 to 0.87 (n=52). This heterogeneity in P_1 could be fully explained by a variation in P_{ves1} (0.04–0.94) and a variation in the average size of the pre-primed pool (0.4-2.1 vesicles). The great variation in P_{ves} among synapses disappeared after the first stimulus, attaining a value of 0.3-0.4. Following depletion of the pre-primed pool (within 3-6 stimuli at 50 Hz) release was found to be limited by the rate of recruitment/priming of new vesicles. The heterogeneity in facilitation/depression among the synapses was highly dependent on P_{ves1} whereas it was virtually unrelated to the size of the pre-primed pool.

P9 ACTIVITY BLOCKADE INDUCES FORMATION OF FUNCTIONAL SYNAPSES IN THE DEVELOPING RAT HIPPOCAMPUS

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Background. Spontaneous electrical activity is suggested to be necessary for the proper formation and refinement of synaptic contacts. In the neonatal hippocampus activity is characterized by repetitive bursts of synchronous neuronal firing. Here we have studied the role of the endogenous activity on the assembly of developing synapses in P3-P4 rat hippocampus *in vitro.*

Results. Inhibition of activity by tetrodotoxin for 14 h resulted in a 161% increase in synaptic density in the CA3 *stratum radiatum,* revealed by stereological counting of synaptic densities from electron micrographs. Further, an increase in the immunoreactivity against synaptophysin (349±55% of control) and dendritic GluR1 (280±50%) was seen. These changes were correlated with increased protein expression of synaptophysin and GluR1 detected in Western blots. Concurrently, the frequency and amplitude of mEPSCs were increased as a result of TTX incubation providing a functional correlate for the observed structural changes. Similar results were obtained upon blockade of the synchronous activity by the GABA-A agonist muscimol thus suggesting a critical role for the GABAergic inhibition in the formation of developing hippocampal network.

Conclusions. The synaptic connectivity in the newborn hippocampus is controlled by the characteristic network activity. In the developing hippocampal circuitry high-frequency bursts serve to maintain the dynamic balance between synaptic assembly and disassembly.

P 10 AGE DEPENDENT IMPAIRMENT IN SPATIAL LEARNING OF APP+PS1 MICE

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Background. Mutations in the genes encoding amyloid precursor protein (APP) and presenilin 1 (PS1) lead to increased beta amyloid (Aβ) accumulation and development of amyloid plaques in the brain in the familial form of Alzheimer´s disease (AD). Several transgenic mouse lines carrying these mutations have been shown to be cognitively impaired especially in spatial learning.

Methods. In this study, we investigated the effect of aging on cognitive functions in APP/PS1 double transgenic male mice by using the Morris water maze and the position discrimination task in a T-maze. Transgenic and control mice were tested at the age of 4 and 11 months. Previously, it has been shown that these mice have elevated Aβ levels in the brain and develop amyloid plaques starting at age of 9 months. Early in the process, the amyloid plaques are most common in the hippocampus and the subiculum.

Results. At the age of 11 months, the APP/PS1 mice were clearly impaired in the Morris water maze compared to the wild type mice. However, the task performance of the mouse lines did not differ at age of 4 months. By contrast, APP/PS1 were as good as the wild type mice at either age in the position discrimination task which is not sensitive to hippocampal damage.

Conclusions. The task specific and age dependent impairment in spatial learning in the APP/PS1 mice corresponds to the progressive amyloid pathology that is most severe in the hippocampus. Therefore, these data suggest that amyloid accumulation in the hippocampus is closely associated with the cognitive impairment in these mice.

P 11 INCREASED Aβ ACCUMULATION IN THE HIPPOCAMPUS OF APP/PS1 MICE CORRELATES WITH IMPAIRED WATER MAZE PERFORMANCE.

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Background. Several mutations in the genes encoding the amyloid precursor protein (APP) and presenilin 1 (PS1) genes lead to increased beta amyloid (Aβ) accumulation and development of amyloid plaques in the brain, and lead to familial Alzheimer's disease. The double mutant APP/PS1 transgenic mice used in this study have elevated brain Aβ levels and develop amyloid plaques at the age of 9 months. At the early phase of the pathology, the plaques are largely confined to the hippocampus and subiculum.

Methods. We investigated the performance of 11 months old APP/PS1 male mice in two cognitive tasks, the Morris water maze and position discrimination in the T-maze. Furthermore, we examined the relationship between hippocampal Aβ levels, number of amyloid plaques, and the performance in the behavioral tests.

Results. Compared to wild type mice, APP/PS1 mice were impaired in spatial navigation that requires normal functioning of the hippocampus, but not in habit learning in the T-maze that is not compromised by hippocampal damage. The impaired water maze performance of the APP/PS1 mice correlated with the increased levels of hippocampal Aβ40 and Aβ42.

Conclusions. These results suggest that amyloid accumulation in the hippocampus in the APP/PS1 mice accounts for their deficits in spatial navigation. This mouse model offers a reasonable tool to study pathological changes in the hippocampus.

P 12 THE ELECTROPHYSIOLOGICAL CHANGES IN APP/PS1 DOUBLE AND SINGLE MUTANT TRANSGENIC MICE

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The mutations of certain familiar forms of Alzheimer's disease (AD) involve three genes: amyloid precursor protein (APP), presenilin 1 (PS1) and 2 (PS2). In order to find electrophysiological characteristics of these mutations we used transgenic mice carrying human APPswe and PS1-A264E mutations to record EEG and auditory evoked potentials (EP).

In APP/PS1 double mutant mice there were significant differences in cortical theta ($P<0.01$) and beta ($P<0.01$) power in EEG and in cortical P35 latency ($P<0.05$) when compared with wild-type mice (mice age was from 7 months to 13 months). Also cortical P35 latency increased significantly during age in both groups ($P<0.01$). But there were no differences in hippocampal theta ($P>0.05$) and hippocampal frequency of maximum power ($P>0.05$). For further study we recorded cortical EEG and P35 latency in APP, PS1 single mutant mice comparing APP/PS1 double mutant and wild-type mice when mice age was 8 months. The results showed that there were differences between the groups in cortical theta ($P<0.05$) and beta ($P<0.01$) and in cortical P35 latency ($P<0.001$). APP single mutant mice were different from PS1 ($P<0.05$) and wild-type mice ($P=0.05$) but no difference from APP/PS1 mice ($P>0.05$) in cortical theta power. In cortical P35 latency APP mice also showed difference from wild-type mice ($P<0.05$) but no difference from APP/PS1 mice ($P>0.05$).

These results suggest that APP/PS1 double mutant mice have quite different electrophysiological features and that it is mainly the APP mutation that induces the changes in EEG and P35 latency. These findings could be useful indicators for early clinical diagnosis of certain familiar AD.

P 13 THE EFFECTS OFFIMBRIA-FORNIX LESION ON AMYLOID PATHOLOGY AND SPATIAL LEARNING AND MEMORY IN APP AND PS1 DOUBLY MUTANT TRANSGENIC MICE

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Background: The characteristic pathological features of Alzheimer's Disease (AD) include the extracellular deposition of amyloid β peptide (Aβ), intracellular formation of neurofibrillary tangles (NFTs) and the loss of basal forebrain cholinergic neurons. It is still not understood how cognitive deficits, amyloid pathology and cholinergic degeneration may be interrelated.

Methods: To investigate whether a compromised cholinergic system may influence the APP metabolism and the processes of learning and memory, male APP and PS1 doubly mutant transgenic (AP) mice and wild type controls (WT) were fimbria-fornix (FF) or sham lesioned at the age of 7 months. After 10 months recovery, the mice were tested for spatial working memory in T-maze and 8-arm radial maze (RAM), and for spatial reference memory in Morris water maze. Following the behavioral tests, the mice were sacrificed for biochemical or histological study.

Results: Extensive amyloid depositions were found throughout the hippocampus and cortex; but no difference of amyloid plaques count was found between FF-lesioned and non-lesioned AP mice. Compared with WT mice, AP mice showed an impaired long-term memory as revealed in the follow-up of RAM task and spatial reference memory deficits in performing water maze task. However, AP mice did not show any working memory impairments on performing the delayed alternation task in T-maze and RAM. FF-lesion severely impaired the performance of both AP and WT mice in the delayed alternation task in T-maze and RAM and significantly increased the latency of mice to find the platform in the water maze. Nevertheless, no interaction between FF-lesion and transgene was revealed in this study.

Conclusions: Our results indicate that amyloid deposition in mice impairs hippocampal dependent spatial long-term memory but not working memory. The cholinergic denervation of hippocampus alone is not sufficient enough to influence the APP processing and consequently accelerate the amyloid deposition. Thus, the relationship between cholinergic activity and APP processing is still unclear.

P 14 AMYLOID β_{1-42} ACCUMULATION DOES NOT AFFECT REGENERATIVE SPROUTING IN APP/PS1 MUTANT MICE.

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It has been demonstrated by many studies that, following entorhinal cortex ablations, the dentate gyrus shows a sprouting response of non-lesioned axons. We hypothesized that this response would be altered in transgenic mice expressing AD mutations (i.e., PS1 [A246E mutation] and $APP_{swe)}$, at 15 months of age when they show severe AD pathology. At this age the $A\beta_{1-42}$ levels were 40 μg/g for the AD animals.

We unilaterally lesioned the entorhinal cortex by injections of ibotenic acid in these mice, young double mutant mice, and in age-matched control animals. Four weeks later the animals were sacrificed and transcardially perfused, the brains were cut and stained for "sprouting" markers, the most consistent changes were present in the material stained for AChE and for synaptophysin, a protein that marks presynaptic terminals.

Following the lesions the ipsilateral hippocampus demonstrates sprouting, i.e., an increased expression of synaptophysin was present in the outer molecular layer of the dentate gyrus. The young control, and the young AD mice displayed a robust sprouting response to the lesion. Surprisingly, neither the age-matched control or the AD mice showed any diminishment in the sprouting response compared to the young animals.

The presence of the high levels of Aβ in these mice did not change the response of the brain to lesions compared to age-matched control mice, i.e., no change in plasticity.

P 15 MIDLIFE VASCULAR RISK FACTORS INCREASE THE RISK OF LATE-LIFE ALZHEIMER'S DISEASE. A LONGITUDINAL, POPULATION-BASED STUDY.

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Background*:* Vascular risk factors may play an important role as risk factors for Alzheimer's disease (AD), but their role is still generally undetermined. The aim of this study was to examine the relation of midlife elevated blood pressure (BP) and serum cholesterol levels to late-life Alzheimer's disease (AD).

Methods*:* Subjects were derived from random, population-based samples previously studied in one of the surveys carried out in 1972, 1977, 1982 and 1987. After an average follow-up of 21 years, a total of 1449 (73%) subjects aged 65-79 years participated in the re-examination in 1998.

Results: After adjustment for age, body-mass index, education and vascular events, subjects with high systolic BP (SBP) (≥160 mm Hg) [odds ratio (OR) 2.3, 95% confidence interval (CI)1.0-5.5] or high serum cholesterol level (≥6.5 mmol/l) [OR 2.1, 95% CI 1.0-4.4] at midlife had a significantly higher risk of late-life AD compared to subjects with normal SBP or serum cholesterol. Subjects with combined risk factors of high SBP and cholesterol at midlife had a significantly higher risk to develop AD also compared to the individuals with either of these risk factors alone [OR 3.2, 95% CI 1.5-6.8].

Conclusion: Both elevated SBP and serum cholesterol levels at midlife, and in particular the

combination of these risks, increase the risk of late-life AD. These findings point to a role for elevated SBP and serum cholesterol levels in the pathogenesis of AD, and indicate that more emphasis should be placed on observation and appropriate treatment of these conditions as potential preventive measures.

P 16 SEVERE WEIGHT LOSS AS A COMPLICATION OF THE EXPERIMENTAL STROKE MODEL IN RATS

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Transient middle cerebral artery occlusion (MCAO) is the most common method used to produce corticostriatal infarcts in rats. This experimental stroke model, however, induces a severe loss of body weight (approximately 16 %) immediately following surgery, which might affect the outcome of behavioral tests used to assess functional recovery of ischemic rats. The purpose of this study was to investigate whether the weight loss after MCAO is associated with dehydration or stress. The right middle cerebral artery of male Wistar rats was occluded for 120 min using the intraluminal filament method. Sham-operated rats were used as controls. Body weight and limb-placing were measured after surgery. Rats were sacrificed 3 or 12 h, 1, 2, 3, 7, 14, or 28 d after surgery and blood samples were taken by cardiac puncture. Plasma osmolality was analysed to determine the level of hydration. Plasma corticosterone levels, determined using radioimmunoassay, were used as an index of stress. The plasma osmolality did not change after surgery, indicating that the rats were not dehydrated. Plasma corticosterone levels were higher 3 and 12 h after surgery in both ischemic and sham-operated rats, followed by a decrease to the preoperative level within 24 h. These data suggest that weight loss after MCAO in rats is not associated with dehydration or long-lasting postoperative stress.

P 17 AGING, ESTROGEN, AND PLACE CELLS

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Background: Hippocampal place cells represent a window into the declarative memory of a rat. We looked through this window at the workings (on the single cell level) of spatial memory from both aging male rats and estrogen-manipulated female rats. We therefore used rat hippocampal place cells in order to examine the workings of spatial memory in relation to two current issues of Alzheimer's, aging and ovariectomy. Aging is known to cause spatial memory deficits in rats, while estrogen-level has been shown to modulate hippocampal physiology.
Methods: In the first experiment we recorded place cells from 9 aged Long-Evans males (27 months old) and 6 young males (7 months old); in the second experiment we recorded from 7 ovariectomized, aged Wistar females (20 months old), 6 ovariectomized-plus-estradiol-injected, aged females, and 7 sham-operated, aged females. These five groups of animals were all recorded in two environments, alternating between a familiar one and a novel one for a total of 5 times, each recording taking 7 minutes.
Results: Rats from all groups had almost equal numbers of stable / unstable fields in the familiar environment; instead, the most discriminating parameter was the response to the change in environment. Place cells of the young male rats generally exhibited stable, new fields in the new environment, while aged male rats, particularly those who had performed poorly on the spatial water maze task, had place fields which remained rigid despite changes in the surrounding environment. Of the female, aged rats, a sizable portion from all groups showed this rigidity as well.
Conclusions: Aged female rats had place cell characteristics which resembled those of aged male rats, and thus estrogen manipulations neither accelerated nor allayed the spatial memory deterioration of aging.

P 18 Interaction between Estrogen and NMDA Receptors on Synaptic Plasticity in the Mouse Hippocampus.

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Background: There is strong evidence that estrogen induces structural and functional changes in hippocampus. In rodents, ovariectomy attenuates NMDA-mediated neurotransmission, which can be reversed by estrogen replacement therapy (ERT). To test the interaction between estrogen and NMDA receptors, we compared the dose-response curves for a competitive NMDA antagonist in ovariectomized (OVX) mice with or without ERT.

Methods: Female ovariectomized C57Bl/6J mice were used in both behavioral (12 months old) and electrophysiological (7 months old) studies. Half of the mice were given ERT in the form of a subcutaneous pellet. The mice received either (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) at 0.5, 2.0, or 5.0 mg/kg or saline intraperitoneally during behavioral studies, and direct infusion of drug (CPP, 5.0, 10.0 uM) to the incubation fluid during slice studies. Spatial learning and memory was studied in the water maze (WM) and field potentials (EPSP) in hippocampal CA1 area *in vitro*.

Results: CPP impaired finding of the hidden platform (WM) dose-dependently. During the last day of testing, the impairing effect of CPP was more pronounced in the OVX mice than in the OVX+ ERT mice. Also in the probe trial with the platform removed, OVX+ERT mice receiving CPP at 2 or 5 mg/kg spent more time in the former platform location than the OVX mice. The initial induction of LTP without CPP did not differ between the OVX and OVX+ERT groups. However, CPP at 5 µM blocked LTP in the OVX but robust LTP was still observed in the OVX+ERT mice. We found a significant difference in the EPSP amplitude and slope between the groups at 15 and 30 minutes after LTP induction.

Conclusion: The data is consistent with the hypothesis that estrogen affects the number of NMDA receptors in the hippocampus, because a higher dose of CPP was needed in the ERT group than in the control group to depress learning and LTP.

P 19 THE CORRELATION BETWEEN COGNITIVE PERFORMANCE AND THE NUMBERS OF CHOLINERGIC NEURONS IN THE BASAL FOREBRAIN OF OVARIECTOMIZED AND ESTROGEN TREATED MICE

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Background. Malfunction of cholinergic system has been shown to correlate with cognitive decline observed in Alzheimer's disease. Recent epidemiological studies have indicated that estrogen replacement therapy (ERT) may improve cognitive abilities in post-menopausal women. The experimental studies suggest that ERT may enhance the function of cholinergic projections to the hippocampus and cortex, and thus improve cognitive performance. In this study, we investigated whether ovariectomy and ERT influence the numbers of basal forebrain cholinergic neurons and cognitive performance in mice, and whether there is any correlation between these two parameters.

Methods. Aged (22-month old) female C57BL/6J mice were either Sham-operated; ovariectomized (OE) 2 or OE 6 weeks before behavioral testing; and OE 2 weeks before testing and treated with ERT (OE+ERT; s.c. estradiol pellets). Mice were tested in the radial arm maze (RAM), T-maze, Y-maze and water maze. The brain sections of these animals were immunostained for choline acetyltransferase. Cell counting was performed using unbiased stereological methods.

Results. There were no statistical significant differences either in behavioral studies or in numbers of cholinergic neurons between the four groups. However, the number of reference memory errors in RAM correlated with the numbers of cholinergic cells in the medial septum/vertical diagonal band in Sham-operated and ERT mice groups.

Conclusions. The findings give evidence that in mice having normal physiological level of circulating estrogen, cholinergic system can play a significant role in certain cognitive performance.

P 20 Retrograde amnesia for spatial memory induced by NMDA receptor-mediated long-term potentiation

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Background

If information is stored as distributed patterns of synaptic weights in the hippocampal formation, retention should be vulnerable to electrically induced long-term potentiation (LTP) of hippocampal synapses after learning.

Methods

Rats were implanted with electrodes in the angular bundle and the dentate gyrus, and trained over 5 days in a spatial watermaze task. After a spatial probe test, we delivered bursts of high-frequency (HF) or control stimulation to the perforant path in the angular bundle, before retention was tested again.

Results

High-frequency stimulation induced LTP in the dentate gyrus and probably also at other hippocampal termination sites. Retention was disrupted after HF stimulation. When the competitive NMDA receptor antagonist CPP was administered prior to the high-frequency stimulation, watermaze retention was unimpaired. CPP administration blocked the induction of LTP.

Conclusions

High-frequency stimulation of hippocampal afferents disrupts memory retention only when it induces a change in the spatial pattern of synaptic weights. The NMDA receptor dependency of this retrograde amnesia is consistent with the synaptic plasticity and memory hypothesis.

P 21 Role of NMDA receptor mediated neurotransmission in the regulation of spatial working memory.

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Introduction: Blockade of glutamatergic N-methyl-D-aspartate (NMDA) receptors has a disrupting effect on spatial learning and memory. In a cellular level, antagonising the NMDA receptors prevents the induction of long term potentiation (LTP) in the hippocampus, a phenomenon considered to be a synaptic model for neural plasticity and learning. It is possible, that NMDA receptors are also important in the regulation of working memory.

Methods: Male Han:Wistar rats aged 10 months were used in the experiment. Working memory performance was measured using delayed non-matching to position task, that monitors also non-mnemonic behavioural variables. CPP, a non-competitive NMDA receptor antagonist, scopolamine, a non selective muscarinic antagonist, and pirenzepine, a selective m1 receptor antagonist, were used to compare the effects of NMDA receptors and muscarinic receptors in the regulation of spatial working memory. The drugs were I.C.V. administered.

Results: Scopolamine (3 and 10 µg), pirenzepine (10 µg) and CPP (0.3 µg) all decreased % correct choices delay-independently. In addition scopolamine and CPP reduced motivation and motor activity. Combined administration of subthreshold doses of scopolamine (1 µg) and CPP (0.01 and 0.03 µg) resulted in non-memory-specific behavioural disruption, i.e. increased the omissions and the latency of sample press. Subthreshold doses of pirenzepine (10 µg) and CPP (0.03 µg) together did not affect any of the measured parameters significantly.

Conclusion: The present data suggests that both the blockade of central muscarinic acetylcholine and NMDA receptors disrupts non-mnemonic aspects of the delayed non-matching to position task performance. In addition, NMDA and muscarinic receptors do not jointly modulate working memory *per se*.

P 22 Analysis of the hippocampal place cells activity during free exploration.

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Background: Most of the studies of hippocampal place cells (PC) employ foraging or other forms of reinforced behaviour. The relative importance of free exploration on the activity of the PC is still unclear. We have studied the emergence and stability of the CA1 place cells activity after repeated exposures to a new environment, preceded with recordings in the home-cage.

Methods: Single microelectrode recordings were made in the CA1 field of the hippocampus. Behavioural testing in the open-field apparatus was commenced only after the complex spike cells were recorded in the home cage on two consecutive days. Data was clustered by using Off-line Sorter and processed by dedicated software (Sess-Analysis).

Results: The PC were recorded from the very first exposure to a new environment, some of those cells could also be identified in the home cage. New PC have appeared in the course of repeated exposures. Analysis was focussed on the properties of individual PC recorded over long period of time (weeks). Firing stability and place-field properties were evaluated by several parameters (like spatial selectivity, spatial coherence etc.). Some PC displayed many firing fields, which appear to vary their characteristics over the course of testing.

Conclusions: The present study provides evidence that free exploration task reveals the existence of PC with multiple firing fields. Furthermore, the PC can dynamically modulate their firing properties during the course of exploration.

P 23 Place fields of hippocampal pyramidal cells in the watermaze

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To provide a background for studying place-related activity in hippocampal neurones during spatial learning, we compared the activity of hippocampal place cells in an annular watermaze and an analogous land-based task.

Rats were implanted with tetrodes in area CA1 of the hippocampus. To make sure that the rat traversed the apparatus in a uniform manner, we constrained swimming to a circular corridor.

Pyramidal cells had robust place correlates in both conditions, and a significant proportion of the cells had place fields at the same locations. Firing rates were slightly higher in water than on dry land. The place fields were under stronger directional modulation during swimming. Directional sensitivity appeared regardless of whether the animals were trained to find a platform or not. There were directionally modulated units also in the open watermaze, but the number was smaller than in the corridor.

Place fields in the watermaze appear to be largely controlled by the same factors as on dry land, in spite of the differences in kinesthetic and vestibular input. Differences in firing rate and directional control may depend on the geometric and cognitive structure of the task rather than the medium on which the rats are moving.

P 24 Place representation in hippocampal area CA1 in the absence of input from area CA3

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Background

The indirect pathway to CA1 from the entorhinal cortex (EC) through the trisynaptic circuit has been regarded as the main excitatory input to CA1. However, anatomical evidence suggests that the role of the direct pathway from EC to CA1 may have been underestimated. We examined whether pyramidal cells in area CA1 exhibit location-specific activity in the absence of the indirect input from CA3.

Methods

Localized knife-cuts were made along the longitudinal axis of the hippocampus to disconnect CA3 from CA1, and microdrives with four tetrodes were then implanted in CA1. To avoid input from the contralateral hippocampus, the contralateral hippocampus was removed by ibotenic acid. CA1 pyramidal cell activity was recorded from the rats while they walked on a linear track or in a square black box ($1m^2$) with a white cue card on one of the sidewalls.

Results

CA1 cells showed place fields both on the treadmill and in the box, and many fields remained stable between trials. Histological examination revealed that the cut had separated the CA1 from the CA3 along most of the dorsal hippocampus.

Conclusions

The indirect pathway to CA1 through CA3 may neither be necessary for establishing nor maintaining place fields in single CA1 pyramidal cells. Control experiments with retrograde tracer injections in CA1 are being conducted in order to confirm that the recording area is disconnected from CA3.

P 25 Information Transfer Between Rhythmically Coupled Networks: Reading the Hippocampal Phase Code

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Background: There are numerous reports on rhythmic coupling between different brain networks. It has been proposed that this rhythmic coupling indicates exchange of information. So far, few computational models have been proposed which explore this principle and its potential computational benefits. Recent results on hippocampal place cells of the rat provide new insight: it has been shown that information about space is encoded by the firing of place cells with respect to the phase of the ongoing theta rhythm: phase coding. A network reading the hippocampal output must inevitably also receive an oscillatory theta input in order to decipher the phase coded firing patterns.

Methods: A computational model of two coupled networks has been constructed. The first network, modeling the hippocampal CA3 region, produces phase coded information by repeated sequence read out. The second network reads and decodes the hippocampal firing patterns. Both networks receive rhythmic theta drives with a variable phase difference. The network neurons are modeled as integrate-and-fire units.

Results: By changing only the phase of the theta input to the decoder qualitatively different information is transferred from the encoder: the theta phase determines whether representations of current or upcoming locations are transferred to the decoder. Several brain regions (entorhinal, cingulate and/or prefrontal cortex) could make use of the hippocampal phase decode as will be discussed.

Conclusion: The proposed mechanism provides a computational principle for information transfer between oscillatory networks and might generalize to brain networks beyond the hippocampal region.

P 26 CORRELATES OF COMPLEX SPIKES IN CA1 PYRAMIDAL CELLS

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Pyramidal cells in the hippocampus are known to fire to distinct types of spiking events: single spikes and complex spike bursts (Ranck, 1973). A complex spike is a series of action potentials separated by short (3-5 ms) intervals. Complex spikes might serve a role in neuronal signalling, by allowing more reliable transmission across axonal branching points and weak synaptic connections. Bursts may also serve a role in memory formation, as post-synaptic bursting may potentiate synapses.

We recorded the activity of CA1 pyramidal cells as trained rats swam in a corridor-constrained version of the water-maze. Spiking events of isolated units were classified as single or complex spikes on the basis of inter-spike intervals, which were required to be less than 15 ms within bursts. We observed that each unit displayed a characteristic amount of bursting, typically in the range of 10-50% of the spiking events, and this proportion was consistent across several recording sessions. Place fields of single and complex spikes were co-localised in space. Place fields of complex spike events were less sparse than the field of single spikes. A subset of cells with fields on the platform burst on nearly all events.

These results are consistent with the idea that complex spikes may serve a role in neuronal signalling during memory processing.

P 27 Subcortical modulation and local axon collaterals of septally projecting non-principal cells in the rat hippocampus

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We aimed to study the morphology and pharmacology of non-principal cells that give rise to the hippocampo-septal GABAergic projection. Fluorescent microbeads were injected into the medial septal area of young rats (PD 16-17) in order to retrogradely label the hippocampo-septal projecting cells (HS cells). After 2 days of survival, in vitro hippocampal slices were prepared. The HS cells were identified in the CA1 area str. oriens with a fluorescent microscope using a CCD camera. Cell-attached and subsequent whole-cell recordings were performed to investigate the response properties of HS cells to different drugs. The effect of several receptor agonists (Ach, 1mM; ACPD, 10 μM; 5HT 10 μM) has been tested on the cells using the U-tube application system (1-3sec application of the drugs) in the presence of ionotropic glutamate and GABA receptor blockers. In the cell-attached mode, the pharmacological modulation of the cells' firing was tested. In the whole-cell mode (Vh = -60mV), the underlying agonist-induced currents were measured. During the recordings the cells were filled with biocytin through the recording pipette. They were then visualized using the ABC method and reconstructed using camera lucida.
In the vast majority of cells all 3 agonists increased the firing of the cells and induced an inward current. The HS cells had local axon collaterals in strata oriens and radiatum, three of them even projected across the hippocampal fissure into the dentate hilus. Therefore, they are most probably distinct form the well-characterized O-LM (oriens/lacunosum-moleculare) cells, and likely correspond to the backprojection neurons.

P 28 BACKGROUND, BUT NOT FOREGROUND, CUES ANCHOR PREFERRED DIRECTIONS OF ANTERODORSAL THALAMUS NEURONS IN RAT.

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Background. A likely neural substrate for spatial learning are head direction (HD) cells. In the anterodorsal (AD) thalamic nucleus they discharge selectively when the rat orients its head in a 'preferred' direction (PD). Salient visual cues strongly influence the PDs of HD cells. Here we study which cue properties engage the HD cells, in particular relative distance to background.

Methods. In a square room bordered with black curtains, rats foraged on a table in the presence or absence of a black cylindrical enclosure. Three distinct objects were placed at equal distances against the enclosure. PDs were tested before and after rotating the objects by ±120°. Then the cylindrical enclosure was removed but the objects maintained in their same positions. Again, preferred directions were determined before and after object rotation. Between manipulations, the rats were disoriented in darkness.

Results. While the cylindrical enclosure was present, the PDs of all 30 HD cells rotated by the same angle as the objects. But after the enclosure was removed, the preferred directions remained fixed relative to the room - even though the objects remained salient (since the rats contacted them frequently).

Conclusions. These data are consistent with the hypothesis that the head direction system is preferentially influenced by visual cues in the background regardless of their actual distance from the animal.

Support: CNES, Cogniseine, GIS, FRM.

P 29 Does the hippocampus have a role in fear expression?

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Background

Converging evidence suggests that the hippocampus is essential for spatial and contextual learning in particular and episodic or declarative learning more generally. Connections between the hippocampus and several nuclei of the amygdala and hypothalamus suggest that the hippocampus may also influence non-mnemonic functions such as emotional behaviour and internal state.

Methods

To examine whether the hippocampus has a role in expression of unconditioned fear, we made ibotenate lesions of the hippocampus and measured unconditioned fear during exposure to an unfamiliar elevated plus-maze with two open arms and two closed arms.

Results

Animals with hippocampal damage exhibited less fear behaviour in the elevated plus maze. Whereas the sham-operated control animals spent most of the trial time within the closed arms, rats in the hippocampal group visited the open arms both more often and for longer periods. They also spent more time in the central area. The groups did not differ significantly in total path length, total time moving or freezing. There was no differential preference on a subsequent test with all four arms closed. The rats with hippocampal damage had longer escape latencies in a watermaze reference memory task and failed to show any preference for the platform area on the probe trials.

P 30 Involvement of HB-GAM and N-syndecan in learning and memory pathways: evidence from genetically modified mice.

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Background. HB-GAM (heparin-binding growth associated molecule) is a secretory, extracellular matrix-associated protein promoting neurite outgrowth. The cellular effects of HB-GAM are dependent on binding to the transmembrane heparan sulphate proteoglycan N-syndecan. The role of HB-GAM and N-syndecan in the long-term potentiation (LTP) has been studied in hippocampal slices from genetically modified mice. Inhibition of the LTP was observed in HB-GAM transgenic mice, whereas the knockout mice had a decreased threshold for the induction of LTP. N-syndecan knockout mice displayed enhanced LTP.

Methods. We employed a large-scale battery of behavioural tests to characterise the mutant mice. Learning and memory was assessed by means of Morris water maze.

Results. We have not observed any gross abnormalities in neurological functions, locomotor activity or co-ordination of the mutant mice. However, striking differences between the genotypes were established in learning paradigms. HB-GAM transgenic animals displayed faster learning and better memory than wild type mice in the initial phase of training. On the other hand, HB-GAM knockout mice learned initial position of the escape platform at the level of controls, but were impaired in subsequent reversal learning. N-syndecan knockout mice learned slower than respective controls.

Conclusion. HB-GAM and its receptor N-syndecan are involved in synaptic plasticity and in spatial learning. Our results do not suggest any straightforward relationship between LTP and the ability of spatial learning. For example, in the case of HB-GAM knockout mice the enhanced synaptic plasticity may lead to "overimprinting" of the learned tasks which leads to cognitive rigidity. Further testing is required to establish the consequence of gene effect more explicitly at the behavioral level.

P 31 A perirhinal cortex activation during visual associative encoding: An fMRI study

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Background: The entorhinal and perirhinal cortices (PrC) are located in the medial temporal lobe (MTL). The involvement of the rhinal cortex in declarative memory encoding and association formation is well established from rat and non-human primate studies. Little is known, however, about the function of the rhinal cortex in humans *in vivo*. Therefore, functional magnetic resonance imaging (fMRI) was used to examine the participation of the rhinal cortex in visual associative encoding.

Methods: A total of 12 subjects (6 males, mean age = 25) participated in this study. fMR-imaging was performed on a Siemens Vision 1.5 T scanner (gradient-echo EPI sequence; 128 sets of 16 5-mm axial slices). Image processing was performed using MEDx software. Results: The rhinal cortex was activated in 8 of the 12 subjects ($p \leq 0.00001$); the location of the rhinal cortex activation was in the medial bank of the collateral sulcus, in the posterior PrC. The hippocampus, posterior parahippocampal gyrus, and the temporo-parietal association cortices were also activated.

Conclusions: To our knowledge, this is the first fMRI study reporting activation in humans in this medial part of the PrC during visual associative encoding.

P 32 Latency shift of auditory event-related potentials as a model for short term memory.

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Background: In human psychophysical studies, the latency to respond in a short-term memory task is proportional to the working memory load up to 7+-2 items. We wanted to assess if memory load also affects latencies of auditory ERPs using the mouse a model.

Methods: Eight C57BL female mice were used in experiment. The 3 different sounds (all 3 complex sounds or one complex and two pure tones 3 and 5 kHz) were presented randomly in sequence, about 133 times each. The inter-stimulus interval (ISI) was 1,3 seconds. All sounds were 30 ms in duration with 5 ms onset/offset. ERPs were recorded between a parietal cortical screw electrode, and between two hippocampus electrodes (different between tips about 0.5 mm). A frontal cortical screw served as the common reference electrode.

Results: The latency of the ERP was dependent on the number of stimuli between repetitions of the same stimulus. When the sound was repeated instantly, the averaged ERP peak latency (mean 56 ms after onset) was significantly shorter than averaged ERP after one or more intervening sounds. The latency shifted as a function of the number of intervening sounds from one (mean 67 ms) to three or more intervening sounds (mean 88 ms). The averaged ERP latencies after one and two intervening sounds were significantly different from averaged ERPs after three and more intervening stimuli. In addition, the latencies after one and two intervening sounds were significantly different from each other.

Conclusion: The ERP peak latency is function of the number of intervening stimuli. This finding is consistent with the notion that repeated stimuli induce a memory trace to which incoming information is compared with. Our finding suggest that mice can keep a list of three sound items in memory and compare every incoming information with all items in the list starting from most recent.

P 33 STIMULATION FREQUENCY SPECIFIC GAMMA SPIKES IN THE CA1 AREA OF THE HIPPOCAMPUS *IN VIVO*.

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Background. Gamma oscillation (25-80 Hz) is associated with sensory binding and memory. We studied whether electrical stimulation induced gamma oscillation is maintained after the stimulation and, more specifically, whether the exact frequency is retained.

Methods. Thirty 250-350 g Wistar rats were urethane anesthetized and extra- and intracellular responses from CA1 were recorded during and after electrical fimbria fornix stimulation. The stimulation consisted of four 0.2 ms pulses delivered at frequencies from 30-60 Hz with an underlying theta frequency. The intervals between gamma stimulations patterned normal hippocampal electrical activity and provided a timeframe for internally generated events. Each animal received multiple stimulation frequencies.

Results. Gamma stimulation directly evoked population spikes followed by? single or repetitive spikes, not time-locked to the stimulus, at the stimulation frequency. Additionally, fast gamma stimulations often resulted in spikes at only half the stimulation frequency, but still within the gamma frequency band. Furthermore, in 40% of the animals a short gamma population burst of two to eight spikes appeared at the stimulation frequency 400-600 ms after the stimulation.

Conclusions. We conclude that the gamma stimulation caused short lasting frequency specific changes in the CA1. These alterations can result from sustained network resonance or a change in synaptic weights.

P 34 DISTINCT EARLY PHASE-LOCKED GAMMA RESPONSES EVOKED BY SPEECH AND NON-SPEECH SOUNDS IN HUMANS

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Background:

In humans an early phase-locked gamma response is evoked by visual and auditory stimuli at latencies 20 to 100 ms after stimulus onset. The dependence of the response on stimulus parameters and the functional role of the response have remained unclear and controversial.

Methods:

We used magnetoencephalography (MEG) to measure evoked responses to the speech sound /pa/ and its complex non-speech acoustical counterpart. We computed time-frequency representations and estimated peak latencies, amplitudes and frequencies for both hemispheres.

Results:

Gamma-responses to the speech and non-speech sounds differed in their latency and amplitude distributions. Gamma-responses to the speech sound peaked earlier in the left than right hemisphere, whereas the reverse was true for the non-speech sound. The gamma amplitude for the non-speech sound was right-lateralized at all frequencies, whereas for the speech sound the amplitude was right-lateralized only at high frequencies (45-70 Hz).

Discussion:

Gamma oscillations and synchronous firing have been hypothesized to underlie feature binding. According to previous studies, high-level-object representations are needed already 120-150 ms after stimulus onset and thus we propose that already the early phase-locked gamma oscillation may underlie a bottom-up neuronal representation of the presented stimulus.

P 35 Pre-attentive memory storage of temporal relations between sounds – differences between musicians and non-musicians.

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Background

The mismatch negativity (MMN) is a pre-attentive electrical brain response, which is elicited when an incoming acoustical event does not correspond to the memory of the preceding sounds. We investigated whether this pre-attentive memory system can encode temporal relations between tones and whether musicians differ in this respect from non-musicians.

Methods

We measured the MMN in response to continuous streams of tones presented to subjects who were watching a silent video and disregarding the sound stimuli. Within the stream of tones, four subsequent tones of the same pitch were most of the time followed by four tones of another pitch. Occasionally, there were five tones of the same pitch. These fifth tones violated the length of the repetitive four-tone sequences. We recorded musicians and non-musicians with a slow paced version and non-musicians with a faster paced version.

Results

Musicians showed an MMN to the occasional fifth tones in the slow paced version, whereas non-musicians did not. Non-musicians did, however, show an MMN in the fast paced version.

Conclusions

The MMN data indicate that both groups are able to pre-attentively store temporal relations between sounds. What distinguishes musicians is that they can store temporal relations over a longer time span.

P 36 Brain oscillations in the 20 Hz band are modulated by audiovisual speech perception - a MEG Study

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Background: The so-called "McGurk effect is a robust finding demonstrating that the perception of acoustical utterances is modified by viewing the speaker's lip movements. The aim of this study was to explore how cortical rhythms are modulated by auditory utterances which match the lip movements (congruent) as compared to utterances which do not (incongruent).

Methods: We employed four different types of audiovisual utterances. The standard utterances (85%) were congruent /ipi/ utterances, the deviant stimuli were either congruent (5%) or incongruent (auditory /iti/ and visual /iti/ (5%)) /iti/ utterances. Five percent of the stimuli were congruent /ivi/ utterances (auditory /ivi/ and visual /ivi/) which the subjects were instructed to silently count. Brain activity was measured using a 306-channel whole-head magnetometer. To characterize the frequency content of the signals following the presentation of the syllables, time-frequency representations (0-40 Hz, -550 - 1050 ms) were calculated for each trial and then averaged.

Results: We observed a decrease in beta band (18-24 Hz) power after the presentation of both congruent and incongruent audiovisual deviant stimuli at ~200-400 ms after sound onset. An increase in the rhythmic activity in the beta band (18-24 Hz) ~500-1000 ms was observed after the presentation of congruent deviant stimuli only.

Conclusions: The dissociation of rhythmic responses in the beta band between the presentation of congruent and incongruent audiovisual utterances might reflect differential processing of congruent and incongruent audiovisual information. If an increase in beta rhythm reflects inhibition or disengagement of cortical sensorimotor networks, then the lack of increase in the beta rhythm observed after incongruent utterances might signify that sensorimotor networks are involved in the post-processing of conflicting audiovisual stimuli

P 37 DEVIANCE IN VISUAL SPEECH ELICITS MISMATCH FIELDS

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Background: Seeing incongruent articulatory gestures may change the auditory percept phonetically (McGurk and MacDonald 1976). We studied the neural basis of auditory modifications caused by visual speech with a 306-channel whole-head magnetometer by recording neuromagnetic mismatch fields (MMFs), which are known to be elicited in the auditory cortices by an occasional change in a sound sequence. We wanted to find out whether a change in the visual features of speech could also be detected in the auditory cortices and thus elicit MMFs.
Method: We presented among standard speech stimuli, which were congruent audiovisual /ipi/ utterances, both congruent (acoustic /iti/, visual /iti/) and incongruent (acoustic /ipi/, visual /iti/) deviants. Both deviant stimuli were perceived as /iti/.
Results: The results from 7 subjects show that both congruent and incongruent deviants elicited MMFs. Thus, even when there was no acoustical difference between the deviant and standard, MMF was elicited, in agreement with Sams et al. (1991). The sources of both MMFs seem to be in the auditory cortices
Conclusion: Our results show that a change in the visual features of speech stimuli is detected in the auditory cortices. This indicates either (1) that the sensory memory trace represents both acoustical and visual features of standard speech stimuli or (2) that the visual features modify auditory features before the sensory input is compared with the sensory memory trace.

P 38 Long-range temporal correlations in the somatosensory mu oscillations during rest and median nerve stimulation

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Background Spontaneous mu oscillations fluctuate erratically in amplitude during resting-state conditions, and are also modulated by somatosensory stimuli. Recently, we found that in the condition of rest, the mu oscillations exhibit long-range temporal correlations of a power-law form, indicating that the underlying neural networks carry a memory of their own dynamics for hundreds of seconds [1]. Here we investigate the effect of somatosensory stimuli on the temporal correlations of the mu oscillations.

Methods Spontaneous activity was measured with MEG for 30 min in five subjects. The left median nerve was electrically stimulated every 3 s. The temporal correlations of the mu-oscillation amplitude were quantified with detrended fluctuation analysis (DFA) [1].

Results In the rest condition, the mu-oscillation fluctuations exhibited power-law scaling behavior with temporal correlations characterized by the DFA exponent $a = 0.68 \pm 0.06$. In the stimulation condition, power-law scaling with a smaller DFA exponent ($a = 0.60 \pm 0.04$) was observed.

Conclusion We conclude that power-law scaling behavior of the somatosensory mu oscillations may be preserved despite its perturbations by median nerve stimulation, although the strength of the temporal correlations decreased. We propose that the processing of the somatosensory stimuli breaks up parts of the intricate spatial patterns of correlations created continuously by the spontaneous network oscillations.

[1] Linkenkaer-Hansen *et al., J. Neurosci,* February 15, 2001.

P 39 High-resolution magnetic recordings of spike-like (600 Hz) SEF bursts

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Background

As a response to electric nerve stimulation, the human brain generates a high-frequency (600 Hz) burst superimposed onto the N20m response from the primary somatosensory cortex. This burst is supposed to reflect the timing of rapidly repeating population spikes in thalamo-cortical afferents and/or intracortical neurons.

Methods

We used a whole-head magnetoencephalographic system comprising magnetometers and gradiometers to record high-resolution SEF to electric median nerve stimuli (ISI 110 ms, acquisition passband 1 Hz - 1.2 kHz, 3-kHz sampling rate, 25000 responses averaged, offline upsampling to a rate of 24 kHz). After high-pass filtering at 300 Hz we calculated the signal-to-noise ratio (SNR) for each channel.

Results

The high-frequency burst was detected with an SNR of 17 (12) in the best magnetometer (gradiometer) channel. Optimized noise reduction increased the SNR of the best magnetometer channel to 25. The magnetic burst field maps were found dipolar (resembling the N20m maps) at the peaks and troughs of the oscillatory cycle but showed a complex sequence of rotations and even quadrupolar patterns in-between.

Conclusions

In the present experimental setting magnetometers clearly outperformed gradiometers in terms of burst SNR. The exceedingly high SNRs for a large number of magnetometer channels allowed to reveal a complex and non-stationary burst field pattern indicating that multiple and/or moving sources contribute to these spike-like SEF components.

P 40 QUANTIFICATION OF DYNAMIC PHASE SYNCHRONY ACROSS FREQUENCY BANDS

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Background Synchronous neuronal oscillations at a number of frequency bands are a hallmark of the mammalian brain and have been proposed to relate eg. to a variety of perceptual and mnemonic functions. Little, however, is currently known about their mutual dynamics and cross-frequency phase correlations.

Methods Convolution of a signal with a Morlet (or Gabor) wavelet of some center frequency f yields the phase of the signal at the narrow frequency band as a function of time. The phase-difference f_{xy} of signals x and y at frequencies f_x and f_y is given by $f_{xy} = mq_x - nq_y$, where integers m and n give the ratio of the frequencies: $mf_x = nf_y$. The degree of phase-locking can be estimated with a phase-locking factor and statistically tested with shuffled data.

122-channel MEG and 64-channel EEG were recorded from 5 subjects in an attentive audiovisual paradigm.

Results Complex temporal patterns of robust phase correlations between theta, alpha, beta and gamma frequency bands were found to follow the audiovisual stimuli.

Conclusions We have developed a novel wavelet-based method with optimal time-frequency resolution for the quantification of phase synchrony across frequency bands. The method is conceptually straightforward and computationally efficient. The neuronal functions and cognitive correlates of the patterns of phase synchrony across frequency bands merit further investigation.

P 41 Combined Analysis of MEG and fMRI using Minimum l1-Norm Estimates

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The combination of MEG and fMRI, the former providing precise temporal information and the latter high spatial resolution, may form an effective non-invasive functional brain imaging tool. However, because these two imaging modalities reflect different aspects of brain function, they may indicate activity in different areas. Moreover, with the traditional analysis methods the combination of the two sets of data is not straightforward.

Here we present a method to combine MEG and fMRI results by using the fMRI data as spatial a priori information in the analysis of the MEG data. First, the average activation during the fMRI was estimated, the results were smoothened, and the activity was used as a weighting factor in a minimum-norm estimate of the MEG results. We applied an minimum l1-norm estimate, because it yields results where closely spaced small source areas can have independent activity. The estimates were calculated using a three-dimensional grid of points within the subject's brain. The active areas were projected to the surface of the brain and color-coded for visualization purposes.

The method was evaluated by analyzing MEG and fMRI measured during tactile stimulation of the palms. The results obtained from the combination were clearer than the results from either modality alone and were in accordance with the earlier studies.

P 42 Bursting activity in responses of visual neurons from the cat's cortex lateral suprasylvian area

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Background. Analysis of neuronal responses from different sensory systems has demonstrated existence of so-called bursting activity, which is related to various parameters of stimuli. Aim of this study was to investigate precise temporal structure of responses of visual neurons from the cat's lateral suprasylvian area and to relate it to changing characteristics of visual stimulus.

Methods. Data was collected from extracellular recordings from the cat's lateral suprasylvian area neurons. Two types of stimuli were used- stationary flashing light slit of different orientations and moving in different directions light slit and spot. Directions of moving and stationary stimuli have been subdivided into optimal and non-optimal based on the level of neuron responsiveness. Neuronal response was subdivided into bursting and isolated spikes and bursts of spikes based on value of critical interspike interval calculated from histogram of interspike intervals.

Results. Analysis of data from 22 neurons revealed that: 1) bursting activity is more pronounced in responses to stimuli of optimal orientations/directions as compared to non-optimal orientations/directions; 2) moving stimuli elicit longer bursts in responses then stationary flashing stimuli; 3) number of bursts composed of 2-5 spikes directly depends on type of stimulus used and optimality of orientation/direction, whereas number of longer bursts doesn't.

Conclusion. Bursts of spikes in neuronal responses from the cat's lateral suprasylvian area might be related to transmission of information about parameters of visual stimuli**.**

P 43 DISTINCT NEUROCHEMICAL FEATURES OF RETICULAR THALAMIC NEURONS IN THE ANTERIOR POLE OF RETICULAR NUCLEUS.

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The anterior pole of the reticular nucleus projects to medial thalamic nuclei (anterior group, intralaminar and mediodorsal nuclei), which play a crucial role in various aspects of learning and memory as well as in conscious experiences. We demonstrate here that the expression of two functionally important membrane proteins (K-Cl cotransporter, KCC2; and type 2 muscarinic acethylcholine receptor, m2) is different in the anterior pole of the reticular nucleus than in the rest of the nucleus.

i; Chloride extrusion mediated by KCC2 plays an important role in the regulation of GABA-A receptor-mediated responses. In the present study the neuropil of all relay nuclei was found to display intense KCC2 immunostaining, while the majority of reticular neurons were negative for KCC2. In the anterior pole of the reticular nucleus, however, KCC2 immunostaining was comparable to the relay nuclei.

ii; Muscarinic hyperpolarization of reticular cells via m2 receptor-mediated mechanisms plays a major role in the attenuation of GABAergic inhibition during arousal. m2 receptors are strongly expressed in the majority of reticular cells. Two-thirds of the calretinin-positive neurons in the anterior pole of reticular nucleus lack m2 immunoreactivity.

Our results suggest that the lack of KCC2 in the majority of reticular cells sets EGABA-A to produce shunting fast IPSPs in these cells, whereas the presence of this major chloride extrusion protein in reticular cells with medial thalamic projection most likely results in hyperpolarizing IPSPs. The lack of m2 receptor in many calretinin-positive reticular cells indicate that in contrast to other relay nuclei, inhibition is not entirely attenuated during aroused brain states in the medial thalamic nuclei.

P 44 GLUTATHIONE RECEPTORS IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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BACKGROUND: γ-Glutamylcysteinylglycine (GSH, reduced glutathione) is present both intra- and extracellularly in the mammalian central nervous system (CNS). Intracellular GSH is an antioxidant which protects cells but the role of extracellular GSH is less well known. Already almost 50 years ago GSH was suggested to play a role in signal transduction in Hydra and recently extracellular GSH has been shown to modulate the functions of ionotropic N-methyl-D-aspartate (NMDA) and (S)-2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and metabotropic group III glutamate receptors.

RESULTS AND CONCLUSIONS: We have now found in pig cerebral cortical synaptic membranes two (high and low-affinity) binding sites for GSH not displaceable by glutamate, GABA and glycine receptor ligands. However, many analogs of cysteine and glutathione (e.g., cysteine, cysteamine, cystamine, cysteinylglycine and glutamylcysteine) effectively inhibit the binding of labeled GSH. The binding is strongly allosterically activated by thiokynurenate. These specific binding sites for GSH thus differ from any known excitatory or inhibitory amino acid receptor. In the rat cortical ”wedge” preparation, GSH evokes a Ca^{2+}-independent depolarizing potential probably by enhancing Na^{+} influx. The effect is not blocked by the antagonists of AMPA and NMDA receptors 6,7-dinitroquinoxaline-2,3-dione (DNQX) and L(+)-2-amino-5-phosphonovalerate (L-AP5), respectively, but again greatly potentiated by thiokynurenate. The cysteine moiety is mandatory in eliciting excitation since dipeptides or tripeptides containing neutral amino acids instead of cysteine give no depolarizing responses. While GSH modulates glutamate receptors via its γ-glutamyl moiety, GSH binds to and activates these sites of its own via the cysteinyl moiety. Our results evidence the presence of specific ionotropic excitatory GSH receptors involved in synaptic transmission in the mammalian CNS. Supported by the Medical Research Fund of Tampere University Hospital and ALS Association, Canada.

P 45 THE TRIGGERING ROLE OF DOPAMINE IN NEUROTOXIC DAMAGE CAUSED BY AMPHETAMINE

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Background: The breakdown products of dopamine (DA) are potentially neurotoxic but neuronal damage due to amphetamine (Amph) treatment may not only be produced by DA radical metabolites. The aim of present study was to explore the relationships between extracellular DA, glutamate (Glu) and taurine (Tau) and hydroxyl radical ($OH^{\bullet}$) generation during subchronic Amph treatment (5 mg/kg, 4 injections i.p. with 2-hour intervals).

Methods: The extracellular levels of DA, Glu and Tau were estimated by means of HPLC and the generation of $OH^{\bullet}$ with salicylate method in the rat neostriatum upon microdialysis.

Results: Amph caused an immediate increase in the DA concentration up to 950%, which effect was quickly reduced to the baseline values. The subsequent Amph injections were followed by a much smaller increase in the extracellular DA concentration (about 300%). Amph produced marked increase in the $OH^{\bullet}$ generation, the first wave was observed 80 min after the second injection and persisted during 2 hours (up to 700%), and the second increase incurred after the 4-th injection (up to 400%). Amph (5 mg/kg) caused a marked gradual increase in the Glu and Tau levels (up to 500 and 450% of the predrug value, respectively) by the end of experiment.

Conclusion: Our results suggest a triggering role DA in neurochemical changes which lead to neuronal damage. The changes in extracellular DA, Glu, Tau and $OH^{\bullet}$ reflect different subsequent phases of Amph neurotoxicity.

P 46 Neuroelectromagnetic telecommunication.

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Introduction: Biotelemetry, neurological communication and control method has been at use for decades, and its mental and physical health effects after millenium with newest technology are discussed.

Objectives: To study whether behavior modification and influencing brain functions and mental and emotional processes along with bodily functions remotely and wirelessly through computer satellite links has been successful

Methods: Literature surveys and experimentation.

Results: Astronauts were implanted with a microchip to monitor their body functions, feelings, thoughts, dreams and even subconscious from earth. Supercomputer screen of over 200 billion bits/s override human thought of 5000 bits/s. 15-2 μm diameter chip also creates a computer generated visualization based upon the user's request. They are usually situated in cochlea or optic nerve or temporalfrontal lobe or subcutanously. The laser machine in Discovery emitted 10 pulses per s, which is within frequency of the human alfa range. Microchip operates with a low frequency radiowave aimed at it through satellite. The implanted person can be located anywhere, and two-way radiocommunication controls the subject.

Conclusions: Neuroelectromagnetic wireless control is successful technology and shows that microwaves can cause healthy persons to hear voices and motion, emotion, and that behavior can be directed by electrical forces.