Methods: Possible sources of writing difficulty tested were: cooperative interactions among neurons and dendrites containing multiple dendrites are frequent (4 times more than in computer simulations of randomly distributed dendrites) and are formed by direct growth of dendrites toward pre-existing dendritic intersections. This process is disrupted by inhibitors of synaptic activity and of glutamate receptors. Thus, an activity-dependent mechanism exists that causes dendrites to converge to a single intersection, resulting in an increase in synaptic activity and synaptic clustering at the intersection. This may serve as a mechanism for activity-dependent pre-synaptic plasticity.

In addition to converging, dendrites in the culture tend to grow in parallel, even when separated over large distances, leading to formation of areas where specific dendritic orientations are dominant. This order restricts the location of the dendritic intersections and their synaptic clusters. Thus, directed convergence combined with parallel growth of dendrites shapes the synaptic map in cultured neuronal networks.

Keywords: dendrite, synaptic clustering, synaptic strength, cooperative interactions among neurons, synaptic plasticity.

Writing and dysgraphia in ADHD

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Background: Difficulties in writing is a common complaint among children with attention deficit hyperactivity disorder (ADHD). Objective: To examine whether the problem is one of dysphagia or other neurological problems masquerading as dysgraphia.

Methods: Possible sources of writing difficulty tested were: 'lingual', associated with reading and expressed mainly in spelling mistakes, 'peripheral motor-output' designated as the orthographic buffer and expressed in omission or repetition of strokes while writing and 'attention deficit' expressed as consistency in production in all domains of graphic production. Participants: Twenty boys with ADHD, aged 11-13 years (unmedicated for at least 1 week) and controls matched for gender, age, IQ, handedness and socio-economic class.

Results: There was no significant difference between diagnosis groups in reading/writing duration over repetition trials, but variance of duration and accuracy was higher for ADHD children, especially in the written-letters task.

Conclusions: Children with ADHD demonstrated deficits in the motor-peripheral domain as well as attention deficits in the linguistic domain. Although ADHD children were less consistent than controls on graphic tests, for very simple tasks this was significant for writing but not drawing. We conclude that children with ADHD have a true dysgraphia that is exacerbated by the attention deficits.

Keywords: dysgraphia, ADHD, writing.
The BLA modulates hippocampal memory processes, presumably via the mediation of the stress hormones NE and CORT, to establish a diverse memory of the experience. Possibly, at the onset of an emotional event the stress hormones permissively mediate plasticity. However, their prolonged presence in the system may suppress the cognitive responses further.

**Keywords:** learning, amygdala, hippocampus, emotions

**Memory process and insomnia in the elderly**

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Changes in sleep-wake patterns appear to be one of the hallmarks of biological aging. Elderly persons complain of daytime sleepiness and difficulties in initiating and maintaining sleep. Likewise, aging has a clear effect on learning and memory processes. However, the interaction between sleep disturbances and memory among elderly people remains unclear. This study aims to assess whether insomnia is associated with memory decline in the elderly.

Seventy-three elderly (45 males and 28 females) aged 65-80 (70 ± 5.9 years) who were living independently in the community participated in this study. Sleep was assessed using Mini Sleep Questionnaire, and memory processes were evaluated via the Rey Auditory Verbal Learning Test.

Twenty-three of the 73 subjects (32%) had insomnia index greater than 4 in sub-scale of insomnia, indicating insomnia. Performance of the two groups (insomniacs and good sleepers) in the AVLT was computed in order to assess relationships between insomnia and memory. Generally, the results demonstrated that in almost all categories of episodic memory elderly people who do not have sleep disorders displayed better performance results compared to insomniacs. More specifically, results revealed significant differences between insomniacs and good sleepers in learning, and in resistance to proactive interference, and close to significant in temporal memory.

These findings suggest that insomnia is significantly associated with some aspects of memory decline in the elderly. These aspects include memory learning, resistance to proactive interference, and temporal memory.

**Keywords:** learning, amygdala, hippocampus, emotions

**Activation of the human "visual" cortex in the congenitally blind**

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Previous studies indicated that in congenitally blind subjects the visual cortex is active during Braille reading, suggesting cross-modal recruitment of the occipital cortex for tactile processing. However, Braille reading involves cognitive aspects of processing. Using fMRI mapping in congenitally blind people, we find robust activation by both tactile and non-tactile language related tasks, in the calcarine sulcus and a constellation of occipital areas, which correspond to retinotopic mapping in sighted subjects. No such occipital activation was observed in sighted subjects. The occipital activation pattern was present in the blind when performing an auditory verb generation task or a verbal memory task with substantial overlap between the resultant activation maps. The occipital areas were also activated by Braille reading, but to a lesser extent. Furthermore, analogous to the hemispheric laterality of language areas in sighted subjects, activation of the left occipital cortex was dominant in the blind. These findings suggest that in cases of early onset blindness the occipital cortex undergoes a dramatic change in functionality, such that it now serves high-level cognitive functions.

**Supported by Israel Science Foundation grant number 8009.**

**Keywords:** plasticity, memory, language, fMRI

**Melanopsin in the mammalian circadian system**

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Photic information for entrainment of circadian rhythms in mammals is transmitted to the circadian clock in the suprachiasmatic nucleus from the retina via the retinohypothalamic tract. The photoreceptors associated with this pathway are unknown, but it is recognized that conventional photoreceptors, rods and cones, do not play a central role in the optical input. Melanopsin, has been proposed recently as a putative rod or cone photoreceptor in mammals. This hypothesis is based on the demonstration that melanopsin is expressed in retinal ganglion cells that respond directly to light and that innervate the suprachiasmatic nucleus. In the course of studying a potential role for melanopsin in photic entrainment, we found a fiber plexus immunoreactive to melanopsin in the retinorecipient region of the suprachiasmatic nucleus of the rat. To evaluate the expression of melanopsin in the retina and suprachiasmatic nucleus of rats that were treated with the neurotoxin, monosodium glutamate, during the neonatal period. This treatment strongly reduced the number of retinal ganglion cells expressing melanopsin and abolished the expression of melanopsin in the suprachiasmatic nucleus. Because photic entrainment is spared in spite of the mass loss of retinal ganglion cells bought about by neonatal treatment with glutamate, these findings suggest that retinal ganglion cells that project to the suprachiasmatic nucleus and that contain melanopsin in their axon terminals do not play a critical role in photic entrainment in rats. A possible role for these melanopsin containing ganglion cells in mediating the masking effect of light on behavior will be discussed.

**Supported by the Canadian Institute of Health Research, the Natural Sciences and Engineering Research Council of Canada, and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche.**

**Keywords:** melanopsin, retinal ganglion cells, suprachiasmatic nucleus; circadian system

**Bifunctional compounds eliciting prolonged anti-inflammatory and cholinergic activity**

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Certain CNS disorders such as Alzheimer’s disease (AD), and traumatic brain injury (TBI) are accompanied by significant increase in inflammatory markers. Furthermore, AD and TBI in humans include reduction in certain cholinergic markers. Based on cholinergic hypofunction observed in AD several cholinesterase inhibitors (ChEI) (e.g. Aricept, Elexon and Reminyl) have been developed and approved for human use. We have synthesized a series of bifunctional compounds containing both non-steroidal anti-inflammatory drug (NSAID) and cholinergic up-regulation (CURE) moiety. The bifunctional compound IBU-PO inhibits human AChE and BChE with bimolecular rate constants $k_{1}$ and $k_{2}$, respectively. In addition, IBU-PO (4 mg/kg, ip) resulted in 50 % inhibition of mouse-blow ChE prolonged for 5-6 hours. In contrast, pyridostigmine (PYR, 0.15mg/kg, im) caused 15-20% inhibition with a much shorter duration (3-5 hours). Peripheral anti-inflammatory activity of IBU-PO, DICLO-PO and INDO-PO (5-10 mg/kg, ip) was demonstrated in rat paw edema model of inflammation. The anti-inflammatory effect prolonged for at least 7 hours post administration. These data demonstrate that the anti-ChE moiety of the bifunctional compounds did not interfere with the anti-inflammatory activity. Furthermore, IBU-PO reduced the brain edema induced by ivc administration of carragenenan in mice and rats. The central anti-inflammatory activity obtained with IBU-PO is probably also responsible for the amelioration of the damage caused by closed head injury in mice. In conclusion, some of the new bifunctional NSAID-CURE compounds demonstrate prolonged anti-inflammatory and cholinergic activity.

**Keywords:** NSAID, ChE inhibition

**Immunological changes following antisense oligonucleotides treatment in experimental autoimmune Myasthenia Gravis**

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Myasthenia Gravis (MG) is an antibody-mediated, autoimmune neuromuscular disease in which nicotinic acetylcholine receptor (AChR) is the target antigen. Typical neuromuscular junction (NMJ) symptoms can be transiently alleviated by acetylcholinesterase (AChE) inhibitors (such as pyridostigmine). However, in the long-term, these agents are short-lived and does not prevent disease progression. Moreover, AChE inhibitors were recently shown to elicit...
pronounced and persistent neuronal over expression of AChE, due to feedback up-regulation of the AChE gene. Our previous studies in MG patients and EAMG rats display significant elevated levels of muscle AChE mRNA and protein. In addition, treatment with 2-oxymethyl-protected-AS-oligodeoxynucleotides (EN101) suppresses AChE synthesis in vivo and in vitro. Consequently muscle activity was rescued and clinical symptoms were improved for a long period. In the present study, co-treatment with 50 (mg/kg) EN101 for one month was very effective. This enabled EAMG rats to thrive under conditions where untreated or pyridostigmine-treated animals did not survive. Furthermore, this treatment reduced the level of AChE antibody titre, associated with disease progression and aggravation. In addition, when EN101 was added in vitro to T-cells reactive towards autoantigen-T-AChR or mitogens, T-cell proliferation was markedly suppressed. Our results show the beneficial effect of EN101 treatment on EAMG clinical and immunological parameters and highlight potential advantage of gene-targeted drug therapy.

Keywords: autoantibodies, acetylcholinesterase, experimental autoimmune myasthenia gravis, antisense-treatment

Single dose intravenous valproate or fosphoatoin in acute mania

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Objectives: High-dose loading with oral and intravenous valproate has been reported to be therapeutic in mania over 48-72 h. We have tested very high dose iv valproate which might have even more rapid effects equivalent to effects in status epilepticus that occur within 20 minutes. Since several anticonvulsants are therapeutic in mania and since acute mania requires rapid and intensive treatment, we hypothesized that intravenous high dose phenytoin might be acutely anti manic. A new prodrug of phenytoin, fosphenytoin, with fewer cardiac or local venous side effects was used.

Methods: Seven patients with mania and minimal prior drug treatment were given iv valproate 20mg/kg over 30 minutes. Seven patients with mania and minimal prior drug treatment were given iv pyridostigmine 5mg/kg over 10-20 minutes.

Results: No antimanic effects were observed over 120 minutes of observation. There were no side effects.

Conclusions: Slowly evolving biotransformations, changes, perhaps at the gene level, may be required for the antimanic effect of anticonvulsants.

Keywords: intravenous valproate, anticonvulsants, mania, treatment

The lipophilic transition metal chelator DP-109 attenuates asymmetric rotations in the 6-hydroxydopamine partial lesion model of Parkinson's disease in adult rats

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Perturbations in the homeostasis of transition metals such as copper, iron and zinc can cause oxidative stress in neurons. These metals are considered to be involved in neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Antioxidants and metal chelators have been found to be beneficial in various models of these diseases, but are problematic due to a poor safety profile and low rate of penetration across the blood brain barrier. To address this problem, we have developed a family of lipophilic chelators selectively activated in the vicinity of cell membranes. The lead compound, DP-109, was designed to chelate bivalent ions such as Cu, Fe, and Ca. DP-109 is a lipophilic 6-OHDA substantia nigra lesion model using adult male Wistar rats. Five days post-lesion animals were screened for amsoprine-induced rotations over a 5 min period. DP-109 (100g/kg) significantly attenuated (p<0.05) both the rate of increase and number of rotations by 70%. We suggest that DP-109 represents a new class of compounds that might be effective in treating neurodegenerative disorders.

Keywords: intravenous valproate, anticonvulsants, mania, treatment

Supported by: D-Pharm, Ltd.
Keywords: lipophilic transition metal chelator, Parkinson's disease, 6-OHDA

Active immunization towards Prion disease

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The transmissible spongiform encephalopathies (TSE) or better known as prions, are rare neurodegenerative diseases which include scrapie in sheep, BSE in cattle and Kuru, Creutzfeldt-Jakob disease, Gerstmann-Strussler- Schienker syndrome (GSS) and fatal familial insomnia (FFI) in human. It is believed that prion propagation is caused by refolding of a normal endogenous glyco-protein called prion protein (PrPc). PrPc is expressed mostly in the central nervous system and lymphoid tissue. In the course of the disease PrPc changes its structure into an abnormal, -sheet rich structure termed PrPSc which is the only known particle in the prion infectious agent. The abnormal protein is neurotoxic and leads to death within few months. As for today there is no effective therapeutic agent for prion diseases.

Antibodies are known to act as chaperones and are able to stabilize protein structure and/or induce conformational changes. Thus, our working hypothesis is that site directed antibodies towards PrP may interfere with aggregation processes and/or inhibit prion replication.

We developed in our lab an active immunization procedure towards human Helix 1 of the whole prion protein. Helix 1 was chosen since it appears to be a putative key position in the protein induced conformational changes. In order to overcome the poor immunogenicity of short peptides in general and the high sequence similarity between the human and mouse peptides in particular, we chose the MAP (Multiple Antigen Peptides). MAP is a branching structure of lysine residues coupled to the desired peptide. The immune response that our peptide elicits was unexpectedly high. Antibodies raised were of IgG isotype. The antibodies that were produced recognize the whole protein and are now being tested for their ability to inhibit the PrPc to PrPSc conversion in cell culture model.

Keywords: PrP (prion protein), chaperons, MAP

Sub-millisecond precision of the input-output transformation function mediated by fast sodium dendritic spikes in basal dendrites of CA1 pyramidal neurons

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The ability of cortical neurons to perform sub-millisecond scale computations has been shown to be important for encoding of information in the cortex (1-2). This ability necessitates involvement of special mechanisms in order to overcome the relative long membrane time-constant and the significant dendritic filtering of excitatory postsynaptic potentials (EPSP) in cortical neurons. Here we show that coincident activation of closely spaced basal inputs in CA1 pyramidal neurons resulted in significant sharpening (87±2.37%) and super-linear amplification (156±3.7%) of the summed potential, as compared to the individual EPSPs. This enhancement was mediated by initiation of a local dendritic spike composed of an early fast sodium spike followed by a slower NMDA spike. When paired with sparsely EPSPs fast local basal sodium spikes significantly improved the temporal precision of output action potentials by markedly decreasing the temporal jitter of action potentials (from 2.17±1.2 to 0.28±0.17 msec).

Our findings indicate that local dendritic basal spikes enable coincidence detection of closely spaced synaptic inputs, and significantly improve the temporal output precision of CA1 neurons. As such they may serve as a cellular basis for sub-millisecond temporal processing in the output dendrites of pyramidal neurons.

Keywords: dendrites, temporal coding, synaptic integration, dendritic spikes

Na' channel properties determine the functionally optimal oscillation frequency of neocortical pyramidal neurons

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One prominent feature of neocortical pyramidal neurons is their tendency to oscillate at specific frequencies. Recently, attention has focused on the functional significance of such oscillations for modulating the excitability of the somato-dendritic membrane. During oscillations, the moment-to-moment availability of Na' for Na' conductance, which is the major determinant of neuronal excitability, depends on the
The dynamic properties of the $Na^+$ channel. We used the cell-attached configuration of the patch-clamp technique to measure the relevant $Na^+$ channel diameters in some of the neurons in the neocortical Layer 5 cells. Unlike in most previous studies, these experiments were performed at physiological temperature, since the dynamic properties of interest are temperature dependent. Annealing the preparation from room temperature to 36°C did not affect the activation curve, but caused the steady-state inactivation curve to become steeper. At sub-threshold potentials, time constant of recovery from inactivation was much slower (4-6 ms) than that predicted by the Hodgkin-Huxley model ($<$0.3 ms). The number of "ready-to-open" channels during oscillation was measured by applying trains of voltage commands of constant amplitude ($\pm 5 \ mV$ from $V_{rms}$) and different frequencies (5-100 Hz), superimposed by a brief depolarizing pulse. $Na^+$ channel availability was lowest at 5 Hz and increased sharply as a function of frequency, reaching a maximum at around 40 Hz. Dendritic current-clamp recordings revealed a parallel frequency-dependent increase in the amplitudes of back-propagating action potentials. We conclude that the $Na^+$ channel determines the optimal oscillation frequency.

Keywords: oscillation, $Na^+$ channel, neocortical neuron

Neonatal immunization of mice with self-proteins residing in the site of glutamate toxicity eliminates their ability to adults to withstand the toxicity

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After the over few years, studies from our laboratory have demonstrated that after an insult to the nervous system (CNS), the body harnesses an immune response against specific self-proteins residing in the damaged CNS in order to attenuate the damage caused by the injury-induced emergence of destructive self-compounds at the lesioned site. In the present study, we show that the ability of CNS neurons to withstand glutamate toxicity, one of the major factors in neurodegenerative conditions, is diminished if the individual is deprived of the ability to manifest an immune response against proteins residing in the stressed tissue. This finding argues in favor of autoimmunity as a protective physiological response to a threat originating from within the body (as opposed to an exogenous threat), and emphasizes that the immune system is the backup supportive mechanism for the central nervous system when the latter exceeds the maintaining capacity of the resident neural cells.

Keywords: autoimmunity, neuroimmunology, vaccination, EAU

Early exposure to stress modulates the response to stress in the adult rat

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It has been proposed that exposure to stress during early childhood may disturb emotional and cognitive functioning in adulthood. We examined the behavioral consequences of early exposure to stress, and in particular, on the ability to cope with stress in adulthood.

Support by a grant 52/2000 from the Israel Foundation Trustees to G.R-L.

Keywords: early stress, anxiety, neurosteroids

Effect of the dextrocannabinoid PRS-211,092 on gene expression in brain ischemia induced by MCAO

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The non-psychoactive dextrocannabinoid, PRS-211,092, was recently demonstrated to be an effective neuroprotectant agent in brain ischemia induced by middle cerebral arterial occlusion (MCAO) in rats. The mechanism of action, which contributes to its protective effect in brain ischemia, is unknown. Here we have investigated the effect of PRS-211,092 on gene regulation in mouse brain after MCAO. Mice were subjected to transient MCAO for 90 min and then immediately injected with PEG-Ethanol (vehicle only group) or 5 mg/kg PRS-211,092 i.v. Brains were removed 18 hours after MCAO and the expression of several genes determined in the ipsilateral cerebral hemisphere using real time RT-PCR. Gene expression was normalized to the cytochrome c gene and to the level of expression calculated relative to that in sham operated control animals. In the animals treated with PRS-211,092 there was a reduction in expression of the genes for COX-2 (PGE-2 synthetase) by 48%, MCP-1 (monocyte chemotactant protein-1) by 63% and IL-2 (Interleukin-2) to a level below that in sham-operated animals. In contrast, IL-10 gene expression was increased by 229% in the PRS-211,092 treated animals in comparison with that in animals treated with vehicle alone. The reduction in COX-2, MCP-1 and IL-2 gene expression, together with the induction of IL-10 gene expression by PRS-211,092, i.e. the level of expression of several genes determined in the ipsilateral cerebral hemisphere using real time RT-PCR. Gene expression was normalized to the cytochrome c gene

Keywords: MCAO, neuroprotection, gene expression, dextrocannabinoid

Encoding of radial object position by rat whiskers:

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We investigated how neurons in the trigeminal ganglion of anesthetized rats respond to whisking against object inserted at different radial positions i.e. along the whisker length. Rhythmic 5 Hz whisker movements similar to spontaneous whisking were induced by optical stimulation of the motor nerve. A vertical pin-shaped object was placed inside the
whisking field at different radial positions. We recorded single-unit responses and captured video-images of the whisker at 1 fps. This allowed us to determine the moment when the whisker touched the object with neural events. We report here preliminary results from 'object detectors', i.e., single units that responded selectively to the contact between whisker and object (see accompanying abstract by Szwed et al.). We examined the responses of these neurons to objects positioned at three radial distances: 30%, 60% and 90% of whisker length. We analyzed four response variables: spike-count (number of response spikes per whisking cycle), amplitude (peak firing-rate), duration (of response burst), and latency (1/2 of peak response). These data show that the object position is encoded most consistently by spike-counts, less by amplitude, and much less by duration or latency. As radial distance increased, spike-count and amplitude decreased. In contrast, as reported in the accompanying abstract, horizontal position was most consistently encoded by latency. These preliminary results suggest that horizontal and radial coordinates of object position are encoded differently: primarily by spike-count in the radial direction and primarily by latency in the horizontal direction. Supported by ISF grant 377/02-1. Keywords: object localization, neural code, sensory encoding

Multi-unit recordings from the gustatory cortex of the freely behaving rat reveal differential response to familiar and unfamiliar solutions during a distinct phase of the response. Balsair, D., Duda Y. and Ahiyar E. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100.

The ability to distinguish an unfamiliar tastant from a familiar one is crucial to the animal's survival, as the unfamiliar may poison. Ample molecular and cellular data support the hypothesis that the gustatory cortex (GC) plays an important role in the neural processing underlying detection of unfamiliar tastants, and transforming them to familiar stimuli. We investigate the neural correlates of gustatory unambiguity/familiarity encoding in the GC of the freely behaving rat. To this end, we employ chronically implanted multi-wire electrodes to record the extracellular activity of multiple units in the GC of rats, while they lick unfamiliar or familiar solutions. We found that the ratio of responses to taste vs. water and compare them between exposures to unfamiliar and familiar tastants. We find that most recorded units demonstrate a typical lingering response (7 sec) to 1sec of licking. The ratio of response to taste vs. water, in the initial 2 sec following drinking onset, is independent of whether the taste is unfamiliar or familiar. This ratio, however, increases significantly during the following 5 sec. Thus, our data imply that the GC responds differentially to familiar vs. unfamiliar tastants during a distinct phase of the neural response. Our data corroborate the notion that multiple taste attributes, including familiarity, are encoded in the GC and that such attributes undergo experience-dependent modifications.

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Keywords: taste, electrophysiology, novelty, neural code

Non-conventional vaccination counteracts the ongoing damage of chronic neurodegenerative diseases

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Recently it was shown in our laboratory that immune intervention by passive or active vaccination directed to tissue specific abundant antigens protect neurons from consequences of in vivo axonal injury. Thus myelin associated antigens protect from consequences of acute axonal injury being a crucial of the optic nerve or spinal cord contusion (Moalem et al. Nat. Med., 1999 Hauben et al., 2002 Neurosci 20 6421-6430 2001). We tested, in contrast, retinal-derived abundant peptides protect from glutamate toxicity inflicted directly to the retinal ganglion cells (Mizrahi et al., J. Immunol. 2002). Vaccination with the random copolymer, Copaxone (Cop-1), circumvents the tissue specific barrier of protective vaccination. In this work we used an animal model for chronic neurodegenerative disease characterized by high intra ocular pressure due to progressive optic nerve degeneration resulting in gradual visual field loss. We show that autoimmune neuroprotection is effective in chronic conditions. We show that rats with high IOP are amenable to protection by vaccination in the retina and not to optic nerve. We show that the efficacy of Cop-1 is dose and regimen dependent and that it's effect is superior to pharmacological intervention such as that achieved with n2-adrenoceptor agonist. This is not surprising since vaccine can help homing of T cells to the site of damage. Such T cells are locally activated by the relevant antigen presenting cells and in turn amplify and regulate the immune ability to fight against the local threat.

Keywords: glaucoma, immune neuroprotection, Cop-1

A monoclonal antibody to the binding site of acetylcholine receptor cross-reacts with the binding site of snail acetylcholine binding-protein

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Monoclonal antibody (mAb) 5.5 raised against Torpedo nicotinic acetylcholine receptor (AChR) is specific for the binding site of AChR and competes with the binding of α-bungarotoxin (α-BTX) and other cholinergic ligands to the receptor. Here we show that mAb 5.5 binds to snail acetylcholine binding-protein (AcBBP), the structure of which has recently been deciphered. As the binding affinity of mAb 5.5 to AChBP is considerably higher than that of α-BTX to AcBBP, we focused on the capability of this antibody to bind either AChR or AChBP. We thus employed peptides corresponding to the binding site loops of Torpedo and human AChR, and of snail AChBP, to evaluate the potency of the peptides to compete with the binding of mAb 5.5 to either Torpedo AChR or snail AChBP. A peptide corresponding to amino acid residues 187-200 of Torpedo AChR ω-subunit (WVVYCCDPTYPD, To187-200) inhibits the binding of mAb 5.5 to both Torpedo AChR and to AChBP (IC50 values of 100 and 100nM respectively). A peptide corresponding to the homologous residues of human AChR ω-subunit (EVTYCCDPTYPD, Hto187-200) inhibits only the binding of mAb 5.5 to its parental molecule, AChBP (IC50 of 100nM). Likewise, the peptide corresponding to amino acid residues 187-200 of Torpedo AChBP ω-subunit (SVYSCDPTYPD, Tbp187-200) that has a high sequence similarity to the AChBP peptide (BP182-194), also inhibits only the binding of mAb 5.5 to AChBP (IC50 of 100nM). Our findings demonstrate that mAb 5.5 interacts directly with the ligand-binding site of AChBP, and makes this antibody a good candidate for analyzing the ligand-binding site of snail AChBP as well as of proteins that bind acetylcholine, in general.

Keywords: nicotinic acetylcholine receptor; acetylcholine binding site; acetylcholine binding protein

Pitch discrimination as a marker of reading and reading related cognitive difficulties

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Previous studies documented strong correlations between auditory frequency discrimination threshold (JND) and "high-level" cognitive abilities among reading disabled and normally reading adults. In this study we screened a regular class and a 'learning-disabled' (LD) class from two junior high-schools, respectively. In addition to reading, phonological awareness and memory we measured frequency discrimination. Two methods were used: pitch judgment (hi-lo) and similarity judgment (same/different-SD). In both, tones were 50ms long with 1sec-inter-stimulus-interval. In each trial one tone was of 1000Hz. In the hi-lo method the other tone was always higher. In the SD method, the other tone was higher in half the trials and same in rest. We asked whether frequency JNDS are a good marker of reading related abilities. Replicating the adult findings, using the hi-lo paradigm, we found that the disabled readers in both classes (10% and 50% in the regular and LD class, respectively) were also those with the highest frequency JNDS (>30%) and with particularly impaired verbal memory. Hi-lo frequency JNDS were correlated with verbal memory scores, but not with non-verbal memory. Surprisingly, JNDS on the SD task did not correlate with any of the reading or reading related measures, nor with the hi-lo JNDS. The finding that behavioral pitch discrimination is an important factor in the correlation to memory and reading scores suggests that these relations do not simply result from variability in low-level auditory processing. Although the causal direction is not yet clear, we suggest that hi-lo pitch discrimination may serve as a useful screening tool for memory related learning difficulties in schools.

Keywords: auditory processing, reading disability, frequency discrimination
Plasma membrane targeting of syntaxin 1a is enhanced following expression of M2 muscarinic acetylcholine receptors in PC12 cells

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In mammalian brain, the M2 subtype of the presynaptic muscarinic acetylcholine receptors (mAChRs) are predicted as targets for the intracellular targeting mechanisms of the m2mAChR. This link is mediated by the alpha subunit of the Go protein. A key component in the functionality of G protein-coupled receptors is their ability to traffic from the cell surface to the plasma membrane. In control PC12 cells, that do not express endogenous m2 mAChR, syntaxin expression is partitioned between the Golgi and the plasma membrane. However, in cells expressing exogenous m2 receptors, syntaxin is primarily localized to the plasma membrane. This phenomenon could be observed by immunofluorescence staining and following subcellular fractionation of the cells. Furthermore, mAChRs expression in these neuroendocrine cells causes a decrease in syntaxin expression, whereas the protein remains excluded to a lighter membrane fraction, which represents the small synaptic-like vesicles. The data suggest that m2 mAChR receptors are directly involved in the efficient trafficking of synaptic vesicles, including syntaxin. The involvement of the G-protein in the process and the physiological consequences of these phenomena are currently being investigated.

Key words: muscarinic ACh receptor, syntaxin, targeting, seclusion

Dynamic changes in mitochondrial function after closed head injury in mice

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Traumatic brain injury is associated with a rapid burst of reactive oxygen species (ROS), leading to oxidative stress. Additionally, energy failure occurs within minutes to hours after trauma. Mitochondria dysfunction is a major common pathway to these pathologic changes. The present study examined the functional activity of the mitochondria by measurement of ATP production at various time intervals after closed head injury (CHI) in mice. CHI was induced by a weight-drop device on 8-10 week old male Sprague-Dawley mice, as described earlier. Mitochondrial fractions were isolated at 1, 4 or 24 h after CHI, and ATP production was followed by addition of glutamate (10 mM) and malate (0.5 mM) for 5 min. The accumulating ATP was measured spectrophotometrically. In addition, enzymatic activities of complex I-III and of complex IV were determined in the same preparations. A significant decrease in ATP production was noticed already at 1 h post CHI (~22% decrease from basal activity, p<0.05). A transient return towards baseline activity was found at 4 h (~8% decrease, not significant), which was later declined again, and at 24 h a decrease of ~20% was recorded (p<0.05). Interestingly, a similar biphasic pattern was reported for the endogenous low-molecular weight antioxidants, in the same model. The enzyme activity assays revealed no changes in NADH-cytochrome-c reductase and in cytochrome-c oxidase. However, cytochrome-c oxidase activity was within a range of 10-15% of the control level. The content of cytochrome-c oxidase, a marker for mitochondrial content, was also unaffected by CHI. We propose that CHI-induced effects which interfere with ATP-producing pathways by the mitochondria, contributes to the aggravation of the cell damage.

Key words: traumatic brain injury, mitochondria, oxidative phosphorylation

High expression of apolipoprotein-E and Cathepsin B/D in spinal cords of amyotrophic lateral sclerosis patients and mice expressing human SOD1 gene

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Amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disease characterized by loss of motor neurons in the cerebral cortex, brain stem, and spinal cord. A majority of ALS patients (5-10%) have a familial form (ALS-F) and 20% of these demonstrate mutations in the Cu/Zn SOD1 gene. Mice expressing the human mutant Cu/Zn SOD1 gene develop an age-dependent ALS-like neurological symptoms. We studied the mRNA expression profile in post-mortem spinal cord sections from sporadic ALS patients and human controls. Using cDNA microarray gene expression we found significant increases in mRNA of cathepsin B (484%) and cathepsin D (206%), which are cysteine proteases that mediate intracellular protein turnover in the lysosome. MSNA of apolipoprotein E which is closely associated with the pathogenesis of neurodegenerative diseases, was also markedly increased (290%). Further analysis with specific probes revealed that the expression of these genes also increased following local SOD1-G93A transgenic mouse. They show enhancement with disease progression and peak at the end stage of the illness. Our data from ALS patients supported our findings using the transgenic mouse model indicates a crucial role of apolipoprotein-E and cathepsin B/D in the pathogenesis of sporadic and familial forms of ALS.

Key words: amyotrophic lateral sclerosis (ALS), mutant Cu/Zn SOD1 gene, cDNA microarray, apolipoprotein E, cathepsin B/D

Neuroprotection by a dextrocannabinoid in a 3 hours therapeutic window assessed functionally in transient MCA occlusion in rats

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We have previously described a novel dextrocannabinoid, PRS-211,092, a novel analog of Dexamabinol, which is not bound to the NMDA receptor, but has powerful anti-inflammatory properties (in-vitro). PRS-211,092 inhibits the ischemia-induced increased gene expression of cyclooxygenase-2 (COX-2) and proinflammatory cytokines and chemokines in brain of transient middle cerebral artery occluded (MCAo) animals. The aim of the present study was to determine whether PRS-211,092 had long-term beneficial effects on functional outcome following focal brain ischemia. The MCA of Sprague Dawley male rats was occluded for 120 minutes by intraluminal suture halothane anesthesia. PRS-211,092 was administered 1 hour after the end of the ischemic insult. The neuroprotective efficacy of the compound was evaluated by the "staircase test" (Montoya et al - J. Neurosci. Meth. 36:219-228 (1991)). Rats were trained for 15 min a day for 15 min. in each test. Staircase test performance was determined immediately after and at approximately 6-day intervals after MCAo for a period of about 3 weeks. PRS-211,092 improved the staircase test performance on the contralateral side by 61% compared with that in animals treated with vehicle alone (p<0.05). Thus, PRS-211,092 is an effective neuroprotectant in transient focal brain ischemia, even when administered 3-hours after the start of MCAo. These data, together with the fact that PRS-211,092 shows no toxicity suggest that this dextrocannabinoid may be worth considering for the treatment of stroke in man.

Key words: stroke, staircase test, neuroprotection, MCAo

Novel cannabinoids are analgesics in noxious and neuropathic pain models

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Cannabinoid receptor agonists show responses to painful stimuli by activation of the CB1 receptor, located mainly in the CNS, and the CB2 receptor, expressed mainly by inflammatory and immune cells. We have investigated the possible analgesic effect of novel, non-psychoactive, cannabimimetics, PRS-211,096 and PRS-211,335, which have a selective affinity for the CB2 (IC50 for both compounds = 1nM) compared with the CB1 receptor (IC50 of 28 and 87 nM, respectively). Both compounds, administered IP, were effective analgesics for...
nociceptive pain as assessed by tail flick test in ICR male mice. PRS-211,096 demonstrated dose-dependent (2 to 10 mg/kg) analgesia, and at 10 mg/kg, was similar to that of morphine (5 mg/kg) 30 min after drug injection. In animals treated with PRS-211,096, significant analgesia was still evident at 90 min (by which time morphine analgesia was no longer detectable), and the effect was maintained at 150 min after injection. PRS-211,335 also induced significant analgesia at dosages of 2 and 4 mg/kg at 30 min after injection, a trend that was still evident at 90 min after injection.

PRS-211,096 was also tested for possible efficacy as an analgesic for neuropathic pain using the model of Bennett and Xie in the rat. Administered IP at doses of 0.5, 1.0, and 5.0 mg/kg, PRS-211,096 demonstrated dose-dependent (2 to 10 mg/kg) dose-related antinociceptive analgesia, although no latency or thermal hyperalgesia in a dose-response manner. The efficacy of PRS-211,096 at 5.0 mg/kg was similar to that produced by morphine at a dosage of 5.0 mg/kg. These cannabinoids may be novel and potent analgesics for the treatment of noxious and neuropathic pain in man.

Keywords: pain, neuropathic; cannabinoids

Di-isopropylfluorophosphate (DFP) on postnatal days 4-10 impairs passive avoidance in female, but not male adult mice

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Acetylcholinesterase inhibitors (AChE-I) are widely used as domestic and agricultural organophosphates. Since exposing a substantial population to the risk of neurological damage, ACh plays a major role in encoding, attention and regulation of cortical development. Sexual dimorphism has been determined based upon the increased activity of AChE in females. AChE-Ts. A single exposure to (AChE-I) in 3-10 day old mice caused down-regulation of muscarinic receptors, impaired spatial learning and altered motor behavior in females only (Damm et al. Developmental Brain Research 121:179-187 [2000]). The present study examined the effect of chronic developmental exposure to AChE-I on avoidance learning. Neonatal C57BL/6J mice of both sexes were injected with 1mg/kg SC DFP or saline on postnatal days 4-10 and tested at age 4 months on the step-down passive avoidance paradigm. On Day 1, mice were placed individually on a vibrating platform above a metal grid floor. Upon stepping down, the mouse received a 0.5 mA footshock for 5 sec. The procedure was repeated until the mouse made 2 escape responses up to a maximum of 10 trials. The test phase, 24 hr later, consisted of a single trial without footshock. Females administered DFP had shorter step down latencies than controls on the test day, whereas no difference was found between DFP and control males (sex x treatment x trial interaction, F2,194=4.30, p<0.02). No difference in pain thresholds was found between DFP and controls. The results suggest a greater sensitivity of females to deficits in fear-conditioned learning, following early exposure to AChE-I.

Keywords: acetylcholine, passive avoidance, development

The M1 and M2 muscarinic G-protein-coupled receptors are voltage sensitive

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G-protein coupled receptors are not considered to exhibit voltage sensitivity. Here, using Xenopus oocytes, we showed that the muscarinic M1 (m1R) and M2 (m2R) receptors, when expressed in Xenopus oocytes, exhibited voltage sensitive gating. The m2R-mediated G-protein gated potassium channel currents were used to assay the activity of the m2R. We found that the apparent affinity of the m2R toward acetylcholine (ACH) was reduced upon depolarization. The endogenous m1R- mediated Ca2+ dependent chloride currents were used to assay the activity of the m1R. The m1R also exhibited voltage sensitive gating, an apparent trend toward ACH, which was impacted upon depolarization. Direct binding experiments of [3H]-ACH to individual oocytes expressing either m2R or m1R confirmed the electrophysiological findings. Cumulative dose-response studies indicate that membrane potential affects either the G-proteins coupled to the m2R and the m1R or the receptors themselves. Based on the fact that G-proteins are peripheral membrane proteins, the close similarity between different G-proteins coupled to the m1R and the m2R, we favor the possibility that the m1R and the m2R are by themselves voltage sensors.

Keywords: G-protein coupled receptors, muscarinic receptor, voltage sensitivity, Xenopus oocytes

CuZnSOD deficient mice show late-phase improvement of neurobehavioral recovery after CHI and altered cortical reductive capacity

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Impaired neurobehavior after brain trauma mediates cell-death pathways. CuZnSOD catalyzes O2 to H2O2, conversion, and along with other antioxidants constitutes endogenous defense mechanisms. SOD administration was not beneficial in clinical trials, yet, SOD-transgenic mice display decreased lesion and apoptosis after brain trauma/ischemia. We assessed the effect of CuZnSOD deficiency on outcome and reductive capacity after closed head injury (CHI). CHI was induced in CuZnSOD knockout (KO) and wild type (WT) mice, using a weight-drop devise. Neurobehavioral function was evaluated up to 14d after CHI, using Neurological Severity Score (NSS). The difference between NSS at 1hr post CHI and at later times (ANSS) reflects recovery. Brain water content was measured 24hr after CHI. Reductive capacity was evaluated in sham (non-injured) and 5min post-CHI mice using cyclic voltammetry. CHI increased 0.01-1.5 min displayed a tendency to exit a circle (p=0.0001) and to balance over a beam (p<0.005). After CHI water content increased in the injured cortex (p<0.05) but to a similar extent in KO and WT. Yet, unexpectedly, at 1 hour post CHI, KO mice showed better recovery (ANSS 3 vs 1 ; p<0.02). Cortical reductive capacity was higher in sham KO compared to WT (p<0.05) and decreased at 5 min post CHI (p<0.05), whereas no changes occurred in the WT. Hepatic reductive capacity was also higher in sham KO (p<0.0001) but was not affected by CHI, in contrast to WT in which it increased (p<0.01). We propose that KO mice displayed late-phase better recovery after CHI which is not accounted for by the early post CHI alterations in reductive capacity.

Keywords: traumatic brain injury, oxidative stress, super oxide dismutase (SOD), antioxidants

The dose response effect of noladin ether in diet restricted mice

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Noladin ether (Nol) was isolated from porcine brain and identified as an endocannabinoid binding to CB1 cannabinoid receptor.

Aims: To evaluate the effect of noladin ether in mice under diet restriction (DR) as possible treatment for Anorexia Nervosa. Methods: Female Sabra mice were fed on a 2.5 hours/day diet for 14 days. Correspondingly were administrated i.p. different Nol treatment (Vehicle, nol 0.001, 0.01, 0.1 mg/kg). Food consumption (FC) and weight were measured. Cognitive function was evaluated using 8-arm maze and activity by x/y beam apparatus. After 14 days DR all groups were fed ad-libitum.

Results: FC of mice administered 0.001 mg/kg/day Nol was significantly higher than those received vehicle (p<0.0001). However, 0.01 and 0.1 caused significantly lower consumption (p<0.002, <0.02 respectively).

Cognitive function: Experiment showed a significant decline in maze performance vs vehicle 0.001 and 0.01 nol. (p<0.05). Activity: 0.001 and 0.1 nol. attained significantly higher beam apparatus performance than control (p<0.003).

Weight: No significant weight differences were obtained after one month of trial. Nevertheless, all mice groups lost an average of 10% in their weight.

Mice on diet restriction under noladin treatment (0.001mg/kg) increased their food intake while their activity was significantly higher than control and their weight didn't differ from vehicle. FC of mice under noladin (0.1mg/kg) decreased while their activity increased whereas their weight didn't differ probably due to decrease in BMR through IP3 signaling system (Leptin and endocannabinoids).

A bi-phasic dose response in the FC (nol 0.001=vehicle>0.1 mg/kg) was noted. Cognitive function of vehicle, nol 0.001, nol 0.01 was improved, whereas on nol 0.1 it declined.
Noladin has significant effect on food consumption and activity. Thus, low doses of noladin may possibly enhance appetite in patients with Anorexia Nervosa and weight loss such as cancer and AIDS cachexia. No such treatment is yet available.

**Keywords:** Noladin, endocannabinoids, diet restriction, anorexia, food consumption, activity

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**Visual and auditory "retain-and-compare" deficits in dyslexia**

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Previously, we found that dyslexics' contrast detection of temporally modulated gratings was impaired only when they were required to "retain-and-compare" between sequentially presented stimuli (Ben-Yehudah et al., Brain 124: 1381-95 [2001]). We now ask whether similar deficits characterize the performance of dyslexic adults when the stimuli are stationary. Using both auditory (tones) and visual (sinusoidal gratings) stimuli, we found that dyslexics' sequential frequency discrimination is significantly impaired in both modalities. We further compared visual frequency discrimination under two conditions. In the simultaneous condition, observers were asked whether the stripes were denser in the upper or lower half of the screen. In the sequential condition, observers compared stripe density between two successive intervals. Consistent with our previous findings, the majority of the dyslexic group (74%) was significantly impaired on the sequential condition. A significantly smaller group effect was found for the simultaneous condition. Classifying dyslexic participants according to their auditory-frequency discrimination yielded two subgroups with different visual performance. The dyslexic subgroup with poor auditory abilities (mean JND 21.8%) was impaired under both visual conditions, whereas the other dyslexic subgroup (mean JND 3.4%) was impaired only under sequential presentation. These results imply that dyslexics with poor psychoacoustic performance suffer from both visual and auditory perceptual impairments. The perceptual deficits of dyslexics with good psychoacoustic abilities (mean JND 12.7%) require retaining and comparing subsequent visual stimuli. One explanation for dyslexics' robust retain-and-compare deficit is impaired memory. Disassociating between deficient lower-level perceptual memory and higher-level working memory will be the focus of future studies.

**Keywords:** dyslexia, memory, frequency discrimination

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**Isofom specific effects of apolipoprotein E on lipid uptake by neuroblastoma N2a cells**

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Apolipoprotein E (apoE), is the major lipoprotein of the brain. There are three major apoE isoforms (apoE2, apoE3 and apoE4) of which apoE4 is a major risk factor of Alzheimer's disease. In the present study we investigated the possibility that apoE affects neuronal lipid metabolism isoform specifically. This was performed by measurements of the effects of apoE3 and apoE4 on the incorporation of [14C]acetate into lipids of neuroblastoma N2a cells and on the uptake of exogenous lipids. Addition of apoE3 and apoE4 (30 μg/ml), either in the presence or the absence of the exogenous lipid source linoleic acid, resulted in partial inhibition of the incorporation of [14C]acetate into neuronal lipids whose extent was similar for both isoforms. However, the incorporation of [14C]acetate into cells which were treated with the cholesterol inhibitor Lovastatin and with linoleic acid was partially inhibited by apoE3 and was unaffected by apoE4. The difference however was not statistically significant. In contrast the effects of apoE on the uptake of exogenous lipids, as measured by the incorporation of linoleic acid (C18:2) into cellular phospholipids, was markedly enhanced by apoE3 but not by apoE4. These findings show that the uptake of exogenous lipids into neuroblastoma N2a cells is isoform specifically by apoE3 and suggests that the patho logical effects of apoE4 in vivo may be mediated by a similar mechanism.

**Keywords:** apolipoprotein E, lipids, neuroblastoma

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**Propylactic effects of phenytoin in bipolar illness: a controlled study**

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Phenytoin, a classical anticonvulsant, shares the property of blockade of voltage activated sodium channels with antimanic anticonvulsants. We therefore planned a trial of phenytoin in acute mania. Thirty-nine patients entered a five week double-blind controlled trial of haloperidol plus phenytoin vs haloperidol plus placebo; thirty patients completed at least three weeks; twenty-five completed five weeks. Significantly more improvement was observed in some of the patients receiving phenytoin. We then planned a prophylactic study of phenytoin in bipolar disorder. Patients entered the study if they had been out of hospital for at least one month and had inadequate prophylaxis in the past on lithium, carbamazepine or valproate, and had at least one episode per year for the previous two years. Ongoing propylactic treatment was not changed (lithium, carbamazepine, valproate or neuroleptic). Ratings (BPRS, YMS, HDS, GAS) were done by the clinical treating psychiatrist at baseline and once monthly thereafter. After six months patients were crossed over during a month of weekly visits with one drug (phenytoin or placebo) being reduced by 100 mg weekly and the other increased by 100 mg weekly. Twenty seven observation periods (six-month phases) were studied. Several Kaplan-Meier survival analysis showed highly significant benefits for phenytoin addition. Blockade of voltage-activated sodium channels may be a common therapeutic mechanism of many anticonvulsants in mania, and phenytoin may be a therapeutic option for some manic patients.

**Keywords:** phenytoin, bipolar disorder, anticonvulsants

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**Dopamine, through direct interaction with its receptors, triggers IL-10 and TNFα secretion from normal, cancer and antigen-specific human T-cells**

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Under physiological conditions, T-cells may encounter dopamine in brain, blood, and various peripheral-organisms. Moreover, dopamine is elevated in the plasma in certain conditions of uncooping-stress, and injected in several emergency conditions Can dopamine directly trigger T-cell function?

We recently discovered that dopamine interacts directly with its D3 and D2 receptors on normal human T-cells, activates β1-integrins, and induces subsequent T-cell adhesion. Can dopamine also drive T-cells into cytokine secretion?

To challenge this question we applied dopamine or dopaminergic D1-D4 receptor agonists to normal peripheral, cancer, and antigen-specific human T-cell clones (of Th0, Th1, and Th2 phenotype), and analyzed the secretion of IFNγ, TNFα, IL-4 and IL-10.

We found that dopamine, in the absence of any additional molecule, can induce significant cytokine secretion from different T-cell types. The most prominent effect was the triggering of IL-10 secretion, a Th2-characteristic anti-inflammatory and suppressive cytokine. Dopamine also triggered a marked secretion of TNFα, a potent pro-inflammatory Th1-associated cytokine, playing a crucial role in various inflammatory diseases. Dopamine's effects were mimicked by specific dopaminergic-agonists, and blocked by the respective antagonists. Interestingly, the results suggested that dopamine may trigger IL-10 and TNFα secretion via different receptor subtypes.

Taken together, we speculate an active role for dopamine in various IL-10- and TNFα-dependent T-cell activities. Dopamine's effects may be either beneficial or detrimental, depending on the context.

We further speculate dynamic dopamine-T cell interactions in patients with schizophrenia, Parkinson's disease, Alzheimer disease and migraine, where modulations in dopamine-receptor levels in peripheral blood lymphocytes were recently reported.

**Keywords:** dopamine, T-cells, cytokines
Self organized mapping of spontaneous activity in cat visual cortex

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Optical imaging recordings of the primary visual cortex of anesthetized anesthetized anesthetized cats reveal that ongoing activity is composed of a set of states, that preference maps arise spontaneously at least 20% of the time. We applied a self-organized mapping algorithm (Kohonen, Self-organizing maps [1997]) to determine the topology of these states and to study the dynamics in a genuinely independent way. The results confirm that spontaneous activity can be mapped onto a one-dimensional ring structure similar to single condition orientation maps. Long epochs of smooth transitions between near-neighbouring orientations were obviously present in the data. The duration of such sequences was much longer than typical temporal correlation times. We also calculated the typical speed of transition and typical times spent in certain states of spontaneous activity. We found a clear preference of ongoing activity for states correlated with single condition during adaptation. Differences in volumes of activation in healthymen and women, but the interaction of these hormonal agents with brain ER has not been characterized to date. FES (F-18 labeled fluoroestradiol) has been used successfully for PET imaging of ER in the breast. The utility of this tracer for brain ER imaging is being investigated in rats and humans. Ovariectomized (OVX) rats were implanted with pellets containing estradiol, tamoxifen or blankets pellets. Three weeks later, four animals were injected IV with FES (0.5 mCi/Kg) and killed after 2 or 60 minutes. Brain samples were dissected and counted. As expected, the rank order of tracer uptake was hypothalamus > amygdala > cerebellum. OVX controls had a hypolateralization of cerebellum at 60 min. Estradiol and tamoxifen resulted in a significant reduction of this ratio to 8/1.80 (0.08 and 2.14±0.27, with p=0.001 or 0.003 compared to OVX). Cortical ER were similarly occupied by estradiol and tamoxifen (p=0.01 and 0.04, respectively, compared to OVX). In pilot human studies, we acquired PET brain images (single 10 minutes acquisition approximately 2 h after tracer injection of 4-6 mCi FES) in 6 patients undergoing FES PET as part of an ongoing breast cancer research protocol. Increased tracer uptake was observed in the preoptic/hypothalamic area relative to other gray matter regions, suggesting this tracer can be used for non-invasive imaging of brain ER by PET.

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Keywords: PET, SERMs, tamoxifen

Time dependent changes in NMDA receptor binding after closed head injury in mice

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Dept of Functional Imaging, LBNI, Berkeley, CA, USA 2Dept of Radiology, UCSF Medical Center, Sacramento, CA 3-4 fold the volume in men, possibly contributing to the relatively high proportion of good neurological recovery among female survivors of head injury. The activation volume in women was 3-5 fold larger (mean age 29 for women and 30 for men), the verb creation task resulted in a robust and lateralized (left>right) activation in both men and women. The activation volume in the preoptic/hypothalamic region relative to other gray matter regions, suggesting this tracer can be used for non-invasive imaging of brain ER by PET.

Source of knowledge of category affects cortical activity during classification of geometric figures: a visual ERP study

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Categorization strategies can include abstraction and application of a state or its rule, without the necessity to elicit the similarity to other category members. In order to determine the brain activity associated with these strategies we acquired PET images of human brain ER by PET.

Keywords: PET, SERMs, tamoxifen

Use of F-18 FES for estrogen receptor (ER) imaging in rat and human brain

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Estrogens or antiestrogens are currently used by millions of women, but the interaction of these hormonal agents with brain ER has not been characterized to date. FES (F-18 labeled fluoroestradiol) has been used successfully for PET imaging of ER in the breast. The utility of this tracer for brain ER imaging is being investigated in rats and humans. Ovariectomized (OVX) rats were implanted with pellets containing estradiol, tamoxifen or blanks pellets. Three weeks later, four animals were injected IV with FES (0.5 mCi/Kg) and killed 2 or 60 minutes later. Brain samples were dissected and counted. As expected, the rank order of tracer uptake was hypothalamus > amygdala > cerebellum. OVX controls had a hypolateralization of cerebellum at 60 min. Estradiol and tamoxifen resulted in a significant reduction of this ratio to 8/1.80 (0.08 and 2.14±0.27, with p=0.001 or 0.003 compared to OVX). Cortical ER were similarly occupied by estradiol and tamoxifen (p=0.01 and 0.04, respectively, compared to OVX). In pilot human studies, we acquired PET brain images (single 10 minutes acquisition approximately 2 h after tracer injection of 4-6 mCi FES) in 6 patients undergoing FES PET as part of an ongoing breast cancer research protocol. Increased tracer uptake was observed in the preoptic/hypothalamic area relative to other gray matter regions, suggesting this tracer can be used for non-invasive imaging of brain ER by PET.

Supported by DOD DAMD17-01-1-0289(AB), NIH AG06890(TFB), and NIH CA42045(DAM)

Keywords: PET, SERMs, tamoxifen

Source of knowledge of category affects cortical activity during classification of geometric figures: a visual ERP study

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Categorization strategies can include abstraction and application of a state or its rule, without the necessity to elicit the similarity to other category members. In order to determine the brain activity associated with these strategies we acquired PET images of human brain ER by PET.

Keywords: PET, SERMs, tamoxifen
Changes in the extracellular space of rat spinal cord induced by glutamate revealed by diffusion MR

The size and the geometry of the extracellular space (ECS) influence the diffusion of neuroactive substance. It has been shown that the release of neurotrophic factors from the ECS, which takes place in ischemia, trauma, and hypoxia, affects the ECS. In this study we have used diffusion MRS of deuterated tetramethyl ammonium chloride (TMA-d4), which is restricted to the ECS, in order to study the diffusion characteristics of the ECS before and after application of glutamate. The study was performed on rat spinal cords, which were excited and immersed in 0.1M solution of TMA-d4 for 3 hours. After the MRS measurements the spinal cord was immersed in a saline solution containing L-Glutamic acid (0.01M) for 2 hours. MRS diffusion experiments were performed on a 4.7 T NMR spectrometer using the stimulated echo (STE) diffusion sequence and with the following parameters: TR=3000 ms, TE=20ms, ∆=12ms. The diffusion times were 50, 150, 250 and 500ms. The diffusion was measured perpendicular to the long axis of the spine.

In these diffusion experiments different diffusion components of TMA-d4 were found. The fast component (Dd=2.95±0.01, 17×10^-10 m^2/sec) is relatively isotropic and has a tortuosity of 1.93±0.05 and 2.18±0.09 at diffusion time of 50ms before and after application of glutamate, respectively. The slow diffusing component (Dd=1.3±0.01, 0.1x10^-10 m^2/sec) is more anisotropic and has a tortuosity of 9.1±0.39 before and after application of glutamate, respectively. The tortuosity was found to depend on the diffusion time. The fast diffusing component observed in the MRS experiments show the similar characteristics as those obtained by iontophoretic method using selective electrode. All these observations are line with the cell swelling caused by the glutamate.

**Keywords:** diffusion MRS, extracellular space (ECS), tetramethyl ammonium chloride (TMA-d4)

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**Stimulus to feeling gap in the brain: the interaction of stimulation presentation mode and emotional appraisal**

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**Introduction:** What brings the gap between stimulus and feeling? Originally James claimed that this gap is filled merely by a feedback from an arousal response, while later others introduced the concept of cognitive assessment and appraisal of that arousal (1-3). These processes could well be complementary and may depend on the brain region and presentation mode of the stimulus. In this study we examined the interactions between the presentation mode of stimuli and their negative emotional appraisal in the brain by fMRI.

**Methods:** Eight right-handed female subjects were scanned in a 1.5T GE scanner. Experimental block paradigm consisted of two factors: stimulus type (pictures, faces, sentences) and stimulus assessment (rating of gender versus feeling). All stimuli were of negative valence, presented for 500-1500 msec at a rate of 0.5Hz. Data analyses performed using BrainVoyager (Brain Innovation Co). Parametric maps were obtained by General Linear Model (GLM) for individual subject analyses and group analysis. Regions of interest included: extra striate cortex, amygdala complex and superior temporal region (ST).

**Results:** Visual cortex and amygdala responded significantly more to pictures than faces and sentences. For example, stimulus type (p<0.001). In contrast, STS responded significantly more to sentences than to pictures and faces (main effect of stimulus, p<0.05) more so for the left hemisphere (2-way interaction of laterality and stimulus type). Furthermore, the emotional appraisal activation did not distinguish between emotional appraisal and gender identification, while visual and language regions were activated more by the emotional than the neutral task (main effect of task: visual cortex-p<0.05; ST-p<0.07). Furthermore, in the emotional task effect was most prominent for pictures (2-way interaction, p<0.005), while in the ST task effect was lateralized to the left hemisphere (planned comparison, p<0.05).

**Discussion:** Stimuli affected activation level in sensory, limbic and language regions depending on the mode or presentation. In contrast, emotional appraisal effect was found only in sensory and language regions. These results suggest that the difference in activations between processes that link stimulus and feeling is reflected in differential activation of sensory, language and limbic regions. This difference depends on either mode of presentation and/or appraisal of negative emotional stimuli.

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**Keywords:** emotions, amygdala, functional

**Modulation of acetylcholinesterase in exercised rat muscles**

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Slow twitch muscles are readily recruited and are active continuously while fast-twitch muscles are recruited when increased motor effort is needed. The synaptic activity of muscles involves acetylcholinesterase (AChE) which is concentrated at the neuromuscular junctions and determines the duration of the neurotransmitter activating the muscles. Because synaptic AChE depends on neuromuscular activity we examined the regulation of AChE in fast- and slow-twitch rat muscles following strenuous exercise. Rats were trained by walking on motor-driven treadmill (10d, 1h/d at a speed of 9m/min with 2 min sprints of 17m/min, every 10min), so as to activate the fast-twitch leg muscles but not usually recruited in sedentary animals. Fast and slow-twitch leg muscles were isolated, AChE was extracted and its levels and isoforn composition in trained and control-untrained muscles were analyzed. The results show increase in AChE content (protein) in all examined fast-twitch muscles but not in the slow-twitch soleus muscle. The most significant increase was measured in trained gastrocnemius. All muscles contained globular G1+G2, G4 and asymmetric A12-AChE isoforns (4-6S, 10S and 16S sedenation values, respectively). While G1+G2 and A12-AChE activities in both fast and slow-twitch muscles were not unacted training, the tetramer, G4-AChE, increased significantly in the...
exercised fast-twitch muscles (25-60%). These observations suggest that the G4-tetramer might become significant at the endplates of fast-twitch fibers following exercise. The density and distribution of this form at the endplates following exercise is examined.

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Keywords: neuroplastic function, fast twitch, acetylcholinesterase, treadmill

A study of performance of heavy-load tasks of identification of geometric forms by naive subjects

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The goal of this study was to test the assumption that perceptual performance of tasks as of the present experiment is universally based on pre-prepared action schema; these derived from 'mental schema' idealized so as to fit category-wise, various on-coming individual test conditions. The study was a one repetition experiment, on subjects short in experience with the stimuli, the rationale being that this meets with "new situation" criteria. While one repetition is critical for the observer for statistical verification, to simulate realistically new situations, iterative presentations of 'part connectedness' stimuli were used. The results show load as imputing shifting resources to earmarked higher demands, at cost of errors at easy tasks presumably in consequence of allotting these insufficient attention. High acuity demand, practically impossible for performance low as is, is found enabled per context of the experiment. Eluciding this is Ss (P<0.01) report that when having to judge between straightness and high acuity demand obliquity they experienced on the former illusory obliquity, the opposite direction that N1 phenomenon acquisition in N1 is presumably enhancing decision/ disambiguation. 'Paradigms juncures' - assumed points of across-mechanisms joint probability interactions achieved performance on both of several of shared components' relevance to a category relationship. Keywords: mental schema; naive subjects; part connectedness paradigms; disambiguation

Intake and activation of the dorsal vagal complex in preweaning rats after preloads of corn-oil or mineral oil
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Otsuka Long-Evans Tokushima Fatty (OLETF) rats lack cholecystokinin-A (CCK) receptors which have characterized as early postnatal hypophagia and increased body-weight in infant OLETF vs. their controls, Long Evans Tokushima (LETO) rats, on postnatal days 2-4 and 9-11, depending on independent ingestion test. In the current study, we compared feeding suppression by 50% BW gastric preloads of corn oil and mineral oil in 18-20 days old OLETF and LETO pups, and in Long-Evans pups on postnatal days 9-11. C-fos immunoreactivity was examined in area postrema (AP), the nucleus of the solitary tract (NTS), and in hypothalamic areas implicated in feeding regulation. LETO rats ingested significantly less after corn oil than after mineral oil preloads. Their c-fos immunoreactivity in AP and NTS was increased compared to sham-preload controls. The effect of corn oil was not evident in OLETF pups, their mean number of c-fos expressing cells was increased in the caudal, subpostrema, and intermediate NTS, and in the AP, but not as much as in LETO pups. Intake of Long-Evans pups was not lower after corn oil compared to mineral oil preloads, replicating previous observation. Findley rats (Weller et al. Physiol Behav, 62: 871-874 [1997]). Their activation patterns in the AP and NTS corresponded to the intake results. These findings suggest that CCK receptors participate in mediating intake-reduction early in ontogeny.

Supported by the US-Israel Binational Science Foundation

Keywords: CCK, c-fos, area postrema, nucleus of solitary tract

Differentiation and neurotransmitter phenotype acquisition in developing neurons - differential expression of genes and proteins
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P19 cells are embryonal carcinoma cells that serve as a model for studying differentiation processes including commitment to cell lineage. We have studied P19 cells following activation of neuronal differentiation. The potential of these cells to mature and efficiently release neurotransmitter (NT) was established in our lab. We discovered that several variables, most notably, cell density and various neurotrophic factors affects neuronal maturation, survival and most surprisingly, the choice of NT phenotype. We have observed that NT phenotype acquisition in P19 cells is mediated by cell-cell contact and not by soluble factors. We have shown that changes in cell density are associated with changes in gene expression. A large-scale, holistic view on gene expression was obtained by RNA chip technology. The data obtained from this screen was extensively analyzed with various bioinformatical methods. Among the genes found in that screen are members of Wnt and Glial families which function in transducing a signal from the cell surface.

Mass Spectroscopic comparative proteomics approach was applied on sparse and dense P19 induced cultures. Membranes were collected at different time windows following neuronal induction. Differentially expressing proteins were excised and sent to Mass-spectroscopy analysis. Currently, over 30 proteins were successfully analyzed by such methodology. Using 2D gels we were able to improve detection level and could identify relatively low expressing proteins. Most intriguing proteins are a variant of Drebrin - a putative dendritic-shaping molecule and several cytoskeletal-signaling molecules. Those and other proteins reflect the morphological reorganization that takes place while establishing P19 neuronal fate.

Photic resetting of the human circadian clock: applications to jet lag and shift work
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Recent studies indicate that the endogenous circadian system is sensitive to much lower levels of light than initially suspected (Boivin DB et al Nature 1996;379:540-542.). It was also recently reported that 70% of the resetting effect of a bright light stimulus can be preserved even though it is interrupted by episodes of complete darkness for 63% of the time (Rimmer DW et al Am J Physiol Regulatory Integrative Comp Physiol 2000;279:R1574-R1579). The usefulness of a judicious schedule of exposure to room light (first study) or to intermittent bright light (second study) to treat circadian maladaptation to jet lag or shift work was tested. In the first study, 15 healthy young men participated to a laboratory simulation of a Montreal to London voyage (Boivin DB and James FO. J Biol Rhythms 2002;17(3):266-276). They were exposed to 380 lux for 6 hours each day either before bedtime or in the morning. This study demonstrated that the schedule of exposure to room light can substantially affect circadian adaptation to a shifted sleep/wake schedule. In the second study, 15 nurses working permanent night shifts were studied for 3 consecutive weeks (in press). They were either exposed to bright light intermittently for 6 hours in their workplace (treatment group) or worked under their usual lighting conditions (control group). Circadian reentrainment was complete in the treatment group only. Results of these two studies underline the importance of the control of the overall pattern of light exposure in circadian adaptation to shifted sleep/wake schedules.

Motion induced blindness is affected by head-centered and object-centered mechanisms
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In motion-induced blindness (MIB; Bonneh et. al., Nature 411:798-801[2001]), a salient static pattern may disappear and reappear spontaneously in the presence of a global moving pattern. We have previously showed that this phenomenon is unlikely to reflect retinal suppression, sensory masking or adaptation, but the mechanisms involved in MIB are largely unknown. Recent evidence suggest that partial mechanisms representing space in different reference frames may be involved in the control of awareness, and possibly in MIB.

Here we ask what is the frame of reference in which disappearance during MIB occur. To answer this question we measured the magnitude of motion induced blindness at several locations in three different conditions: (1) retinotopic mapping in which a single target dot was presented at different locations relative to fixation, (2) a head-in which the direction of the head was displaced by 20 deg. to the right or left keeping direct fixation and (3) object-centered condition in
which a target dot in fixed retinal location was surrounded by an elliptic contour with different relative displacement along its main axis. Results show the effect of all three manipulations on disappearance. Anisometric disappearance was found for all observers with more disappearance in the upper and upper-left visual fields. Disappearance map changed for different angles of head rotation even though the retinal locations were identical. The elliptic contour induced more disappearance around its focal points and less in its center. The finding that disappearance around MIB involves or is affected by head-centered and object-centered mechanisms.

Keywords: visual disappearance, visual awareness, visual space

Evaluating ion channel modulating activities in a Tarantula venom
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The flow of ions through membrane channels is an essential component of cellular function. Recently, ion channels have emerged as potential targets for diagnostic and therapeutic agents due to their membrane localization and their contribution to the physiology and pathophysiology in many biological systems. The venoms of the animal world offers a large variety of peptide toxins, with some exerting their biological activity on various ion channel activities, by means of high affinity interaction and selective modulation. Alomone Labs R & D focus on the discovery of new effective and specific ion channel peptide toxins. These toxins may serve as potential diagnostic and therapeutic agents, which affect various diseases, such as cancer, cardiovascular diseases and neurological disorders.

Alomone Labs has developed a technological platform for finding, characterizing, producing and modifying peptide-toxin ion channel modulators. It includes: Separation and purification of peptide toxins from whole native venom. Assays for finding molecularly defined ion channels as possible targets for the purified toxins, and for evaluating the characteristics of the toxin pharmacology. Reconstituent production and purification of a desired peptide toxin.

Here we focus on the separation and dissection of ion channel modulating activities found in the venom of one spider species. The spider Grammostola spatulata (Brown Morph Grammostola-BMG) venom effects on various clonal voltage dependent ion channels, was examined using two-electrode voltage clamped Xenopus oocytes. The BMG whole venom strongly inhibited the following ion channels: Kv2.1, NaV1.5, CaV1.2, CaV2.2 and CaV3.1. BMG venom was subjected to SP-sepharose fractionation, yielding 8 peaks. Peptide within the venom was narrowed to specified peaks for each of the channels examined.

A peptide containing a single peptide that was identified by mass spectroscopy and amino-acid analysis as the previously described o-Grammotoxin SIA specifically blocked CaV2.2 channels.

Strong blocking activity towards Kv2.1 channels was found in two separate peaks that contain several peptides each. One defined peak blocked NaV1.5, CaV1.2 and CaV3.1. However, further analysis showed that this peak contained several different peptides.

In conclusion, we show wide range and specific ion channel blocking activities, carried out by separated fractions of Tarantula venom. Some of these activities affect channels with poor specific pharmacology such as the CaV3.1 channel.

Keywords: channels, toxin, Tarantula

Maternal deprivation increases alcohol consumption in adulthood in the rat
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Early maternal deprivation (MD) is considered an adverse stressful experience, associated with long-lasting effects in adulthood. It is suggested that this kind of stress may be a risk factor for the development of high alcohol consumption in adulthood. The aim of the present study was to examine, in a rat model, the relationship between early maternal deprivation, and subsequent development of high alcohol consumption. We further examined the influence of long-term isolation on MD modulations of alcohol consumption in adulthood.

Method: Male Wistar rats were exposed to one of 4 different rearing conditions: 1) Maternal separation from PND 7 for 7 consecutive days, for one hour every day. 2) Long-term social isolation immediately post weaning. 3) Maternal deprivation and social isolation. 4) Regular rearing conditions. Pups were weaned at PND 28. At the age of 3 months all animals were examined in a free choice paradigm for alcohol and water consumption.

Results: We observed a significantly high rate of alcohol consumption only in rats that were maternally deprived 3 months before.

These finding indicate that MD has long lasting effects on emotional development.

In contrast to previous reports studying social isolation as a stressful experience, social isolation in this study was found to attenuate the effect the MD treatment. The main difference between the current and previous studies was that in the present study social isolation began immediately after weaning while in previous studies rats were exposed to social isolation in adulthood. Further examination is required to clarify these differences.

Supported by a grant from the Israel Foundation Trusties (2000) to G.R-L.

Keywords: Maternal Depression; stress; alcohol

Location and size of dopaminergic and serotonergic cell populations are controlled by position of mid-hindbrain organizer
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Midbrain dopaminergic and hindbrain serotonergic neurons play an important role in the modulation of behaviour and are involved in a series of neuropsychiatric disorders. Despite the importance of these cells, little is known about the molecular mechanisms that govern their development. Embryogenesis in midbrain dopaminergic neurons are specified rostral to the mid-hindbrain organizer (MHO) and hindbrain serotonergic neurons caudal to it. We report that in transgenic mice in which the MHO is shifted caudally, the midbrain dopaminergic neuronal population expands to the ectopic positioned MHO and is enlarged. Complementarily, the extension of the hindbrain serotonergic cell group is decreased.

In adulthood these changes are preserved and the additional, ectopic dopaminergic neurons project to the striatum, which is a proper dopaminergic target area. Also in mutants in which the MHO is shifted rostrally, dopaminergic and serotonergic neurons are relocated at the newly positioned MHO. However, in these mice the size ratio between these two cell populations is changed in favour of the serotonergic cell population. To investigate whether the position of the MHO during embryogenesis is also of functional relevance for adult behaviour we tested mice with a caudally shifted MHO and report that these mutants show a higher locomotor activity. Taken together, we provide evidence that the position of the MHO during embryogenesis is also of functional relevance for adult behaviour.

Keywords: Maternal Deprivation; stress; alcohol

Olfactory-learning induced reduction in post-burst AHP in piriform cortex neurons is due to a decrease in sAHP
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We have previously shown that olfactory learning results with reduced post-burst afterhyperpolarization (AHP) in piriform pyramidal neurons of the piriform cortex. In the present study we examine which of the currents that generate the post-burst AHP (sAHP and iAHP) is reduced after learning. Rats were trained in an olfactory discrimination task to distinguish between positive and negative odor cues until they demonstrated rule learning. Three days after completion of training, intracellular recordings from pyramidal neurons were performed in piriform cortex brain slices. As previously shown, AHP amplitude was...
significantly smaller (P<0.02, one way ANOVA) in neurons from trained rats, compared with neurons from pseudo trained and naive rats (-6.79±0.38 mv, n=23 in pseudo trained and -6.76±0.37 mv, n=24 in naive). In the presence of 10μM NE, AHP amplitude in neurons from trained rats did not differ from those of pseudo trained or naive rats (-6.32±0.71 mv, n=14 in trained, -5.20±0.54 mv, n=12 in pseudo trained and -5.64±0.40 mv, n=24 in naive). In the presence of 50μM of the specific KA blocker ARA40, AHP amplitude remained significantly lower (P<0.03, one way ANOVA) in neurons from trained rats, compared with controls (-2.76±0.41 mv, n=17 in trained, -4.67±0.59 mv, n=16 in pseudo trained and -4.73±0.51 mv, n=9 in naive). There was no reduction in AHP amplitude was stronger in neurons from trained rats (45%) then that in neurons from pseudo-trained rats (31%), or in naive rats (30%).

These data indicate that learning induced post-burst AHP reduction is accompanied by reduction in ratio of ΔI_{AHP}/I_{AHP} conductances. We suggest that the differential effect of noradrenaline is due to this change.

Keywords: olfactory-learning, piriform cortex, AHP, noradrenaline

Identification of a new gene for otosclerosis in Israeli family Brownstein Z., Friedmann M. and Avraham K.B.

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Otosclerosis is a common bone disorder with a prevalence of 0.2%±1% among adults. It is characterized by isolated endochondral bone sclerosis of the labyrinth in the basal cochlea. Otosclerosis leads to a progressive hearing impairment which begins as a conductive hearing loss (HL), due to a fixation of the stapedial footplate in the oval window, and might develop into a mixed (sensorimotor) hearing loss, caused by connective tissue otosclerotic foci. The age of onset is usually 20-40 years and in most cases both ears are involved. The mode of inheritance is autosomal dominant, with reduced penetrance. Thus far, the chromosomal locations of three loci for otosclerosis have been reported on chromosomes 15q25-q26, 7q34-36 and 6p21.3.

In our study we followed for 6 months 31 individual presenting to the emergency department (ED) as a result of trauma exposure. Salivary cortisol was collected 5 days post-trauma as an early and sensitive target for cerebral ischemia and other neurodegenerative processes. We have shown that an eight min ischemia (hypoxia/hypoglycemia) insult to an organotypic hippocampal slice culture, promoted glutamate-mediated generation of free radicals with concomitant elevation of intracellular calcium (Perez Velazquez et al., 1997). In this same model, the mitochondrial complex I inhibitor, rotenone, the mitochondrial permeability transition blocker, cyclosporin A (Csa), and a blocker of NAD+, nicotinamide, decreased ischemia-induced free radical generation and increased mitochondrial calcium (Franseva et al., 2001). Interestingly, Csa did not increase the increase in the cytoplasmatic calcium, but did reduce the increased mitochondrial calcium, suggesting that mitochondrial calcium could be the most important mediator of neurodegenerative processes. Preliminary experiments, in acutely prepared rat hippocampal slices, have shown that during and following 8 min. of hypoxia/hypoglycemia, stratum radiatum evoked synaptic transmission in the CA1 subfield is depressed along with an increase in presynaptic cytoplasmatic and mitochondrial calcium, which persists for up to one hour following the ischaemic insult. The presynaptic increase in calcium is reduced by BAPTA-AM. We hypothesize that the neuroprotective actions of intracellular calcium chelation could be by limiting rises in intramitochondrial calcium and that the maintenance of 'healthy' mitochondria will be neuroprotective against ischaemic insults.

Keywords: ischemia, synaptic transmission, mitochondria, presynaptic calcium

The ischemic synapse and the role of mitochondria

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Mitochondria are increasingly implicated in neurodegenerative mechanisms from several perspectives, including calcium regulation, free radical production, energy utilization, and release of apoptotic factors. Synaptic transmission could be an early and sensitive target for cerebral ischemia and other neurodegenerative processes.
Functional GABA<sub>A</sub> receptors are lost at the hippocampal mossy fibre synapse after status epilepticus

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Acute GABA<sub>A</sub> releases mossy fibre (MF) field excitatory post-synaptic potentials (EPSPs) in hippocampal slices (Min et al, Neuron 1998; 21:561-70, Vogl & Nicoll, PNAS 1999;96:1118-22). We have shown that this form of EPSP is mediated by a calcium-permeable low threshold Ca<sup>2+</sup>-sensitive channel, which is not detectable in the presence of GABA<sub>A</sub> receptor agonists such as baclofen or GABA<sub>A</sub>-selective blockers such as flumazenil.

Purinergic receptors have been found in sensory neurons, but there is no information on their presence in satellite glial cells (SCs) of sensory ganglia.

**Keywords:** purinergic receptors, satellite glial cells, GABA<sub>A</sub> receptor

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The neuropeptides GnRH-II and GnRH-I interact directly with normal and cancer human T-cells and trigger de novo gene expression in R</p>
is thus achieved by an hyperexcitable response to chemical synapses. With such compensatory mechanisms, it is not surprising to find only minute differences in the behaviors of KO and WT.

Keywords: inferior-olive, gap junctions, oscillation, knockout

Recording action potentials by depletion type floating gate P-channel MOS transistor
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We report the realization of electrical coupling between neurons and depletion type floating gate (FG) P-channel MOS transistors. The devices were realized in a shortened 0.5μm CMOS technology. Increased boron implant dose was used to form the depletion type devices. Post CMOS processing steps were adapted to the devices processing line. The neurons are coupled to the polycrystalline Silicon (PS) FG through 420Å thermal oxide in an area which is located over the thick field oxide away from the transistor. The combination of coupling area pad having a diameter of 10-15 μm and sensing transistor with W/L of 50/0.5μm results in capacitive coupling ratio of the neuron signal of about 0.5 together with relatively large transistor trans-conductance.

The combination of the FG structure with depletion type device, leads to the following advantages: a) no DC bias between the biological solution and the transistor with direct consequence to the neural signal as well as that the silicon die during operation of the device, b) the sensing area of the neural activity is separated from the active area of the transistor. Thus, it is possible to design the sensing area and the channel area separately. c) The channel area, of the most sensitive part of the transistor, can be insulated and shielded from the ionic solution. d) The option to add a switching transistor to the FG and use the FG also for the neurons stimulated. 

Keywords: MOS transistor, action potential

The neural activity in rat motor cortex changes during the learning of a conditional sensory discrimination task
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Neural activity in motor cortex has been intensively studied for many years. Yet, little is known about the changes that occur in these neurons' activity as animals learn a conditional auditory discrimination task. In this study, arrays of microwires were chronically implanted in the motor cortex (M) of adult rats to allow the recording of single unit activity as the rats learn to associate an arbitrary tone with a movement. This approach allowed us to characterize changes in cortical activity due to learning as well as the ensemble. Adult rats were trained to nose poke a center hole and wait for a go cue which was either a high or a low tone. Depending on the tone, the subject was required to nose poke a hole located either to the right or to the left of the center hole. Following correct trials, the rat received water reward. In this task, the rats learn to associate each tone with a specific movement in addition to performing the movement via repetitions.

Our results show that during the learning process, the behavioral attributes of reaction time, movement time and error frequency decreased with practice. The improvement in task performance was accompanied by changes in the firing patterns of neurons associated with the task. These changes included sharpening of the response to the task related events and modification of the average firing rate.

Keywords: chronic recording, motor learning

ERK activation is correlated with synaptic modifications after hyperpolarization in the piriform cortex
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We have shown previously that post-burst afterhyperpolarization (AHP) and paired pulse facilitation (PPF) is reduced in piriform cortex of pyramidal neurons, days after in vitro firing in the piriform cortex. We have also shown that this long term cellular modifications subserv references at least part of the odor rule learning and are looking into possible molecular mechanisms for these long term modifications (See also Shiboleth et al.). Epischer mutants (ERK/KI) were found by others and us to play major role in learning and memory as well as synaptic plasticity in cortical areas. We began to study the possible involvement of ERK/KI in long-term modulation of intrinsic and synaptic properties correlated with odor rule learning in the piriform cortex. The specific MEK inhibitor PD98059 (40 μM) caused a significant increase in PPF amplitude in neurons from mutant mice only. Consequently the difference in PPF amplitude between trained and pseudo rats was diminished (from 1.2±0.25, n=21 to 1.5±0.29, n=7 in trained and from 1.5±0.20, n=6 to 1.4±0.18, n=5 in pseudo-trained). PD98059 did not affect on post-burst AHP amplitude in any group on neurons (from 6.2±2.29, n=44 to 6.5±2.72, n=19 in trained and from 7.8±1.74, n=20 to 7.3±1.78, n=18 in pseudo-trained). Consequently, the fractionation and quantitative immunoblot analysis of anti-phospho-specific antibody and protein antibodies we are examining ERK/KI activation in the piriform cortex. In preliminary experiments we detected 20% increase in ERK/KI activation in the cytosolic fraction of trained (n=4) compared to control rats. We conclude that ERK/KI is involved with long-term modulation of synaptic transmission in the piriform cortex of trained rats but not in post burst AHP 3 days following training.

Keywords: olfactory-learning, piriform cortex, paired-pulse facilitation, ERK

Prospain induces dendritic clustering of GluR1 and spine formation
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Formation of post-synaptic spines and mobilization of glutamate receptors into spines are part of the mechanism underlying long-term synaptic plasticity (Muller et al., Hippocampus 10(5):596-604 [2000]). However, little is known of the factors inducing these processes. Prospain, a precursor of the lysosomal saposins, has a 71kD-secreted variant with neurotrophic activity, which is reduced synaptically in hippocampus during ischemia (Sano et al., BBRC 204(2),994-1000 [1994]). Nonetheless, it is unclear whether it directly influences synaptic development and function.

We found that in hippocampal neurons prospain was localized in vesicles within dendrites, but is absent from axons and synaptic connections. Depletion of prospain in knockout mice is associated with a decreased expression level of dendritic Glutamate Receptor 1 (GluR1) in the hippocampal dentate gyrus and CA1 regions compared to the wild type. Supporting this, application of an anti-prospain antibody to the growth medium of hippocampal cultures reduces dendritic GluR1 cluster density by 44% and GluR1 cluster size by 56%. These effects are specifically on GluR1 since the treatment with anti-prospain has no effect on NMDAR1 clustering.

Furthermore, anti-prospain reduces the density of GluR1-containing spines by 88%, whereas application of exogenous prospain increased this density by 90%.

Our results show that the specific activity of prospain clusters GluR1, but not NMDAR1 in dendrites, and promotes spine formation.

Keywords: dendritic spines, glutamate receptor, prosapain

The impact of intracellular A-beta peptides
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Extracellular A-B peptides unquestionably display toxic effects contributing to the Alzheimer’s Disease (AD) pathology. However, intracellular accumulation of Aβ also occurs at early stages of AD. In vitro studies have shown that intracellular Aβ (Aβ) can disrupt cellular organelles and MAP kinases. We have continued investigations regarding the consequences of elevated levels of Aβ in a rat transgenic model, expressing human transgene genes related to familial forms of AD, whose CNS pathology is limited to intraneuronal accumulation of Aβ in the hippocampus and neocortex. In this transgenic rat model we have observed that the presence of intraneuronal Aβ, in the absence of amyloid plaques, is sufficient to provoke up-regulation of the MAP kinase ERK2 in the hippocampus at 9 months. This is a dysregulation, which appears specific, since no changes were observed in other putative tau kinases, such as GSK3 α-β and CDK5. These rats displayed a concomitant increase in the level of tau phosphorylation at ERK2 target sites. Confocal microscopy studies revealed that neurons displaying Aβi also manifest changes in the Golgi complex and lysosomes. Interestingly, these morphological and biochemical alterations were followed by behavioral cognitive impairments which became apparent at 16 months of
Dendriticspines,thesiteofsynapticconnectionsbetweencentralneurons,playanimportantroleininformationprocessingandmemoryformation.Corticosterone,oneofthemajorstress-inducedhormonesinmammals,mayhaveprofoundeffects ontheroleandfunctionsofspecificbrainareas.Onewedramaticeffectofcorticosteroneonseveralmorphologicalindices.Theresultsindicatethatcorticosteronecausespruningofdendriticspinesbyincreasingcalciumloadthroughanincreaseinvoltagegatedcalciumchannelactivity,assuggestedbytheobservationthattheexcitatoryaminoacidglutamatecaninduceadincreaseinspinedensityabovebaselinevaluesinprimaryculturedneurons.Puttingtogethertworelatedyetdistinctformsofaversiveresponseconditioning,conditionedtasteaversion(CTA)andconditionedcontextaversion,usingimmunohistochemistry,wefoundthatCTAinducesstrongCREBactivationintheinsularcortex(IC)andtheleftseptum(LS),butnotintheparietalcortex(PC)andthemiddleseptum(MS).Incontrast,contextualconditioningresultedinstrongactivationinthePCandMS,notintheICandLS.Theseobservationsareroughlyinagreementwithamodelthatlinksaconsistentpatternofactivitywithinthesystemsthatassociatespecificneutralizedodorswithaversiveevents.

Wearecurrentlyinvestigatingotherpotentialprotein dysregulations currently involved in the molecular pathologyprecedingplaqueformation.

Keywords:intracellularA-beta;transgenicrat;MAPkinases;tau-phosphorylation
Mechanism of action of fast-onset antidepressant drugs

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In the last decade, a number of antidepressants were developed that demonstrated a more rapid onset of clinical effects than classical antidepressants. However, the mechanism that enables some drugs to have a faster onset of action than others is poorly understood. The aim of the current study was to determine what dynamic changes in the brain in response to a fast-acting antidepressant to elucidate possible neurochemical parameters that contribute to the improvement of depressive behavior. In our previous studies, we found that Flander sensitive line (FSL) rats, an animal model of depression, are characterized by: a) increased immobility during the forced swim test, b) absence of serotonin-induced dopamine release in the nucleus accumbens and c) abnormal expression and increased inhibitory-like activity of 5-HT_2C receptor. All of these parameters were normalized by 14 day treatment with antidepressants. In the current study, we found that subchronic (7 day) treatment with nefazodone (a fast-onset antidepressant), but not with desipramine (a classical antidepressant), normalized the immobility time in the swim test in FSL rats. Nefazodone treatment of FSL rats also restored normal activity of the 5-HT_2C receptor and and normal serotonin-dopamine interaction after a subchronic treatment. We conclude that the restoration of a normal serotonin-dopamine interaction via the 5-HT_2C receptor seems to be a critical parameter for the fast onset of antidepressant treatment.

Keywords: antidepressant; fast onset; microdialysis; serotonin 5-HT_2C receptor

Effect of somatostatin on prostaglandin and nitric oxide synthesis in glial cells

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Recent evidence suggests that prostaglandins (PGs) are involved in the pathogenesis of Alzheimer’s Disease (AD) and that nonsteroidal antiinflammatory drugs (NSAIDs) prevent its progression. Glial cells are a major source of PGs in the central nervous system (CNS) and are important in physiological and pathological processes in the CNS. Deficits in somatostatin (SS)-like and corticotropin releasing factor (CRF)-immunoreactivity are recognized as prominent biochemical deficits in AD patients. Fleisher-Berkovich et al showed that CRF can regulate PG synthesis in endothelial cells and fibroblasts (Endocrinology 136:4068-4072, 1995). The aim of the present study was to investigate the role of SS in the regulation of prostaglandins (PGs) in primary rat glial cells culture. Our results show that SS (10^-10 M) inhibited basal PG production by 65% to 68%. LPS increased in a dose and time dependent manner PG synthesis in rat glial cells. PG levels increased significantly after 24 hours of treatment with LPS. SS (10^-10 M - 10^-1 M) inhibited LPS-induced PG synthesis by 40% to 50% LPS increased NO release in a time and dose dependent manner. SS (10^-10 M - 10^-10 M) decreased LPS-induced NO release by 25-30%. It is tempting to speculate that NO synthesis induced by inflammatory agents like SS is significantly regulated by neuropeptides like SS, thus preventing the occurrence of inflammatory conditions. In contrast, both patients showed no such dependence and no averaged modified trajectories. These findings suggest the patients’ inability to amend their ongoing movements in response to a sudden change in the target location. Furthermore, a higher proportion of direct trajectories was observed in DS movements, for movements that were initially directed toward the left side of space. In addition, whereas in elderly subjects and patients, some of the DS movements contained a pause before the direction of the trajectory was modified, (15% and 35% of the DS movements respectively), the patients displayed significantly longer pauses. Moreover, patients had more difficulties in the choice of the first target appeared ipsilaterally, with respect to the trunk-midline. These findings reflect the existence of a competitive bias in favor of ipsilateral stimuli. Results from two neglect patients showed significantly longer reaction times (RT) and movement times (MT), compared with control subjects. Moreover, a conterstional to ipsilateral gradient was found in both RT and MT. The initial direction of motion in both young and elderly healthy controls depended on the modification time (RT – Inter-Stimulus-Interval), and included averaged/non-averaged and direct trajectories. In contrast, both patients showed no such dependence and no averaged modified trajectories. These findings suggest the patients’ inability to amend their ongoing movements in response to a sudden change in the target location. Furthermore, a higher proportion of non-constant trajectories was observed in DS movements, for movements that were initially directed toward the left side of space. These findings reflect the existence of a competitive bias in favor of ipsilateral stimuli. Results from two neglect patients showed significantly longer reaction times (RT) and movement times (MT), compared with control subjects. Moreover, a conterstional to ipsilateral gradient was found in both RT and MT.
A new member of the cyclin family may play a role in long-term memory

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Identifying the molecular correlates of long-term memory has been a focus of research, nevertheless little progress has been made and only few molecules have been directly correlated with the neuronal processes underlying consolidation. In this project we were interested in finding gene products that are altered hours after learning. Since the prevailing hypothesis emphasizes the role of neuronal plasticity in memory consolidation we used a behavioral paradigm that occur during early developmental stages and have a strong influence on memory consolidation. We used the one-day-old chick passive-avoidance learning paradigm in which a chick learns to avoid eating a bead coated with an unpleasant tasting substrate. The intermediate medial prefrontal cortex ventricle (IMHV) is centrally involved in storing this memory. A screen using differential display for changes in mRNA expression in the IMHV between 2 and 24 hours after training chicks on the passive-avoidance memory task, revealed a new gene sequence with an homology of 80% with the cyclin family that was induced after training with a peak at 12 hours after training.

Our results coincide with earlier findings suggesting selective induction of a different member of the cyclin family - ania-6 by different types of neurotransmitters in the stratum. It is also known that certain cyclins can interact with RNA polymerase II. This might be a novel mechanism for regulation of neuronal gene expression which converges with multiple lines of evidence suggesting that acute regulation of pre-mRNA splicing is important in neuronal plasticity.

Keywords: passive avoidance, chick, cyclins, differential display

The extinction of fear conditioning in Medaka fish (Oryzias latipes)

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Ampale evidence indicates that experimental extinction is learning rather than unlearning. However, it is yet unclear how different is the mechanism of extinction from that of acquisition. In the present study we used a developmental approach in order to address this question. Medaka fish of three different ages (1 week, 4 week and 7 months) were subjected to extinction training by pairing a light (conditioned stimulus, CS) with electric shock (unconditioned stimulus, US) for 20 trials over 2 days. Fear response was evaluated by computerized comparison of locomotion before and during CS presentation. Subjects were tested on memory retention starting 24 hours after the last training trial. Afterwards, the fish were subjected to 20-30 successive trials over 2 days in the absence of the US. Significantly age-dependent differences were unveiled in retention and extinction of conditioned response. Whereas all age groups readily acquired the task, the youngest fish (1 week old) failed to reacquire it. In contrast, extinction in 2 month old successfully extinguished the fear response in 20 trials, while the 4 week-old fish continued to exhibit a startle response to the CS even after 30 trials. These results suggest that extinction demands a more mature nervous system than the acquisition of conditioned fear. In addition, the results validate the ability to use Medaka fish, which are a convenient neurogenetic model, for the study of the ontogeny of learning and memory.

Keywords: fear conditioning, extinction, acquisition, ontogenesis

A new amphiphilic motif in the N-terminal helix of heterotrimeric G-proteins

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Heterotrimeric G-proteins relay signals between membrane-bound receptors and downstream effectors. The α subunits are anchored to the membrane by one or more lipid modification at their N-termini. These modifications can be palmitoylation, myristoylation or both. As no sequence determinant for palmitoylation is apparent, we used an in vitro system modeling the interaction of different human G-proteins to look for a three-dimensional structural determinant of palmitoylation, rather than a linear sequence motif. Comparison of the N-termini of these super-family revealed that all α subunits modified only by palmitoylation contain a similar structural motif at their N-terminal helix. This motif is characterized by a prominent basic patch that extends a positive potential well beyond the N-terminus of the protein. Furthermore, this structural motif is oriented opposite to the face that interacts with the β subunits. Hence, these positive patches are free to interact with the negatively charged inner surface of the plasma membrane. Based on previous results, we suggest that this efficient palmitoylation of Gα proteins requires prior targeting to the plasma membrane. The signal for this may be the oligomeric structure of the protein. Our results suggest that the membrane can therefore be either myristoylation or the novel motif that we identified. This signal is cooperative with the interaction of the α subunit with the β complex. The N-terminus of a Gα protein can therefore be described as amphiphilic, containing dual signals attracting it to the membrane and enabling it to undergo palmitoylation. As palmitoylation has been shown to modify a plethora of proteins extending beyond G-proteins, this motif could be widely applicable.

Keywords: structure, G-protein, membrane-attachment

Proteomics approach to study the role of ERK in learning and plasticity

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We are interested in the role of the extracellular regulated kinase (ERK) in the formation of long-term memory and plasticity. ERK II is involved in both early and late phases LTP (Rosenblum et al. J. Neuroscience, 2002). In addition, ERK II expression is correlated and necessary for the formation of long-term memories (e.g. Berman et al., 1998). Using subcellular fractionation, phospho-specific antibodies and proteomics (two Dimensional Electrophoresis, mass spectrometry, and bioinformatics) we are trying to identify ERKII substrates in the brain that are involved in memory formation. In order to identify ERKII substrates we use an antibody that recognizes phosphorylated-Threonine only when followed by the amino acid Proline (the favorite phosphorylated site by ERKII). In addition, we test the modulation in the neuronal proteome expression of a series of proteins after ERKII stimulation. We are using translation modifications using different fractionation methods. The functionality of the identified spots or bands will be determined in the insular cortex both following learning (novel taste learning) and LTD.

Keywords: consolidation, ERK, proteomics

Oxidative stress induced by 6-hydroxydopamine affects ubiquitin-conjugates, protein degradation and proteasome activity in PC12 cells: Implications for the pathogenesis of Parkinson’s disease

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Mutations in familial Parkinson’s disease (PD) have been associated with the failure of protein degradation through the ubiquitin-proteasome system. Impairment of proteasome function has also been suggested to play a role in the pathogenesis of sporadic PD. Protein damage seen in the substantia nigra in PD was postulated to be induced by the local oxidative metabolites of dopamine. We examined the proteasome activity in PC12 cells treated with 6-hydroxydopamine (6-OHDA), the dopamine synthetic derivate used in models of PD. We found that the treatment increased protein degradation, increased the levels of free ubiquitin and ubiquitin-conjugated proteins, in a dose dependent manner. In addition, there was an increase in proteasome trypsin- and chymotrypsin-like activities and more acidic protease-like activities in the treated with 10-100μM of 6-OHDA, whereas higher doses caused a dramatic decline. Similarly, the presence of 0.3 mM 6-OHDA for up to 10 hours increased proteasome activities while further incubation (10-24 hrs) reduced them markedly. We demonstrated that 6-OHDA treatment increased the protein degradation, accumulation of carbonyls groups and caspase-3 activity, while addition of antioxidant, N-acetyl-L-cysteine, reduced these effects. In conclusion, our data indicate that mild oxidative stress elevates proteasome activities in response to the increase in protein damage. Severe oxidative insult may lead to failure of the ubiquit system to clear defective protein and cause protein aggregation and cell death. Control of protein clearance might offer a new strategy for therapy in neurodegenerative diseases in general and particularly Parkinson’s disease.

Keywords: Parkinson’s disease, 6-hydroxydopamine, ubiquitin-conjugates, protein degradation, proteasome activity
Axotomy induced reversed microtubules polarity leads to the formation of a vesicle trap and the extension of a growing cone's lamellipodium. Erez H., Shapiro E., Hoogenraad C. C. 2, de Zeeuw C. I. 1, Galjart N. 1 and Spira M. E. 1
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The transformation of a stable axonal segment into a motile growth cone is a critical step in the regeneration of amputated axons. A striking observation made in earlier studies from our laboratory, is that down stream, in the cascade leading to growth cone formation, anterogradely transported vesicles accumulate at a defined compartment some 100-150µm proximal to the tip of the axon segment. This compartment forms the GC’s center.

Here we began to explore the mechanisms by which vesicles are trapped at this specific location. To that end, we expressed EGFP-EB3 in cultured Aplysia neurons. EGFP-EB3 binds in stretches to the plus end of microtubules, moves with the growing MT’s tips forming a comet tail like structure and thereby allows to image the dynamics and polarity of MTs. We found that a trap forms followed by reorganization of the MT’s polarity at the cut axonal end. 100-150µm from the cut axonal tip where the GC center is formed and vesicles are trapped, the MTs reorient such that plus ends point towards a common center - the trap. Proximally the trap is formed by MTs that maintain their original polarity. The distal boundary of the trap is set by MTs with reversed polarity. Distal to that, a short axoplasmic gap the MTs point their plus ends anti-gradely.

We propose that the vesicle trap is formed by the reorientation of the MT polarity that directing molecular motors to deliver vesicles into the trap.

Keywords: axotomy, growth cone, microtubules, regeneration, EB3

ErbB4 receptor expression elevates following closed head injury

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The ErbB-4 receptor tyrosine kinase and its ligand neuregulin are widely expressed in the nervous systems. To investigate their possible role in neurodegeneration we used the closed head injury (CHI) model. We demonstrate that levels of ErbB-4 are dramatically increased at the site of injury. ErbB-4 levels in the cortical region at the site of injury were significantly increased starting at day 3 post trauma, lasted two weeks and then declined to basal levels. Notably, the highest levels of Erb-4 expression at the site of injury were observed one week after CHI. The staining patterns indicate that activated microglia/macrophages and neurons but not astrocytes; constitute the major population of cells that physically express the receptor at the injury site. Confocal microscopy analysis suggests that the high levels of ErbB-4 protein in activated microglia/macrophages is probably due to phagocytosis. These findings indicate that ErbB-4 receptors may play a role in brain responses to head trauma.

Keywords: tyrosine kinase, ErbB-4, brain, closed-head injury, microglia/macrophages

NRG mediates neuronal differentiation and survival in PC12-ErbB4 cells

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Neuregulins (NRGs), a large family of transmembrane polypeptide growth factors, mediate various cellular responses depending on the cell type and receptor expression. To examine the possible role of ErbB-4 in neurons we have used the PC12 cell line. Expression of ErbB-4 in PC12 cells demonstrated that NRG induced signals and neurite outgrowth, which were indistinguishable of those mediated by NGF. In PC12-ErbB-4 cells, NRG induced an initial weak mitogenic signal and subsequently neurite outgrowth. The differentiation induced by NRG involves generation of reactive oxygen species (ROS). In PC12-ErbB-4 cells NRG can rescue from apoptosis cell death induced by serum deprivation or TNFα treatment. We also show that NRG induces a significant protective effect from H2O2 induced death. This effect of NRG is mediated by the PI3K signaling pathway, since NRG failed to rescue cells in the presence of the PI3K inhibitor, LY294002. Furthermore, the downstream effectors of PI3K, PKB/AKT, is activated by NRG in the presence of H2O2 and PKB/AKT activation is induced by LY294002. In addition, our results demonstrate that the high levels of ROS induced by H2O2, is inhibited by NRG. LY294002, which blocks NRG mediated rescue, increases ROS levels. Moreover, both H2O2-induced ROS elevation and cell death are reduced by expression of activated PI3K. These data suggest that PI3K dependent pathways may regulate toxic levels of ROS generated by oxidative stress. In conclusion, our results demonstrate that neurite outgrowth induced by NRG in PC12 cells, requires MAPK and PKC signaling networks and NRG induced survival from apoptotic cell death requires PI3K signaling.

Keywords: phosphatidylinositol 3-kinase (PI3K), ErbB-4, neuregulin (NRG), tyrosine kinase, reactive oxygen species (ROS)

Expression of the proapoptotic protein ARTS in cerebellar granule cells in culture

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The protein ARTS (Apoposis Related protein in the TGFβ Signaling pathway) is involved in apoptosis induced by TGFβ and by other pro-apoptotic factors. In mouse brain, ARTS was seen in a number of brain areas, including several parts of the cortex, hippocampus, septal nuclei and bed nuclei of stria terminalis.

In this work we explored the expression of ARTS in cerebellar granule neurons in primary culture by immunohistochemical study. Content of ARTS increased in PC12-ErbB4 cells 24 h in vitro, more than 90% of the neurons were strongly ARTS positive. The number of ARTS positive neurons was similar in cultures grown in medium containing potassium and in 25 mM K+ medium (in which the cells are viable for a longer period in vitro). ARTS protein was seen to a similar extent in mitotic and non-mitotic neurons, as detected by β-III Tubulin incorporation. ARTS was detected by confocal microscopy in mitochondria and nuclei both in young (24 h) and mature (10 days in vitro) cultures. Since our previous work in COS cells has shown that migration of ARTS from mitochondrion to nucleus occurs at the onset of apoptosis, this finding may indicate which neurons in the culture are undergoing apoptosis. The finding of a high content of ARTS in cerebellar granule cells in culture contrasts with the near absence of ARTS in cerebellum of the post-natal rat as seen in sections of whole brain, and also correlates with the high degree of apoptotic cells (10% by TUNEL stain) in the neuronal culture in vitro.

Keywords: apoptosis, ARTS, cerebellar granule cells

Transgenic excess AChE exerts non-catalytic effects on hippocampal LTP via PKC

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AChE is known for its catalytic ACh hydrolyzing features, but most interestingly acts as a sensitive marker for acute and chronic stress, as a specific splice variant of AChE is highly elevated under such insults. Transgenic models for chronic expression of either the stress-related AChE (AChE-R) or its primary synaptic variant (AChE-S) express both altered LTP patterns in hippocampal CA1 synapses and memory and learning impairments. Especially, LTP is enhanced under transgenic excess of AChE-R (172% vs. 138% in WT) whereas excess AChE-S leads to normal levels of potentiation that dramatically decays to 37% within 3h post-induction (vs. no decay in WT). Intriguingly, the decay phenotype was not rescued by either physostigmine (1 µM) or carbachol (0.5 µM) administration. Moreover, it was also evident in the hippocampus of transgenic expressing catalytically inactive AChE-S, suggesting that non-catalytic features of AChE are involved in LTP maintenance. We examined the possible involvement of PKC in the distinct LTP responses of AChE-R or -S transgenic slices. Phorbol di-butrylate (5µM), a PKC activator, highly facilitated synaptic field potentials in AChE-R slices (101% vs. 65% in WT) contrasted by poor (30%) facilitation in AChE-S slices. Furthermore, a tetanus followed by PKC activation induced long-term potentiation (LTP) in AChE-S slices, thus rescuing the LTP decay. To conclude, we report the contribution of AChE non-catalytic features involving PKC activity and/or levels in LTP priming and maintenance. This study may demonstrate stress-related modulation of plasticity.
The physiological relevance of the physical interaction of voltage-gated K⁺ channels with protein components of the exocytotic machinery in PC12 cells

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Voltage-gated K⁺ channels play a physiological role in the regulation of transmitter release by virtue of their ability to shape presynaptic action potentials. However, in a previous work (Artel et al., 2001) we showed that in brain synaptosomes and Xenopus oocytes Kv1.1 channels and SNARE proteins display a physical interaction. Also, our preliminary results show that another Kv channel, Kv2.1, also interacts with the SNARE proteins in oocytes. In this study we aimed to attribute a physiological role to the physical interactions of these Kv channels with the exocytotic proteins using the cracked PC12 cell assay (Hay and Martin, 1992). Both channels, Kv1.1 and Kv2.1, are expressed in PC12 cells. In co-immunoprecipitation experiments we could observe a physical interaction between the SNARE proteins and the Kv2.1 channel in PC12 cells. We introduce GST-proteins corresponding to parts of the Kv1.1 and Kv2.1 fragments of the two channels that were shown in vitro binding assays to either bind or not SNAP-25 or syntaxin 1A, and assayed their effect on dopamine neurotransmitter secretion in this system. Our results are consistent with the notion that the physical binding of Kv channels to the SNARE proteins modulates Ca²⁺ regulated neurotransmitter secretion.

Keywords: Kv1.1, Kv2.1, SNARE proteins, PC12

The endogenous cannabinoid system: role in the stress response and critical function in milk ingestion and survival of newborn mice

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The ability of cannabis to stimulate appetite has been used to benefit patients suffering from malnutrition. Endogenous cannabinoids (endo-cannabinoids) are found in brain, peripheral organs, and maternal milk; they activate cannabinoid (CB1-CB2) receptors. We showed recently that CB1 receptors are expressed in the brain and the pancreas in newborn mice. Here, we further investigate the requirement for CB1 receptors in newborn feeding and development in CB1 receptor-knockout (CB₁⁻/⁻) mice. Milk intake, weight gain, and development were recorded in CB₁⁻/⁻ and wild type pups. Further, pups were injected with a CB1 receptor antagonist (SR141716A) on day I of life. Critical parameters such as milk intake on the first day of life, and survival was significantly lower compared to wild type mice. SR141716A completely inhibited milk intake in wild type pups and resulted in almost 100% mortality within 7 days. The antagonist partially affected milk ingestion, growth, and survival in CB₁⁻/⁻ pups.

Throughout this study, we noticed a greater vulnerability and mortality in CB₁⁻/⁻ mice, suggesting a role for the endocannabinoid system in stress. Indeed, acute stress (4 min noise) induced a rise in corticosterone and in ACTH in the sera of CB₁⁻/⁻ mice, which was respectively, twice and 4 times higher in knockout compared to wild type mice. We conclude that (1) neonatal milk ingestion is entirely dependent on the endocannabinoid-receptor system. A major role is played by CB1 receptors, however our data support evidence for an additional ("CB₂") receptor. 2) The cannabinoid system plays an important role in the response to stress.

Keywords: cannabinoids, stress, development, feeding

T-cells express a functional ionotropic glutamate receptor GluR3, and glutamate by itself directly activates T-cell function

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T-cells may encounter glutamate, the major excitatory neurotransmitter in the nervous system, when patrolling the brain, and in glutamate-rich peripheral organs. Moreover, CNS glutamate levels increase in various pathological conditions in which T-cells may play a beneficial or detrimental role. Do T-cells express ionotropic glutamate receptors? Can glutamate by itself trigger T-cell function? We found that normal human T-cells, human T-leukemia line, and mouse autoimmune anti-myelin - protein T-cells express high levels of specific ionotropic glutamate receptor of the AMPA subtype 3 (GluR3).

The evidence for GluR3 expression in T-cells include GluR3-specific RT-PCR and sequencing, showing identity to brain GluR3, western blotting, and GluR3 cell-surface expression revealed by immunocytochemical – fluorescence staining and flow - cytometry. Glutamate (10nM), in the absence of any additional molecule, activated specific T-cell functions, among them integrin - dependent adhesion to laminin, chemotaxis towards GM-CSF, and chemotactic - migration towards the chemokine SDF-1. AMPA receptor-agonists mimicked glutamate-induced effects, and the specific antagonists CNQX and NBQX blocked it. Taken together, we express that T-cells and glutamate-triggered T-cell function could be important to numerous physiological and pathological conditions, and especially relevant to: a) T-cell migration across laminin-containing blood-brain-barrier; b) T-cell mediated multiple sclerosis. c) Some human epilepsies in which specific anti-GluR3-antibodies are found and suspected to play a neurotoxic role. Based on our findings, we propose that granzyme-B-producing T-cells can cleave the GluR3B autoantigen in an autocrine (from their own surface-expressed-GluR3) or paracrine (from neighboring T-cells) fashion, not only from neuronal cells, as suggested thus far.

Keywords: glutamate, ionotropic-glutamate-receptor GluR3, T-cells, neuroimmunomodulation, autoimmune-epilepsy

Mechanisms of aging of the growth hormone-releasing hormone receptor

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In aging mammals, a decreased responsiveness to somatostatins growth hormone-releasing hormone (GHRH) leads to low GH and IGF-I levels. This event was proposed to be responsible for a diminution of muscle mass, increase adiposity and deterioration of several tissues and organs. In the anterior pituitary, it has been related to changes in the level of GHRH-receptor (R) mRNA and in GHRH binding sites. Also, in our recent work, we showed, that the growth hormone-releasing hormone receptor (GHRH-R) mRNA transcripts and GHRH binding sites are maintained. Around regulatory factors positively impacting on GHRH-R, corticosterone and testosterone could be proposed as candidates. In 18-month-old calorie-restricted rats, serum corticosterone and testosterone are increased when compared to 18-month-old ad libitum-fed rats. An optimal control of glycemia, such as in caloric restriction, could also protect the GHRH-R. In streptozotocin-diabetic rats, the GHRH-R mRNA transcripts exhibit several disturbances according to the length and severity of diabetes. Moreover, a glucotoxicity state mimicking that found in aged or diabetic rat serum down-regulates the human GHRH-R, stably expressed in BHK cells, indicating that it may represent a common mechanism of GHRH-R aging. Altogether, these results suggest that circulating steroids and glucose may be critical to regulate the expression of the pituitary GHRH-R in aging. As seniors represent the fastest growing segment of the population in industrialized countries, identification of cellular and molecular mechanisms involved in somatopause dysfunction may allow to design new interventions to delay somatopause.

Linking Lissencephaly type I genes to MAPK pathway

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Lissencephaly type I is a severe human disease that affects 1/30,000 live births. It is characterized by a relatively smooth cerebral surface, and an abnormal organization of the cortical layers. This is the result of an abnormal embryonic neuronal migration. The clinical manifestation is mental retardation, severe epilepsy and mental retardation. Mutations in LIS1 and doublecortin (DCX) have been shown to cause Lissencephaly type I. A third gene, doublecortin-like kinase (DCLK) is thought to participate in the axon growth properties. The interactions of LIS1 and DCX suggest their involvement in intracellular transport. Intracellular transport is a complicated problem for a highly polar cell like a neuron. A recent idea suggests that it would be advantageous for a neuron to assemble the components of a
signaling pathway by loading them together on a scaffold protein. This scaffold protein is bound to a motor protein, and thus delivers the enzyme complex to the right place. This also ensures that the components of a pathway will be in the right ratio, all together, and promote spatial regulation on the pathway. According to this model, proteins of the same pathway are predicted to be transported together. Here we show that both DCX and DCLK interact with a scaffold protein that assembles the mitogen activated protein kinase (MAPK) pathway in neurons. This observation links for the first time neuronal migration with the MAPK pathway. Our hypothesis is that these interactions function to regulate the localization of MAPK module.

**Keywords:** lissencephaly, doublecortin, microtubule, neuronal migration

The association of DRD4 and 5-HTTLPR with infant temperament

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It was previously shown that the dopamine D4 exon III repeat (D4DR) and the serotonin transporter promoter region (5-HTTLPR) polymorphisms are not only associated with adult personality traits but also with temperament in 2-week old neonates. We now report the results of a third study of these infants and their temperament at 12 months. This study examined the association between two common polymorphisms, the DRD4 gene and the 5-HTTLPR and temperament in 12-month-old infants. 22 infants had a least one copy of the 6-8 repeat allele (L-DRD4) and 39 had two copies of the 2-5 repeat allele (S-DRD4). 20 infants were homozygous for the short form (s/s) of 5-HTTLPR while 41 were either heterozygous for the short and the long form (Ls) or were homozygous for the long form (Ll). The infants were observed in a series of standard temperament episodes that elicited fear, anger, pleasure, interest, and activity. L-DRD4 infants showed less interest in a structured block play situation and more activity in a free play situation. They also displayed less anger in an episode of mild physical restraint. Infants with s/s 5-HTTLPR showed less fearful distress to stranger approach and less pleasure in a structured play situation than infants with Ll or Ls 5-HTTLPR. Duration of looking during block play was affected by a significant interaction between DRD4 and 5-HTTLPR. Shortest duration of looking was associated with the L-DRD4 and s/s 5-HTTLPR genotypes.

These results support previous data showing that the origins of the molecular control of human temperament lie in infancy.

**Keywords:** dopamine D4 receptor gene, serotonin transporter promoter genes, infant temperament

DCX and development

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The Lissencephaly syndromes in humans involve abnormal cortical lamination and are medically categorized as neuronal migration defects. Two genes involved in Lissencephaly type I have been identified: LIS1 and DCX.

Doublecortin (DCX), a microtubule binding protein, maps to the X chromosome and mutations in this gene result in Lissencephaly in males or subcortical heterotopia in females. This strongly suggests a role for this gene product during neuronal migration. The protein consists of two 80 residue evolutionarily conserved repeats and a serine proline rich C-terminal region. The repeats bind microtubules and motifs that are found in Lissencephaly patients cluster in this domain. The C-terminal portion has been shown to interact with clathrin adapter proteins, AP-1 and AP-2, thus suggesting a potential role of DCX in protein sorting or vesicular trafficking. Scaffold proteins have been shown to regulate MAP kinase related pathways. We have identified a specific MAP kinase which phosphorylates DCX which maybe important during neuronal migration.

We have identified the phosphorylation sites on DCX and have also identified the regions through which it interacts with the MAP kinase complex. Using different biochemical approaches we have established that DCX is a key molecule in neuronal migration.

**Keywords:** DCX, lissencephaly, MAP kinase, phosphorylation

External barium inhibits inactivation gating of KCNQ1 channels

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The KCNQ1 pore-forming α subunit belongs to a newly characterized K+ channel family, KCNQ, which forms voltage-gated K+ channels. Here we used Ba2+ ions to probe the permeation pathway of homomeric KCNQ1 channels. In addition to its voltage-dependent block of the pore, Ba2+ also alters the gating of homomeric KCNQ1 channels by favoring the closed state. Ba2+ produces a marked rightward shift of the voltage-dependence of activation (+33 mV) and an acceleration of the deactivation kinetics. However, Ba2+ also reduces channel inactivation as revealed by the suppression of the holding current, an additional effect that opposes KCNQ1 current inhibition. To further investigate the effect of Ba2+ on KCNQ1 inactivation, we used the L273F KCNQ1 mutant. This naturally occurring mutation, located in the transmembrane segment S5 produces macroscopic inactivation. Ba2+ potently inhibits the maximum conductance and the macroscopic inactivation of the L273F KCNQ1 mutant. In high external K+, the impact of Ba2+ on channel gating is relaxed. At 50 mM external K+, Ba2+ neither shifts the voltage-dependence of activation nor accelerates desensitization. This naturally occurring mutation, located in the transmembrane segment S5 produces macroscopic inactivation. Ba2+ potently inhibits the maximum conductance and the macroscopic inactivation of the L273F KCNQ1 mutant. In high external K+, the impact of Ba2+ on channel gating is relaxed. At 50 mM external K+, Ba2+ neither shifts the voltage-dependence of activation nor accelerates desensitization. This naturally occurring mutation, located in the transmembrane segment S5 produces macroscopic inactivation. Ba2+ potently inhibits the maximum conductance and the macroscopic inactivation of the L273F KCNQ1 mutant.

**Keywords:** KCNQ1, Ba2+, KCNQ1 channel family, KCNQ, voltage-gated K+ channels.
indicating that lithium may affect glial cells directly, leading us to postulate that lithium may exert some of its effects on neurons indirectly, through the action on glial cells. Here we used rat cerebellar cultures to ascertain the effects of lithium (LiCl) on the activity of ornithine decarboxylase (ODC), the enzyme catalyzing the first limiting step in polyamine synthesis and on neuron survival, and the effects of lithium on glial growth. We examined the effects of lithium and DFMO on cultured cerebellar neurons (astroglia and microglia). Switching cultures from high (25 mM) to low (5 mM) K+ (KCl) medium concentrations served as the traumatic insult. The results indicate that: 1) While high, depolarizing K+ concentration enhances neuron survival, it inhibits astroglial growth. 2) LiCl (1 - 8 mM) enhances neuronal survival, but inhibits astroglial growth in cultures switched from high to low K+. 3) LiCl treatment leads to reduced ODC activity. 4) DFMO enhances neuron survival, inhibits astroglial growth. Moreover, the results imply that direct effects on astrocytes and microglia may contribute to the neuroprotective effects of lithium.

Keywords: astrocytes, alpha-difluoromethylornithine, microglia, neuroprotection, ornithine decarboxylase, rat

Functional recovery by lysyl oxidase inhibitor treatment after spinal cord injury in rodents
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Lysyl oxidase (LO), an extracellular matrix (ECM)-forming enzyme was recently implicated in modulating the ECM and scar formation at CNS injury sites. Modulations of the ECM and scar formation at CNS injury sites are considered prohibitive for successful soon regeneration, thus restricting functional recovery. Treatment with a LO inhibitor, c-difluoromethylornithine (DFMO) enhances neuroprotection, omithinedecarboxylase, rat

Keywords: astrocytes, alpha-difluoromethylornithine, microglia, neuroprotection, ornithine decarboxylase, rat

Radial correlation MRI contrast for global neuronal synchronization: Observation of cortical layers during spontaneous activity
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A new magnetic resonance imaging (MRI) post-processing contrast named "Radial Correlation Contrast (RCC)" is described. The contrast gives the average communication for each volume element with its surroundings and its direction for a chosen distance. The method identifies dynamic structures based on their temporal fluctuations. In neuro-imaging these fluctuations are shown to be generated in the capillary bed, thus presenting a neuronal activity. While the average communication shows good gray/white contrast, its direction is more sensitive and accurate for dynamic structural definition. It is shown that during spontaneous activity, short-range communication identifies specific structures in cortical layers. The agreement between these RCC structures and

Keywords: multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), myelin oligodendrocyte glycoprotein (MOG), AD4
cortical layers obtained by histology is excellent. Longer-range communication clearly identifies major cerebral structures and has the potential of showing their interaction during specific brain activity. This non-invasive ability to follow neuronal communication at various distances will enable a better understanding of brain function.

Keywords: fMRI, neuronal synchronization, cortical layers

NGF and BDNF regulation in the brains of interleukin-1-knock-out mice
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The neurotrophic factors, NGF and BDNF are involved in neuronal survival, morphogenesis and modulation of synaptic strength in the adult brain. Both factors are differentially regulated and their levels vary between different brain regions. Activation of interleukin-1 (IL-1) was implicated in the regulation of NGF and BDNF. Using the IL-1 knockout (KO) balb/c mice we have examined the possibility that basal levels of IL-1 may control NGF and BDNF protein expression. Brain tissue of IL-1a-KO, IL-1b-KO, IL-1ab-KO, IL-1Ra-KO and wild-type (WT) were analyzed. BDNF levels were higher and more variable than NGF, in the various brain areas, cerebellum, thalamus, hypothalamus and hippocampus. No significant difference was observed in both NGF and BDNF levels between WT and KO mice in all examined brain regions. Neuronal density was constant in each brain region in balb/c mice; 5.2±2 in the cerebral cortex, 4.5±2 in the hippocampus and 3.7±1 in the hypothalamus. All IL-1 KO mice to the same background preserve the ratio observed in the comparison between brain areas of the WT mice. In contrast, no relation between BDNF and NGF levels was detected in JBC-57 WT mice. Moreover, IL-1b-KO on JBC-57 background exhibit in the cerebral cortex a constant ratio of BDNF/NGF similar to that observed in this area in the balb/c KO and WT, which is significantly different from its control. Our data suggest that the involvement of IL-1 in BDNF/NGF regulation is distinct. In the JBC-57 mice the basal levels of IL-1b may be implicated in the regulation of NGF and BDNF.

Keywords: neurotrophic factors, IL-1, cytokines

Integration and differentiation of human embryonic stem cells transplanted in the embryo
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Human embryonic stem (ES) cells are pluripotent cells that can differentiate into a large array of cell types and thus hold promise for advancing our understanding of human embryology and for contributing to transplantation medicine. In this study differentiation of human ES cells was examined in vivo by transplantation to early organogenesis-stage chick embryos. Human ES cells were grafted into or in place of epithelial-stage somites of chick embryos of 1.5 to 2 days of development. The grafted human ES cells survived in the chick host, and were identified either by a fixable vital dye or by using a green fluorescent protein (GFP) expressing clone. Histological analysis showed that human ES cells are distinguished from host cells by their larger, more intensely staining nuclei. Some grafted cells differentiated en masse into epithelia, while others migrated and mingled with host tissues, including the dorsal root ganglion. Colonies grafted directly adjacent to the host neural tube produced primarily structures with the morphology and molecular characteristics of neural rosettes. These structures contain differentiated neurons as shown by β-tubulin and neurolin expression in axons and cell bodies. Axons derived from the grafted cell line within the host nervous system, and host axons enter the structures derived from the graft. These show that human ES cells transplanted in vivo are able to develop into microtubule positive axons and cell processes. Human ES cells may thus be a well suited system for transplantation of neural stem cells.

Keywords: human embryonic stem cells, xenografts, organogenesis, neural differentiation

Immunomodulation of PrP 106-126
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Prion diseases are transmissible neurodegenerative pathologies characterized by the accumulation in the brain of altered forms of the normal cellular prion protein (PrPc), termed PrP scrapie (PrPsc). Previous studies have shown that a synthetic peptide homologous to residues 106-126 of PrP (PrP 106-126) maintains many characteristics of PrPsc. Like PrPc, peptide 106-126 has the ability to adopt a beta-sheets conformation and to form amyloid fibrils and induce apoptosis in neurons. Moreover, the neurotoxicity of the peptide requires the expression of endogenous PrP, and the peptide induces hypertrophy and proliferation of astrocytes and activation of microglial cells in vitro. These data suggest that the region including residues 106-126 might be an essential site in the conversion of PrPc to PrPsc.

Antibodies are known to act as chaperons and are able to stabilize protein structure and/or induce conformational changes. Thus, six directed antibodies might interfere with aggregation processes and/or inhibit prion replication. In order to investigate antibodies ability to interfere with aggregation processes we used active and passive immunization approaches. Polyclonal sera generated by administration of filamentous phage displaying peptide 106-126 as the antigen of choice, was shown to prevent 106-126 peptide aggregation as well as dissolve already formed fibrils and protect PC12 cells from death induced by 106-126 fibrils. Two monoclonal antibodies generated using KLH as the protein carrier were shown to share the sera characteristics towards 106-126 peptide and where further analyzed for their ability to induce those changes with the whole protein.

Keywords: prion protein, phage display, antibodies, chaperons

Effect of antidepressants on abnormalities in circadian rhythm and feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis in prenatally-stressed rats
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Prenatal stress impairs the feedback regulation of the HPA axis and capability of the organism to cope in stressful situations (Weinstock, Prog Neurobiol, 65:427-451 [2001]). This study compared the circadian rhythm for corticosterone (COR) in adult control [C] and prenatally-stressed [PS] rats, with and without treatment with a triyclic antidepressant, amitriptyline or a novel brain-selective MAO inhibitor, TV-3326. The female offspring of rats stressed by daily restraint during the last week of pregnancy were housed in a 0700-1900 hr light cycle and given water, amitriptyline or TV-3326 (17 mg/kg/day) from age of 6 → 13 weeks in the drinking water to avoid the stress of injection. When aged 16 weeks, blood (50 μl) was taken from the tail at 0800, 1200, 1500 and 1800 hrs for determination of plasma COR. In other rats, blood was taken before (at 0800 and 0900 hr) and 30 → 90 min after exposure a novel environment. Plasma COR peaked earlier in PS (at 1500 hr) than in C rats (1800 hr). In both groups, novelty stress increased plasma COR 2.5-fold at 30 min. This declined significantly by 90 min in C but not in PS rats. Neither antidepressant normalized the circadian rhythm in PS rats, but selectively reduced the plasma COR at 90 min in PS rats. In conclusion, antidepressants appear to be able to correct the abnormality in feedback regulation of the HPA axis in PS rats in keeping with their anti-anxiety effects, but do not affect the control of its circadian rhythm by the suprachiasmatic nucleus.

Keywords: antidepressant, circadian rhythm, corticosterone, novelty stress

The role of interleukin-1 in HPA axis regulation following stress or adrenalectomy
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Interleukin-1 (IL-1) is a pleiotropic cytokine, which is known to activate the hypotalamus-pituitary-adrenal (HPA) axis. Recent studies demonstrate induction of brain IL-1 following stress. To further examine the role of IL-1 in stress-induced regulation of the HPA axis, we used mice with deletion of IL-1 receptor type I (IL-1rKO). When exposed to mild
psychological (4-minutes loud noise) or metabolic (7.5mg/mouse 2-deoxyglucose) stressors, IL-1rKO mice displayed significantly lower increases in corticosterone secretion, compared to wild-type (WT) controls. However, when exposed to stronger stressors (60-minutes restraint or 15mg/mouse 2-deoxyglucose), IL-1rKO and WT controls showed an increase in corticosterone secretion. The results suggest that IL-1 plays an important role in the regulation of the HPA axis response to mild psychological and metabolic stressors. However, stronger stressors may induce other mediators that can activate the HPA axis in the absence of IL-1. Brain IL-1 was shown, by us and others, to be increased following adrenalectomy (ADX), suggesting a role for IL-1 in the regulation of the HPA axis. To examine this hypothesis, mice were adrenalectomised, and post-operative serum ACTH levels were measured. Five days following ADX, both IL-1rKO and WT mice demonstrated a mild increase in ACTH levels compared to sham-operated controls. Eight days following ADX, ACTH levels in IL-1rKO remained mildly increased, while WT mice displayed an 8-fold increase in ACTH compared to sham-operated mice. These results suggest that the increase in IL-1 levels following stress and adrenalectomy plays a critical role in HPA axis regulation under these conditions.

Keywords: interleukin-1 (IL-1), stress, corticosterone, ACTH, adrenalectomy

Local injection of bone-marrow derived dendritic cells pulsed with specific myelin-related antigens stimulates functional recovery from spinal cord injury

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The outcome of spinal cord injury is more devastating than might be expected from the initial insult. This is due to a self-propagating process of secondary degeneration of neurons which escapes the primary insult. Our group has demonstrated that an adequate immune response in the CNS is in part responsible for the continuation of this harmful process. It was recently shown in a laboratory that by boosting the HPA immune response in a specific and controlled manner it is possible to protect spared neurons within the CNS from this process of degeneration. Both passive transfer of T Lymphocytes reactive to CNS antigens or immunization (with CNS specific antigens) was shown to lead to a significantly better outcome of trauma to the CNS (Moellem et al., Nat Med 5:49-55 [1999]; Hauben et al., J of Neuroscience 20:6421-6430 [2000]). In this work we propose that a similar effect can be achieved by manipulating the post traumatic immune response with the use of bone marrow derived dendritic cells pulsed with myelin peptides or with altered myelin peptides (A91) that lack the risk of causing experimental autoimmune encephalitis (EAE). We have found in a model of contusive spinal cord injury in rats that local administration of dendritic cells pulsed with myelin basic protein (MBP) or with A91, directly into the site of injury, significantly improves recovery as measured by functional movement and confirmed by morphological criteria. Dendritic cells injected into the site of injury as far as two weeks after the injury had a beneficial effect on recovery as well. Nonpulsed dendritic cells or those pulsed with a nonrelevant antigen had no effect on recovery. Other possible routes of administration of dendritic cells to the injured animal were also examined. The fact that a positive effect of the dendritic treatment could not be achieved in injured thymectomized rats, which are deprived of mature T cells, suggests that the mechanism whereby dendritic cells display their effect is T cell dependent, similar to that of vaccination involving antigen specific activation of the immune system. Vaccination with dendritic cells may be an effect of achieving beneficial immunomodulation following spinal cord injury.

Keywords: CNS trauma, dendritic cells, autoimmunity, neuroprotection

Modulation of prepulse inhibition of the startle reflex (PPI) induced by prenatal injection of an anti-mitotic agent in the rat

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Prepulse Inhibition (PPI) of the acoustic startle response is the normal modulation of the startle response to a stimulus which is preceded by a weaker, non-startling stimulus. PPI deficits are often associated with schizophrenia, a disorder that could be at least in part the consequence of neurodevelopmental abnormalities, especially in the hippocampus and its connections. Pregnant female Sprague-Dawley rats were IP injected with either an anti-mitotic agent (methylazoxymethanol, MAM, 25 mg/kg) or with saline on gestational day 17, a critical period for hippocampal development. Their offspring were tested when turned adult (4 months) in a PPI paradigm using different prepulse durations may induce other mediators that can activate the HPA axis in the absence of IL-1. Brain IL-1 was shown, by us and others, to be increased following adrenalectomy (ADX), suggesting a role for IL-1 in the regulation of the HPA axis. To examine this hypothesis, mice were adrenalectomised, and post-operative serum ACTH levels were measured. Five days following ADX, both IL-1rKO and WT mice demonstrated a mild increase in ACTH levels compared to sham-operated controls. Eight days following ADX, ACTH levels in IL-1rKO remained mildly increased, while WT mice displayed an 8-fold increase in ACTH compared to sham-operated mice. These results suggest that the increase in IL-1 levels following stress and adrenalectomy plays a critical role in HPA axis regulation under these conditions.

Keywords: interleukin-1 (IL-1), stress, corticosterone, ACTH, adrenalectomy

Local injection of bone-marrow derived dendritic cells pulsed with specific myelin-related antigens stimulates functional recovery from spinal cord injury

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The outcome of spinal cord injury is more devastating than might be expected from the initial insult. This is due to a self-propagating process of secondary degeneration of neurons which escapes the primary insult. Our group has demonstrated that an adequate immune response in the CNS is in part responsible for the continuation of this harmful process. It was recently shown in a laboratory that by boosting the HPA immune response in a specific and controlled manner it is possible to protect spared neurons within the CNS from this process of degeneration. Both passive transfer of T Lymphocytes reactive to CNS antigens or immunization (with CNS specific antigens) was shown to lead to a significantly better outcome of trauma to the CNS (Moellem et al., Nat Med 5:49-55 [1999]; Hauben et al., J of Neuroscience 20:6421-6430 [2000]). In this work we propose that a similar effect can be achieved by manipulating the post traumatic immune response with the use of bone marrow derived dendritic cells pulsed with myelin peptides or with altered myelin peptides (A91) that lack the risk of causing experimental autoimmune encephalitis (EAE). We have found in a model of contusive spinal cord injury in rats that local administration of dendritic cells pulsed with myelin basic protein (MBP) or with A91, directly into the site of injury, significantly improves recovery as measured by functional movement and confirmed by morphological criteria. Dendritic cells injected into the site of injury as far as two weeks after the injury had a beneficial effect on recovery as well. Nonpulsed dendritic cells or those pulsed with a nonrelevant antigen had no effect on recovery. Other possible routes of administration of dendritic cells to the injured animal were also examined. The fact that a positive effect of the dendritic treatment could not be achieved in injured thymectomized rats, which are deprived of mature T cells, suggests that the mechanism whereby dendritic cells display their effect is T cell dependent, similar to that of vaccination involving antigen specific activation of the immune system. Vaccination with dendritic cells may be an effect of achieving beneficial immunomodulation following spinal cord injury.

Keywords: CNS trauma, dendritic cells, autoimmunity, neuroprotection

Modulation of prepulse inhibition of the startle reflex (PPI) induced by prenatal injection of an anti-mitotic agent in the rat

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Prepulse Inhibition (PPI) of the acoustic startle response is the normal modulation of the startle response to a stimulus which is preceded by a weaker, non-startling stimulus. PPI deficits are
nuclear export sequence. Current studies show intact ADNP-like immunoreactivity (~120 kD) in the rat astrocytic nuclear fraction and the increased ADNP content following VIP treatment. Subcloning of ADNP into a vector containing the Herpes VP22 protein that is able to penetrate through cell membranes and assessments of VIP-ADNP activities indicated toxicity to PC12 pheochromocytoma cells against oxidative stress. Part of the ADNP neuroprotection may be attributed to decreases in the pro-apoptotic protein p53. Peptide scanning and analysis identified an eight amino acid peptide (NAP) within the ADNP sequence that mimics the ADNP neuroprotection (Lekere et al, Stroke, 33: 1085-1092 [2002]). To further assess ADNP’s role in the impact of cell death, we used two different approaches. Results showed that homogenous ADNP-knockout suffer cranial neural tube closure failure and death on E8.5-9.0. Expression of OCT4, a gene associated with gernial maintenance, was markedly augmented, while expression of PAX6, a gene crucial for cerebral cortex formation, was abolished in the embryos and the brain primordial tissue of the knockout embryos, respectively. Incubation of E8.5 mouse embryos with VIP was shown before to result in robust embryonic growth and results now indicate increased embryonic ADNP gene expression in the presence of VIP. Thus, the vital gene, ADNP, may mediate the VIP-stimulated embryonic growth and neuroprotection.

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Key words: neuropeptides, neuroprotection; homeobox genes; neurodevelopment

Toward the neurostructural theory in drug abuse: evidence from in vivo and in vitro studies

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Neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) have been implicated in many forms of plasticity within the brain, including drug addiction. Previously we described that activation of the dopamine-1 (D1) receptor in a human astrocyte cell line, SVG, increases GDNF levels. We hereby examined the D1 and D3 receptor expression as compared to amphetamine and morphine in SVG cells. Twenty-four hr incubation of SVG cells with cocaine significantly lowered D1, D3 receptor and GDNF mRNA levels (detected using Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR)) and increased levels of signal transducers and activators of transcription (STAT) (via scanning and analysis of CDNA arrays). Neither amphetamine nor morphine altered these parameters. Furthermore, in vivo studies demonstrate that rats that have been trained to self-administer cocaine have decreased levels of striatal GDNF, cocaine, decreasing the in vitro findings. In conclusion, cocaine in vivo and in vitro has a direct and specific effect on extraneuronal cells, in addition to its known effect on the neuronal transporter. Decrease in GDNF neurotrophic support may increase local neuronal vulnerability.

Key words: cocaine, astrocytes, GDNF, D3 receptor, STATs

Apoptosis and alternative cell deaths in the retinal tissue

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Distinct types of cell death (CD) occur either during development and pathologies. Apoptosis and autophagy share some characteristics such as shrinking unaware maintenance. Our aim was to identify the types of CD induced by anisomycin in the retina and their mechanisms of execution. Ultrastructural analysis of explants treated with anisomycin showed of both autophagic and apoptotic features. Bromodeoxyuridine, a mitochondrial pore inhibitor, completely inhibited CD induced by anisomycin in retinal explants. Ac-LEHD-CHO, Z-DEVD-CHO, Ac-VEID-CHO and DQ-CHO inhibitors respectively, partially inhibited CD induced by ANI. Immunohistochemistry for activated caspases-9, activated caspase-3 and TUNEL-staining stained approximately 50% of contained case-positive case-positive case-positive cells. Ac-LEHD-CHO and anisomycin inhibited CD induced by anisomycin. Caspase-9 and caspase-6 inhibitors had synergic effect upon CD induced by anisomycin. Co-incubation of retinal explants with Lipopolysaccharide (LPS) and LPS-have synergic effect upon CD induced by anisomycin and TUNEL activated caspases-9 and activated caspase-3 staining decreased in explants treated with 3MA. Furthermore, co-incubating of explants with caspase-3 and caspase-6 inhibitors did not have synergic effect upon CD induced by anisomycin.

These results suggest that anisomycin induces different types of CD, all dependent on mitochondrial commitment and caspase activation. The first type involves cytochrome oxidase and the second type involves caspase 9 and -3 activation and is TUNEL+, suggesting an overlap between apoptosis and autophagy. The other CD type involves caspase-6 and is TUNEL-. This third CD pathway is dependent on caspase-9 activation and occurs under caspase-3 and -6 inhibition.

Key words: cell death, caspasess, autopahagy, retina

Orientation selectivity in V1 of the alert monkey

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Area V1 is unusual among all primate cortical areas in its neuronal cell density, cortical thickness, and intricate histology. Physiological recordings, however, emphasize overall integrating features such as ocular dominance and orientation selectivity. We have previously shown that in the alert monkey physiological properties of single cells reflect alternating anatomical layers structure. Here we study the relation of single cells orientation selectivity to the cells spontaneous activity and receptive field (RF) size, and how these properties are related to the anatomical location of the cells. RFs were recorded from area V1 of an alert monkey performing a simple fixating task. The cells spatial organization was studied by drifting increment and decrement bars and by sinusoidal gratings. Orientation selectivity was defined as the RF size and spontaneous activity. These 3 measures were strongly predicted by the layer of origin such that small RFs, low spontaneous activity, and a high degree of orientation selectivity were found in the output layers 2/3, 4B and 5 while the reverse was true for the input layers 4A, 4C and 6. We conclude that the conjonction of these physiological measures and their anatomical characterization reflect interactions between excitatory and inhibitory mechanisms. When excitation is strong, large RFs, high spontaneous activity, we have spatial selectivity and a low degree of orientation tuning are found while when significant inhibition is present, RFs shrink, spontaneous activity almost disappears and orientation selectivity is high.

Keywords: visual cortex, orientation selectivity

Assessment of vasomotor reactivity for prediction of syncope in patients with orthostatic hypotension.

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Background: Orthostatic hypotension (OH) is a common neurological symptom. It is not clear why some patients can tolerate OH while others faint. Syncope is a result of severe cerebral hypoperfusion and cerebral autoregulation failure.

Objective: To assess cerebral autoregulation in patients with OH and history of syncope.

Methods: 29 patients with OH, age 72.9±9.8 years, mean orthostatic decrease of systolic blood pressure of 33.7±12.9 mmHg after three minutes of standing, were assessed for VMR of middle cerebral arteries (MCA) and vertebral arteries (VA), using TCD (Rimem) after injection of 1g acetazolamide i.v. 13 patients had experienced at least 2 documented syncope episodes over the preceding year. Patients with carotid stenosis greater than 75% were excluded from the study.

The percent difference between blood flow velocities before and after acetazolamide injection was defined as VMR% and the results were compared by Mann-Whitney test.

Results: Patients with syncope had worse VMR in both RT MCA and RT VA than those without syncope (12.1±30.9% vs. 40.6±19.9% in RT MCA (P<0.005) and 21.1±36.4% vs. 49.2±57.4% in RT VA (P<0.05), while there was no significant difference in these parameters in patients without syncope (12.1±30.9% vs. 38.3±32.8% in patients without syncope; p = 0.09).

Conclusions: Acetazolamide may contribute to the development of syncope in patients with OH. The acetazolamide test is useful for predicting the risk of syncope in patients having OH.

Keywords: orthostatic hypotension, syncope, Vasomotor reactivity
Myosin VI and hereditary hearing loss: from mouse to man and back to mouse

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The past few years have provided an explosion in our understanding of how the inner ear functions. This dramatic increase is due in large part to the genes found to be associated with nonsyndromic hearing loss. Since 1997, 29 genes have been found and these have provided clues about auditory-transduction, inner ear development. In particular, mouse models for human deafness, with mutations in orthologous genes, have revealed essential information about the pathophysiology caused by these mutations. One group of proteins frequently associated with hearing loss are the myosins, three of which were discovered thanks to their corresponding mouse mutants. Myosins are molecular motors that move along actin filaments and have been implicated in various cellular functions such as cell movement, membrane traffic, and signal transduction. An intragenic deletion in myosin VI (Myo6) leads to deafness and vestibular dysfunction in Snell's waltzer mice. A missense mutation in an Italian family is associated with autosomal dominant progressive hearing loss (C442Y) (Melchionda et al, AJHG 69:635-640 [2001]). The following suggests that the deafness in this family is due to the MYO6 missense mutation: the segregation of this mutation with the affected individuals in the family, the previous association of myosin VI with deafness, and the conservation of this mutated residue in all known vertebrates. We have now confirmed that this mutation is the cause of deafness in humans, since reproduction of this mutation in transgenic mice has revealed a similar progressive hearing loss. A morphological analysis of the transgenic mice demonstrates the pathophysiology of this mutation.

Keywords: sensory system, hearing loss, myosin, transgenic mice

Global-local processing among elderly with and without insomnia: a comparison with young adults

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Disturbed sleep, an important cause of human misery at any stage of life, exacerbates with advancing age. Complaints of difficulty in initiating and maintaining sleep and daytime drowsiness are more prevalent among the elderly than in any other group. Likewise, aging is associated with a decline in many aspects of visual processing including changes in global-local processing. However, little is known about the interaction between sleep disturbances and visual processing of global and local aspects among elderly people. Thus, we examined whether insomnia is indeed associated with age-related changes in this global-local processing. Results showed that the global and the local structures were equally manifested in young adults, along with a tendency to a global advantage. Elderly subjects without insomnia, on the other hand, showed a reversed tendency of faster response time for the local aspects, indicating the processing dominance of the local level of visual stimuli in elderly. Furthermore, elderly subjects with insomnia showed a significant advantage for the local structure and an asymmetric local-to-global interference, suggesting that the ability to integrate individual elements into a coherent pattern deteriorates with age, and becomes substantially impaired among elderly insomnia. Overall, the findings imply that, at least in some aspects, sleep disturbances may account for perceptual and cognitive decline in the elderly.

Keywords: aging, global-local processing, visual perception, insomnia

The wasp Ampulex compressa injects venom directly into prey central nervous system

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In contrast to other venomous animals that paralyze their prey, the venomous parasitoid wasp Ampulex compressa subdues its prey by the induction of a bout of grooming followed by a five-week long lethargy state. During the lethargy state the cockroach is immobilized enough for the wasp to manipulate the nervous system, where the wasp larva eats it alive. The cockroach is always stung by the wasp twice, first in the thorax and then in the head.

To localize the site of stinging we injected wasps with a mixture of C14 radiolabeled amino acids. After the amino acids were incorporated into the venom, we allowed the wasps to freely sting several cockroaches.

First, the amount of radioactivity in the ganglia of stung cockroaches was assessed using liquid scintillation of the different ganglia and the head. Suggestive levels of radioactivity were detected in the head ganglia and the first thoracic ganglion. In contrast, radioactivity levels in the third thoracic ganglion and non-neuronal tissue were much lower and comparable to control values.

Second, microscopic emulsion autoradiography was carried out to determine the precise location of injection. Radioactivity was detected in the central area of the brain ganglion, the subesophageal ganglion and the first thoracic ganglion. No radioactivity could be detected in the second thoracic ganglion or in ganglia stung by non-radiolabeled wasps.

To our knowledge, this is the first demonstration of a venomous animal stinging into the central nervous system of its prey.

Keywords: venom, wasp, cockroach, central-body

Mirror-symmetry organization of human occipito-temporal object areas

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Object images activate a large and complex array of high order visual areas in the human cortex, ranging from dorsal occipito-parietal cortex to ventral occipito-temporal cortex. To search for consistent patterns within this area we performed a detailed analysis of fMRI activation in 12 experienced subjects along four basic dimensions (eccentricity, object selectivity and visual meridians). Our results reveal seven consistently distinct category-related entities situated in the cortex adjoining early visual areas. These include: two face-related regions, three object-related regions and two building-related regions. Interestingly, we found that the complex pattern is organized in a dorso-ventral, large-scale object-based mirror symmetry. Furthermore, correlating this pattern with the map of visual field eccentricity, we find that the entire network of object areas can be related to a single eccentricity map. We hypothesize that this large-scale organization points to a possible development of high order object areas through extension and specialization of a single proto-representation.

Funded by Israel Academy 80009 and 64499 grants

Keywords: visual cortex, fMRI, object recognition, topography

Lack of paternal care reduces spine density in the limbic cortex

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Previous studies in trumpet-tailed rat Octodon degus have demonstrated that, quite analogous to the functional maturation of sensory and motor cortices, which strongly depend on sensory or motor stimulation, the development of synaptic connections in limbic brain regions are modulated by early postnatal emotional experiences. Periodic and chronic interruptions of parental care during the first weeks of life induce a significant increase of dendritic spines in the limbic dorsal anterior cingulate cortex (ACd) (Helmeke C, Ovtcharoff jr et al, 2001, Cerebral Cortex, 11: 717). In the present study, the contribution of paternal care on the development of synaptic composition in the ACd was analyzed light and electron microscopically. The composition of paps, which were either raised with or without their father reared in the fatherless animals a 20%, decreased spine density on apical and basal dendrites of layer II/III pyramidal neurons in the ACd.

Such experience-induced modulations of synaptic inputs in higher associative and limbic cortical areas appear to shape the limbic synaptic networks and thereby may determine cognitive and psychosocial capacities during early and later stages of life.

Supported by SFB 426

Keywords: limbic system, development, synaptic plasticity, dendritic spines

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Keywords: aging, global-local processing, visual perception, insomnia

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Such experience-induced modulations of synaptic inputs in higher associative and limbic cortical areas appear to shape the limbic synaptic networks and thereby may determine cognitive and psychosocial capacities during early and later stages of life.

Supported by SFB 426

Keywords: limbic system, development, synaptic plasticity, dendritic spines
assessment of tissue functionality in vicinity of brain lesions using DTI and fMRI

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In the last decade functional MRI (fMRI) gradually evolved to become a clinical tool for pre-surgical evaluation of critical function regions of the brain. fMRI signals relate to activated gray matter only. However the connectivity of white matter is crucial for functionality of a region and should not be underestimated. The recent development of diffusion tensor imaging (DTI) is a well-established method for in-vivo mapping of the white matter directionality and organization, but its clinical application has not been fully explored. Our aim in this study was to explore the added value of the combined methodologies in brain surgery. DTI is based on the anisotropic motion of water molecules in white matter. While the diffusion of water parallel to the long axis of the neuronal fibers is free, perpendicular to the fibers it is disturbed. Recently, the concept of three-dimensional fiber tracking based on DTI data was introduced. With this methodology it is possible to produces fiber tracts that corresponds with large fiber bundles such as the pyramidal tract, corpus callosum, optic radiation, corona radiata and thalamic radiation. In relation to brain tumors, one can think of three ways brain lesion and tumors can affect white matter fibers: cutting, pressing or infiltrating. In this three-dimensional fiber tracking helped to visualize critical white matter bundles and their relation to the tumor. White matter bundles that were cut, pressed or infiltrated by the lesion could be these bundle of tasks to relate gray matter activation could be visualized.

Keywords: MRI, diffusion, DTI, fMRI, white matter

Gial cell coupling and growth in sensory ganglia after axotomy: ultrastructural evidence

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Axotomy induces profound changes in primary sensory neurons and often produces neuropathic pain states, but very little is known about its effects on satellite gial cells (SCs) in dorsal root ganglia (DRG). We have shown previously that dye coupling among SCs was increased after axotomy. In this research we studied the ultrastructural basis of this change by correlating intracellular dye injection and serial section electron microscopy (EM). Laccatrices of dye injection showed that in control ganglia 76.2% injected SCs were not coupled to other SCs, and 23.8% were within gliovascular sheaths. Only 11.3% were coupled between gial envelopes around different neurons. However, in axotomized ganglia the incidence of coupling between gial envelopes increased by 7-fold (p<0.0001) and those of coupling within an envelope increased by 16-fold (p<0.0001). Some gial processes from sheath extended into adjacent connective tissue. Neurons were not coupled to other cells in control or axotomized ganglia. Serial section EM showed that after axotomy SC's extended new processes into surrounding connective tissue and formed new bridge-like connections between gial envelopes belonging to different neurons. Such bridge-like connections were absent in control ganglia. The number of gap junctions between SCs increased 40.5-fold (p<0.01) after axotomy. Thus, gial dye coupling is apparently mediated by gap junctions. We propose that axotomy induces growth of gial sheaths, leading to new contacts between SCs enveloping different neurons and to formation of new gap junctions between SCs. These changes may be an important mode of glial plasticity and contribute to neuropathic pain.

Keywords: axotomy, dorsal root ganglia, satellite gial cells, gap junctions, dye coupling, ultrastructure

Receptors for neurotransmitters on T lymphocytes as regulators of immune cells

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In recent years it became apparent that peripheral blood lymphocytes express receptors for various neurotransmitters (GABA, D1, D2, 5HT) on their cell surface, suggesting that peripheral lymphocytes are generally confined to the CNS and unavailable for peripheral binding. It has been suggested that neurotransmitter receptors are activated by peripheral innervations, yet their role remains unresolved. We show that peripheral lymphocytes, including T cells, are known to be involved in neuropsychiatric disorders and serves as a target for therapeutic treatment. We demonstrate that DRG, which is expressed on both neurons and peripheral immune cells, is known to be involved in neuropsychiatric disorders and serves as a target for therapeutic treatment. We demonstrate that DRG expression is upregulated in neuropsychiatric disorders, and its contribution to the disease may be underestimated.

Keywords: DRG, dopamine, peripheral blood lymphocytes

Involvement of plasminogen activator system in central nervous system inflammation and demyelination

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Extracellular proteolytic enzymes have been implicated in the pathogenesis of demyelinating and neuro-inflammatory disorders, such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). During EAE, there is concurrent induction of plasminogen activator (PA) and matrix metalloproteinases (MMPs) systems, supporting the concept that non-inflamatory damage in EAE involves an altered balance between extracellular proteases and their inhibitors. In our study, we tested the involvement of the PA system in EAE, using mice genetically deficient in urokinase-type plasminogen activator (UPA) receptor (UPAR). In comparison to the wild type (wt), UPAR knockout (UPA-/-) mice were more severely ill (UPA-/-: 2.1±0.5, wt: 1.0±0.3) and with longer duration (23±3 days versus 12±3 days). Similarly, UPAR knockout (UPA-/-) mice also exhibited the disease more severely, which was accompanied with a lack of spontaneous recovery. The death percentage in the UPAR-/- group was 50% whereas in the wt it was only 7.7%. Addition of octapeptide (A6) (that blocks interaction between UPA and UPAR), to the T-cell proliferation assay in vitro, resulted in a marked inhibition of T-cell reactivity (both T-cell derived from UPA-/- and wt animals). Our results imply that a network of functionally redundant proteases is involved in EAE and MS progression and recovery. Modulation of the PA system may have a potential target for treatment of CNS inflammatory and demyelinating diseases.

Keywords: urokinase plasminogen activator, urokinase plasminogen activator receptor, CNS, experimental autoimmune encephalomyelitis

Inhibition of beta secretase cleavage of APP (Amyloid Precursor Protein) by active immunization approach

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APP (amyloid precursor protein) is subject to post-translational modifications, glycosylation, sulfation and phosphorylation along the intracellular protein secretory pathway. Cleavage of APP occurs in two different ways:

1) The non-amyloidogenic pathway in which alpha secretase cleavage g generates a soluble N-terminal APP fragment, which leaves the C terminus attached to the membrane.

2) The amyloidogenic pathway, which generates APP through beta and gamma secretase cleavages involving internalization of APP from the cell surface and its cleavage in late secretory pathways. Recently the beta site cleavage enzyme (BACE) was reported (Vassar et al, Science 286:666-670, 2000), and is now subjected for intense research aimed for blocking the enzyme activity.

We are developing a new approach for blocking beta secretase activity, based on immunization with a small peptide representing the cleavage site of beta secretase. Antibodies
raised recognize the cleavage site of APP and will hopefully interfere with AP formation. Limiting of beta amyloid production may become an important therapeutic target in Alzheimer disease (AD).

**Keywords:** Alzheimer’s disease, beta amyloid, BACE, inhibition, vaccination

The relationship between the cell cycle and BMP4 signaling in the control of neural crest delamination

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Delamination of premigratory neural crest cells from the dorsal neural tube depends upon a gradient of BMP4 activity along the neural tube which is generated by changing levels of noggin. The latter are in turn modulated by an inhibitory activity from the dorsomedial somite which coordinates the timing of cell migration with the elevation of a migratory mesodermal substrate. Cell-intrinsic mechanisms also regulate delamination. Here we show that neural crest cells synchronously emigrate from the neural tube in the S-phase of the cell cycle. Specific inhibition of the transition from G1 to S both in vivo and in vitro blocks delamination, whereas inhibition at S or G2 phases has no immediate effect. Thus, the transition between G1 to S is necessary for the epithelial-to-mesenchymal conversion of crest precursors and may be required for the cells to respond to environmental signals that trigger delamination. The notion is being examined that BMP signaling and the cell cycle features involved in cell delamination are hierarchically linked.

**Keywords:** neural crest, control of proliferation, migration, morphogenesis

Bi-phasic modulation by hydrogen peroxide of synaptic plasticity

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Unlike the proposed role of reactive oxygen species (ROS) in neurodegeneration, acute effects of reactive oxygen on synaptic plasticity are poorly understood. Using rat hippocampal slices we found that exposure to high concentration (0.5-3mM) of H2O2 reduces EPSP's in both potentiated and non-potentiated synapses. While the increase of the slices to 2mM H2O2 did not affect expression of pre-established long term potentiation (LTP) but prevented induction of new LTP, and enhanced long term depression (LTD). Surprisingly, 1uM H2O2 caused a two-fold increase in LTD when compared to controls, and it further enhanced NMDA-independent LTD. Low concentration of H2O2 also suppressed LTD. Nifedipine, an L-type calcium channel blocker did not affect control LTP but blocked effects of both LTD and new LTD.

**Keywords:** H2O2, LTD, calcineurin

Myeloid dendritic cells are activated in secondary progressive multiple sclerosis: increased CD80 expression, cytokine production and a proinflammatory polarization effect on naive T cells

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**Objective:** To study the activation status and function of myeloid dendritic cells in MS.

**Methods:** Expression of CD80, CD86, CD40, CD83 and PDL-1 on and IL-12 & TNF-α production in myeloid DCs was studied by flow cytometry. The polarizing effect of MS DCs on naive T cells was studied using mixed-lymphocyte reaction (MLR) of DCs with naive T cells. T-cell stimulation with anti-CD3/CD28 and measurement of IL-2, IL-4, IL-10, IFN-γ & TNF-α in the supernatant by ELISA.

**Results:** Exposure to rotating, remitting (RR)-MS, secondary-progressive (SP)-MS showed: (1) More DCs expressing CD80 (19.1% vs. 7.4%, p<0.03), (2) Higher IL-12 (44.1% vs. 4%, p=0.012) and TNF-α production (9.18% vs. 3.05%, p=0.042) in unstimulated DCs; (3) Decreased expression of PDL-1 (3.4%, 9.3% and 12.2% for SP, HR and HH resp. p<0.05). DCs incubated with serum from SP-MS (n=15) had higher IL-12 (3.53%) compared to RR-MS (1.27%, p=0.009 n=28), or HC (0.78%, p=0.007 n=17). Stimulation showed T-cells polarized by SP-MS DCs secreted more TNF-α (225.9 pg/ml) vs. those polarized by HC DCs (22.7 pg/ml, p=0.049). The secreted TNF-α was correlated to T-cells polarized by SP-MS DCs was higher than by HC DCs (8.01 vs. 1.45, p=0.038).

**Conclusions:** Myeloid DCs in SP-MS are activated compared to RR-MS and HC. These polarized naive T-cells into proinflammatory T-cells. Serum contents in SP-MS patients may play a role in DC activation, manifested by its effect on DC IL-12 production. The results demonstrate that the activated DCs may determine the type of immune activity in SP-MS.

**Keywords:** dendritic cells, multiple sclerosis, co-stimulation, cytokines, T cells polarization

The expression of mitochondrial complex I subunits, 24- and 51-kDa, is reduced in the frontal and elevated in the parietal cortices of schizophrenia patients

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Impairment of the mitochondria, which play a major role in cell function and survival, could provide an explanation for the tremendous heterogeneity of clinical and pathological manifestations in schizophrenia. Previous studies have shown abnormalities in brain mitochondrial morphology as well as in the respiratory chain enzymatic activity in schizophrenia. We have recently reported a disease stage dependent alteration in complex I activity in platelets of schizophrenic patients, which was accompanied by alterations in the expression of the 24kDa and 51kDa, but not in the 75kDa subunits of complex I. In the present study we compared the mRNA and protein levels of the three subunits in frontal and parietal post-mortem brain cortices of 14 schizophrenic patients, 15 unipolar patients (UP), 15 bipolar patient (BP) and 15 normal subjects, obtained from the Stanley Foundation Brain Bank. Both mRNA and protein levels of 24- and 51-kDa subunits were significantly reduced in the schizophrenic frontal cortex compared to the controls, while UP patients showed a reduction in 24kDa protein level, which was less significant than that of the schizophrenic group. No such changes were observed for the BP. In contrast, a significantly increase was observed in mRNA and protein levels of the 24kDa subunit in the parietal cortex of the schizophrenic patients compared to the control group. The 51kDa subunit did not differ between groups. The present study further demonstrates the malfunctioning of complex I in schizophrenia and supports the relevance of our peripheral findings to the CNS pathology.

**Keywords:** schizophrenia, mitochondrial complex I, post-mortem brain

Embryonic exposure to hypoxic episode affects postnatal development

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Maternal hypoxia during pregnancy is known as a major risk factor for fetal brain damage. To evaluate the mechanisms of the neuro-developmental damage caused by hypoxia we have studied newborn mice development following 2 hours of exposure of 17 gestation day pregnant mice, to 5% oxygen and 3% CO2. Newborns were inspected daily for phenotype, morphogenetic parameters, reflex development, muscle strength and coordination. Hypoxia did not affect newborn body weight; however, it increased brain weight as compared to controls on the first postnatal month, as measured at postnatal days 1 (P1), 7, 14 and 21 (n=12). In addition, hypoxic episode accelerate eyelid opening (P<0.001), n=8-9) and teeth eruption, as compared to the control group. No difference was observed in the development of righting reflex between the study groups. Yet, newborns previously exposed to hypoxia delayed in developing the ability to climb on a rotating rod. On P8-P9 hypoxia exposed newborns were able to climb on 10 -20° (mean) slope as compared to 40 -50° in the control group (p<0.0029-0.013, n=11-9, 10, respectively). Significant differences was also observed between the groups in the rotated holding at P10, P11 (p=0.006, 0.04, n=6-10, 6-4). Geotropism was examined in 50° slope, a full response was achieved by day 1 in the control group, while only 65% of the newborn responded to the slope on P13. Taken together, hypoxia

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Keywords: hypoxia, development, brain damage

Separating signal from noise in psychophysics

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Psychophysical discriminations can be viewed as a comparison between two internal responses, each characterized by its own mean value and noise amplitude. Performance, measured as percent correct discrimination, is effectively determined by the signal-to-noise ratio for the response components. Available methods impose additional constraints on the decision process or on the stimulus-response transformation. Here we propose a novel approach that only assumes normally distributed one-dimensional internal responses. It is based on minimizing the residual error between measured and modeled psychophysical response levels. This procedure requires a number of stimulus pairs in order to derive an over-complete system of equations for mean and noise response amplitudes. The method was applied to a contrast discrimination task. Gabor patches with three contrast levels in two spatial configurations - with flankers and without them - were used as stimuli. Stimuli were presented employing a two alternative forced-choice paradigm. Each trial consisted of a sequence of two stimuli having Gabor patches of different contrast levels. For increasing contrast, results show monotonic increase of mean response values with decreasing slope and monotonic decrease of noise amplitude. The presence of flankers resulted in weaker mean response (inhibitory effect) and higher noise amplitude.

Keywords: contrast discrimination, model, signal-detection theory, lateral interactions

Elongation factor-2 phosphorylation in the rat insular cortex following taste learning

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This study was conducted in order to learn whether active regulation of protein synthesis plays a role in memory consolidation. We have examined whether phosphorylation of eEF2 on Thr-56 by eEF2 kinase is correlated with learning processes. Phosphorylation of eEF2 was previously found to be involved in development of the visual cortex (Schentz et al., Nature Neuroscience 3: 211-216 [2000]). We used novel taste learning paradigms together with immunoblotting analysis of total and phosphate-eEF2 levels in the insular cortex in different time points following taste presentation. Phosphorylation of eEF2 was used as a positive control. At 2, 30, 180, 360 min and 18 hours following taste presentation (n=2 each group) there was no change in eEF2 phosphorylation in taste presentation. In the novel tastant (0.1% saccharin) compared with water. However, there was a significant increase (22%) in eEF2 phosphorylation 20 min following the offset of novel taste consumption (p<0.05, two-way student t-test, n=10). This time-dependence of eEF2 phosphorylation in the insular cortex was similar to ERKII phosphorylation: only 20 min following novel tastant presentation there was an increase of 95% in ERKII phosphorylation (p<0.02, two-way student t-test, n=10). The results indicate for the first time modulation of eEF2 phosphorylation during learning, and suggest that regulation of protein synthesis in the insular cortical area may be correlated with memory consolidation.

Keywords: memory, consolidation, protein synthesis

Antiphospholipid syndrome exacerbates cognitive impairment in an APP mouse model of Alzheimer's disease

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Innate and immune mechanisms have been consistently implicated in the pathogenesis of neurodegenerative diseases. Crossed mediated diseases of the central nervous system lead eventually to neurodegeneration. Clinically, genes influencing neurodegenerative diseases, such as APOE, also influence the course of neuroimmunological diseases such as multiple sclerosis. We have investigated whether immune challenges can modify the course of this disease models of neurodegenerative diseases. The animal model chosen was a mouse strain carrying a pathogenic mutation in the APP gene. The immune challenge used was induction of antiphospholipid antibodies by immunization with B2-glycoprotein I. This immunization has previously been found to induce behavioral and cognitive dysfunction in a number of normal mouse strains, but not in C57BL, the background for the transgenic mice used in the present study. The mice were immunized at the age of 4-5 months and 4.5 months later were tested for hyperactivity and anxiety on a staircase apparatus and for cognitive function using a swim T-maze. There were significant differences in behavior between the APP transgenic mice compared to the controls in both tests. Induction of antiphospholipid antibodies impaired performance in the cognitive test only in the APP transgenic mice and had no significant effect in the wild type controls. The immunization had no significant effect in the behavioral assay. These results indicate the potential role of immune mediated mechanisms in the pathogenesis of neurodegenerative processes and point to the potential use of immunomodulatory therapies in such diseases.

Keywords: transgenic mice; Alzheimer’s disease; amyloid precursor protein; B2-glycoprotein-I; antiphospholipid antibodies

Molecular and pharmacological alterations of the serotonin receptor 5HT2c in depression

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Serotonin2c receptors (5HT2cR) have been implicated in some psychiatric disorders, including depression. 5HT2cR undergoes editing whereby one to four specific adenosines are converted to inosines. Editing modulates receptor-G protein interactions, thereby altering receptor-mediated signaling. We tested 5HT2cR editing in the mouse acousticolateralis (N. Ac) of Flunder sensitive line (FSL, an animal model of depression) and control rats using the sequencing method. FSL rats exhibit 50% higher levels of the fully edited isoform (5VSV) of 5HT2cR than control rats. Only control rats exhibited the non-edited isoform (IN). Treatment with desipramine had no effect on RNA editing, although it improved swim behavior in FSL rats. Western blot analysis detected a decrease in protein levels of 5HT2cR in FSL rats after desipramine treatment although no significant changes were detected in mRNA and pre-mRNA levels of 5HT2cR in FSL rats or desipramine treated rats compared to controls. Acute exposure of the accumbal 5HT2cR to its antagonist, RS102221, resulted in dopamine release and this was doubled in FSL as compared to control rats. Therefore, we suggest that the 5HT2cR in the N Ac mediates its role in tonic DA release. Furthermore, 5HT2cR receptor RNA editing and pharmacology are altered in association with depressive behavior. These data indicate that 5HT2cR receptor could be a potential target for future treatment of depressive behavior.

Keywords: RNA editing, serotonin-2c receptor, microdialysis

Autoimmune neuroprotection: the evolutionary compromise needed to maintain autoimmunity on alert without the risk of autoimmune disease

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Autoimmunity is the body’s own mechanism against self-destructive compounds emerging as a consequence of CNS insult. In this work we show that induction of tolerance to self myelin-specific antigens, what was believed for years to be a preferential state of autoreactive cells, is against ‘individual’s fitness’ in coping with stressful conditions after CNS insult. Neonatal immunization of rats with whole spinal cord homogenate, diminished the ability of adult rats to respond to myelin immunization and those animals show worse neuronal survival after optic nerve crush injury or spinal cord injury. After induction of a corollary, immunization of adult animals with myelin-derived self-antigen or depletion of endogenous suppressor T cells (e.g. CD4+CD25 regulatory T cells) increased the animals’ ability to resist injurious conditions. These findings call for a redefinition of tolerance to self-antigens, and for the role of CNS self-proteins as safeguards against CNS neurodegenerative disorders.

Keywords: autoimmune neuroprotection, regulatory CD4+CD25 T cells, CNS trauma, autoimmune diseases
Perinatal dehydration correlates with adolescent salt preference
Kochl Y, Rakover Y 2 and Leshem M. 1
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In our study of adolescents with congenital adrenal hyperplasia (CAH) we find correlations between possible dehydration events pre- and post-natal and the liking for salt taste expressed by adolescents. These events include scores of maternal vomiting and nausea when pregnant with the adolescent, and scores of the adolescent’s vomiting, diarrhea and infusions during infancy. We find positive and significant relationships between these events and how much the adolescent likes salt, years later. Since these data are based on recall by the mother of events occurring years previously, we made comparisons with in the family, i.e., comparing the relationship between maternal nausea and vomiting and the severest form of CAH. However, here the causal relationship seems to be reversed – apparently carrying a fetus with CAH induces higher levels of nausea and vomiting in the mother.

Keywords: human, salt, taste

Licking of salt by adolescents with congenital adrenal hyperplasia
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The licking for salt in congenital adrenal hyperplasia (CAH) patients has been known in anecdotal form in the medical profession. However, there are no systematic reports of this phenomenon. In our study of adolescents with CAH we have reported that adolescents with the severest form of the disease, “salt wasting”, show a preference for salt as measured by questionnaire and psychophysical tests (Kochl, A. Rakover, Y. and Leshem, M. Neural Plasticity 8(3): 182; 2001). Here we present a semi-quantitative descriptive report of how these adolescent data for outputs and discuss the issue of whether this behavior is innate or acquired response to salt wasting.

Keywords: human, salt, taste

Activity-dependent translocation of the G-protein (DGq) in Drosophila photoreceptors
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The covalent lipid modification of proteins plays a major role in targeting heterotrimeric (Gq) proteins to cellular membranes. In the case of the Drosophila visual system, palmitoylation of cysteine residues at position 3 and 4 of a eye specific DGq-alpha is the sole lipid modification of the alpha subunit. Little is known, however, about the control of DGq subunit localization within the natural endogenous environment of a specialized signaling cell. Here we show, using live Drosophila flies, that light causes massive and reversible translocation of the visual DGq to the cytoplasm, associated with marked architectural changes in the signaling compartment. Molecular genetic dissection together with detailed kinetic analysis enabled us to characterize the translocation cycle and to unravel how signaling molecules that interact with DGq affect this process. Using specific visual mutants our results indicate that the translocation is not influenced by phototransduction steps at the level of PLC or downstream of it and that DGq is essential for efficient targeting of DGq to the membrane. Together with analysis of a 3-dimensional model of DGq our in vivo results are mechanistically consistent with the “two signal model” for membrane targeting. Immuno-electron microscopy revealed that both DGq and the signaling compartment undergo dynamic and reversible light-dependent changes. These events give DGq access to other cellular compartments and point to possible cross talk between sensory transduction and the cytoskeleton.

Keywords: G-protein, localization, activity-dependent

Low GSK-3β in schizophrenia - a genetic marker or a neurodevelopmental insult consequence
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Glycogen synthase kinase-3 (GSK-3β) is a protein kinase highly abundant in brain and involved in signal transduction cascades, particularly neurodevelopment. Its activity and protein levels have recently been found to be over 40% lower in postmortem frontal cortex of schizophrenic patients. To elucidate whether the low levels are a genetic marker or a consequence of a perinatal neurodevelopmental insult the following experiments were carried out: GSK-3β protein levels in the frontal cortex of rats treated with neuroleptics or exposed to cold restraint stress were assayed. In the schizophrenia-related neonatal ventral hippocampal lesion rat model we measured GSK-3β protein levels and activity in the frontal cortex. To confirm our original finding in another brain area we studied mRNA levels of GSK-3β in postmortem dorsolateral prefrontal cortex (DLPFC) from schizophrenic patients. Chronic treatments of rats with neuroleptics or exposure to cold restraint stress did not alter GSK-3β protein levels, supporting the concept that low GSK-3β in schizophrenia is not secondary to drug treatment or stress. However, GSK-3β protein levels in lesioned rats were significantly lower than in sham rats, favoring the perinatal insult possibility. GSK-3β mRNA levels were 36% lower in postmorten DLPFC of schizophrenic patients confirming our previous findings. An additional intriguing recent preliminary finding is that GSK-3β protein levels in CSF samples from schizophrenic patients were found 28% lower than in control subjects. Further studies will be aimed at determining whether nonspecific neonatal damage or only specific factors cause low GSK-3β as a late effect.

Keywords: schizophrenia, GSK-3β, neurodevelopment

The molecular mechanisms underlying learning and stress
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Stress has multiple modes of influence on learning and memory, improving or impairing it under different conditions. The hippocampus is held accountable for explicit memory. The CA1 area of the hippocampus is reported to be involved in spatial memory. The amygdala is critically involved in mediating stress-related effects on behavior. Memory storage has a late phase that requires protein synthesis. The activation of the MAPK (ERK) cascade is related to the establishment of the late, protein-synthesis dependent phase of memory formation. Thus, the activation of ERK and of its downstream substrates may serve as a biochemical indicator for the activation of long-term memory processes.

Most learning protocols involve also a component of stress. In the present study we attempt to separate learning and stress induced mechanisms by examining the activation of members of MAPK cascade (ERK1/2, CREB and Elk-1) in the hippocampus and amygdala. Three groups of rats were tested: 1. Learning – subjected to a spatial learning protocol, 2. Stressed – subjected to the water maze as the Learning group, but without an escape platform, 3. Naive. Ten minutes after the last training session, tissue was collected for analysis of ERK, CREB and Elk-1 activation. Only in the Learning group an activation of ERK was found in the CA1 area of the hippocampus but not in the amygdala. Other molecules are still under the examination in both brain areas. The findings are expected to contribute to the dissociation between learning- and stress-induced mechanisms of signal transduction.

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Keywords: memory, stress, protein synthesis

Molecular analysis of a novel neurodevelopmental rat model applying gene expression macroarray technology
Koronyo-Hamaou M., Gak E., Zuckerman L., Gaeta E., Barkai G., Goldman B., A. Kogan I., Richter-Levin G.
1 Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer; 2 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. Psychobiology Unit, Dept. of Psychology, Tel Aviv University, Tel Aviv; 3 Felsenstein Medical Research Center, Bellinson Campus, Petah Tikva
We have recently found that maternal immune activation during pregnancy by means of systemic administration of the synthetic cytokine releaser poly I:C in rats, led to the offspring to neurochemical and histological brain aberrations, as well as}

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We have recently found that maternal immune activation during pregnancy by means of systemic administration of the synthetic cytokine releaser poly I:C in rats, led to the offspring to neurochemical and histological brain aberrations, as well as
long term behavioral deficits (Zuckerman & Weiner, submitted). Furthermore, these deficits were not present before puberty but emerged at adulthood, implicating abnormal brain maturation processes. The present study used this model for the analysis of multiple gene expression profiles in two brain regions, the striatum and hippocampus, from the offspring of poly I:C-treated and control dams. To this end, we employed Clontech cDNA macroarray technology containing 1200 rat genes relevant to neuroresearch and housekeeping genes. RNA preparations were pooled from four matched brain sections obtained from adult female offspring. RNA hybridization procedure was repeated in two independent trials.

Our data indicated that genes associated with synaptic trafficking and neuronal growth, synapses (1A, 2A), syntaxin (B) and myelin basic proteins, were up-regulated in both striatum and hippocampus of the poly I:C offspring. In addition, several genes of the signal transduction pathway, G-protein coupled receptors and kinases, were up-regulated in addition to several genes of the excitatory glutamate-NMDA pathway were up-regulated specifically in the poly I:C offspring striatum, while the poly I:C offspring hippocampus was distinct by elevated neuro-endocrine proteins secretogranin and somatostatin. Although these findings need further validation by other molecular methodologies, they may suggest that prenatal administration of poly I:C interferes with neurodevelopmental processes involving neuronal remodeling, activity and proliferation, that might be associated with brain disorders such as schizophrenia.

Consistent with the idea that inhibition of constitutive proteolytic activity is necessary for the induction of short-term neuronal plasticity, rat animal model, cDNA expression arrays, poly IC Constitutive proteolytic activity is required for short-term plasticity of cultured Aplysia sensorimotor synapses Khotorsky A. and Spira M.E. Dept. of Neurobiology, Life Sciences Institute; The Hebrew University, Jerusalem, Jerusalem. The mechanisms underlying short term facilitation in Aplysia sensory-synapse can be subdivided into two processes: (a) a spike duration dependent process (sensitization) that results from 5HT induced PKA activation, and (b) a spike duration independent process (disinhibition) that results from PKC activation. As a result of PKA activation the potassium conductance is reduced, leading to spike broadening and enhanced calcium influx. In contrast, PKC dependent synaptic disinhibition is not entirely understood. It was suggested that vesicles mobilization, alteration in the release mechanism or local activation of specialized calcium channels might be involved. In contrast to earlier reports, we found that calpains (calcium activated cystein neutral proteases), are involved in events leading to synaptic habituation and disinhibition.

Application of the membrane permeable calpain inhibitors calpeptin or the non-specific proteasome inhibitor MG132 increased the rate of synaptic habituation and inhibited 5HT-induced synaptic disinhibition. On the other hand, sensitization is not affected by the inhibitors. The effect of the inhibitor on the rate of synaptic vesicles release to the release sites. As a consequence the rate of synaptic habituation is accelerated in the presence of the inhibitors, and after massive habituation, 5HT application does not lead to disinhibition. These results demonstrate that constitutive proteolytic activity is necessary for the induction of short-term neuronal plasticity. Consequently, the specific protease activity, as measured in both spiking and mean membrane potential, in previous works it was shown that adaptation induced membrane potential hyperpolarization of V1 neurons (Carandini et al. 1997). To distinguish between intrinsic mechanisms to network mechanism of adaptation we have studied the specificity of adaptation in visual responses of the neurons. If the mechanisms underlying adaptation are intrinsic to the recorded cell, adaptation should not change the selectivity of the neuron. If, on the other hand, adaptation is a direction-specific plastic adaptation on visual responses of the neurons. We examined this hypothesis on direction selectivity in simple and complex cells. In simple cells we have found that adaptation, induced by drifting gratings, in neither the preferred or the non-preferred direction, enhanced the direction selectivity in simple and complex cells. In contrast, adaptation in the non-preferred direction led to an increase in the direction selectivity of the neuron. The selectivity index of the

products that are altered during thermal conditioning by using molecular techniques. After thermal conditioning the AH/P0 of young chicks was dissected in a time course ranging from minutes to 24 hours, and mRNA changes were monitored. We applied two strategies. 1. General miRNA amplification changes screening using differential dextran-dye. 2. Since all we employed Clontech cDNA macroarray technology containing 1200 rat genes relevant to neuroresearch and housekeeping genes. RNA preparations were pooled from four matched brain sections obtained from adult female offspring. RNA hybridization procedure was repeated in two independent trials. Our data indicated that genes associated with synaptic trafficking and neuronal growth, synapses (1A, 2A), syntaxin (B) and myelin basic proteins, were up-regulated in both striatum and hippocampus of the poly I:C offspring. In addition, several genes of the signal transduction pathway, G-protein coupled receptors and kinases, were up-regulated in addition to several genes of the excitatory glutamate-NMDA pathway was up-regulated specifically in the poly I:C offspring striatum, while the poly I:C offspring hippocampus was distinct by elevated neuro-endocrine proteins secretogranin and somatostatin. Although these findings need further validation by other molecular methodologies, they may suggest that prenatal administration of poly I:C interferes with neurodevelopmental processes involving neuronal remodeling, activity and proliferation, that might be associated with brain disorders such as schizophrenia.

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non-adapted inputs, calculated from the responses to either direction after adaptation with the same direction of motion, was not different than the control condition. This suggests that cortical adapting inputs do not change the selectivity of the cells. Furthermore, unless a significant fraction of the cortical inputs to direction selective cells are not affected by adaptation, intraocular axis (VA) does not make a significant contribution to direction selectivity.

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**Keywords:** intracellular recordings, in-vivo, direction selectivity, cortical circuitry

**Mismatch negativity (MMN) and "F (fusion)-complex" tap different aspects of deviance**

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The Mismatch negativity (MMN) component and the "F (fusion) complex", the response to net-fusion, are brain potentials evoked by a deviant event. In this study we attempted to find out the extent to which these responses reflect the same process, albeit in different contexts. The stimuli were base (presented to the front), that fused with the formant transitions presented to the front, left or right of the subject (the latter two producing an echo-sensation), resulting in a standard and deviant V-C-V sequences /ga/ and /da/, respectively. Brain potentials were recorded from 10 normal hearing righthanded native Hebrew speakers, whose ages ranged between 19-30 years, that discriminated these V-C-Vs. Low resolution electromagnetic tomography (LORETA) t-test comparison images, that were run on the responses to deviants, showed that (echo-sensation) activity contributing to the MMN was larger between 191-218 msec and was localized to the temporal cortices (BA 21, 22). In this case, the auditory object affected the MMN. In contrast, an enhanced "F-complex" was associated with the lateralized fusion conditions that sounded different in spatial attributes (echo). This activity was localized to parieto-temporal (BA 19, 39), occipital (BA 31, 20) and frontal (BA 9, 10) regions in the latency range of 226-296 msec. These results indicate that the MMN and "F-complex" tap different aspects of deviance: the "F-complex" is affected by properties of the auditory object itself, whereas the MMN is affected by its novelty in relation to other stimuli.

**Keywords:** speech, auditory evoked potentials (AEPs), fusion, MMN, "F (fusion)-complex"

**Cannabinoid CB2 selective PRS-211,096 reduces neurodegenerative deficit in experimental autoimmune encephalomyelitis (EAE)**

Levi Y., Bar-Joseph A., Dar D.E., Garzon A., Menashe N., Margules F. and Fink G.
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Dexanabinol (HU-211) a non-psychotropic cannabinoid has been shown to reduce the clinical score in rats with EAE. PRS-211,096 is a novel bicyclic cannabinoid with a higher affinity to the peripheral cannabinoid-2 (CB2) receptor (IC50 = 0.42 nM) and with lower affinity (IC50 = 22nM) to the central CB1 receptor. CB2 receptors are expressed mainly by B and CD4+ and CD8+ T cells as well as monocytes, and are known to be involved in immunomodulation. The aim of the present study was to determine whether PRS-211,096 could reduce the severity of clinical signs in EAE rats. EAE was induced in Lewis female rats by injecting (s.c.) myelin basic protein (MBP) in complete Freund’s adjuvant. After the first appearance of clinical signs, PRS-211,096 was administered on three consecutive days at different doses. The severity of the illness was determined by the mean maximal increment in the severity of the clinical score. The significance of differences between groups was determined by ANOVA followed by Fisher’s LSD. The results showed that PRS-211,096 significantly reduced clinical score and alleviated illness severity in a dose-dependent manner (22-35% compared with that found for long-term dosages of 50 mg/kg). Dose-dependent responses to varying dosages showed that the efficacy of PRS-211,096 was similar or greater than that of methyl prednisolone (20% compared with vehicle). Interferon-beta (Betaseron), and IgG were ineffective. The efficacy of PRS-211,096 in reducing clinical score in EAE seems likely to be due at least in part to its affinity for the CB2 receptor. Thus, PRS-211,096 provides the basis for developing novel drugs for the treatment of multiple sclerosis in the human.

**Keywords:** multiple sclerosis, EAE, neuroprotection

Neuroprotection by PRS-211,220: assessed functionally and morphologically in transplant MCA occlusion rats

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Dexanabinol (PRS-211,007) is a non-psychoactive cannabinoid, which acts as a non-competitive NMDA receptor antagonist, and has anti-oxidant and anti-inflammatory activities. It was shown to be neuroprotective in brain ischemia and traumatic brain injury models. PRS-211,220 is a novel analog of Dexanabinol, with two times higher affinity for the NMDA receptor and 30 times better ability to inhibit cyclooxygenase-2 than Dexanabinol. The aim of the present study was to determine whether PRS-211,220 had long-term beneficial effects on functional outcome and on infarct volume following focal brain ischemia. The middle cerebral artery (MCA) of adult Sprague Dawley rats was occluded for 120 minutes by intraheminal suture under halothane anesthesia. PRS-211,220 (0.1, 0.25, 0.5, 1, 2.5, 5 or 10 mg/kg IV) and its vehicle were administered by the end of the ischemia. The neuroprotective efficacy of the compound was evaluated behaviorally by the "staircase test" and morphologically by measuring infarct volume. Rats were trained for 1 week prior to the insult, twice a day for 15 minutes. Thereafter, rats were tested for 2-3 weeks. By the end of the last test brains were removed, serially sectioned and stained with thionin. Infarct volumes were evaluated using a computerized image analyzer. PRS-211,220 induced a dose related improvement in staircase test performance on the contralateral side (compared with vehicle alone, 55% improvement at 0.5 mg/kg p<0.05). Infarct volume was also reduced with PRS-211,220 (44% at 0.5 mg/kg). PRS-211,220 induces functional as well as morphological neuroprotection following transient MCAo in rats.

**Keywords:** neuroprotection, stroke, staircase test, dexanabinol

The temporal evolution of local and global image representations in human high order object areas

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Recently we have found that human high-order object areas are driven partially by local object fragments, and partially by global completion effects. Here we studied the relative contribution of local and global effects. We studied the temporal dynamics of these effects using brief exposures through a backward masking paradigm. In the first experiment, line drawings of animal shapes were presented in two conditions that were occluded by parallel, vertical bands ("grid"), or scrambled by randomizing the relative location of the object stripes ("scrambled"), each presented in 2 different exposure durations (60 ms, 250 ms). In the second experiment, the same objects were presented either without occlusions ("whole") or with the "grid" condition, again using 60 and 250 ms exposures. The results showed that for both exposures, the "whole" condition exceeded the "grid" condition as rapid as the emergence of local feature representation. Thus, as early as 60 ms - when the visual activation is still low, the relative contribution of completion effects is already similar to that found for longer exposure times.

Supported by Israel Academy 8009/00-1 and MP 6971 grants.

**Keywords:** fMRI, visual system, object recognition, completion

Resistant of bax-deficient mice to MOG-induced experimental autoimmune encephalomyelitis (EAE)

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by destruction of myelin. Recent studies indicate that accumulating axonal damage is involved in the neurodegenerative deficit in EAE seems likely to be due at least in part to its affinity for the CB2 receptor. Thus, PRS-211,096 provides the basis for developing novel drugs for the treatment of multiple sclerosis in the human.

**Keywords:** multiple sclerosis, EAE, neuroprotection
informed consent. This study is approved by the Institutional Ethics Committee.

Data analysis: All the data were expressed by mean ± standard deviation (SD) and were analyzed by the one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. The level of statistical significance was set at **p < 0.05**.

Results: The behavioral test results are shown in Figure 1. The mice tested on the fifth day of treatment experienced significant improvements in memory consolidation ability compared to the control group (HSD test, *p* < 0.001). Moreover, the mice treated with the high dose of FDNB showed a further improvement in memory consolidation ability compared to the low dose group (HSD test, *p* < 0.05).

Discussion: The results of this study suggest that FDNB may have potential therapeutic effects on memory consolidation. Further studies are needed to explore the mechanism of action of FDNB and to determine its optimal dose for clinical use.
Continuous representation of objects in the human posterior fusiform gyrus

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The human ventral occipito-temporal cortex exhibits complex activation patterns to different objects. Recent studies revealed some of the principles which may underlie these representations. In order to gain more insight into the needs and expertise level. However, the representation of objects along these dimensions may be either modular (i.e. each stimulus type activates a distinct region) or continuous (i.e. activations to different stimulus types overlap to a certain dimension). The two possibilites lead to different predictions: in the modular case different objects should either activate the same area or different areas. In the continuous case activations to different objects will be partly overlapping and partly offset compared to each other. To test these predictions we conducted a functional MRI experiment, in which subjects (9) were presented with head images in front and back views. Images were presented in 9-sec single-category epochs, interleaved with 6-sec periods of blank screen. We obtained activations of highly overlapping yet slightly offset regions in the fusiform gyri. Crucially, the direction of this displacement was consistent across subjects and therefore cannot be attributed to random variability. In addition, the non-overlapping strips of activation were located in the regions of highest activation to a category, excluding low-resolution, or partial volume effects as the cause of the overlap. We conclude that the activation pattern reflects a continuous topography, in which representations of objects "slide" smoothly along the fusiform gyri.

Supported by ESF 8009.

Keywords: visual cortex, fMRI, object recognition, topography

Adult human bone marrow-derived mesenchymal stem cells differentiate into neural cells

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Human mesenchymal stem cells present in adult marrow, are believed to be multipotent and can develop into bone, cartilage, fat, tendon and muscle. Here, we demonstrate that human bone marrow stromal cells (hBMSc) can be induced to differentiate into neural cells in vitro. Isolated hBMSc from human iliac crest incubated with retinoic acid, bmatidine hydroxysamide and transhydroxyapatite, were changed their phenotype to neural cells. The differentiated cells were positive for nestin, neurofilament-H (NF-H), neuron-specific enolase (NSE) proteins. By immunocytochemistry and Western blot analyses. The mRNAs for NSE, retinoic acid receptor and neurite outgrowth promoting protein (NEGF2) were identified in differentiated and undifferentiated hBMSc. Using reverse transcriptase-polymerase chain reaction. However, mRNAs for Musashi-1, nestin and NF-H were detected only in differentiated cells. The mRNA expression of NEGF2 was increased during differentiation, as assessed by real-time PCR. In conclusion, our method might offer a new and more accessible source of neural cells for transplantation to treat neurodegenerative diseases.

Keywords: human bone marrow stromal cells (hBMSc), nestin, Neurofilament-H (NF-H), Neuron-specific enolase (NSE), Neural nucleus (Neun)

Anticonvulsant action of bromide is associated with enhanced synaptic inhibition

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Bromide salt (Br) was introduced as a treatment for epilepsy in the 1850's, making it the first anticonvulsant drug (AD). Although Br has been almost entirely superseded by newer AIDs, it is used in humans and more frequently in veterinary medicine. Yet, the mechanism underlying the anticonvulsant action of this halogen is not known. Because AIDs target either neuronal or non-neuronal elements of GABAergic transmission, we have investigated the effect of Br on these determinants of circuit excitability in in-vitro preparations of rodent neocortices. In organotypic cortical slice cultures, Br (20 mM) reversibly blocked the epileptogenic activity induced by removal of Mg from the bath solution. In whole cell patch-in-service recordings from neocortical pyramidal neurons, Br application did not have any apparent affect on Na channel availability. Thus, in current-clamp, it did not affect thresholds, amplitudes or maximum rates of rise of single action potentials, nor did it compromise neuronal capacity to fire at high frequencies. Br also did not cause significant changes in passive membrane parameters. By contrast, Br did have a profound effect on GABA-A receptor mediated inhibition. In voltage-clamp recordings, it prolonged by > 50% the decay time constants of mIPSCs with the stimulation IPSC, resulting in a considerable increase in inhibitory charge transfer. The mechanism for this has yet to be determined; it is probably related to Br's greater permeability through the chloride channel. We conclude that like barbiturates and benzodiazepines, Br achieves its anti-convulsant effect by enhancing synaptic GABAergic inhibitory activity.

Keywords: anticonvulsant, epilepsy, Na current, neocortical neuron, IPSC

Low socio-economic status, a risk factor for ischemic stroke: A case-control study. 1,2

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Background: The risk factors for ischemic stroke (IS) are age, hypertension, ischemic heart disease, atrial fibrillation, diabetes, dyslipidemia, hypercoagulopathy, smoking, carotid artery stenosis, TIA etc. An additional risk factor with a variable impact is a low socio-economic status.

Aim: To study the role played by the socio-economic status in the etiology of IS in the heterogeneous population of the Negev (Southern Israel).

Methods: 102 acute ischemic stroke patients were compared to 102 sex, age and country of origin matched patients admitted to different surgical departments. All the patients were interviewed about medical history, habits, socio-economic status, education, past and present occupation, income, living conditions, property ownership, medical insurance, social support. STATA was used for the epidemiological analysis and univariate and multivariate analysis, X2, t test, univariate and multivariate Logistic regression with Odd Ratio and 95% CI calculation. Results: A significant association of low socio-economic status and IS incidence was found. On univariate analysis a significant positive association was found with spouse low educational level (p=0.05), spouse employment in blue-collar occupation (p=0.047) and with low income (p=0.0017). The IS patients were less property owners (p=0.000) and lived in crowded conditions (p=0.027), without additional medical insurance (p=0.023) and with a decreased social support (p=0.037) and without friends (p=0.000). On multivariate analysis, systolic hypertension heart disease, diabetes, family history of hypertension and low income were found to be risk factors for IS.

Conclusion: Low socio-economic status is associated with an increased risk for IS. People with a low level of education and income is the target population for intervention on known medical risk factors to prevent IS.

Keywords: ischemic stroke, socio-economic status, income, risk factors

Assay of GTP hydrolysis by G-proteins

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G-proteins are transducers of a wide range of cellular transactions, including transmembrane signaling, cell proliferation, intracellular transport and modulation of cytoskeletal organization. Despite their functional diversity, all members of the G-protein family share a common regulatory mechanism, the so-called regulatory GTPase cycle. The interaction of G-protein, with GTP, causes activation of the G-protein, which is determined by the conformational state of the G-protein, which is influenced by the identity of the guanine-nucleotide in the binding site. When charged with GTP (guanosine triphosphate), the G-protein is in the "on" state, capable of acting on its downstream effectors. Hydrolysis of the bound GTP to GDP (GTPase), causes the G-protein to lose this ability, and "turns the protein off". This cycle constitutes the G-protein's biochemistry switch. In designing a GTPase assay is to enable us to determine the net
rate of GTP hydrolysis. This is not a trivial undertaking because both the on and off reactions occur simultaneously and GTP hydrolysis is not necessarily the rate-limiting step. Here we present the development of a GTPase assay that overcomes these difficulties. It allows us to investigate the effect of different molecules and protein modifications on the rate of GTPase hydrolysis by G-proteins.

Keywords: signal transduction, ras protein, one cycle GTPase assay

Computation By Ensemble Synchronization In Recurrent Networks With Synaptic Depression

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While computation by ensemble synchronization is considered to be a robust and efficient way for information processing in the cortex (C. Von der Malsburg and W. Schneider Biol. Cybern. 54: 29-40 [1986]; W. Singer Inter. Rev. Neuro. 37: 153-183 [1994]; J.J. Hopfield Nature 376: 33-36 [1995]), the neuronal mechanisms that might be used to achieve it are yet to be uncovered. Here we analyze a neural network model in which the computations are performed by near coincident firing of neurons in response to external inputs. This near coincident firing is enabled by activity dependent depression of inter-neuron connections. We analyze the network behavior by using mean-field approximation, which allows predicting the network response to various inputs. We demonstrate that the network is very sensitive to temporal aspects of the inputs. In particular, periodically applied inputs of increasing frequency result in different response profiles. Moreover, applying combinations of different stimuli lead to a complex response, which cannot be easily predicted from responses to individual components. These results demonstrate that networks with synaptic depression can perform complex computations on time-dependent inputs utilizing the ability to generate temporally synchronous firing of single neurons.

Keywords: mean field, population spike (PS), recurrent network, synaptic depression

Behavioral responses to pain of individuals with cognitive disability

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Introduction: Individuals with cognitive impairment as well as the entire population are exposed to painful medical procedures and painful conditions. However, since many of them cannot communicate verbally with the surrounding, they are frequently not treated for pain. Pain behavior scales that are not applicable in assessing pain may be used for this population. Our aim was to study whether the behavioral responses to pain differ according to the level of cognitive impairment, and whether these are in accordance with verbal reports of pain.

Methods: 108 cognitively impaired individuals (22 mild; 43 moderate; 33 severe and 22 profound) participated. They were videotaped before and during a flu vaccination. Two examiners, using two behavioral scales, analyzed pain behavior: NCCPC-R (general pain behaviors) and FACS (facial expression of pain). Subjects with mild and moderate cognitive impairment were also asked to rate their perceived pain on a VAS scale.

Results: Pain behavior, measured with NCCPC-R, was significantly increased in all groups during the injection (p<0.001-0.01), while facial expression of pain (FACS) was only significantly increased in the mild and moderate retardation groups (p<0.01). 30% of individuals with severe and profound retardation responded with a "frees" during injection compared to only 4.5% of individuals with mild to moderate retardation. There was a good correlation (r=0.76) between the VAS and the behavioral scores only in "individuals with mild retardation. In addition, there was a good to high correlation between the two methods (r=0.60-0.87) and a high correlation between the two examiners (r=0.79). Conclusion: By using NCCPC-R and FACS reliably detect changes in pain intensity in individuals with mild to moderate retardation but NCCPC-R is more sensitive to these changes compared to FACS in severe retardation. Both scales have high inter rater reliability. The response to acute pain differs according to the level of retardation.

Keywords: pain, mental retardation, pain behavior

The neurosteroid DHEA attenuates cocaine-seeking behavior in rats

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The term “neurosteroids” refers to steroids that can be synthesized de novo in the nervous system from sterol precursors. The group includes pregnenolone (PREG), dehydroepiandrosterone (DHEA), their sulfates (PREG-S and DHEA-S) and reduced metabolites such as tetrahydroprogesterone (allopregnanolone). In addition to the well known genomic effect of steroids via nuclear receptors, neuroactive steroids can also act as allosteric modulators of neurotransmitter receptors, such as GABA, NMDA, and sigma receptors, and thus affect behavior. Indeed, clinical studies in humans have associated some of these hormones with a sensation of "well-being", but also with reward-related processes, mood and motivation. Neurosteroids thus may play a role in substance abuse. In the present study, we tested the hypothesis that the neurosteroid DHEA attenuates cocaine self-administration. Rats were pretreated with either DHEA (2 mg/kg/day i.p.) or vehicle solution, and were then trained to self-administer cocaine (1 mg/kg/intake) on a fixed-ratio schedule of reinforcement, while continuing hormone treatment. DHEA significantly reduced cocaine-seeking behavior. These results suggest that DHEA affects cocaine reward. We further analyzed brain DHEA levels in non-treated rats after a single pre-treatment. DHEA levels increased in DHEA levels in different mesolimbic brain areas of rats that maintain drug-seeking behavior compared to sham-operated controls.

This suggests that changes in brain concentrations of neurosteroids may play a role in the modulation of psychological states, including cocaine dependence.

Keywords: neurosteroids, DHEA, cocaine, self-administration

Neurosteroids modulating the antidepressive activity of paroxetine

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Although it is known that selective serotonin reuptake inhibitors (SSRIs), as other antidepressants, elevate mood only after 3-4 weeks of treatment, the mechanism responsible for this delay is not understood. SSRIs have been demonstrated to alter the levels of neurosteroids such as allopregnanolone (THP) which possess anxiolytic and mood elevating properties. We compared the effect of 9 and 21 days i.p. administration of paroxetine, a potent SSRI, on the synthesis of THP and its precursor, 5α-dihydroprogesterone (DHP) in the mouse cortex, hypothalamus and olfactory bulb. Cortex, olfactory bulb and hypothalamus synthesized levels of DHEA were significantly raised after 9 days of paroxetine administration. A significant rise in the THP synthesized level was observed only after 21 days of treatment. Peripheral synthesis of DHP measured by the level in serum, significantly increased after 9 days, but reverted to normal levels after 21 days. THP was detected in serum THP levels either after 9 or 21 days' treatment. Differences in peripheral and brain synthesis indicates independence in brain synthesis. The data indicate that paroxetine administration differentially increases [3H]DHP and THP content, depending on the duration of the treatment.

Our results suggest that brain THP may be involved in the antidepressive and anxiolytic activity of paroxetine.

Keywords: paroxetine, neurosteroids, allopregnanolone (THP)

Nicotine switches on "silent" synapses in the developing hippocampus

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The hippocampus of new-born rats, increased release of glutamate rather than insertion of new postsynaptic AMPA receptors accounts for switch on silent synapses and for LTP maintenance in both CA1 and CA3 areas (Gasparini et al. Proc. Natl. Acad. Sci. USA 97, 9741-9746 [2000]). Neuronal nicotine receptors (nAChRs) activation is known to enhance neurotransmitter release. Single
fibre whole-cell EPSCs, elicited by Schaffer collateral stimulation (double pulse protocol), were recorded in pyramidal neurons in hippocampal slices from P1-P5 old rats. Bath application of nicotine (1 μM for 3 min) increased the probability of glutamate release converting silent synapses into functional. The percentage of successes to the first and second stimulation was increased by 3% and 5%, respectively, before and after nicotine respectively. This effect which was long lasting (for up to 2 hours) was prevented by the nicotine receptor antagonist mecamylamine. This effect was mimicked by activation of nAChRs following stimulation of cholinergic fibers, in the presence of atropine (1 μM) to block muscarinic receptors. This mechanism which is relevant for early LTP may be contributing synaptic contacts and for rewiring immature hippocampal circuitry.

Keywords: EPSC; nicotine; cholinergic pathway; glutamate release

Green tea polyphenol (-)-epigallocatechin-3-gallate regulates secretion of non-amyloidogenic precursor protein via protein kinase c pathway in vitro and in vivo

Keywords: EGCG; sAPPa; PKC; Alzheimer's disease

Neuroprotection and cell survival/cell cycle gene expression by green tea polyphenol (-)-epigallocatechin-3-gallate: involvement of protein kinase C activation

Keywords: Post-traumatic Stress Disorder, animal model, pre-traumatic stress disorder, anxiety, stress, maladapted, well-adapted

Exposure to acute stress blocks the induction of long term potentiation at amygdala-prefrontal cortex synapses in vivo

Most studies of stress-induced synaptic plasticity impairments have focused on the hippocampus. The present study examined whether stress, which impairs hippocampal long-term potentiation (LTP), also affects LTP at amygdala-prefrontal cortex synapses in vivo. After undergoing an inescapable stress experience, rats exhibited markedly impaired LTP without having effects on baseline transmission. In contrast, unstressed control animals showed robust LTP that persisted during the recording period. Similar to stress, LTP at the amygdala-prefrontal cortex synapses was completely blocked when the competitive NMDA receptor antagonist CPP was injected prior to the tetanus. These results demonstrate that similar to the hippocampus, amygdala-prefrontal cortex synapses is blocked after exposure to stress and the induction of LTP in this pathway is an NMDA receptor-dependent process. The present data suggest the amygdala-prefrontal pathway is amenable to long lasting plastic changes, behavioral stress and administration of NMDA-receptor antagonist CPP attenuate LTP in this pathway. Taken together these data suggest that the amygdala-prefrontal cortex pathway is relevant to stress-mediated impairments in LTP, and this pathway may have an important role in emotional memory processes.

Keywords: EGCG, apoptosis, neuroprotection, PKC

The relevance of differential response to trauma in an animal model of post-traumatic stress disorder

Post-traumatic Stress Disorder affects 20-30% of those exposed. Clinical studies employ stringent inclusion/exclusion criteria, yet animal studies include the entire exposed population as the study population. We examined the effect of grouping prestressed rats according to severity of response on the statistical analysis of results. The effects of exposure to a cat on behavioral measures in rats were demonstrated. Response severity was assessed and used to divide the animals into “diagnostic” groups. The two extremes were studied, i.e. those clearly “maladapted” and those clearly “well-adapted”, using arbitrarily selected “Cut-off Behavioral Criteria” (CBC). The middle group was discarded for reasons of clarity. The hypothalamic-pituitary-adrenal axis and heart-rate variability were subsequently analyzed for the entire exposed population and then according to the CBC. A single ten-minute exposure to a predator caused anxiety or fear-related behaviors. However, only 25.3% of exposed rats were affected. Compared to controls and to “well-adapted” exposed rats, “maladapted” rats exhibited significantly higher plasma corticosterone and ACTH concentrations, increased sympathetic activity, diminished vagal tone and increased sympathovagal balance. These differences were significantly more obvious when data were analyzed according to CBC. Animals respond to stress heterogeneously, resembling humans. Overlooking this heterogeneity may significantly affect the results of bio-behavioral data analyses, and animal models can (and probably should) be divided into distinct groups according to their response.

Keywords: Post-traumatic Stress Disorder, animal model, anxiety, stress, maladapted, well adapted

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Imaging dementia using b value q-space analyzed diffusion magnetic resonance imaging

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High b-value diffusion weighted magnetic resonance imaging is highly sensitive to white matter (WM) changes. Diffusion images, when analyzed using the q-space approach, show areas of abnormal WM in multiple sclerosis (MS) not detected by other MRI methods like T2, FLAIR, and DTI (Assaf et al., Magn. Reson. Med. 47:115-126 [2002]). When applied to vascular dementia (VaD) patients, high-b-value diffusion analyzed detected areas of abnormal WM extending further than T2 apparent hyperintensities. In the images, when analyzed using the q-space approach, show areas of abnormal WM in multiple sclerosis (MS) not detected by other MRI methods like T2, FLAIR, and DTI (Assaf et al., Magn. Reson. Med. 47:115-126 [2002]). When applied to vascular dementia (VaD) patients, high-b-value diffusion analyzed detected areas of abnormal WM extending further than T2 apparent hyperintensities. In the areas that presented hyperintense signal in the FLAIR and T2-weighted images, the WM density, as assessed with voxel images, was reduced. These observations point to extensive nerve fiber loss in the WM in VaD. Analysis of high b-value, q-space analyzed diffusion images of Alzheimer's (AD) patients also contributed information not apparent from other MRI methods. AD patients presented less marked WM density decline in the parietal lobes than VaD patient but similar WM loss in frontal areas. Interestingly, diffusion images of AD patients also showed marked effects in gray matter areas, consistent with the pathologic changes in AD that consist of accumulation of neurofibrillary tangles and senile plaques along with neuronal and synaptic atrophy. To summarize, high b-value q-space diffusion MRI shows high sensitivity to WM changes and may be useful to characterize tissues and AD, as well as to the differentiation between those two pathologies.

Keywords: Alzheimer's disease, vascular dementia, q-Space, diffusion MRI

Dexanabinol and its analog PRS-211,092 inhibit LPS-induced brain inflammation

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Dexanabinol, a nonpsychotropic cannabinoid, and its novel analog, PRS-211,092, are neuroprotective in brain ischemia induced by middle cerebral artery occlusion. Both compounds were previously shown to reduce PGE-2 secretion in LPS activated macrophages in vitro. The aim of the present study was to assess whether Dexanabinol and PRS-211,092 inhibit central neuroinflammation. C57BL mice were injected intra-cerebro-ventricularly (i.c.v.) either with 250 ng LPS (dissolved in 5μl PBS) or 5μl PBS as sham. The mice were immediately injected IP with either cremophor-ethanol (vehicle) alone, Dexanabinol (20 mg/kg) or PRS-211,092 (20 mg/kg). The mice were killed under anesthesia 24 hours and 3 days after LPS injection. IL-1β gene expression was determined in brains extracts from animals killed at 24 hours using RT-PCR. Analysis of activated microglia was performed after 3 days using immunohistochemistry. Treatment with Dexanabinol or PRS-211,092 reduced the number of microglia immunoreactive cells surrounding the hippocampus by 40% and 47% respectively, compared with that in animals treated with vehicle alone. IL-1β mRNA level was reduced by 50% in the brains of mice treated with PRS-211,092. Similar results were obtained in LPS induced mouse macrophages (RAW 264.7). These results show that Dexanabinol and PRS-211,092 can inhibit CNS microglial activation as well as IL1β gene expression, properties that may play an important role in the neuroprotective effect of these compounds in ischemia and brain injury.

Keywords: inflammation, gliosis, gene expression

Interaction of syntaxin 1A and SNAP-25 with the C-terminus of the voltage-gated potassium channel Kv2.1 underlies their functional effects on the Kv2.1 current

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The voltage-gated potassium channel, Kv2.1, showing a delayed rectifying-type of current, exhibiting slow inactivation, is widely distributed in neuroendocrine and endocrine cells. Previously we showed that Kv2.1 physically interacts with SNAP25 proteins, particularly, with syntaxin 1A and SNAP-25. We also described the functional implication of the interaction in Xenopus oocytes. Here we present further investigations that attempt to elucidate the structural basis of such interactions. We found that nearly complete deletion of the C-terminus of Kv2.1 channel (delC416) abolished Syntaxin 1A induced left shifts of the channel steady state activation and inactivation curves. It also abolished SNAP-25 induced increase of the sustained current and right shift of the current-voltage curve. The slowing down of the onset of inactivation by SNAP-25 disappeared too. Similarly to delC416, deletion of the last two-thirds of the C-terminus (delC351) abolished the effects of syntaxin on the curve, but in contrast to delC416, only partially affected the effects of SNAP-25, diminishing the increase in the sustained current without significant effect on the steady-state inactivation curve. Based on these data we suggest that: i) syntaxin 1A interacts with the proximal half of the C-terminus; ii) SNAP-25 has two sites of interaction, one, high affinity, at the proximal half, deletion of which cancels the right shift of the steady-state inactivation curve; iii) SNAP-25 binds to the high affinity, at the distal half of the channel, responsible, mainly, for the sustained current effect. These data concur with in vitro binding assays obtained in our lab (see the abstract of S. Tsuch). Keywords: Kv channels, syntaxin 1A, SNAP-25, C-terminus, SNARE complex

Fusion of speech elements: Early auditory cortex involvement

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Purpose: To define the auditory cortex's capacity to fuse of speech elements, and suggest plausible speech processing stages.

Methods: Stimuli were monaural formant transition and base, that were leading to absolutely or partially formed vowel-consonant-vowel-syllable /ada/. Right-handed, adult, native Hebrew speakers listened to 2/sec presentations. Brain potentials from Cz during the from CV transition onset to the responses to transition, to the fused word and to base alone were recorded. The net-fusion response was extracted by subtracting the sum of potentials to the base and the formant transition from the potentials to the fused sound.

Results: Auditory middle-latency components (20-45 msec), comprising of nine peaks and troughs were recorded in response to the base, to the formant transition and to the fused /ada/. In general, the responses to the fused object were significantly smaller in peak amplitude and in total activity (area under the curve) resulting in the difference waveform of the net-fusion response that also included 9 peaks, but with opposite polarities.

Conclusion: auditory cortex is involved in the definition and clustering of sounds elements as speech, as early as 30 msec after stimulus onset. This early processing involves both inhibition and occlusion, and precedes the later stages of discrimination and meaning analysis.

Keywords: auditory object, streaming, cortex

In vivo two-photon imaging of dendritic stability in identified glomeruli of the mouse olfactory bulb

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Ongoing sensory neuron and granule cell neurogenesis results in turnover of both excitatory and inhibitory inputs to mitral cells, the main output neurons of the mammalian olfactory bulb. To evaluate the long-term stability of mitral dendrites in the face of these ongoing changes, we used two-photon microscopy to image mitral cell dendrites over prolonged periods in adult mice in vivo. Using transgenic mice expressing Yellow Fluorescent Protein in roughly 1/3 of the mitral cell population (Feng et al., Neuron 28:41-51 [2000]), we imaged the same dendritic trees in identified glomeruli over intervals of up to two weeks. We first examined the stability of mitral dendrites over short and long periods. Randomly chosen areas of the dendritic arbors were reconstructed in three dimensions and compared at different time intervals. The overall arborization of dendrites and their dimensions and compared at different time intervals. At the end of the second week, all but the smallest distal dendritic segments remained extremely stable over periods of hours, days and weeks (imaging intervals: 1 hour, n=3; 24 hours, n=6; 2 weeks, n=3). We next used intrinsic signal imaging of the dorsal surface of the olfactory bulb to identify individual glomeruli activated by a specific odorant. The sizes of glomeruli stimulated by 0.1% butanal (n=5) were almost identical to the glomerular sizes imaged by the smallest distal dendritic segments with somewhat reduced signal intensity. Given that dendritic stability of mitral cells serves as a solid baseline in control mice, we are currently using this combined approach to assess the effects of odor exposure and odor-based learning on morphological stability in identified glomeruli.

Methods: Stimuli were monaural formant transition and base, that were leading to absolutely or partially formed vowel-consonant-vowel-syllable /ada/. Right-handed, adult, native Hebrew speakers listened to 2/sec presentations. Brain potentials from Cz during the from CV transition onset to the responses to transition, to the fused word and to base alone were recorded. The net-fusion response was extracted by subtracting the sum of potentials to the base and the formant transition from the potentials to the fused sound.

Results: Auditory middle-latency components (20-45 msec), comprising of nine peaks and troughs were recorded in response to the base, to the formant transition and to the fused /ada/. In general, the responses to the fused object were significantly smaller in peak amplitude and in total activity (area under the curve) resulting in the difference waveform of the net-fusion response that also included 9 peaks, but with opposite polarities.

Conclusion: auditory cortex is involved in the definition and clustering of sounds elements as speech, as early as 30 msec after stimulus onset. This early processing involves both inhibition and occlusion, and precedes the later stages of discrimination and meaning analysis.

Keywords: auditory object, streaming, cortex
Key words: olfactory bulb, dendrites, two-photon microscopy, transgenic mice

Pathogenic self-antigens in autoimmune disease are potentially protective: A lesson from the visual system

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Axonal injury in the central nervous system (CNS) leads to primary and secondary degeneration of the fibers, with resulting death of the corresponding cell bodies. Our laboratory recently discovered that CNS myelinated axons, after suffering a mechanical insult such as a crush injury, can benefit from the activation of T cells directed against myelin antigens (self-antigens associated with an autoimmune disease in the brain and spinal cord). Using a rat model of optic nerve crush or glutamate toxicity, we show here that vaccination with peptides derived from either interphotoreceptor retinoid-binding protein or s-antigen (a retinal self-antigen that can cause experimental autoimmune uveitis) reduces retinal ganglion cells (RGCs) loss resulting from either glutamate toxicity or axonal injury. In the case of glutamate insult, no such protection was observed by vaccination with myelin antigens. These results suggest that as in the case of myelinated antigens, the protective antigens for RGCs is identical to the self-pathogen associated with the common autoimmune disease in this tissue (i.e. uveitis). Based on our results we propose a more general phenomenon: pathological immunity in autoimmune diseases may represent the potential protective autoimmune-evoking antigens.

Keywords: autoimmune, neuroimmunology, vaccination, glaucoma, optic neuropathy

Functional properties of the anterior calcarine cortex in the human

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Retinotopic mapping of the human visual cortex has provided detailed information on the sequence of visual areas located ventrally and dorsally to area V1. However, little information is available concerning the functional properties of extra-striate cortex situated anterior to area V1, along the medial wall of the occipital lobe. Here we used Functional Magnetic Resonance Imaging (fMRI) on 15 different subjects, in 5 experiments, in an attempt to define the functional profile of a region we term Anterior-Calcarine (AC) cortex. AC cortex is situated anterior to area V1, and is predominantly located in the ventral and dorsal banks of the calcarine sulcus. This region manifested positive fMRI signals in certain experimental conditions and negative fMRI signals in others. Conditions in which positive fMRI signals were observed included: visual field stimulation, preferential activation to images of building compared to images of faces, and a preference to real-life photographs compared to line-drawings. Negative (inactivation) signals were found for foveal/mid eccentricity visual stimulation, and auditory stimulation by sounds of various objects (both man made objects and animal voices). No consistent meridian maps were observed in the AC. Finally, this region manifested a highly non-linear temporal signal summation so that a four fold increase in the frequency of presentation of visual images led to only a 1.26 + 0.13 fold increase in signal strength. We hypothesize that the AC is a high order area involved in the representation of the peripheral, ambient environment. Supported by ISF 8009.

Keywords: anterior calcarine, visual cortex

Glycogen synthase kinase (GSK)-3 in postmortem hippocampus of schizophrenic patients

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GSK-3 is a protein kinase highly abundant in brain and involved in signal transduction cascades, particularly in neurodevelopment. We have shown that in frontocortical regions obtained postmortem GSK-3β is decreased at the level of protein (41%), activity (45%) and mRNA (34%) in tissue from subjects with schizophrenia (postmortem brains were donated by the Stanley Medical Research Institute in Collection, along with a finding that has been replicated by others at the protein level (Beasley et al., 2001. Neurosci Lett 302:117-20). It has also been shown that GSK-3β is not reduced in occipital cortex of schizophrenic subjects suggesting that the loss of this protein are regional specific. We are now extending these studies to a number of other regions of CNS using tissue from the Autism Research Laboratories, Victoria, Australia, and hippocampus from 15 pairs of postmortem specimens from schizophrenic and controls subjects was studied blind to diagnosis. GSK-3β protein levels were measured using Western blot and GSK-3β activity using in vitro phosphorylation of a GSK-3 specific substrate phospho-CREB by γ[-P]-ATP. These studies do not find that GSK-3β protein levels are altered in the hippocampus from subjects with schizophrenia although there is a trend to a reduction (31%) in GSK-3 activity in that region. We are currently studying the frontal cortical specimens from the same subjects.

Keywords: schizophrenia, glycogen synthase kinase-3, hippocampus, neurodevelopment

Dynamics of cortical responses to tone pairs in relation to task difficulty

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When sounds are presented in pairs with intervals of < 300 ms, the 100-ms cortical response N100m to the 2nd stimulus is enhanced (Loveless et al., 1990). We studied the mechanisms underlying this enhancement effect and its relationship to behavioral performance and task difficulty. Whole-scalp magnetoencephalographic (MEG) signals were recorded from 10 subjects with a 306-channel neuromagnetometer. Sequences of 5 pairs of 50 ms binaural tones were presented with stimulus-onset asynchronies of 150 ms, inter-pair intervals of 1 s and an inter-sequence-interval of 10 s. Subjects were asked to discriminate inter-pair frequencies under 2 conditions: easy (1000 vs. 1100 Hz) and difficult (1040 vs. 1055 Hz). In 50% of the pairs the tones were of the same frequency. All stimuli elicited prominent N100m responses in the AC, peaking at 105 ± 2 ms to the 1st tone and at 155 ± 15 ms to the 2nd. The response to the 1st tone in a pair decreased by 46 ± 5% from the 1st to the following pairs. In contrast, the response to the 2nd tone in a pair gradually increased in amplitude from the 2nd to the 5th pair. Consequently, the ratio of the 2nd and 1st responses increased throughout the five pairs from 0.73 ± 0.07 to 1.23 ± 0.05 (p < 0.02). Task difficulty did not affect the response amplitudes, whereas the response latencies to the 2nd stimulus were 11 ± 4 ms longer for the difficult condition across pairs and hemispheres (p < 0.02), the difference was most prominent (25%) in the 5th pair. Response N100m related to inter-pair frequency discrimination. Task difficulty did not affect the response latencies to the 2nd stimulus was similar to control cells while Munc18 phosphorylation enhanced vesicle pool refilling.

Munc18 phosphorylation enhances vesicle pool refilling

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During repetitive or intense stimulation, neurosecretory cells and neurons undergo use-dependent changes in secretory strength, which are partially mediated by activation of protein kinase C (PKC). However, the molecular targets of PKC and their mode of action remain elusive. In the present study we investigated the effect of Munc18 phosphorylation on catecholamine secretion from chromaffin cells by measuring capacitance measurements following flash photolysis of caged calcium. Munc18 is phosphorylated by PKC in a calcium-dependent manner, which inhibits an interaction with Syntaxin. By mutating the three PKC phosphorylation sites of Munc18 we created the Munc18-3A mutant, which mimics the non-phosphorylated form of the native protein. Overexpression of Munc18-3A enhanced vesicle recruitment under high calcium concentration, similar to the effect of wild-type Munc18 overexpression. In contrast, the effect of a second stimulation was similar to control cells while Munc18...
wild-type overexpressing cells exhibited a larger response. This suggests that vesicle pool refilling is reduced in Munc18-3A overexpressing cells. As was shown previously, during the flash stimulation, high calcium levels activate PKC, which acts on specific targets to enhance vesicle pool refilling. The reduction in vesicle pool refilling observed in the Munc18-3A cells as compared to Munc18 wild-type cells suggests that Munc18 may be one of these targets. Application of phorbol ester demonstrated that PKC in Munc18-3A overexpressing cells could not enhance secretion but only control. These results indicate that phosphorylation of Munc18 in chromaffin cells potentiates vesicle recruitment after emptying of the releasable vesicle pools.

Keywords: Munc18, PKC, secretion, membrane capacitance

Distinct neural activity following an invalidly cued target in a cued attention task

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The cued attention task involves presentation of a cue stimulus, which provides information to direct attention to a target stimulus that follows, to which the subject has to respond. In the minority of cases the cue provides erroneous information about the target (invalid cue). The cue validity effect on brain response to the target was analyzed in the current study, and the brain areas involved were estimated. In previous studies, a P3 component in response to the target was not found. Using a modified cued attention paradigm in the current study, a P3 component was evoked. The distinct neural activity following an invalidly cued target, which provides information to direct attention to a target (invalid cues). The cue validity effect on brain activity (current density) following invalidly cued targets. Those areas include attention related areas (BA), as well as emotion related areas (BA), and, surprisingly, also visual cortex. In summary, distinct and significant activation was found, following identical stimuli, depending on whether they were preceded by invalid compared to valid cues. The additional brain activation in the invalid case may be related to emotional processes, attention shift, and spatial updating.

Keywords: Evoked Potentials, attention, P3, LORETA

Time and the Learning of a Cognitive Skill: The Spacing of Practice Sessions Across Days Improves Performance

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Apolipoprotein E (apoE), the most abundant lipoprotein in the brain occurs as three allelic forms termed E2, E3 and E4, which differ from each other by a single amino acid substitution. Genetic and epidemiological studies revealed that the allele E4 (apoE4) is a major risk factor of chronic neurodegenerative diseases such as Alzheimer's disease (AD) and of acute insults such as head injury. The mechanisms underlying the isoform specific pathological effects of apoE4 and the molecular structural features which differentiate between apoE4 and the other apoE alleles are not known. The present study was directed at the development of mAbs which react specifically with apoE4 in its native form and at their use, together with mAbs which react equally well with the different apoE isoforms, for structural analysis of the apoE molecule. The first approach used was to immunize apoE knockout mice which express human apoE within Y chromosome. The approach yielded several mAbs which bound specifically to apoE4 in ELISA experiments but whose binding characteristics varied in different preparations. In order to overcome this problem we undertook an alternative approach. Fresh rat brains synaptosomes were incubated with apoE specific and panapoE mAb, and the mAbs were isolated and affinity purified. The mAb specific and panapoE mAbs thus obtained were described and their application for structural and functional studies of apoE will be discussed.

Keywords: apolipoprotein E, monoclonal antibodies, Alzheimer's disease, neurodegeneration

The metabolotropic glutamate G-protein-coupled receptor mGluR3 is voltage sensitive

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G-protein coupled receptors (GPCRs) comprise the largest superfamily of proteins in mammalians and are involved in most signal transduction processes as well as in regulation of many fundamental processes such as release of neurotransmitter from nerve terminals. In spite of GPCRs being transmembrane proteins they are not considered, unlike voltage gated channels, to be voltage sensitive. However, it was shown that muncaric receptors, GPCRs, bind ACh in a voltage dependent manner (Ilouz et al, J Biol Chem, 274: 29519-29528 [1999]). Here, we examined whether mGluR3, a preynaptic autoreceptor that mediates feedback inhibition of glutamate release in CNS, exhibits voltage sensitivity. Using fresh rat brain synaptosomes we show that preynaptic glutamate receptors at their physiological environment, similarly to the muncaric receptors, bind glutamate in a voltage dependent manner. Depolarization reduces the maximal binding of [3H]Glu several folds and further analysis revealed that depolarization reduced the fraction of the high affinity glutamate receptors. The experiments with synaptosomes could not discern whether it is the mGluR3 or the NMDA receptors (or both) that is responsible for the voltage dependent binding. However, the results indicate that the mGluR3 toward glutamate was reduced upon depolarization. The results presented here are compatible with the notion that the mGluR3 is by itself voltage sensors.

Keywords: G-protein coupled receptors, metabolic glutamate receptors, voltage sensitivity

Preparation and characterization of monoclonal antibodies directed specifically at the apolipoprotein E isoform

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Aplysia neurons lead to a transient and local elevation of the free intracellular Ca2+ concentration, calpain activation, local protein degradation, the submembrane spine thinning and growth cone formation. Inhibition of calpain by calpeptin prior to axotomy inhibits growth cone formation. Here we investigated the mechanisms by which calpain activation participates in the transformation of an axonal segment into a growth cone that end we compared the ultrastructural alterations induced by...
axonotomy of cultured Aplysia neurons performed under control conditions and in the presence of calpeptin. We identified critical slowdown-dependent cytokinesis, a segment of the transacted axon. This compartment "traps" transported vesicles and serves as a locus for microtubule polymerization. As a result, a pool of vesicles accumulates in close proximity to a segment of the plasma membrane along which the spectrin membrane skeleton was protelyzed by calpain. We propose that this facilitates the fusion of vesicles with the plasma membrane, promoting the extension of the growth cone's lamellipodium. The growth process is further supported by the radial polymerization of microtubules from the growth cone's center.

Keywords: axonotomy, growth cone, microtubules, calpain, regeneration

Opposing effects apoE3 and apoE4 on APP metabolism following closed head injury

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This study examined the possibility that the pathological effects of apoE4 can be reversed by the beneficial effect of apoE3 on APP metabolism following closed head injury (CHI). We investigated the role of apoE on APP metabolism in vitro and in vivo. APP metabolism was assessed using a novel biochemical assay. This assay allows for the determination of APP metabolism in the presence of apoE. We found that apoE3 and apoE4 have opposing effects on APP metabolism. In vitro, apoE3 increased APP metabolism whereas apoE4 decreased it. In vivo, apoE3 and apoE4 had opposing effects on APP metabolism. In the absence of apoE, CHI induced a significant increase in APPs. However, in the presence of apoE, CHI induced a significant decrease in APPs. These findings suggest that apoE has opposing effects on APP metabolism following CHI.

Keywords: apoE, APP, CHI, neuroprotection

Interhemispheric perceptual learning transfer depends on task difficulty

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We previously found hemispheric differences in feature search (Pavlovskaia et al. Spatial Vision 14,151-73, 2001) when search arrays were in one or the other hemifield, rather than when the array was central and target elements lateral central. In parallel, Ahissar & Hochstein (Nature, 387, 401-6, 1997) found perceptual learning transfer across position or orientation depends on task condition. Learning effects transfer when the task is easy (large target-distractor difference; limited target position uncertainty; long test-to-mask delay) and are considerably specific when conditions are harder. These easy vs. hard condition differences were related to cerebral sites of training modification: hard tasks were seen as requiring low-level (specific) representations which were not modified when performing high cortical level mechanisms alone. We ask if inter-hemispheric transfer also depends on task difficulty, since high-level receptive fields include contra-lateral areas. Subjects performed a color search task with either tight or broad lateral targets, each within one hemifield. Sessions were half easy, half hard. Following training, we switched the sides of color and orientation tasks, and found nearly complete transfer for easy conditions, and considerably less with difficult conditions. Our results support the conclusion that easy search depends on high cortical level mechanisms, while hard search is performed by guided return to low-level condition discrimination. Practice improves performance by modifying high or low levels, accordingly. Thus, easy task performance and learning take place at a cortical area sufficient high that its receptive fields are not limited to a single hemifield.

Keywords: visual search, perceptual learning, inter-hemispheric transfer

Neuroscience the science for psychiatry

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Psychiatry was the only medical discipline that had no scientific foundation until recent years. Biological psychiatry began approximately half a century ago, when the effects of corticosteroids on psychiatric symptoms were discovered. The so called "biological" effect of medication on mood affect and behavior initialized an enthusiastic surge of biological investigations in psychiatry. Research was directed to brain biochemistry and biochemistry of neurotransmitters and receptor alterations relevant to medications and their clinical effects. This trend of biological psychiatry is at its peak even nowadays. Our interest is in psychiatry with molecular biology education who advance your career enormously. Disappointing however are the advances for the patients. Even though patients' symptoms have improved, patients are not anywhere near real cure conditions, especially if serious mental disorders such as schizophrenia are targeted. Both the molecular biology as well as the genetic research findings failed to approach the big picture of mental disorders. To put the findings of biological psychiatry in any useful context the brain as a dynamic organization of neural networks has to be addressed. Cutting-edge thinking about mental disorders point to neuroscience as the future science for psychiatry. Mental disorders are thus disturbances in the organization of complex dynamic interacting cell ensembles and ever-changing neuronal networks spread in the brain. The future of psychiatry will require understanding of complex non-linear dynamics. Concepts such as criticality and optimizations, that are currently alien to psychiatrists will be the basis of understanding mental disorders. Instead of a hereditary nomenclature such as "schizophrenia" in an ineffective low-reliability diagnostic system, futuristic psychiatry will diagnose "optimization breakdown" in the brains of patients. Psychiatry treatment will be directed toward re-optimization of neural brain systems for real cure of mental illness.
Keywords: psychiatry, complexity, system theory, functional connectivity

Observation of spontaneous cortical layer activity using physiological MRI noise

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Measuring the functional magnetic resonance imaging (fMRI) blood-oxygenation level dependent (BOLD) signal non-invasively from different layers of the cortex can expand our understanding to the current debate in the fMRI community regarding the neuronal source of the BOLD signal, since the cortex is composed of six layers that differ in their cell body density. Using MRI physiological noise we measure the BOLD fluctuation of the cortical layers during rest. As a model we use the rat’s visual cortex, which has been extensively studied using electrophysiology methods. Two different statistical approaches were used. In the first, for each voxel temporal SD were calculated and averaged together. In the second, the area spatial SD were calculated and averaged along time. Generally, the first approach measures the level of spontaneous neuronal activity, while the second approach measures the homogeneity behavior of the neuronal compartments. Our results show high temporal fluctuations in the deeper layers, indicating high spontaneous activity in these layers and low spatial fluctuations in these layers, indicating high homogeneity behavior: These results are in agreement with previous electrophysiology studies, and demonstrate that the physiology MRI noise is sensitive enough to detect localized neuronal activity and neuronal homogeneity behavior.

Keywords: cortical layers, BOLD, fMRI

Early coassembly of KCNQ1 with KCNE1 or Yotiao but not with an LQT5 KCNE1 mutant prevents channel inhibition by a tetramerization peptide

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The β4 potassium channel complex consists of the heteromeric α and β subunits. Here we show that co-expression of WT KCNQ1 with CAD, a KCNQ1 C-terminus peptide, increases both α and β subunits with KCNQ1 and KCNE1, respectively. Mutations in KCNQ1 and KCNE1 genes produce the long QT (LQT) syndrome, a human genetic cardiovascular disease. We recently identified a small region in the KCNQ1 C-terminus (aa 589-620), which functions as an assembly domain for KCNQ1 α subunits. Here we show that co-expression of WT KCNQ1 with CAD, a KCNQ1 C-terminus peptide (aa 589-620), rescues KCNQ1 channel activity CAD inhibits KCNQ1 currents probably by inhibiting the tetramerization assembly of WT subunits as it encompasses the channel assembly domain and a helix structure that is part of a voltage-sensor domain. Confocal immunocytochemistry and images confirm the virtual absence of KCNQ1 expression in the plasma membrane. Co-expression of WT KCNQ1 with WT KCNQ1 and CAD, fully restores the KC channel activity and the plasma membrane labeling of KCNQ1 and KCNE1 proteins. Conversely, a naturally occurring LQT5 mutation of KCNQ1 (W87R), located at the C-terminus, is unable to fully restores neither functional K+ currents nor the membrane localization of the channel subunits. Yotiao, an adaptor protein that binds to the same KCNQ1 assembly domain could restore like other β subunits (KCNE2 and KCNE3) functional K+ currents when co-expressed with WT KCNQ1 and CAD. In all, these data suggest that the early co-assembly of KCNQ1 with KCNE1 or Yotiao, but not with an LQT5 KCNQ1 mutant (W87R), prevents channel inhibition by the CAD tetramerization peptide.

Keywords: K+ channels, assembly, KCNQ1

Meclofenamic acid and 1-EBIO, novel KCNQ2/3 potassium channel openers: therapeutic implications for epilepsy and neuroprotection

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Voltage-dependent K+ channels play a major role in brain functions. Among them, the M potassium current has profound effects on brain excitability as its low-threshold gating and its subsequent inactivation and deactivation act as a brake for neuronal firing. Recently, the KCNQ2/3 channel complex belonging to the KCNQ family of voltage-dependent K+ channels was identified as the site of the M current. Furthermore, the KCNQ2 and KCNQ3 channel α subunits are mutated in families with benign familial neonatal convulsions, a neonatal form of epilepsy. In this study, we characterize two novel openers of KCNQ2/3 channels, meclofenamic acid and 1-EBIO. Using CHO cells transfected with the KCNQ2/KCNQ3 cDNAs and the patch-clamp technique, we found that meclofenamic acid and 1-EBIO activate KCNQ2/3 channels, by causing a negative shift in the voltage dependence of activation (-23 mV and -8 mV, respectively). In addition, both compounds slow down the channel deactivation kinetics. These openers increase the channel activity amplitude at physiologically relevant potentials (-70 mV to 0 mV). Recording of membrane potential in Xenopus oocytes expressing KCNQ2/3 channels indicates that incubation with 30 μM meclofenamic acid and 1-EBIO for 1 min produces a hyperpolarization from a resting potential of -62 mV to -78 mV. Interestingly, these openers potently and reversibly blocked the evoked and spontaneous firing of rat cortical neurons. In view of the crucial role of KCNQ2 and KCNQ3 channel subunits in epilepsy and neuronal excitability, enhancement of KCNQ2/3 potassium currents by these openers may prove to be an important target for future anti-epileptic and neuroprotective therapy.

Keywords: K+ channels, epilepsy, KCNQ1

Visual deficits associated with object images revealed by fMRI in human amblyopia

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Amblyopia is a visual developmental disorder characterized by abnormal foveal vision due to aberrant early visual experience. In normal subjects foveal vision is linked with the representation of faces (Levy et al. Nature Neurosci 2001). Here we explored possible relationship between the amblyopic deficit and fMRI activation to various object categories. Nine unilateral amblyopic and 3 healthy subjects with normal vision were studied. Three fMRI experiments were conducted using red-green filters for monocular stimulation: (1) Retinotopic mapping of the sound eye (2) Presentation of small and large pictures correlating to visual acuity of 6/6 and 6/60 respectively (3) Line drawings of faces and houses of equal size presented to each eye. In low order visual areas (e.g. retinotopic) the sound eye’s activation was similar to normal subjects. However, in the amblyopic eye, the activation for small pictures was markedly reduced compared to the sound eye, while large pictures’ activation was only slightly reduced. In high order visual areas the sound eye’s activation was similar to normal subjects while the amblyopic eye showed marked activation reductions that appeared to be more emphasized in the fusiform gyrus compared to the collateral sulcus. We conclude that abnormal early visual experience resulting in amblyopia affects in a selective manner the central representation of objects both in low and high order visual cortex regions.

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Keywords: amblyopia, visual system, fMRI

Improving vision in adult amblyopia by perceptual learning

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Practicing certain visual tasks leads to a significant improvement in performance, a process termed “perceptual learning”. Learning was shown to be specific for basic stimulus features such as local orientation, retinal location and eye of presentation, suggesting plasticity of the primary visual cortex in adults. However, it has never been shown that such a low-level learning may have impact on higher-level visual tasks, like recognition. Amblyopia is characterized by several functional abnormalities in spatial vision, including reduction in visual acuity (VA), contrast sensitivity function (CSF), and vernier acuity, as well as spatial distortion, abnormal spatial interactions and impaired contour detection. The visual deficits are thought to be irreparable after the first decade of life once the developmental maturation process is terminated. The visual loss is thought to be due to abnormal operation of the neuronal networks within the primary visual cortex, most notably, orientation selective neurons and their interactions. The perceptual learning procedure described here was designed to train this network by efficiently stimulating these neuronal populations and effectively promoting their...
spatial interactions. Here, using a systematic low-level training of a malfunctioning adult visual system, we show that inducing low-level changes yield significant perceptual benefits and transfer to higher visual tasks. The training procedure produced a two-fold improvement in CSF and letter recognition tasks in the treatment group (N=63). No improvement found in the control group (N=14). The results demonstrate that perceptual learning can improve basic representations within the adult visual system that did not develop during the critical period.

Key words: vision, perceptual learning, development, amblyopia, lateral interactions

The adverse effects of repeated intraperitoneal injections are reduced by a chronic treatment with the antidepressant drug imipramine

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Previous research suggested that repeated handling and intraperitoneal (i.p.) injections constitute a chronic stressor, producing depressive-like physiological consequences. This present study was aimed to characterize further the effects of repeated saline injections in SJL/J mice, particularly in the context of experimental autoimmune encephalomyelitis (EAE). Mice were injected daily with saline (10 mg/kg) for 3 weeks or not handled. Alterations in body weight, spleen weight and blood interleukin-1 (IL-1) protein levels were measured, as well as the mortality rate and susceptibility to the induction of EAE by myelin auto-antigen. Repeated injections were associated with lower body weight and higher levels of IL-1. No effect on spleen weight was recorded. Following immunization with PLP, a common myelin auto-antigen, repeated injections were presented with aggravated clinical symptoms of EAE and higher mortality rate. Chronic treatment with daily injections of the tricyclic antidepressant drug imipramine (10 mg/kg/day) completely reversed the effects of repeated injections on body weight and EAE-associated mortality, and significantly attenuated the effects on IL-1 production and EAE-associated symptoms severity. Oral administration of imipramine (10 mg/kg/day dissolved in the drinking water) had no effect on non-injected EAE mice, indicating that the effects of imipramine on EAE were limited to counteracting the consequences of repeated injections. In conclusion, repeated injections in mice are associated with imipramine-suppressible adverse physiological consequences that may be related to higher production of pro-inflammatory cytokines. These results highlight the importance of control non-handled animals in any study design consisting of repeated i.p. injections.

Keyword: repeated injections, stress, experimental autoimmune encephalomyelitis, interleukin-1

Chronic treatment from adolescence with TV-3326, a brain-selective MAO-cholinesterase inhibitor, abolishes hyperactivity in prenatal-stressed rats

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Chronic treatment with tricyclic antidepressants (TAD) and monoamine oxidase (MAO) inhibitors may be as effective as benzodiazepines in the treatment of chronic anxiety states (Fearghner, J. Clin Psychiatry 60: 22185-22225 [1999]). Studies in animals in which anxiety was induced by acute stress and was sensitive to benzodiazepines failed to demonstrate an antidepressant effect of TADs. Chronic hyperanxiety and depressive-like behaviour can be induced in primates and rodents by prenatal stress. The aim of the present study was to see whether amitriptyline and TV-3326, a novel MAO-cholinesterase inhibitor, had antianxiety effects in the model. Male and female offspring [PS] of rats (at least 9/group) that had been stressed by daily restraint during gestation and of control rats, were given water, amitriptyline (4.5 mg/kg/day) or TV-3326 (17 mg/kg/day) in the drinking water from the age of 6 to 12 weeks. TV-3326 inhibited MAO-A and B in the brain by 70% and 60%, respectively. PS and control rats were tested in the elevated plus-maze, a validated test for anxiety. TV-3326 selectively increased the time spent by PS but not C rats in open arms from 11.9±3.4 to 75.4±15.7 sec (P<0.001), indicating a depressive-like effect in anxiety. It was only possible to detect an anxiolytic effect of amitriptyline in PS rats 2 weeks after cessation of treatment, when its depressive effect on exploration in C rats under continuous treatment was no longer seen. In conclusion, chronic administration of TV-3326, selectively abolishes hyper-anxiety induced by prenatal stress without suppressing behaviour of controls.

Keywords: antidepressant, chronic anxiety, prenatal stress, plus-maze

Reversible internalization of voltage gated channels accompany brefeldin A-induced structural remodeling of cultured Aplysia neurons

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Eukaryotic cells constitutively internalize the plasma membrane. Nevertheless, the morphology and membrane properties of the cells are maintained constant by compensatory constitutive exocytosis. Recently, we reported that the neurites and axons of cultured Aplysia neurons are internalized within 24-48 hr of S(H)gufel brefeldin A injection (Prager-Khoutorsky and Spira 2000, Neuroscience Letters supplement 55, S43). When brefeldin is washed away, the Golgi apparatus reassembles, and the neuron extends new neurites. Here we report that the passive and active membrane properties of the neurons undergo alterations during these restructuring processes. Control experiments revealed that both the cell body and axons are excitable and that the action potentials are not blocked by BFA. We found, that for as long as the axons are not totally internalized (in BFA) depolarization of the neuron evokes action potentials. Nevertheless, when the axons are internalized depolarization of the formal excitable cell body fails to generate action potentials. We thus conclude that the cell body looses its excitability during the axon’s membrane internalization. Cytochalasin B inhibits the effects of BFA. Under these conditions the formal excitability and excitability are not altered. When BFA is washed and the naked cell body begins to extend growth cones, depolarization generates normal action potentials. These observations suggest that the newly formed vesicles carry to the plasma membrane the normal repertoire of voltage-gated channels to generate action potentials. We conclude that regulation of the balance between constitutive exocytosis and endocytosis leads to remodeling of the morphology and the biophysical properties of adult neurons.

Keywords: neuroplasticity, Golgi, endocytosis exocytosis, Aplysia

The effect of external noise on word span

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Previous studies documented strong correlations between auditory perception and working memory in the general population, learning impaired subjects and hearing impaired individuals. We hypothesized that the items retained in working memory are those perceived with sufficiently high signal to noise ratio, with whole words as default candidates. When signal to noise ratio is low, either due to external or to internal noise, the items retained in memory will be sub-word elements and consequently word span will decrease dramatically. We tested this hypothesis in the general population. Subjects were asked to recall sequences of 1-6 two-syllable pseudowords that were presented in quiet and in noise. For the majority of subjects the addition of noise decreased single word repetition accuracy by 10-20%. For these subjects, word span in noise was significantly lower than one would expect had span (memory) and repetition accuracy been independent. When sequences were longer than two words their repetition accuracy dropped dramatically. These results are consistent with our hypothesis that decreased perception (signal to noise ratio) affects level of representation in working memory and consequently decreases memory span.

Keywords: speech perception, verbal memory span, signal to noise ratio

Linking morphology and homeostatic synaptic plasticity using a CAC1 pyramidal neuron model

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A recently discovered plasticity effect, termed 'homeostatic plasticity', takes place when synaptic conductance is adjusted to maintain what seems to be a stable level of activity of the cell. This process is much slower than LTP/LTD (on the order of hours to days) and has been found to not correlate with the level of depolarization. In this study, a detailed model of a CA1 neuron was used to explore the impact of dendritic
morphology on synaptic homeostatic plasticity. The model consists of numerous excitatory synapses that are evenly spread on the dendrites, together with uniformly distributed active channels, giving rise to back-propagating action potentials. Synapses were activated by synaptic inputs. A simple rule was applied to iteratively scale synaptic conductance so as to keep the local dendritic membrane potential at a predetermined level. The synapses progressively reached an average steady state conductance with a bell-shaped profile along the apical dendrites, peaking at about midway from soma to distal tufts. This is reminiscent of recent findings on the distribution of synaptic conductance in CA1 neurons. In the shorter basal tree, only the initial increase in synaptic conductance with distance from soma was apparent. We demonstrate that there is an interesting interaction between specific dendritic morphology, membrane excitability and synaptic plasticity. Together, they shape local voltage perturbation as well as spatial interaction (the "region of influence") of one synapse on others; this in turn affects the global activity of the neuron (e.g., axonal spikes). In consequence, very local plastic rules result in a global cooperative effect.

Keywords: synapse, plasticity, homeostasis, morphology

Protection against soman-induced brain damage and cognitive deficits by an antidotal treatment with anticholinergic and antilutamagetic agents

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The protective effects of post exposure antidotal treatments combined with pyridostigmine pretreatment on the deleterious consequences of soman poisoning were studied. TAB (a mixture of TMBA, atropine and benactyzine) was injected concomitant with the development of toxic signs whereas scopolamine (0.1 mg/kg) or caramiphen (10 mg/kg) were administered five min after soman (1.2 LD50). Atropine (4 mg/kg) was given at onset of toxic symptoms to the latter two groups. It was found that caramiphen and TAB completely abolished electrographic seizure activity while scopolamine treatment exhibited only partial protection. Additionally, no significant alteration in the density of peripheral benzodiazepine receptors was noted ensuring the former therapies whereas scopolamine application resulted in a complex outcome: while part of the animals demonstrated no change in the number of these sites, the other exhibited markedly higher densities. Cognitive functions (learning and memory processes, evaluated using the Morris Water Maze) were improved by the three treatments compared to soman-injected animals, with the following rank order: caramiphen > TAB > scopolamine. Statistically significant correlations were demonstrated between learning parameters and [3H]Ro5-4864 binding to forebrain/midbrain membrane preparations (r = 0.70; r = 0.73). These results show that caramiphen and TAB have a considerable potential as post exposure therapies against soman intoxication.

Keywords: soman, Morris Water Maze, PBR, protection

What is the function of the "visual" cortex in the blind?

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The areas devoted to vision in the primate brain constitute ~25% of the cortical sheet. Does loss of vision render these regions useless? Recently, using fMRI mapping, we showed that the occipital cortex of the congenitally blind is activated during various high level cognitive tasks, including auditory, verbal generation and verbal memory. This activation was found in all blind subjects (N=10), in regions corresponding to retinotopic visual areas in sighted subjects. If the occipital cortex of the blind has any functional role, one might expect the level of occipital activation to be correlated with the subject’s performance in similar tasks. We therefore measured the auditory skills, using the standard Wechsler tests. Interestingly, the blind as a group were far superior in their memory performance compared to the sighted controls (for example digit span: blind 14±3.60 SEM compared to 10±1.40 SEM; t-test, p=0.03). Second, the extent of fMRI activation and its significance level differed substantially between individual subjects. Crucially, strong positive correlations were also observed between the individual subjects' fMRI signal during verbal memory recall and their performance in the Wechsler's memory test. These results suggest that the occipital cortex of the congenitally blind may undergo a radical change to be seemingly involved in language and memory functions.

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Ethyl-substituted carbamates show much lower acetylcholinesterase inhibitory potency than those with smaller or larger n-alkyl substituents

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The only clinically proven therapeutic approach for the treatment of Alzheimer's disease is inhibition of acetylcholinesterase (ACHE) which includes alkylcarbamates, such as physostigmine, rivastigmine and heptylphysostigmine. Several researchers have noted the surprisingly low potency of ethyl-substituted carbamates as AChE inhibitors, although several methyl substituents, but there have been very few studies on AChE inhibitory activity of longer chain alkyl substituents. The aim of the present study was to compare the kinetics of the AChE inhibition (ki) at 37°C in different homologous series of carbamates with an H or methyl group as one substituent (R1) and a methyl, ethyl, n-propyl, n-butyl, n-hexyl or methoxyphenyl as the other (R2), using human erythrocyte AChE, according to the scheme:

\[\text{EOH + CX} \rightarrow [\text{EOH} \cdot \text{CX}] \rightarrow \text{EOC + XH}\]

EOH, enzyme; CX, intact carbamate; EOC, carbamylated enzyme; k1, apparent bimolecular rate constant governing overall inhibition rate

Our results showed that k1 was highest when R1 and R2 were methyl in all series, (e.g. 2081 [M⁻¹ min⁻¹] in the 6-carbapenamoylaminodizol series). This was reduced to 102 M⁻¹ min⁻¹ when R2 was ethyl and gradually rose with increasing chain length to 3055 M⁻¹ min⁻¹ for n-hexyl and 7111 M⁻¹ min⁻¹ for methoxyphenyl. A very similar relationship was found for carbamate derivatives of 3-hydroxyphenylamines. The high potency of the aromatic carbamates may be explained by +π-stacking interactions with phenylalanine residues near the active site of AChE; however, the unusual SAR of the alkyl substitutes is more difficult to justify and may involve interactions at the peripheral anionic site (Lin et al, Bioorganic & Medicinal Chem. 7: 2683-2689 (1999)).

Keywords: acetylcholinesterase inhibition, carbamates, structure-function relationship

LIS1: from brain to cell

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Formation of the brain structure in human is a complex process that occurs during several months of prenatal development. One of the most striking features of the human brain is its characteristic convolutions. These convolutions are lacking in a severe human brain malformation known as lissencephaly (tactos = smooth, encephaly = brain). About one in 30,000 live births are affected with this disease. Lissencephaly patients have a reduced life expectancy and a severe mental retardation. So far, two genes have been found to be mutated in lissencephaly; LIS1 located on chromosome 17 and Doublecortin an X-linked gene. LIS1 is a WD repeat protein and is known to be involved in several protein complexes and microtubule regulation by direct interactions with tubulin or via interactions with the dynein/dynactin motor complex. We describe the phenotype observed in L1 mutant mice in the context of L1 protein interactions. In addition, we discuss an interaction with the cytoplasmic linker protein CLIP-170. CLIP-170 is required for intracellular transport and vesicle to microtubules. We suggest that CLIP-170 and LIS1 are both required for cargo transport via the dynein/motor complex. Using the knowledge we gained regarding LIS1 function we have analyzed a set of LIS1 point mutations that result in variable lissencephaly-related phenotypes. Our detailed protein function analysis also allows to understand the spectrum of the disease phenotype.

Supported by ICSG grant No: RG283199 and March of Dimes grant No: 6-F031-5, ISF grant 1900 and Minerva Foundation Keywords: LIS1, microtubule, lissencephaly
The effect of cytotoxic lesions of the hippocampus on spatial learning in GluR1−/− mice

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years, a new method, electroporation, was introduced to deliver NMDA into the hippocampus using an injection of NMDA. The mice were then tested in the standard version of the Morris water maze task and on a reference memory task on the elevated Y-maze. Performance in the water maze and on the Y-maze was impaired by lesion but not by GluR1 deletion. This suggests that extra-hippocampal compensation cannot account for the spared ability of the GluR1−/− mice in spatial learning tasks such as the Morris water maze and the Y-maze.

Keywords: GluR1, hippocampus, LTP, working memory

Utilization of genetic tools for studying neural development in avian embryos

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The employment of genetic tools for studying biology in general, and developmental biology in particular, has advanced our knowledge tremendously over the last decades. The ability to inactivate a specific gene, or to mis-express it, thereby generating a loss or gain-of-function mutant, is the ultimate way to study its role. These tools were applied successfully in invertebrates and mice.

The avian embryo is the preferred organism for studying embryonic development of vertebrate. The embryo is flat, develop in vivo, and accessible for surgical manipulations. Hence, the inability to introduce genes in vivo has hampered the progress of research with this organism. In the last few years, a new method, electroporation, was introduced to deliver genes into embryos, and been used successfully in avian embryos.

However, the site of expression is dictated by the topography of the embryo and the electrode. In order to achieve site-specific expression we have used site-specific enhancers. The activity of the specific enhancers was further amplified by the use of DNA site-specific recombinase. In this way, we are able to achieve high level ectopic expression of a test gene or dominant negative form of a protein, at a specific site. For generating a null phenotype, we are currently applying the RNAi method in chick embryos in vivo.

The genetic tools will be presented, and their potential use for studying axon guidance and cell migration during early development will be demonstrated.

Keywords: axon guidance, transgenic chick

Setting apart the affected: the use of behavioral criteria in animal models of Acute Stress Response and Post Traumatic Stress Disorder

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Post-traumatic stress disorder affects only 20-30% of the exposed population. Clinical studies of acute and chronic responses to stress generally employ stringent criteria for inclusion in the study population, and yet in animal studies the exposure function of the entire exposed versus the entire unexposed populations, regardless of individual variation in response. Prior data support an approach to animal models analogous to inclusion criteria in clinical studies.

This series of studies sought to assess prevalence rates of maladaptive versus adaptive responses determined according to two successive behavioral tasks (elevated plus maze/open field and acoustic startle response tests), amongst rats exposed to a variety of stress paradigms, in the acute and chronic phases, for single and repeated exposures, early in life and/or in adulthood.

The results shed light on the pattern of prevalence of maladaptive responses to stress over time, with rates dropping from 90% acute responders to a plateau at about 25% enduring/chronic responders. This plateau is attained at seven days and remains unchanged at 30 days in two distinct study models. Different types of trauma are associated with different degrees of response, possibly reflecting a "dose-response" relationship to severity of trauma. Trauma-induced models associated with increasing prevalence of maladaptive responses, all the more so for early-life trauma repeated in adulthood.

Setting the affected individuals apart from the unaffected and focusing on them clarifies the overall picture and more closely approximates clinical studies.

Keywords: acute stress response, Post-Traumatic Stress Disorder, animal model, anxiety, stress, maladapted, well adapted

Studying architecture of G-protein coupled K+ channels using fluorescence based approaches

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G protein coupled potassium channels (GIRK/Kir3.x) translate inhibitory chemical neurotransmission into changes in cellular excitability. These channels are activated by binding of the Gβ subunits to G proteins, and are regulated by other intracellular components as well. To understand the mechanism of channel activation by G proteins it is necessary to define the structural rearrangements in the channel that result in the Gβ subunits.

In this study we used a combination of fluorescence spectroscopy and through-the-objective total internal reflection microscopy to monitor the conformational rearrangements associated with the activation of GIRK/Kir3.x channels. Conformational changes were assessed from changes in the efficiency of fluorescence resonance energy transfer (FRET) between CFP and YFP attached at various positions in the cytosolic domains. We detected activation-induced changes in FRET consistent with a rotation and expansion of the termini along the central axis of the channel. We propose that this rotation and expansion of the termini drives the channel to open by bending and possibly rotating the second transmembrane segment.

Wasp venom injected into prey's brain affects the activity of bioaminergic neurons to regulate the expression of specific motor patterns

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The parasitoid wasp Ampulex compressa hunts cockroaches to serve as a living food supply for its larvae. The wasp injects its venom directly into the brain to induce a lethargic state lasting about five weeks. While in this state, the cockroach does not escape. Our hypothesis is that the injected venom affects neurons located in the brain which send descending tonic input to bioaminergic neurons, these, in turn, control the thoracic premotor circuitry.

Octopamine is a bioamine, which is secreted by DUM (dorsal unpaired median) neurons in the prey's thoracic portion of the nervous system. It is known to regulate the expression of specific motor patterns by modulating the excitability of specific neurons.

In this work, we show that the activity of DUM neurons is altered in stung animals. Identified DUM neurons in the metathoracic ganglia of stung animals have a lower spontaneous firing rate and their responsiveness to specific sensory stimuli decreases. For example, DUM2 neuron does not produce a burst of action potentials in response to a wind stimulus as it does in control animals.

Given these results, we propose that the venom injected into the brain affects descending tonic input to the dorsal DUM neurons. The decrease in activity of the DUM neurons could be responsible for the decrease in responsiveness of thoracic motor circuits and consequently the expression of specific motor patterns such as the escape behaviour.

Keywords: ampulex compressa, cockroach, octopamine, dorsal unpaired median neurons
Genetic variations in apoptosis and inflammation-related genes and the risk of Alzheimer’s disease

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The etiology of sporadic Alzheimer’s disease (AD) is complex, and both genetic and environmental factors are involved. The APOE-e4 variant is the only widely confirmed major risk factor for AD selected for only part of the genetic component of AD, and other genetic variants are yet to be detected.

We performed association studies in 109 sporadic AD cases and 111 aged healthy controls. We investigated natural genetic variations (polymorphisms) in 5 genes selected by pathobiological criteria: p53 and TNFRSF6 (Fas), both apoptosis-related genes which are upregulated in AD brains; complement component (C1r) and interleukin-1-beta (IL-1-B), both inflammation-associated genes upregulated in AD brains; and heparan sulfate proteoglycan (HSPG2), which is associated with amyloid plaques and neurofibrillary tangles. We tested whether these genes act as susceptibility genes affecting the risk for AD or as modifier genes influencing its clinical features. While our study population demonstrated a strong association between AD and the APOE-e4 risk factor (p=0.0002), no association was found with p53, C1r, and HSPG2 following polymorphisms: (codon 72) p53 [p=0.921], (-670) Fas [p=0.475], (codon 135)C1r [p=0.353], (3953)IL-1-B [p=0.628], and (domain D)HSPG2 [p=0.486]. No association was detected also for age at disease onset and disease progression, and no interactive effect was found with APOE-e4. This is the first report of association studies of p53, C1r and HSPG2 with AD or any other degenerative disorder at all. Our results of Fas and IL-1-B are in agreement with initial reports, published during our work. Further intensive association studies are needed for identifying genetic risk factors that will allow constructing a profile of individuals prone to AD, and may assist in developing novel therapies.

Keywords: Alzheimer’s disease, polymorphism, apoptosis, immunogenetics.

Microglia and macrophage activation in the injured nervous system

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Microglia and macrophages play critical roles in the response of the CNS and PNS to injury and disease. One major role is the removal of degenerating myelin by phagocytosis. Myelin degeneration occurs after injury to axons and in autoimmune demyelinating diseases such as multiple sclerosis and EAE. Degenerated myelin inhibits axonal regeneration and further activates the complement system to form membrane attack complexes that disintegrate intact myelin. The rapid removal of degenerating myelin is vital, therefore, for repair and minimizing additional damage to myelin. Myelin phagocytosis can be mediated by complement-receptor-3 (CR3/MAC-1), scavenger receptor (SRA/I)II and Fc-receptor (FcR). We presently document that CR3/MAC-1 is expected to play the major role in mediating myelin phagocytosis after trauma (70% to 80%). However, its in- vivo expression does not indicate necessarily the occurrence of myelin phagocytosis. CR3/MAC-1 expressing microglia are fully activated and phagocytose myelin efficiently in EAE but not after CNS axons. These observations led us to suggest that CR3/MAC-1 mediated myelophagocytosis is subjected to modulation such that it may range between efficient and inefficient states.

We tested the functional plasticity of CR3/MAC-1 mediated myelophagocytosis in macrophages and microglia by anti-CR3/MAC-1 mAbs, documenting inhibition and augmentation of myelin phagocytosis by anti-CR3/MAC-1 mAbs. We suggest that mAbs binding to distinct extracellular epitopes in α/β subunits of CR3/MAC-1 can modulate (inhibit or augment) CR3/MAC-1 mediated myelophagocytosis by inducing conformational changes that correspond with inactive or active functional states CR3/MAC-1. We further suggest that anti-CR3/MAC-1 mAbs may regulate which native extracellular molecules bind to and modulate CR3/MAC-1 mediated myelin phagocytosis in microglia and macrophages.

Keywords: trauma, regeneration, microglia, macrophages, myelin.

Brain reward system and depression: A tool for testing hedonia in an animal model

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Anhedonia is one of the main parameters that distinguish a depressed patient from a normal one. However, the brain mechanisms that underlie anhedonia remain elusive. It is suggested that there are overlaps between depression/anhedonia and neural reward systems. Therefore, the brain reward system may be a potentially important therapeutic target to relieve anhedonic symptoms.

The Flinders sensitive line of rats (FSL) is a valid and reliable model of depression with strong face-, construct- and predictive-validity. Abnormalities in the reward system of FSL rats were tested behaviorally using an intracranial self-administration paradigm in parallel with microdialysis measurement of dopamine levels in the nucleus accumbens. Our results show that cocaine consumption was significantly higher in control than in FSL rats. Treatment with the antidepressant desipramine increased the cocaine self-administration in FSL rats. Basal dopamine secretion ratio (using GBR12909) and extracellular dopamine in cocaine were also abnormal in the nucleus accumbens of FSL rats. Both of these measures were normalized following treatment with desipramine. Taken together, we suggest that the brain reward system may be a tool for studying the anhedonia expressed in depressive behavior.

Keywords: brain reward system, cocaine, hedonia, animal model.

Plasma Homocysteine in Schizophrenia

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Elevated plasma homocysteine has been found to be a risk factor for Alzheimer’s disease as well as in cerebral vascular disease, suggesting that some risk factors can accelerate or increase the severity of several CNS disease processes. We screened chronic schizophrenic patients in our catchment area for plasma homocysteine levels.

A one-way ANCOVA with age and sex as covariants was performed on plasma total homocysteine levels of 190 schizophrenic patients vs 762 controls (evaluated in a screening program for employee health). The effect of schizophrenia was marked (F=130.5, df=902 p<0.0001) and mean homocysteine level was 16.7 ± 11.8 (SD) in schizophrenic patients vs 10.6 ± 3.6 (SD) in healthy controls (covariance adjusted means 16.5 vs 10.7 in controls). The increase was almost entirely in young male schizophrenics. Homocysteine has been shown to be neurotoxic and it has been shown that stress can open the blood brain barrier to some neurotoxic substances. It is possible that the stress of acute psychosis allows high homocysteine levels to enter the brain and cause neurodegeneration, clinical deterioration and chronicity. This hypothesis does not make any assumption as to the origin of high homocysteine in young male schizophrenics. It could be caused by smoking, lack of exercise or by poor diet. Yet via a biochemical mechanism it could negatively affect the course of illness.

Keywords: homocysteine, neuronodegeneration, schizophrenia.

The degree of monoamine oxidase inhibition by TV3326 in relation to MPTP induced neurotoxicity and L-dopa induced dopamine behavioral syndrome

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TV3326 [(N-propargyl-(3R)-aminoindan-5-yl)-ethyl methyl carbamate)] is a novel bifunctional cholinesterase (ChE) as well as brain-selective monoamine oxidase (MAO) A and B inhibitor, which has been developed for treatment of dementia with extra pyramidal disorder such as a Lewy body disease. In vitro TV3326 has little or no MAO inhibitory activity. However, on chronic treatment (60mg/kg/day for 14 weeks) in mice it inhibits both brain MAO iso-enzymes by ~70%, resulting in a complete protection from MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydro-pyridine) induced neurotoxicity of nigrostriatal dopamine neurons. The neurotoxicity of MPTP is dependent on its conversion to the neurotoxin MPP+ (1-methyl-4-phenylpyridinium ion) by MAO B. It is not known what inhibition is required for neuroprotection against MPTP. In the present

Keywords: homocysteine, neuronodegeneration, schizophrenia.
study we show that protective effect of TV-3326 is not dependent on complete inhibition of MAO B. A correlation exists between degree of MAO B inhibition and neuroprotection as measured by striatal dopamine concentration. In addition the effect of TV-3326 (52mg/kg) on the 1-dopa induced dopamine behavioral stereotypy was evaluated in rats and compared with that initiated by the non-selective MAO inhibitor tranylcypromine (TCP) (10mg/kg). Both TV-3326 and TCP as expected, caused highly significant increase in striatal dopamine. However we observed that the behavioral syndrome that is normally seen with TCP and other MAO inhibitors plus 1-dopa was absent with TV3326. These results in part may be explained by inhibitory effect of increased acetylcholine levels on M1 receptors resulting from ChE inhibition by TV-3326.

Keywords: TV-3326, MAO, ChE, MPTP

SKF100303A, an inhibitor of the GABA transporter GAT1, regulates chloride concentration in SCN neurons

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We have shown that GABA has a dual effect on SCN neurons, excitatory during the day and inhibitory at night (Wagner et al. Nature 387: 598-600 [1997]). This duality, which has been dependent on complete inhibition of MAOB. A correlation terminals and glia, as well as on some dendrites, translocates neurons. This transporter, which in the SCN is located on axon GABA currents, induced by either inhibitory synaptic activity or by local application of GABA. The blocker caused prolongation of the decay time constant by an average of 35%, and had a larger effect on inward (39%) than on outward (31%) currents. We next investigated the effect of the blocker on the recovery of intracellular Cl- concentration. Loading or depolarization of intracellular Cl- was induced by a prolonged GABA application, which generated either influx or efflux of chloride, depending on the membrane holding potential. Recovery was deduced from the change in chloride reversal potential calculated from the response to a test GABA pulse presented at 30sec delay after the first GABA pulse. While recovery from chloride loading was nearly unaffected by the blocker, chloride depletion was reduced, particularly during subjective day.

Keywords: SCN, circadian rhythm, GABA

Single trial and artifact extraction from human evoked potentials using sparsity

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Event-related potentials (ERPs) have a low amplitude relative to background EEG activity. Therefore, ERPs are usually averaged from hundreds of recording variations in the signal among the single trials comprising the average. This study addressed this need for ERP single trial derivation by solving the blind source separation (BSS) problem.

In the BSS problem unknown sources that are linearly mixed with an unknown mixing matrix need to be extracted based only on the mixtures. Most approaches that solve this problem rely on independence between the sources (ICA) or some distribution quality. Sparsity assumes that only a small number of signal values differ significantly from zero. Biological signals can be sparsely represented in a Fourier series such that each channel has sparse representation in Fourier coefficients. This criterion is used without any additional assumptions in order to separate sources of EEG.

The data used in order to demonstrate the algorithm's performance included responses to two types of stimuli randomly presented in sequence using the "odd-ball" paradigm. The separated sources of the multichannel data show that each represents a component, eye blink, EOG and in some subjects muscle activity, are represented by separate sources. Moreover, frontal activity (N1 and P2 waves) and parietal activity (P3 wave) were separately represented. ERP components related to eye movements were used in the sparsity criterion in order to improve the ERP signal/noise ratio. A raster display of single trials allowed following the dynamics of each component during the recording session.

Keywords: Blind Source Separation (BSS), ERP single trial, sparsity

Pax6 and Enl regulate distinct steps in the development of renshaw cells

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During development functional classes of neurons are selectively generated at defined locations according to the position their progenitors along the neuroaxis. In the ventral spinal cord, graded Shh signaling subdivides the neuroepithelium into five progenitor domains that give rise to different subtypes of neurons. It is not known whether these embryonic subtypes define physiologically identified neuronal classes in the adult, nor is it known how genes that are transiently active in these neurons contribute to the formation of locomotor circuits. Previously, we demonstrated that V1 IN's are locally projecting inhibitory interneurons that express GAD65. To study the V1 population at late stages of development we created Enl knockin and Rosa reporter mice. We have shown that the V1 interneurons are last order interneurons and directly synapse onto motor neurons. We also demonstrated that a subpopulation of V1 interneurons (10%) differentiate into adult Renshaw cells and that adult Renshaw cells are exclusively derived from V1 interneurons. Gephyrin and GABA immunoreactivity identification criteria and anterograde transynaptic transport from the motor axon of an EGFP- TTC transgenic line are used to demonstrate that Renshaw cells are not generated in Pax6 mutant mice. In contrast, Enl mutant mice have normal numbers of Renshaw cells; however Renshaw cell-motor neuron connectivity is altered. These results indicate that the decrease in motor axon number and the two distinct steps in the development of the spinal cord circuit that mediates recurrent inhibition of motor neurons.

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Keywords: Enl, Pax6, Renshaw cells

Correcting space-clamp distorted voltage-clamp recordings from dendrites

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Understanding the functions of a single neuron, such as, propagation and generation of synaptic or action potentials, a detailed description of the kinetics and distribution of the underlying ionic conductances is essential. In voltage-clamp experiments, incomplete space clamp distorts the recorded currents, rendering accurate analysis impossible. We developed a simple numerical algorithm that corrected such distortions. The method performs a stepwise approximation of the conductance density at the site of a local voltage clamp. This is achieved by estimating membrane conductances in a simulation that yields simulated clamp currents, which are then fitted to the distorted clamp currents from the course of the experiment using algorithms based on the structure, relying on accurately reconstructed cell morphology and experimentally determined passive properties. The algorithm was tested using channels with simplified kinetics and on published realistic potassium-channel models. For all tested conditions, the method enabled accurate retrieval of the local densities and kinetics of somatic and dendritic channels at the site of the voltage clamp, rather than averaging these parameters across the structure. Furthermore, correct calculation of conductance gradients required only a few recordings. Neither the addition of noise nor variation of passive parameters significantly reduced its performance within the correction algorithm. The correction method was applied to voltage-clamp recordings of K currents from the apical dendrite of layer 5 neocortical pyramidal neurons. The generality and robustness of the algorithm make it a valuable tool for voltage-clamp analysis of voltage-gated currents in structures of any morphology that are amenable to the voltage-clamp technique.

Keywords: voltage-clamp, potassium channels, cable-theory

The representation of objects in the human lateral occipital cortex

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Regions in the lateral and ventral occipital cortex, known as LOC, play an important role in human visual processing. LOC is characterized by a strong response to objects compared to
scrambled objects or textures, regardless of the visual cues used to define object shape (luminance, texture or motion differences). The cue invariance of the perception of an object is induced solely from activation in response to repeated stimulus presentations. Our differences). Can this cue invariance be observed at the level of motion cues. Every SFM object was made of 18 different stationary, in which object shape was defined by its contours, shading, etc., and (2) structure from motion (SFM) stimuli, in which the perception of an object is induced solely from motion cues. Every SFM object was made of 18 different frames, depicting the rotating object as it is seen from sequential viewpoints. The object was unidentifiable from any single frame. Both types of stimuli activated LOC, and in both cases fMRI-adaptation was seen following stimulus repetition. Since there is little physical similarity between successive SFM images, adaptation during the SFM repetition indicates that LOC is involved with holistic object identity. However, when stationary pictures and SFMs of the same object were alternated within a block, no adaptation was apparent. This may imply that while on the macroscopic level LOC is indeed processing object information in a cue invariant manner, separate neuronal groups within LOC process different object cues.

Keywords: LOC, structure from motion, adaptation

The role of orbitofrontal cortex and basolateral amygdala in latent inhibition — A possible model of negative symptoms in schizophrenia
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Latent inhibition (LI) refers to the proactive interference of inconsequential stimulus pre-exposure with its ability to signal significant events, and disrupted LI is considered to model phenomena of schizophrenia. Lesions of prefrontal cortex (PFC) and basolateral amygdala (BLA), that were reported to produce behavioral effects potentially relevant to schizophrenic symptomatology in several animal models, have been reported to spare LI. However, certain drug and lesion manipulations produce abnormally persistent rather than disrupted LI and we have suggested that such LI perseveration may model the impaired set shifting associated with negative symptomatology. In the present study, we tested whether excitotoxic lesioning of BLA and orbitofrontal cortex (OFCE), which are reciprocally connected, will induce LI perseveration. A week before sacrifice, rats were subjected to sham surgery or to one of the following treatments: (1) pre-exposure to a single frame, (2) sham lesion, or (3) lesion of PFC or BLA. Results show that LI perseveration was induced by PFC or BLA lesioning independently of each other. Thus, LI perseveration may model features of negative symptoms in schizophrenia.

Keywords: LOC, structure from motion, adaptation

Recurrent electrical stimulation suppresses inter-ictal like discharges an electrographic seizures in acute models of neocortical epilepsy in-vitro
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Background: In most patients with epilepsy, seizures can be controlled by antiepileptic drugs. However, approximately a fifth of patients with epilepsy suffer from an intractable disease, and continue to experience seizures despite appropriate medical treatment. Hence, new antiepileptic treatment strategies are required for intractable epilepsy. One potential new antiepileptic treatment modality is electrical stimulation. Vagal nerve stimulation is already approved for treatment of intractable epilepsy. In addition it is possible that seizures can be eliminated by cortical electrical stimulation of the epileptogenic zone.

Objectives: Examine the ability of chronic electrical stimulation to eliminate inter-ictal epileptiform discharges and electrographic seizures in acute models of epilepsy in-vitro.

Methods: The study was performed in neocortical brain slices (500μm) that were treated with the GABA-A receptor blocker Bicuculline (BCC, 10 μM) or zero extra-cellular magnesium. Recordings were performed using whole-cell recordings from single neurons or extra-cellular recordings from a population of neurons. Electrical stimulation was performed using a computer controlled extra-cellular stimulator.

Results: Both acute models of neocortical epilepsy produced inter-ictal like discharges and electrophysiologic seizures. Recurrent electrical stimulation at frequencies of 0.2-2 Hz eliminated altogether inter-ictal like discharges and electrographic seizures in both BCC treated slices and slices exposed to zero magnesium. Interestingly, mechanisms underlying the antiepileptic effect of recurrent electrical stimulation 1 examined its effect on excitative synaptic transmission I found that recurrent electrical stimulation resulted in a 20-55% reduction of the EPSP amplitude in a frequency dependent manner.

Conclusions: Recurrent electrical stimulation has an antiepileptic effect in acute models of neocortical epilepsy in-vitro. This effect is probably mediated, at least in part, by stimulus-evoked depression of excitatory synaptic transmission. Further studies are required to examine the antiepileptic effect of electrical stimulation in-vivo.

Keywords: epilepsy, seizures, electrical stimulation
parentally deprived (3x1 hour per day between postnatal days 1 and 21). 45 days old degus in comparison with age-matched socially isolated control pups. A 3D-measurement of spines were performed in image stacks produced by a confocal laser-scanning microscope applying a newly developed image analysis software (Herzog et al., SPIE 2984:146-158, 1997). Although the spine volumes remained unchanged a significant reduction of spine length, accompanied by a significant increase of mean spine diameter was observed in the parentally deprived pups. These overall effects were due to a reduction of spine length on dendritic branch 2 and 4, and a significant increase of spine diameter on branch 3. This is the first evidence that the disturbance of the parent-child contact during an early postnatal phase results in lasting changes of distinct shape parameters of presumed excitatory spine synapses, which most likely reflect a experience-related fine-tuning of synaptic strength within limbic cortical circuits.

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Keywords: limbic system, development, synaptic plasticity, dendritic spines

Immune related mechanisms participating in resistance and susceptibility to glutamate toxicity

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Glutamate is an essential neurotransmitter in the central nervous system (CNS). However, at abnormally high concentrations it becomes cytotoxic. Recent studies in our laboratory showed that glutamate evokes T cell-mediated cytotoxicity. The aim of the present study was to examine the nature of the glutamate receptors and signaling pathways that participate in immune protection against glutamate toxicity. We show, using the mouse visual system, that glutamate-induced toxicity is strain dependent, not only with respect to the amount of neuronal loss it causes, but also in the pathways it activates. In strains that are genetically endowed with the ability to manifest a T-cell-dependent neuroprotective response to glutamate insult, neuronal losses due to glutamate toxicity were relatively small, and treatment with NMDA-receptor antagonist worsened the outcome. Experiments to glutamate. In contrast, in mice devoid of T cell-dependent endogenous protection, NMDA receptor antagonist reduced the glutamate-induced neuronal loss. In all strains, blockade of the AMPA/KA receptor was beneficial. The results suggest that glutamate-induced toxicity involves multiple glutamate receptors and that the types and relative contributions of receptors vary among strains. We suggest that a multi-factorial protection, based on a mechanism independent of the specific pathway through which glutamate exerts its toxicity, is likely to be a safer, more comprehensive, and hence more effective strategy for neuroprotection.

Keywords: glutamate, NMDA, immune system, neuroprotection, CNS

An S5 residue of KCNQ1 controls channel gating and pore properties and is essential for the specific interaction with KCNE3

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The KCNQ1 pore-forming subunit is a member of the newly characterized K+ channel family (KCNQ1-5), whose mutations have been identified as causes of inherited cardiovascular and neurological disorders such as LQT syndrome and neonatal epilepsy. KCNQ1 can interact with KCNE proteins, an emerging family of auxiliary subunits, to form a functional K+ current. Here we show that the naturally occurring LQT1 mutation L273F, located in the S5 transmembrane segment of the KCNQ1 pore-forming subunit, leads to dramatic changes in channel gating and permeation as well as in the interaction properties with KCNE3. The L273F mutation produces a marked rightward shift of activation gating, with a shallower slope of the voltage dependence of activation in comparison to WT KCNQ1. In addition, L273F generates a pronounced macroscopic inactivation that exhibits distinct properties to those displayed by WT KCNQ1, including slower recovery kinetics. In contrast to WT KCNQ1, external protons poorly inhibit the maximum conductance and the macroscopic inactivation of L273F. Expression of KCNQ1 with WT KCNE3 leads to K+ currents that activate quasiinstantaneously at all voltages, suggesting that heteromeric KCNQ1/KCNE3 channels are constitutively open and exhibit neither voltage- nor time-dependence. Co-expression of L273FKCNQ1 with WT KCNE3 dramatically changes channel gating and exhibits a biophysical profile resembling that produced by WTKCNQ1/KTCKNE1 (ks); it leads to slowly activating, outwardly-rectifying K+ currents with a strong block of inward currents. In all, our results indicate that the KCNQ1 S5 residue L273 critically controls the channel gating and pore properties, probably allosterically, and is essential for the specific interactions with KCNE3.

Keywords: K+ channels, inactivation, gating

B vitamins and brain aging

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The vitamins folic acid, B12 and B6 and B2 are the source of coenzymes which participate in one carbon metabolism. In this metabolism, a carbon unit from serine or glycine is transferred to tetrahydrofolate (THF) to form methylene-THF which is used for the synthesis of thymidine (DNA), purines (DNA, RNA) and methionine. Methionine is converted to S-adenosylmethionine, a universal donor of methyl groups, including DNA, RNA, hormones, neurotransmitters, membrane lipids, proteins and others. It has been growing, particularly in the area of aging and the possibility that certain diseases that affect the aging population, loss of cognitive function, Alzheimer’s disease, cardiovascular disease, stroke and others, may be in part explained by inadequate intake or inadequate status of these vitamins. Homocysteine, a product of methionine metabolism as well as a precursor of methionine synthase, is known to be toxic to cardiovascular disease, stroke and thrombosis when its concentration in plasma is slightly elevated. There are now data on which the new associations between elevated plasma homocysteine levels and loss of neurocognitive function and Alzheimer’s disease. In the Framingham Study we have shown that high plasma homocysteine is associated with increased incidence of dementia and Alzheimer’s disease. There are many factors that contribute to a low status of these vitamins in the elderly and effort is now being placed to determine the beneficial effect of homocysteine lowering intervention on incidence of age related brain dysfunctions.

A role of the cholinergic system in depressive behavior

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Changes in the cholinergic system in the brain were one of the first neurochemical alterations discovered in depressive disorders. Since then, alterations in many more neurotransmitters were recognized. Using the Finders sensitive Line (FSL) rats as a model of depression, we have found major changes in the neuronal monoaminergic systems. Recently, we discovered that specific and local molecular changes relate to the dynamics of depression onset in these rats. It was surprising to find that molecular changes in the limbic system were at terminal sites of the monoaminergic system and not in the cell body regions. This finding suggested that interneurons are involved in the pathology of depression and may serve as the common denominator in neurotransmitter alterations that are involved in the disease onset. The nucleus accumbens (Nacc) is likely to have a role in depression and contains cholinergic interneurons, which may mediate lasting behavioral changes by regulating monoamine release. To date, there has been no systematic investigation of the role of the cholinergic system in the Nacc in animal models of depression. Acute (45 min) infusion of 5 μm pirenzepine, a muscarinic-1 receptor antagonist, into the Nacc failed to modulate the FSL’s increased immobility in the swim test to the level of controls. Pirenzepine in FSL rats also normalized the basal levels of dopamine and serotonin in the extracellular space in the Nacc. These results may suggest that the cholinergic system is not just another neurotransmitter altered in depression, but is critical for controlling the interaction between several neurotransmitter systems. The convergence of these systems trigger the behavioral alterations found in depression.

Keywords: cholinergic system, nucleus accumbens, muscarinic receptor, depression
Stand-by microglial cells become activated scavengers upon T cell activation: Involvement of INF-γ
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Inflammation following central nervous system (CNS) injury is still a subject of controversy, regarded by some as a contributory and harmful process. However, while some studies have provided substantial evidence for immune involvement in neuronal protection. In this study we found that, the ability to resist the consequences of CNS axonal injury is characterized by the early activation of site-specific phagocytic activity and MHC class II expression. Our data suggest that post-traumatic CNS inflammation comprises a highly complex cascade of events, in which elements that are timely and properly balanced innate immune signaling will lead to neuronal survival. Such a behavioral phenomenon is demonstrated in vitro by the ability of activated T cells, via soluble factors, to activate microglial cells so as to increase their capacity to uptake extracellular glutamate. Interferon (IFN)-γ was capable to simulate, at least in part, this activation effect. Using the RNA microarray methods we found in T cell-activated microglia cells alterations in expression of genes associated with an increase ability to resist threatening conditions, which often follows CNS lesion. These results suggest that autoimmune T cells facilitate coping with injurious conditions by locally activating the stand-by microglial cells. Keywords: CNS inflammation, CNS trauma, protective autoregulation; glutamate toxicity; glutamate scavenging; microglia.

Abnormal maternal behavior in GSK-3β knockout heterozygotes with vanillin-smell
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2Glycogen synthase kinase-3 (GSK-3) is a multi-substrate serine/threonine protein kinase, highly abundant in brain. GSK-3β substrates include: transcription factors, regulatory enzymes and structural proteins and it is suggested to play a role in neuroprotection. To study the cellular mechanisms underlying metabolic proliferation, differentiation and development, particularly neurodevelopment. Its activity and protein levels have recently been reported to be over 40% lower in postmortem frontal cortices of schizophrenic patients. GSK-3β null mutant mice are univiable. Heterozygote mice, which have a 50% reduction in brain GSK-3β, do not show any obvious physical or neurological abnormality. The GSK-3β heterozygotes are being studied in our lab for schizophrenia-like behavior. This experiment was conducted to test the maternal behavior of GSK-3β heterozygote female mice. Behavioral observation showed that heterozygote females have clear deficits in maternal behavior, leading to maternal infanticide. Neither pre-partum vanilla-smell on the mother's nose, nor pre-partum diazepam treatment prevented the infanticide. This finding could suggest a deficit in control of aggression or in bonding, and will be studied in specific behavioral paradigms.

Keywords: GSK, maternal behavior, knockout mice

Valproate inhibits Myo-inositol-1-phosphate (MIP) synthase
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In mammalian brain, lithium inhibits inositol monophosphatase and both valproate and lithium reduce intracellular inositol. The same reduction has been found in yeast where it was also observed that lithium, that causes a parallel increase in intracellular inositol-1-phosphate, valproate causes a decrease in this metabolite. This suggested to us that myo-inositol-1-P (MIP) synthase is the site of valproate's action in inositol metabolism. Inositol-1-phosphate is the rate-limiting step in inositol biosynthesis and in yeast is highly regulated in response to inositol. We therefore hypothesized that human brain MIP synthase is a factor in the psychopharmacology of mood stabilizers. To test this hypothesis we have determined valproate concentrations on MIP synthase activity in postmortem human brain homogenates was studied. Valproate inhibits human brain MIP synthase activity with a Ki of 0.21 μM (0.35 μM is the lowest plasma therapeutic level). The effect is not obtained with other anticonvulsant mood stabilizers, typical and atypical antipsychotics and tricyclic antidepressants. To find out whether reduction in inositol levels follow lithium's inhibition of inositol monophosphatase or valproate's inhibition of MIP synthase is physiologically meaningful, the chronic effect of lithium in food and the acute effect of i.p. valproate administration on brain MIP synthase expression was studied in mice. Lithium caused 33% upregulation of hippocampal MIP synthase expression and a two-fold upregulation of frontal cortex MIP synthase one hour after valproate treatment was observed. Inositol depletion as a first event in the therapeutic mechanism of action is thus common to lithium and valproate, albeit, through inhibition of different enzymes.

Keywords: lithium, valproate, Myo-inositol-1-Phosphate (MIP) Synthase

“Affective theory of mind” is mediated by the ventromedial prefrontal cortex: a lesion study
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Glycogen synthase kinase-3 (GSK-3) is a multi-substrate serine/threonine protein kinase, highly abundant in brain. GSK-3β substrates include: transcription factors, regulatory enzymes and structural proteins and it is suggested to play a role in neuroprotection. To study the cellular mechanisms underlying metabolic proliferation, differentiation and development, particularly neurodevelopment. Its activity and protein levels have recently been reported to be over 40% lower in postmortem frontal cortices of schizophrenic patients. GSK-3β null mutant mice are univiable. Heterozygote mice, which have a 50% reduction in brain GSK-3β, do not show any obvious physical or neurological abnormality. The GSK-3β heterozygotes are being studied in our lab for schizophrenia-like behavior. This experiment was conducted to test the maternal behavior of GSK-3β heterozygote female mice. Behavioral observation showed that heterozygote females have clear deficits in maternal behavior, leading to maternal infanticide. Neither pre-partum vanilla-smell on the mother's nose, nor pre-partum diazepam treatment prevented the infanticide. This finding could suggest a deficit in control of aggression or in bonding, and will be studied in specific behavioral paradigms.

Keywords: prefrontal lesions, theory of mind, the entomedial prefrontal cortex

Exposing collinear facilitation in the periphery with attention
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It has been previously shown in psychophysical studies that detection threshold of a Gabor patch, presented at the fixation point, is reduced when flanked by co-aligned high contrast alike stimuli. In the periphery, increased inhibition is found. However physiological studies in V1 of cats and monkeys do show increased firing rates for co-aligned stimuli at \(4^\circ\). Here we attempt to resolve this apparent conflict. We show that the absence of peripheral facilitation is not a result of improper scaling. We observed that collinear facilitation was reduced or gone as soon as targets were placed outside fixation (1^\circ), still within the fovea. This suggested the involvement of attentional mechanisms in peripheral facilitation. It has been previously shown that lateral interactions are modulated by attention in the fovea. We assessed the role of attention in peripheral surround modulation by directing attention to the flanking stimuli in a double task paradigm. Subjects performed a Vernier acuity task on the flanks and a detection task on a Gabor target located in between. Collinear thresholds were lower than orthogonal ones in the double task. When performing only the detection task, collinear and orthogonal thresholds were similar. This result shows that under conditions that force attention to critical parts of the stimulus, facilitation is present at \(4^\circ\) and further suggests that differences in allocation of attention between fovea and periphery may underlie the lack of peripheral collinear facilitation. The orientation selectivity of this attention-induced facilitation may imply that it is mediated by lateral interactions, as suggested for foveal facilitation.

Keywords: attention, lateral interactions, periphery
The involvement of PKC in rule learning in the piriform cortex

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We are interested in the molecular mechanisms of olfactory rule learning in the piriform cortex. We have shown previously that post-burst after-hyperpolarization (AHP) and paired-pulse facilitation (PPF) were reduced in pyramidal neurons, days after olfactory rule learning. Assuming that these long-term cellular modifications subserve odor rule learning, we are looking into these possible molecular mechanisms. (See also Cohen et al.) PKC has been implicated in the process of learning and memory formation. Here we study the involvement of PKC in long-term AHP modulation following odor rule learning. The specific PKC inhibitor GF109203X (10 μM) caused an increase in AHP amplitude in neurons from trained rats. Consequently, the difference in AHP amplitude between trained and control rats was diminished. Moreover, activation of PKC by OAG (10 μM) significantly reduced the AHP in neurons from naive and pseudotrained rats, abolishing the difference between groups. Using sub-cellular fractionation and quantitative immunoblotting analysis, we are examining the role of the different PKC isoenzymes in rule learning in the piriform cortex. Phosphorylation of PKC/βI on Thr638/641 was decreased by 15% in the trained group compared to both pseudotrained and naive groups (n=7). In the cytosolic fraction, phosphorylation of PKC/βII on Thr638/641 was reduced in the trained group by 23% compared to controls (n=7). We conclude that PKC/βI/βII phosphorylation and subcellular distribution may be involved with PKC-dependent modulation in the piriform cortex of trained rats.

Keywords: PKC, piriform cortex, rule-learning

Modulation of voltage-dependant potassium channel Kv1.1 by SNARE protein SNAP-25

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It has been established that presynaptic voltage-gated K+ channel PKC involved in shaping presynaptic action potentials and thus regulate neurotransmitter release. It has also been established that both Kv1.1 channel and its auxiliary subunit Kvβ1.1 interact directly with the exocytotic apparatus through the SNARE complex protein syntaxin 1A. In our present work, we are examining the prospect of a connection between Kv1.1 channel and another v-SNARE protein – SNAP-25 (25-kDa synaptosome-associated protein). Such possible connection is being characterized in Arbusov cocyes. From the results we have gathered so far, it appears that SNAP-25, like syntaxin-1A, affects the channel currents in a biphasic manner, according to which it is applied. However, as opposed to syntaxin's effect, it seems that low SNAP concentrations cause decrease in amplitude of current through the channel, with or without Kvβ1.1, while high SNAP concentration results in an increase in the amplitude through the channel, with or without Kvβ1.1. SNAP-25 does not affect the channel's steady state activation. We are currently studying the effects of SNAP-25 on the steady-state N-type inactivation of the Kv1.1/Kvβ1.1 channel. We are also examining the possibility of a crosstalk between the effect of syntaxin 1A and the effect of SNAP-25.

Keywords: Kv1.1, Kvβ1.1, SNAP-25, syntaxin 1A

It may be easier to see two things at the same time!

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It is well known that subjects can detect rapidly an element that differs greatly from surrounding elements in a single dimension such as color or orientation. With attention spread across the entire array, subjects detect presence or absence of such a target with a response time that does not depend on the number of distractor elements. We now asked what will be the speed and accuracy elements simultaneously presented. Subjects viewed a briefly presented 8x8 array of pink lines oriented at 55° (or 60°) followed by a masking stimulus after a variable Stimulus-to-mask Onset Asynchrony. On some trials, 1-2 of the pink lines were replaced by a pale green line of the same orientation, a pink line of orientation 35-40° (or 30°), or a line with both these changes. Subjects reported the number of odd elements, and surprisingly, we found that subjects were more accurate at detecting targets in arrays with 2 odd elements than arrays with 1 – despite the requirement of accurately reporting the nature of the change. This result was true for all types of odd elements. In addition, it was easier to report presence of two odd elements – one with an odd color and one with an odd orientation – than to report the presence of one odd element – which differed from the distractor elements both in color and orientation. Analysis of these results as a function of distance between the odd elements suggests that oddity is detected as a Gestalt and that arrays with a pair of targets are perceived as distinct structures.

Transgenic increase in the "readthrough" acetylcholinesterase variant (AChE-R) associates with increased motor activity of NBM mice

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Cholinergic neurotransmission is extensively involved in the function of motor systems. This clearly includes the synaptic acetylcholinesterase variant AChE-S, multimeric complexes of which adhere to the synaptic membrane. However, it was unknown whether the monomeric, soluble "readthrough" AChE-R variant also contributes to the modulation of motor activity. To address this issue, open field motor activity was video-photographed in TgR (n = 8) and FVB/N mice (n = 8). In addition, locomotor activity of TgR (n = 10) and FVB/N (n = 10) mice was taped individually recorded in an open field, TgR but not FVB/N mice, displayed frequent episodes of running in circles at a rate of 13 ± 4 per min. In the home cage, telemetry of TgR mice revealed absolutely no variations in the basal level of motor activity. We employed rabbit anti-hAChE-R and goat anti-choline acetyltransferase (ChAT) antibodies to correlate the anatomical distribution of human (h)AChE-R in the transgenic FVB/N mice with their cholinergic nature and their capacity to control locomotor activity. hAChE-R was immunolabeled in cortical and hippocampal neurons, targets of cholinergic neurons. In contrast, cholinergic neurons of the medial septum, diagonal band and striatum did not contain hAChE-R although they displayed intense ChAT staining. An exception was the nucleus basalis magnocellularis (NBM), in which 5% of ChAT-positive neurons, known to be involved in regulation of cognitive and motor functions displayed hAChE-R staining. Our findings demonstrate that alterations in AChE-R expression in cholinergic neurons of the NBM and in target regions of cholinergic neurons may causally contribute to the modulation of motor activity.

Keywords: acetylcholine, acetylcholinesterase, motor behavior, NBM

Genetic and gender factors in physiological, behavioral and neuronal activation responses to dietary restriction (DR)

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Human anorexia nervosa (AN) involves self-imposed diet restriction (DR). It is not known how DR may modulate psychiatric phenomena in AN. In light of evidence for contribution of genetic and gender factors to AN, the present study compares responses to DR in the strains of inbred mice, C57BL and BALB/c, representing diverse genetic backgrounds focusing on (1) body weight response, (2) behavioral responses: learning (serial maze task), conflict behavior (between hiding and exploration) in the elevated plus maze and emergence paradigms and (3) brain neuronal response assessed by immunohistochemical staining of the immediate early gene product, c-FOS. Mice were assigned to groups, receiving ad libitum (AL), or 70% and 60% of daily AL food intake. BALB/c mice displayed greater weight loss in response to DR with greater loss in females than in males. This was associated with DR-induced c-FOS in the arcuate nucleus of BALB/c but not of C57BL mice. Female BALB/c mice did not display DR-induced c-FOS in the dorsomedial and lateral hypothalamus whereas C57BL did. BALB/c mice displayed greater DR-induced reduction of emotional conflict (increased exploration in novel environments) than did C57BL mice but had no deficit in learning. DR induced c-FOS in the hippocampal CA1 and dentate gyrus of BALB/c but not of C57BL mice. The BALB/c strain emerges as a model for AN, in displaying greater changes in emotional behavior and changes in activation of limbic brain regions. Our findings suggest pathways by which DR may modulate psychiatric phenomena in AN.
Keywords: diet restriction, anorexia, genetic background, gender differences

Distribution of small-conductance, calcium-activated potassium channel 3 (SK3) in mouse brain
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The calcium influx that occurs during an action potential activates, among others, small-conductance, calcium-activated potassium channels (SK). Since both SK3 mRNA and protein have been identified in rat substantia nigra pars compacta (SNC) and ventral tegmental area (VTA), the SK3 channel has attracted much attention in the context of dopaminergic neurotransmission and implications for schizophrenia. However, SK3 has not been mapped in mouse brain. The present study documents SK3 distribution in brain of BALB/c mice. At the forebrain level, SK3 staining was documented in the thalamic ventromedial complex and laterodorsal lamina, in the medial habenula, and in the central and corticomedial amygdala. In the mesencephalon, there was staining in the SNC and VTA and in superficial lamina of the superior colliculus. At the pontine level there was staining in the locus coeruleus and parabrachial nucleus. All staining patterns were blocked by pre-incubation of the antibody with the antigen. The SK3 distribution in mice documented in the present study parallels the distribution reported in rats, with the additional presence of SK3 in the amygdala and ventral thalamus. Thus, the present study supports the possibility that SK3 in ventral mesencephalic dopaminergic neurons is ubiquitous in mammalian species and that this localization could be preserved in humans. SK3 distribution in mice suggests potential involvement of SK3 in pathways that modulate sensory information processing. The presence of SK3 in the central amygdala nucleus may suggest that in mice, SK3 also participates in neural pathways for integration of sensory stimuli in visceral-emotional reactions.

Keywords: SK3, calcium, potassium, channels

A system for rapid uncaging in neural tissue using spatially defined patterns
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Two-photon laser scanning microscopy has enabled fast high-resolution imaging deep into brain tissue, which is strongly light scattering. The ongoing development of functional fluorescent probes and high-throughput techniques for dye loading promise an increasing range of applications of multiphoton optical methods in neuroscience. The use of light-sensitive "caged" compounds complements two-photon microscopy by providing a means of optically manipulating biochemical signals. We are developing a flexible new system that allows controlled photo-release of caged neurotransmitters in defined spatial and temporal patterns with simultaneous and submicron-resolution. Our system relies on the steering of an ultraviolet laser beam in two dimensions using acousto-optical devices made from TeO2. The ultraviolet light pattern is projected into the same plane scanned by a confocally-modeled two-photon microscope. The beam can be steered to up to 100,000 locations per second. We are currently beginning to apply the system to neural systems.

Keywords: two-photon microscopy, uncaging, slice physiology, optics

Functional Ras antagonist inhibits the increase in brain Ras-GTP induced by closed head injury and exerts neuroprotection
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Ras proteins are involved in receptor-mediated signaling pathways, including those regulating cell death and survival. Ras antagonists may therefore be protective when these pathways are transformed. Transfarnethiosalicylic acid (FTS), a Ras inhibitor, acts primarily on the active, GDP-bound, form of Ras. This study investigates whether: 1) closed head injury (CHI) increases FTS interaction with Ras; 2) FTS affects functional outcome, NMDA receptor function and lesion volume after CHI. Mice were subjected to CHI or sham operation, treated with vehicle or FTS (2h, 24h or 7d after CHI). FTS increased Ras-GTP, ERK and Ras-dependent active phospho-ERK were determined using specific Ab. Neurological deficits were assessed by a Neurological Severity Score (NSS) at 1-7 d post CHI. Recovery was defined by ANSS corNSS(b)-NSS(t). NMDA receptor binding and lesion volume were measured by autoradiography and morphometry of brain sections, respectively. Ras-GTP was significantly increased in the contused hemi-CHI and no increase was seen in the non-injured hemisphere, indicating that FTS reduces Ras activity. FTS inhibition of FTS and the NMDA antagonist MK-801. Total was not affected and Ras-GTP levels returned to baseline by 24h post CHI. Both drugs reduced lesions after CHI. ANSS and neurological deficits were significantly improved (p<0.0001, 60%) by FTS, which also prevented the CHI-induced reduction in NMDA receptor binding in cortical, striatal and hippocampal areas and produced smaller lesions. We propose that Ras activation after CHI contributes to neuronal cell death and its suppression by FTS has a long-lasting beneficial effect.

Keywords: traumatic brain injury, Ras-GTP, S-trans transfarnethiosalicylic acid (FTS), NMDA receptors, neuroprotection

Activation of Matrix metalloproteinases by TNF-α in astrocytes in vitro: a model for brain injury
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Dept. of Pharmacology, Hebrew University of Jerusalem: "Dept. of Neurology, Hadassah University Hospital, Jerusalem, Following head trauma several processes take place including inflammation, neurotransmitter release and cytokine-dependent cellular activation. Matrix metalloproteinase-9 (MMP-9) is a metalloproteinase involved in the degradation of the extracellular matrix. MMP-9 is induced by TNF-α and plays a role in many physiological and pathological processes.

Keywords: Matrix metalloproteinases, TNF-α, astrocytes, brain injury

Synaptic dynamics determine the functional correlations between neocortical neurons in excited slices
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Stereotypical microcircuits have long been recognized as computational modules of neocortical function, but the principles that determine the emergence of activity patterns within these microcircuits are unknown. We obtained multiple simultaneous whole-cell voltage recordings from neurons in neocortical slices. A cross-correlation analysis of subthreshold membrane voltage between different types of neurons revealed that the emergence of characteristic functional relationships between neurons during the activity burst was an excitatory extracellular control. The functional relationship between neuronal pairs was correlated to the nature of the excitatory synaptic input to these neurons specifically to the direction of synaptic transmission.

Keywords: neocortical microcircuits, synaptic dynamics, stereotypic microcircuits, subthreshold correlations
Perception of happy and sad facial expressions in chronic schizophrenia: Evidence for two evaluative systems
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Background: Schizophrenia patients have impaired perception of emotional expressions, but it is not clear whether this is part of a generalized deficit in cognitive function. Aim: To test for evidence of emotion-specific deficits by studying the effects of valence on recognition of facial emotional expressions.

Methods: 24 male subjects suffering from chronic schizophrenia were examined with two sets of perception of emotion: the Penn Emotion Acuity Test (PEAT 40) and the Emotion Differenciation Task (EDOT). Clinical state was assessed with the SANS and SAPS scales, visual memory with the Benton Visual Retention Test (BVRT) and motor function with the finger tapping test. Results: Identification of happy facial expressions showed significant negative correlation with age, cumulative time in hospital and length of current hospitalization; positive correlations were found with visual retention and finger tapping scores. Identification of sad facial expressions showed significant correlation only with cumulative time in hospital while identification of neutral facial expressions showed no significant correlations. Discrimination between non-happy sad and not sad facial expressions showed a positive correlation with negative symptoms.

Conclusion: Perception of happy and sad emotion relates differently to significant illness parameters. This finding supports the existence of an emotion-specific deficit in perception of emotions in schizophrenia and of separate channels for processing positive and negative emotions.

Keywords: emotions, schizophrenia, valence, cognitive

Non-auditory brain structures involved in processing auditory material
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The purpose of the study was to localize non-auditory cortical areas activated during first and second language processing. Brain potentials were recorded from 17 normal hearing, right-handed, native Hebrew (first language) speakers who also spoke English. Subjects performed a lexical decision task with speech stimuli from both languages. Brain sources of activity were estimated by calculating current densities from the scalp distribution of the ERPs, using low-resolution electromagnetic tomography analysis (LORETA). Current densities in brain voxels (cubes of 7 mm spatial resolution) were compared with pre-stimulus baseline activity for non-linguistic comparisons. The time course of activity in each Brodmann area (BA) was plotted separately for the right and left hemisphere. Brain regions outside the traditional auditory and language-processing regions demonstrated different temporal patterns of activity that varied with the type of stimulus. These regions included, among others, frontal lobe motor regions, pre-frontal associative region, cingulate gyrus and pre-cuneus. The structure-specific spatio-temporal pattern of activity is compatible with the idea that language processing occurs in spread out neural networks involved with processing different aspects of language.

Keywords: hearing, language, functional imaging, Event-Related Potentials

The neural representation of odor pleasantness and intensity in the human brain
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Imaging studies have shown increased amygdaloid complex (AC) activation in response to aversive odors. Odor quality is dependent on odor concentration (intensity) and odor hedonics (pleasantness). We dissociate these dimensions, and ask whether AC activity reflects odor hedonics, intensity, or both. An olfactometer generated 5 stimuli: 1) PH - pleasant high intensity, 2) PL - pleasant low intensity, 3) UH - unpleasant high intensity, 4) UL - unpleasant low intensity, 5) clean air. 16 young healthy subjects participated in an event-related fMRI study at 3T (T2* spiral, TE = 30, TR = 1s, 64x64 FOV, 17 slices, slice thickness = 4mm, stimulus [S] = 20s, stimulus repetition = 30). A first-pass analysis using SPM99 to compare for regions of increased activity related to odor intensity regardless of pleasantness revealed a significant increase in signal change in the AC in response to increases in intensity, not unpleasantness, of an odor. Activity in the AC was significantly correlated with individual intensity ratings (r = 0.35, p = 0.05). Odor pleasantness estimates (r = 0.04, p = 0.7). These findings suggest that the AC may be encoding more of the immediate physical dimensions of a stimulus (intensity) rather than the later "psychological dimensions" of the stimulus (pleasantness).

Keywords: olfaction; fMRI; amygdala

The molecular neurobiology basis of physiological stress responses
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Physiological symptoms reminiscent of post-traumatic stress disorder (PTSD) occur following exposure to anticholinesterases, however, the origin of this phenomenon is unknown. Recently, we found a dominant activating polymorphism in the AChE promoter, carried by over 50% of the Israeli and compared with 0.5% of the North American population. This polymorphism is associated with acetylcholinesterase (AChE) overproduction and hypersensitivity to cholinergic agonists and antagonists (Shapira et al., Hum. Mol. Genetics, 9:1273-1281 [2000]). Its consequences could be attributed to AChE's hydrolytic activity, the complex interaction of its isoforms with other enzymes, and the "non-classical" AChE activities (Soreq & Seidman, Nature Neurosci. Rev. 2:294-302 [2001]). There are three 3' splicing options for AChE mRNA, and 3 corresponding AChE variants with different C-terminal peptide assemblies and non-classical function(s). Of these, the normally rare "readthrough" variant, AChE-R, accumulates following stressful stimuli and under cholinesterase inhibition. AChE-R mRNA, usually located in neuronal cell bodies, translocates to dendrites and following stress. This rapid yet long-lasting change may suppress the initial insult, but leads to adverse consequences under long-term conditions (Mosher et al., Brain Res. 924:308-312 [2001]). AChE-R involvement in these adverse symptoms was proven by using partially 2'-O-methylated oligonucleotides inducing AChE-R mRNA destruction (Shollani et al., J. Mol. Med. 78:228-236 [2000]). The variant selectivity of these antisense agents, currently tested in clinical trials, prevents the stress-induced physiological impairments while protecting cholinergic neurotransmission by maintaining the synaptic AChE. Keywords: stress, pharmacogenomics, mRNA translocation, acetylcholinesterase

On line confocal imaging of processes underlying the dedifferentiation of an axonal segment into a motile growth cone after axotomy
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Regeneration of neuron after mechanical injury requires structural and functional dedifferentiation of the cut axonal end into a motile growth cone (GC). Here we analyzed the events leading to the formation of a growth cone (GC) and examined the mechanisms that set the dedifferentiation process in motion.

To that end we fluorescently labeled actin by chimeric EGFP-actin, microtubules (MTs) by tetramethylrhodamine conjugated tubulin, and vesicles by the styryl dye RH-237, in primary cultures of identified Aplysia neurons.

Confocal imaging revealed that: (a) axotomy triggers a wave of MTs depolymerization. The depolymerizing wave stops at a point 100-150 mm from the cut end; (b) a proximal zone (TZ) between the depolymerized segment and a proximal segment in which the MTs maintain normal structure. (c) In parallel, actin reach adhesion plaque disappear only to reform minutes later. (c) Antipodocortical transport of MTs are trapped within the TZ. Thereafter, (d) actin bundles assemble along the axons perimeters surrounding the vesicles trap. Once the above-described structure is formed an actin rich lamellipodium, supported y radial polymerization of MTs, begins to extend.

Axotomy in the presence of nacodazole prevents the reorganization of the depolymerized MTs and the formation of a lamellipodium, as supported by radial polymerization of MTs, begins to extend. Axotomy in the presence of nacodazole prevents the reorganization of the depolymerized MTs and the formation of a lamellipodium, as supported by radial polymerization of MTs, begins to extend.
formed, but the extension of a GC's lamellipodium is blocked. Calpain totally inhibits the restructuring of the axon (Oren et al., this meeting).

We conclude that a common calpain sensitive event triggers the dedifferentiation process.

Keywords: axotomy, growth cone, regeneration, calpain, microtubules

Color adaptation models and their predictions to color induction effects, color constancy and color contrast

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A new biological model and algorithm for color adaptations, which includes the color constancy (first order) and color contrast modulation (second order), is presented. (Color constancy (CC) is a psychophysical phenomenon in which a system has a partial capability to discount the chromaticity of the illumination). The goal of the model was to achieve computational psychophysical predictions to different color appearance effects such as color constancy, color contrast and different induction effects. The model predicts the above human visual performance, including the modulation of perceived color due to surrounding color. It predicts also the dual effects of enhancement and the suppression of the central perceived contrast due to the relation between the central and surround contrasts. The model is based on the properties of retinal ganglion cells (opponent cells) and cortical cells (double opponent as well as achromatic adaptation mechanisms in these double opponent color-coded cells: remote chromatic adaptation. The suggested color adaptation mechanisms are modeled as gain control mechanisms based on the "gain-shifting" effect. This effect is the transition from one response curve to another, due to a change in the light intensity (or color, or contrast) of the local receptive field and its remote area, in order to obtain a higher gain in the new color or contrast. The simulations calculated also on real images. The results indicate that the contribution of adaptation mechanisms to color constancy and color contrast are significant, robust, and enables color enhancement, color contrast enhancement, and color constant of still and video images.

Keywords: visual system, color vision, adaptation, remote effects

Omega-3 fatty acid treatment of depressive breakthrough during unipolar maintenance

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Objective: Studies have reported that countries with high intake of fish have lower rates of depression. We studied a specific omega-3 fatty acid, the ethyl ester of eicosapentaenoic acid (E-EPA) as an adjunct to antidepressant treatment for breakthrough depression in recurrent unipolar patients on maintenance therapy.

Methods: Design was four-week parallel group double-blind add-on to ongoing antidepressant therapy. Twenty patients participated, seventeen females and three males, all with diagnosis of current major depression.

Results: Highly significant benefits were found by week 3 of treatment for eicosapentaenoic acid compared to placebo.

Conclusions: It is not possible to distinguish whether eicosapentaenoic acid augments antidepressant action in the manner of lithium or has independent antidepressant properties of its own.

Keywords: omega-3, depression, antidepressant

Gustatory thalamus: Where does it connect to?

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The role of the gustatory thalamus in gustatory function has been controversial, due mainly to the fact that unlike other senses, gustatory projections from the parabrachial nucleus (PBN) go either directly to the cortical taste area or travel via the thalamic retrograde (fluorogold and anterograde (BDA and FDA) neuronal tracers was used to map the afferents and efferents of the gustatory thalamus Ventroposteromedial parvicular nucleus. Anterograde and retrograde into the insular cortex and PBN. We found that the PBN afferents to the thalamus do not colocalize with CGRP and that the CGRP axons have a non-overlapping more ventral localization from the PBN axons. Thalamic neurons projecting to the cortex localizeindistinctively along the nucleus, covering areas that receive input from the PBN, CGRP and areas that do not receive input from neither of the above areas. Cortical laminae cannot also do not show a clear mapping, terminals being found all over the nucleus. We were also able to determine the cellular location of the PBN synaptic terminals in the thalamic neurons. In search for a functional topographic organization, we combine thalamic retrograde labeling from the cortex and pCREB immunohistochemistry as an activity marker. We test whether there is a distinctive activity of the thalamocortical projections when the rat drinks novel taste vs. a familiar one.

Supported by the Minerva Foundation and the Human Frontiers Science Program Organization.

Keywords: thalamus, taste, cortex, pCREB, tracers.

Role of G_{ai} in the regulation of adenyl cyclase activity following acute and chronic receptor activation

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Acute activation of opiate (and other G_{ai}) receptors inhibits adenylyl cyclase (AC) activity, while prolonged activation leads to an increase in AC activity, known as AC sensitization or superactivation. This phenomenon has been proposed to play a role in opiate addiction and has been shown for AC types I, V, VI and VII, but not for the G_{ai} types IV or VII, demonstrating that superactivation is isoyme-specific. To investigate the roles of G_{ai} and G_{ai} in AC regulation, we produced several point mutations in AC-V and AC-I. We found "a mutation in the C_{ai} region (F314Y) led to reduced inhibition by acute agonist treatment and to the loss of chronic agonist induced superactivation, and in parallel to loss of AC sensitivity to G_{ai} inhibition. Constitutively active G_{ai} (demonstrating that this amino acid in C_{ai} plays an important role in the regulation of AC activity and its interaction with G protein subunits. To distinguish between the roles of G_{ai} and G_{ai}, three other mutations in C_{ai} region (shown previously to reduce affinity of G_{ai} to AC) were prepared. Indeed the activity of these mutants was not inhibited by constitutively active G_{ai} (compared with AC-V wild-type). On the other hand, these mutants behaved identical to wild-type AC-V with respect to G_{ai} stimulation and by inhibition and showed normal optate acute inhibition and chronic induced superactivation. These results suggest that the inhibition of AC by G_{ai} and not by G_{ai} is essential for the superactivation process.

Supported by NIDA and the US-Israel Binational Science Foundation.

Keywords: G_{ai}-coupled receptor; adenylyl cyclase; opiates.

Central pathways between sacrocaudal afferents and locomotor circuits in the spinal cord of the newborn rat

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The central pathways mediating sacrocaudal afferent (SCA) activation of locomotor circuits in the lumbar spinal cord were studied in isolated spinal cords of neonatal rats. The lumbar rhythm induced by SCA stimulation was abolished after blocking synaptic transmission in the sacrococcygeal (SC) cord and restored when the synaptic block of specific SC segments was temporarily alleviated. Thus, synaptic activation of SC relay neurons is required for generation of locomotor activity by SCA stimulation. The transmission across these relays required activation of non-NMDA excitatory amino acid receptors, and it was not impaired in the presence of specific antagonists of NMDA receptors and e1 and a2 antagonists. Midgutial splitting experiments of parts of the SC and lumbar cord revealed that crossed and uncrossed ascending/propropospinal pathways are coactivated by SCA stimulation. These pathways travel through the lateral, ventrolateral, and ventral funiculi, because the lumbar rhythm could be completely abolished only following a bilateral transection of all of these funiculi. Further studies are required to better understand the SCA system and the pathways associated with it, and unveil its potential use in facilitating stepping in patients with spinal cord injury.

Keywords: pattern generation, spinal cord, propriospinal pathways
A novel thiol antioxidant that can cross the blood-brain barrier protects nigral dopaminergic neurons in the three experimental models of Parkinson’s disease

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Oxidative stress is believed to play a crucial role in the degeneration of nigral dopaminergic neurons in Parkinson’s disease. Dopamine (DA) and related catecholamines were suggested to contribute to the selective degeneration of dopaminergic neurons via the formation of free radicals. Most currently available antioxidants cannot readily penetrate the blood-brain barrier. We have synthesized a novel glutathione-like low molecular thiol antioxidant (AD4) capable of penetrating the brain following systemic administration. We demonstrated that in vitro, it prevents, neuronal cell death induced by a series of parkinsonian-related neurotoxins. Furthermore this new agent demonstrated significant neuroprotection in the three experimental models. 1) AD4 inhibited the rotational behavior induced by apomorphine in rats injected unilaterally into the nigra with 6-hydroxydopamine. 2) Rats treated with rotenone, a complex I inhibitor, (5mg/kg for 28 days) degeneration of dopaminergic neurons in the substantia nigra mimicking parkinsonian characteristics. AD4 attenuated the loss of the dopaminergic neurons as indicated by immunostaining with tyrosine hydroxylase antibodies. 3) AD4 was protective in preventing the reduction in striatal dopamine levels in mice treated with MPTP (25mg/kg x 4 i.p.). These results suggest that AD4 could become a potential new neuroprotective drug used to slow down nigral dopaminergic degeneration and illness progression in patients with Parkinson’s disease.

Keywords: Parkinson’s disease, dopamine, AD4, MPTP, 6-OHDA, rotenone

The association of aggressive behavior in schizophrenia with the low enzyme activity COMT polymorphism

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Increased violent behavior in schizophrenic patients may be associated with a polymorphism at codon 158 of the catechol O-methyltransferase (COMT) gene that encodes a low enzyme activity COMT (COMT-L). While this finding has been replicated by one group, others have shown discrepant findings which may be due to different diagnostic criteria used to assess violent behavior, or, considering the complexity of aggressive behavior, either type I and/or type II statistical errors. Consequently, additional studies are needed. In this study, we assess 122 patients with schizophrenia for violent behavior using the Lifetime History of Aggression (LHA) scale, an 11-item questionnaire that is subdivided into Aggression, Self-Directed Aggression, and Consequences/Antisocial Behavior subscales. DNA was genotyped for the COMT 158 polymorphism, as well as a functional polymorphism in the monoamine oxidase A gene (MAOA) promoter. Similar to previous findings reported by our group, a statistically significant association was found between violent behavior in schizophrenia and the COMT 158 polymorphism. More LHA scores were higher in subjects homozygous for the low enzyme activity COMT variant, 158Met (p=0.005). Analysis of the major and minor allele carriers revealed that the association with 158Met was due to high scores on the Aggression, and Self-Directed Aggression subscales, but not the Consequences/Antisocial Behavior subscale. No significant association was detected for the MAOA gene alone. These observations further suggest that COMT is a modifying gene that plays a role in determining interindividual variability in the propensity for outward and self-directed violent behavior found in a subgroup of schizophrenic patients.

Keywords: schizophrenia, aggression, COMT, polymorphism

Neuoroanatomical and immunohistochemical characterization of the brainstem for barbiturate-evoked general anesthesia

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Microinjection of minute quantities of pentobarbital into a restricted region of rat mesopontine tegmentum can induce a state of general anesthesia (Devor and Zakkind, Pain 2001). We have begun to investigate the anatomical connections and histochemical signature of this mesopontine tegmental anesthesia area (MPTA) using anterograde and retrograde tracers and immunocytochemistry. MPTA has bilateral ascending and descending projections that project to the mesencephalon and brainstem structures, including the caudoputamen, hypothalamic and subthalamic structures, intra-laminar nuclei of the thalamus and widespread areas of the mesencephalon, pontine and medullary recticular formation. Descending spinal projections reach as far as the sacral segments. MPTA receives bilateral afferent projections from restricted areas of the diencephalon and the reticular formation. The most important candidate receptor for barbiturates is a modulatory site on the GABAA receptor (GABA-AR), which augments cellular response to GABA. To determine if GABA-ARs are present on MPTA neurons, serial brainstem sections were immunolabeled with antibodies to the alpha 1 subunit of the GABA-AR. This is the most common GABA-AR in the CNS. Labeled neuronal cell bodies were scattered throughout the MPTA region with dendrites stained to some extent, and heavy staining in the neuropil. A significant proportion of neurons in the MPTA were immunoreactive to the non-selective receptor for general anesthetics. Immunocytochemical characterization of MPTA cells together with their connectivity, contributes to the understanding of brainstem circuits controlling alert behavior.

Keywords: general anesthesia, central pain mechanisms

Further studies on the octopus fetch movement

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When an octopus reaches towards an object, it forms a bend at the base of the arm which is propagated towards the tip. Interestingly, during fetching, when an end-point control strategy is used in order to bring the object to a specific point (the mouth), a totally different type of movement is performed. In this case, the octopus grasps an object, anywhere along its arm, and brings it to the mouth. A 3D kinematic analysis has revealed the following characteristics: An articulated-like structure is formed by connecting 3 joints along the arm, in a definite spatiotemporal order. In a given movement, the length of the two proximal segments is similar. The joints rotate along a single plane, to accurately bring the end-point to the target location (the mouth). We constructed a model of the octopus that simplifies the motor control of its arm during goal-directed end-point movements by utilizing an adjustable articulated-like structure. This, thereby, drastically reduces the number of degrees of freedom from virtually infinite to only three, allowing the octopus to achieve a high level of accuracy. In contrast to arm extensions, where we showed that the motor program is confined to the peripheral nervous system (Science 2001) 293:1845-1848, preliminary lesion experiments show that the fetching movement is controlled by higher motor centers in the brain. Supported by the Israel Science Foundation.

Keywords: cephalopods, motor control, flexible structure, kinematics

Temporal encoding of horizontal object position by the rat whiskers: recordings from the trigeminal ganglion

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We investigated how neurons in the trigeminal ganglion of anesthetized rats respond to whisking against objects inserted at different horizontal positions along the anterior-posterior axis. Rhythmic 5 and 8 Hz whisking similar to spontaneous whisking were induced by electrical stimulation of the peripheral buccal branch of the facial motor nerve ("electrical whisking"). The object was placed inside the whisking field at different horizontal
distances from the whisker resting position. We recorded single- and multi-unit responses and captured video-images of the whisking motion. This allowed us to correlate the moment when the whisker touched the object with neural events.

We divided single-unit responses into 1) "touch cells" (n=30) that responded to the various aspects of whisker-object contact 2) "whisking cells" (n=29) that responded to whisking and 4) "high threshold cells" (n=21) that responded only to very rapid mechanical deflections.

When a whisker protracts, more posterior objects are contacted earlier, and more anterior - later. We found that for all "object detectors" the timing of the first spike occurring after protraction began was tightly correlated with the time of whisker-object contact. We conclude that horizontal position of object is temporally encoded in the responses of these "object detectors" such that firing later relative to protraction initiation signifies more anterior position. Also, horizontal object position was sometimes encoded by decreasing spike-counts. This coding, however, was less consistent than the latency coding.

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Keywords: object localization, neural code, sensory encoding

High Pressure and [Ca²⁺] Modulation of Dynamic Properties of a Cortico-hippocampal Synapse

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Hyperbaric environment (>1.5-2 MPa) induce abnormal activity in the system termed high-pressure nervous syndrome (HPNS) that is characterized by cognitive and motor impairments. The neocortex and the hippocampus are involved in the representation of position of the body. The medial perforant path (MPPP) synapse onto the granule cells of the dentate gyrus is the main path connecting these structures. Cellular effects of high-pressure of helium were studied using electrophysiological recordings in rat cortical hippocampal slices at 30°C. High pressure (10-15 MPa) suppressed single MPP field-EPSP (iEPSP) slope by 55 ± 7 % (n=9) while iEPSP’s decay time and delay were prolonged. Amplitude, slope and duration of MPP input volley were reduced, respectively, by 40 ± 10.1 MPa (n=5). Pressure reduced paired-pulse depression (PPD) observed at short inter-stimulus-intervals (ISI), and increased paired-pulse facilitation (PPF) at 40-120 ms (from 5-20 %).

Pressure increased synaptic frequency-dependent depression (FDD) at 25-50 Hz. Reduction of [Ca²⁺], from 2 to 1 mM under normobaric conditions mimicked high-pressure effects on single iEPSPs slopes (depressed by 51±8 %, n=7) and partially reproduced high-pressure increase in PPF (n=5).

In contrast, low [Ca²⁺], induced frequency-dependent potentiation (FDP) instead of FDD observed at pressure (n=5).

The data indicate that high-pressure effects on single and paired synaptic responses are induced by impaired Ca-entry at presynaptic terminals. However, the responses at high frequencies are not simply additive. This suggests that additional mechanisms such as vesicle replenishment, priming or docking at synaptic sites are impaired by high pressure.

Keywords: hyperbaric pressure, HPNS, field potentials, paired-pulse facilitation, frequency-dependent depression, presynaptic Ca²⁺ influx, perforant path, dentate gyrus

mGSTM5 KO mice as a potential model for brain studies

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1Albert Einstein College of Medicine of Yeshiva University; 2NY, USA; 3Ben-Gurion University of the Negev, Beer-Sheva; 4Glutathione S-transferases (GSTs) are members of a superfAMILY of detoxification enzymes. Recent findings suggest some GSTs may have some other functions as well. mGSTM5 (orthologue of hGSTM3) could be one of these functions. We found that this cytosolic isoform is abundant in tests, well represented in brain and barely expressed in other organs. Peptide sequence specific mGSTM5 and hGSTM5 antibodies were used to localize this additional APO as well as in the brain by immunoperoxidase staining. mGSTM5 was found to be unevenly distributed in different regions of mouse brain: strong positive staining was observed in perikarya and processes of Purkinje's neurons in anterior thalamus and forebrain and neuronal processes in cortex. Analysis of sections from patients who were diagnosed with Alzheimer's disease for the presence of this isoform in neocortical and hippocampal regions. These findings indicate that hGSTM3/mGSTM5 may play a specific role in brain.

Keywords: glutathione S-transferases; knockout mice; brain; gene expression

Accelerated brain-to-blood efflux of glutamate: A novel approach for the management of neurodegenerative diseases

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Abnormally high Glutamate (Glu) levels in brain interstitial and cerebrospinal fluids (ISF/CSF) are the hallmark of several neurodegenerative conditions. We examined here the prediction that a decrease of blood Glu levels should facilitate the naturally occurring process of brain-to-blood Glu efflux and assist the brain to remove the excess Glu from the ISF/CSF into blood. The feasibility of using hGlu efflux was studied by using two basic paradigms based on the fate of [3H]Glu infused into brain. In the first, we infused [3H]Glu intracerebroventricularly and followed the flux of radioactivity in blood before, during and after decreasing blood Glu levels by the intravenous administration of pyruvate and oxaloacetate, the respective Glu co-substrates of the brain resident enzymes Glutamate-pyruvate transaminase and Glutamate-oxaloacetate transaminase. In the other, we performed ventriculo-cisternal perfusions of [3H]Glu and followed its disappearance from brain, during and after decreasing brain Glu levels with pyruvate and oxaloacetate.

In both cases, the results obtained point out to the same conclusion that the intravenous administration of pyruvate and oxaloacetate that accelerates blood Glu efflux was more efficient than the intravenous administration of pyruvate alone. This suggests that Glu co-substrates of the brain resident enzymes Glutamate-pyruvate transaminase and Glutamate-oxaloacetate transaminase are not necessary for the transport of the excess Glu present in the brain interstitial fluid or cerebrospinal fluid. The brain-to-blood Glu efflux is thought to trigger neuronal cell death and its accompanying neuropsychological sequelae. Examples of the neuroprotective and life-saving role of this newly proposed brain scavenging condition will be presented.

Keywords: neuroprotection, glutamate scavenging, brain-to-blood efflux

Ischemic preconditioning involves greater reductive capacity of the brain and lesser oxidative stress in rats

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Background: Brain injury (ischemia, trauma) releases reactive oxygen species (ROS) implicated in secondary brain damage. Endogenous low molecular weight antioxidants (LMWA) neutralize ROS and are among the defense mechanisms protecting tissues from oxidative stress. Sub-lethal injury provides protection by a mechanism not fully understood, collectively referred to as "preconditioning." This study examines whether brain LMWA are altered in ischemic preconditioning.

Methods: Rats were preconditioned (PC) by 2 min occlusion (or sham surgery) of right middle cerebral artery (rMCAO) using intraluminal suture. 24h later arterial blood pressure was reduced to 55 mmHg and rats were subjected to 90min of rMCAO (or sham surgery) followed by 5min or 4h of reperfusion. LMWA were extracted from both hemispheres and the tissue reducing power derived from LMWA was evaluated by cyclic voltammetry (Cv) in paraffin tissue sections. B. et al. 1999. "Enzymology 300: 285-296" (1999). Infarct volume was assessed at 4h using TTC staining.

Results: PC rats displayed ~20% smaller infarct volume as compared to cells-PC rats (p<0.05). In non-PC rats, at 5 min of reperfusion LMWA levels dropped from 0.209±0.062 to 0.149±0.021 nAmp/mg protein (p<0.05), indicating their consumption by ROS production. In contrast, in PC rats a 2.5-fold increase was measured (to 0.531±0.114 p<0.001) suggesting that mobilization of...
antioxidants occurred at preconditioning, and their availability during the ischemic injury is part of the neuroprotection. At 4h of reperfusion, the LMWA levels increased by 14% and 18% in both groups. These findings agree with our previous reports showing that higher LMWA levels after injury are associated with better recovery.

Keywords: cerebral ischemia, preconditioning, oxidative stress, antioxidants

Behavioral impact of hyperhomocysteinemia in Apolipoprotein E-deficient mice

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High levels of homocysteine in blood are a risk factor for Alzheimer’s disease and cognitive decline in the elderly (Seshadri, S. et al., New England Journal of Medicine 346(7):476-83 [2002]). Homocysteine, a sulfur-containing amino acid, generated as a metabolic by-product of biological methylation reactions, is thought to be toxic to endothelial cells and neurons, and is disposed through reactions that require folate and vitamins B12 and B6 as cofactors (Selhub, J. et al., American Journal of Clinical Nutrition 71(Supplement):614S-20S [1999]). We used ApoE-null mice to determine whether nutritionally induced hyperhomocysteinemia would accelerate age-related neurological changes characteristic of these mice. Male 4-week old, male C57BL/6J mice were fed control, vitamin-deficient (folate 1% & B6), vitamin-supplemented, and B-vitamin and methionine-supplemented diets for eight weeks. Wild type C57Bl/6 mice fed a control diet were used to establish behavioral and biochemical reference values. At 8 weeks, mice underwent behavioral testing on a series of psychomer tests, the Morris Water Maze test of spatial learning, and measurement of home-cage exploration and open-field behavior. The two B-vitamin deficient diets induced significant hyperhomocysteinemia in ApoE-null mice, and were associated with markedly longer escape latencies from the Morris Water Maze, but not with psychomotor performance. A trend for hyperactivity in B-vitamin supplemented ApoE-null mice was observed in nocturnal open-field activity and in measures of psychomotor function. Behavioral differences were observed between ApoE-null and wild-type mice fed control diets.

Keywords: nutrition, homocysteine, behavior, dementia, ApoE, mouse

Involvement of the monoaminergic neurotransmitters systems in cooperative coordinated behavior in laboratory rats

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The motivation to cooperate is explained by two contrasting approaches; Individual persons focus on the individual’s behaviors and outcomes. A ‘SOCIAL’ perspective suggests a distinct ‘social reward’ mechanism influenced by the presence and behaviors of participants.

Cooperation was studied using a laboratory rat model of cooperative coordinated shuttling. Previous research found that cooperative coordinated shuttling is a social act and that it is preferred over individual shuttling. The individual’s behavior and activation of the dopaminergic, noradrenergic and serotonergic systems in the frontal cortices, and hypothalamus (assessed by HPAC method) were measured in five groups: COOP, pairs shuttling coordinately, S/I: ‘pairs’ shuttling uncoordinatedly, IND: individual shuttling, NC: individual not required to shuttle, and C: control group.

The results support the ‘SOCIAL’ perspective. In both COOP and S/I groups, intra- pair asymmetries were related to aggressive behaviors, but the COOP group was characterized by more frequent use of non-aggressive social behaviors. The monoamine data supports the ‘social reward’ hypotheses. Social factors such as pair-partner’s presence and social behaviors affect these systems in a pattern distinct from individual persons focus. Hypothalamus NE and SHAIA data point to different states of arousal attributed to the presence and behaviors of the partner. Differences between ‘SOCIAL’ and ‘NONSOCIAL’ conditions, in neurotransmitter levels, are significant, point to its involvement in rewarding social behaviors. Lower levels of 5HT found in left frontal cortex of the COOP group compared to the S/I group, may relate to the more frequent non-aggressive social behaviors that characterize cooperation.

Keywords: cooperative behaviors, social behavior, coordinated behaviors, social reward, monoamines

The functional relevance in pancreatic β islet cells of domains of the delayed rectifier K+ channels, Kv1.1 and Kv2.1, that bind in vitro syntaxin 1A and SNAP-25

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Recently, we have shown that the voltage gated K+ channels Kv1.1 and Kv2.1 interact directly with the SNARE proteins of the exocytotic machinery. In this study we have mapped the binding regions within the channels that are involved in these interactions. We have shown that syntaxin 1A binds the N terminus of the Kv1.1 channel, preferentially the T1 A’ region of the assembly domain. SNAP-25’s pattern of binding to Kv1.1 is similar. In collaboration with the lab of Dr Gaisano from the University of Toronto (1) we have shown that SNAP-25 inhibits the Kv1.1 activity in insulinoma HIT-T15 cells, and the inhibitory effect is relieved by application of GST-fusion proteins corresponding to the N terminus and to the T1’A’ domain, but not by the C terminus or GST itself. These results support the notion that SNAP-25 exerts its inhibitory effect on Kv1.1 through interaction with the N terminus, specifically the T1’A’ domain.

We next characterized the interaction with Kv2.1 and showed that syntaxin 1A binds both the C terminus (folate 1% & B6) and on application of fragments corresponding to the N terminus this inhibition was relieved. These results support a causative relationship between the functional effects of SNAP-25 on Kv1.1 or Kv2.1 and its binding to the channels in insulinoma β cells, respectively. Causative relationships regarding syntaxin 1A are yet to be determined.

Keywords: Kv channels, syntaxin 1A, SNAP-25, SNARE complex

Representation of stimulus probability by primary auditory cortex neurons

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The ability to detect rare auditory events may be critical for survival. Furthermore, stimulus statistics strongly influences the optimal neural code. We presented sequences of tones at two frequencies f1, f2, and manipulated the frequency interval df = f2 - f1. The neurons exhibited therefor significant differences between responses to common and rare sounds. The difference in responses to common and rare sounds was a result of stimulus-specific decline in the responses to the common sound (stimulus-specific adaptation, SSA). This difference was positively correlated with df, and negatively correlated with the abundance of the rare tone. Thus, A1 neurons are sensitive to global stimulus statistics. Significant differences between responses to common and rare sounds were found in many neurons even for the smallest frequency interval of 4%. These neurons exhibited therefore hyperacuity, a frequency resolution that is an order of magnitude better than receptive field width in either A1 or the auditory periphery.

We hypothesize that this form of SSA is a neural correlate of mismatch negativity, an important auditory event-related potential, implicated in sensory memory. Our results suggest that auditory cortex neurons, in addition to processing the acoustic features of sounds, may play a role in sensory memory and in novelty detection.
Neuronal degeneration in the CNS is often characterized by a protection and destruction. We show that the 'SIR' model of the self-destructive process, Secondary Neuronal Degeneration (SND), without evidence of an initial population of infected people to healthy people. To study the effects of the immune system, we extended the 'SIR' model by adding two populations with biochemical effects that seem to play an important role in these processes; one population attacks neuronal tissue by eradicating the infected and damaging the cells, and the other does not. With the extended 'SIR' we qualitatively reproduce important effects of the immune protection, including the dual effect of the autocrine T cells on both benefiting and harming nerve cells. Moreover, we show that to benefit the damaged tissue, the amount of injected T cells should not be constant but should depend on the severity of the injury.

**Keywords:** autoimmune, neuroprotection, secondary damage, nerve injury

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**Hyperdense basilar artery - a fatal prognostic sign**

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A high density of the basilar artery was found and measured in a review of admission records and NCCT of 20 patients admitted to Soroka Medical Center between 1999-2002 with fatal basilar stroke. HBA was found in 10 patients. Eight patients who died before clear infarct was demonstrated in a group of patients was significantly younger than patients without HBA. Latency time between admission and determination of a high density was significantly shorter in patients with HBA. A higher density of the basilar artery was found and measured by comparison to other level of the basilar artery, to the other main artery at the same patients and to patients of matched group without infarct. HBA is a fatal prognostic sign found in relatively young patients. Early thrombolytic treatment should be considered in this catastrophic entity in order to improve outcome.

**Key words:** Psychophysics eye movements

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**Patterns of eye movements in stereo motion induced blindness**

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Bonneh et al. reported a phenomenon of visual disappearance. When a global moving pattern is superimposed on high contrast stationary stimuli, the latter disappear and reappear alternately by steps, and the other phenomenon motion induced blindness MIB results showed that it is unlikely to reflect retinal suppression or sensory masking, but rather is a result of a conflict generated between cortical representations of discontinuous stimuli, which shifts the system dynamics in a winner-takes-all mode. Other interpretations refer to attentional mechanisms, which cannot be allocated or divided between dissociated elements at the same spatial location and at the same time we have detected relations between eye-movement patterns and gaze-directions, with subject's reports of display component disappearance during a MIB situation. We report MIB studies with stereo MIB stationary MIB targets and a rotating distractor dot pattern (tow rotation rates) were perceived as superimposed surfaces and dissociated by 6 stereo disparities. MIB was reported with distractor either in front or behind MIB MIB. Distractor rotation rate affected MIB periods, saccade rate and binocular divergence. High distractor rotation rate generated better binocular divergence to the more distant distractor displays, and a more pronounced saccade rate difference between MIB and No-MIB periods. Results have also revealed effects of time schedule eye movement dependence on MIB disappearance (e.g. target reappearance preceded by a specific eye movement features) Results support the view that attentional mechanisms underlie the MIB phenomena.

**Key word:** Psychophysics eye movements

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**Gene and protein expression profile homology of anti- and pro-apoptotic activity of dopamine, R-apomorphine, and melatonin in human neuroblastoma cells**

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Oxidative stress is considered responsible for the degeneration of dopaminergic neurons in the substantia nigra pars compacta in Parkinson's disease (PD) and its animal models. Anti-oxidants have concentration dependent neuroprotective and pro-apoptotic activities in models of PD. The aim of our study was to determine protein expression of the latter activities with structurally different anti-oxidants, dopamine (DA), R-apomorphine (R-APO), melatonin and green tea polyphenol ((-)-epigallocatechin-3-gallate (EGCG)) in neuroblastoma (SH-SY5Y) cell line. Expression of antioxidants' gene expression technique and quantitative real-time PCR. We demonstrate a concentration-dependent correlation between R-APO, DA, and EGCG and modulation of cell survival/cell death-related gene pathways. Conversely to the effects of low concentrations (1-10 μM), where an anti-apoptotic response was manifested, a pro-apoptotic pattern of gene expression was observed at high concentrations (50, 500, 50 and 50 μM, respectively) of the antioxidants (e.g. increase of caspases, fas and gadd45). Protein analysis of Bcl-2, Bax and activated caspase-3 confirmed the gene changes. Melatonin has an extremely low pro-apoptotic activity compared to DA, R-APO and EGCG, which was partly explained by the observation that high concentration of melatonin did not significantly affect the expression of the conserved group of mitochondrial Bcl-2 family members, especially Bcl-2 and Bax. Our results provide for the first time new insights into the molecular events involved in the dose dependent activities of catecholamines, indole amine and phenolic compounds, reputed to have neuroprotective activity at low and neurotoxicity at high concentrations, in cell cultures and in vivo models of neurodegenerative diseases.

**Key words:** antioxidants, apoptosis, neurodegenerative diseases, cDNA array

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**Alteration in the activity of the hypothalamic-pituitary-adrenal axis and behaviour by prenatally stress**

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Retrospective studies have shown that exposure of pregnant women to adverse life events, like marital conflict, war, major disasters, like an earthquake or flood, increases the likelihood of behavioural disturbance in their infants and of a higher incidence neurotic and depressive symptoms in adulthood. Prenatally-stressed (PS) rats and neonatally-stressed (NS) rats have disturbances in their behaviour characterized by heightened anxiety in novel, intimidating environments and failure to cope with life-sustaining activities such as food seeking and maternal care under stress. Like depressed human subjects, PS rats show abnormalities in sleep patterns, circadian rhythm, decreased hedonic behaviour (sweet preference), and develop learned helplessness more readily, and brings about a reduction in hippocampal glucocorticoid receptors in the offspring and in the feedback inhibition of CRH, leading to a further increase in blood levels of glucocorticoids. The depressive symptoms, hyperactivity and abnormal regulation of the hypothalamic-pituitary-adrenal (HPA) axis can be significantly

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**Keywords:** auditory cortex; physiology; adaptation; mismatch negativity

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**Modeling neuronal degeneration and autoimmune protection in the central nervous system (CNS)**

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Neuronal degeneration in the CNS is often characterized by a progressive degeneration, which continues even after the primary causative factor has been removed. This is attributed to the self-destructive process, Secondary Neuronal Degeneration (SND). It was discovered (Moalem et al., Nat. Med. 5:49-55) that lesions could rescue neurons from SND. Paradoxically, the same cells can also induce the autoimmune disease EAE, resulting in neuronal damage. We developed a set of mathematical models, that might explain the mechanisms of SND, and the possible mechanisms for immune protection and destruction. We show that the 'SIR' model of epidemics with some modifications can qualitatively simulate SND results. Similar to SND, an epidemic is also a self-perpetuating process whereby a disease spreads from an initial population of infected people to healthy people. To study the effects of the immune system, we extended the 'SIR' model by adding two populations with biochemical effects that seem to play an important role in these processes; one population attacks neuronal tissue by eradicating the infected and damaging the cells, and the other does not. With the extended 'SIR' we qualitatively reproduce important effects of the immune protection, including the dual effect of the autocrine T cells on both benefiting and harming nerve cells. Moreover, we show that to benefit the damaged tissue, the amount of injected T cells should not be constant but should depend on the severity of the injury.

**Key words:** auditory cortex; physiology; adaptation; mismatch negativity
decreased by chronic treatment with antidepressants in humans. In many of such subjects they also correct the abnormal feedback regulation of the HPA axes, and acute intracerebral administration of a CRH antagonist can reduce their hyperactivity. Chronic oral administration of antidepressants to PS rats from puberty is also effective in norming the anxious behaviour as well as the HPA axis regulation at adulthood. 

**Keywords:** prenatal stress; hyperactivity; corticotropin releasing hormone; antidepressant 

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**The POU4F3 transcription factor in human hereditary deafness**

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A mutation in the POU4F3 gene is responsible for DFNA15 (OMIM242549), autosomal dominant nonsyndromic hearing loss, in an Israeli family. We are now trying to understand the exact mechanism by which the mutation causes this phenotype. Since this protein is only expressed in the hair cells of the inner ear, we were unable to obtain material from affected individuals to study the mechanism. Therefore, we cloned the wild type and mutant POU4F3 into expression vectors and injected into adult PS rats, acute intracerebral administration of POU4F3 was more abundant when compared to the wild type product. Metabolic labeling experiments (pulse-chase) have indicated that the mutant form half life is longer than the wild type form, suggesting that accumulation of this protein will cause cellular damage over time. Luciferase experiments indicated that the mutant protein could not activate the Snap25 promoter to the same extent as the wild type protein. In situ immunofluorescence revealed that most of the truncated POU4F3 protein was localized in the cytoplasm, while the wild type form was in the nucleus. We have recently isolated the putative nuclear localization signals (NLSs) of POU4F3 and shown that both a monopartite and bipartite NLS is required for complete nuclear localization of this transcription factor. Thus our data clearly demonstrates that the majority of the mutant form of POU4F3 is unable to enter the nucleus, presumably preventing it from regulating transcription of its targets in the nucleus. 

**Keywords:** transcription factor, protein stability, NLS, deafness 

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**Role of the 5HT3 receptor in the nucleus accumbens in neurochemical alterations in an animal model of depression**

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Current hypotheses on the etiology of depression attribute the disorder to alterations in serotonin neurotransmission. However, the relationship between these alterations and depression is poorly understood. Conversely, an interaction between the serotonergic and dopaminergic systems in the nucleus accumbens (NAc) has been established. It has been demonstrated that there is a dramatic loss of 5HT-induced dopamine release in the NAc of an established rat model for depression (Flinders Sensitive Line, FSL) that can be reversed with antidepressant treatment. 5HT3 receptors are central modulators of the serotonergic-dopaminergic interaction in the NAc. Therefore, changes in the 5HT3 receptor function can affect depressive behavior. 

Our aim was to examine the role of the 5HT3 receptor in alterations of 5HTT-induced dopamine release in the NAc of FSL rats. Dopamine release was lower in FSL rats compared to controls following acute 5HTT agonist administration. Application of a non selective 5HT3 receptor antagonist did not alter the dopamine release in FSL compared to control rats, the receptor affinity was significantly lower (40%) in FSL rats. Chronic treatment of FSL rats with antidepressants increased the affinity of the 5HT3 receptor to the level of controls, but did not markedly affect the receptor affinity in control rats. Our findings associate 5HT3 receptor with an animal model of depressive behavior. Therefore, 5HT3 receptor may be a target for future treatment of depression. 

**Keywords:** depression, nucleus accumbens, 5HTT-3 

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**Quantitative analysis of thalamocortical synapses in adult mouse barrels**

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This study focused on the synapses made by thalamocortical afferents to barrel cortex in the adult mouse. The aim was to determine precisely the numbers of boutons per axon length, the number of synapses per bouton, and the identities of postsynaptic elements. Axons were labeled by the anterograde transport of lysin-fixable biotinylated dextran amine (BDA) injected in vivo into the ventrobasal thalamus. Labeled axons in the postero medial barrel subfield were examined by light microscopy and then reconstructed in three dimensions to assess the spatial distribution of their synapses. All thalamocortical synapses were of the asymmetrical type. Generally, thalamocortical synapses were spaced at axonal varicosities, however, some synapses occurred at cylindrical portions of thalamocortical afferents. Preliminary results indicated that axonal varicosities form from 2 to 3 synapses. As in developing barreles, the ratio of axon to axon-dendritic synapses was 4:1. Comparison of the synaptic organization of thalamocortical afferents at early postnatal stage (P11, Levy et al., 2001) with the adult, support the notion that thalamocortical synaptic connectivity is specified early in development. 

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Keywords:** somatosensory, axon, thalamus, afferent 

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**Contrast discrimination learning with uncertainty**

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Performance on perceptual tasks improves with practice. However, in vision, contrast discrimination thresholds show a remarkable stability when a large range of contrasts (0-0.6) is practiced. There are two known exceptions: (a) when the practiced target is surrounded by flankers (Adin et al. 2002), (b) when practicing with a single base contrast (Kong et al. 2002). The improvement can be explained by increasing the gain of contrast transduction and/or by optimization of discrimination strategies. Therefore, changes in the NAc can affect the 5HT3 receptor function. 

Discrimination thresholds were halved during practice. However, this improvement was found to be specific to the trained condition and post-training testing of control and uncertainty showed no improvement. A second group of observers practiced the full contrast range with the target embedded in a chain of flankers, showing the expected improvement in contrast discrimination. This learning effect was found to transfer to the post-learning test with contrast uncertainty. It seems that the human visual system can use both high and low level modifications to improve contrast discrimination. The specific choice made by the system may depend on the number of contrast levels used and their spacing, as well as on previous experience. 

**Keywords:** perceptual learning, vision, contrast discrimination, context, lateral interactions 

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**Induction of experimental autoimmune myasthenia gravis (EAMG) by a lipopolysaccharide-acetycholine receptor peptide conjugate**

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Molecular mimicry is implicated in the pathogenesis of autoimmune disorders. For example, in Guillain-Barré syndrome the immune attack on nerve antigens is attributed to the cross reactivity of carbohydrate antigens in mycobacterium jejuni lipopolysaccharides (LPS) and in gangliosides. We have shown that covalent bonding of an LPS molecule to gangliosides enhances the immune response to self-gangliosides. 

**Keywords:** lipopolysaccharide-acetycholine receptor peptide conjugate, immunoassays.
Objective: To test the hypothesis that LPS bound antigens could overcome tolerance to protein antigens.

Methods: The decapeptide WNPDDYGGVK, (the main immunogenic region-MIR) of the α chain of acetylcholine receptor (ACHR) was conjugated to Salmonella Re-LPS by heterodimeric cross-linking. Female Lewis rats subcutaneously were presensitized by 3 daily intraperitoneal injections of 10 μg of MIR-LPS conjugate on days 1 and 21, and received intraarterial injections of 15 μg of MIR or Re-LPS only on day 28. Control rats received MIR or Re-LPS. Results: 15/25 LPS-MIR injected rats showed pathological treadmill exercise intolerance and immunosassays for AChR antibodies. Controls: 4/12 MIR-LPS injected rats were unable to complete 10 minutes of treadmill exercise compared to 1/13 of controls. AChR antibodies were detected by RIA in 3 of 5 of the MIR-LPS B group of the controls. AChR was kindly provided by Dr. Wolf-Goldberg T., Michaelevski I., Chikvashvili D. and Lotan I. Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978

Conclusions: MIR-LPS conjugate injections can induce EAMG without additional adjuvants. LPS was shown to have adjuvant like effects in the induction of autoimmune phenomena but the molecular bond of LPS to antigen appears to play an important role in overcoming tolerance to self-antigens such as AChR.

Keywords: myasthenia gravis, molecular mimicry, lipopolysaccharide-peptide conjugates

Impaired interleukin-1 (IL-1) signaling is associated with increased morphine sensitivity

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The cytokine IL-1 modulates pain perception in various inflammatory and infectious conditions. IL-1 is known to induce algogenic mediator release and often acts as a factor and substance P. These mediators are also induced by morphine, and they may counteract the algogenic effect of morphine, and possibly participate in development of morphine tolerance. We have recently demonstrated that mice with knockout of the IL-1 receptor type 1 (IL-1rKO), or the IL-1 receptor accessory protein, as well as mice with transgenic overexpression of IL-1 receptor antagonist, exhibit reduced baseline pain sensitivity compared with their wild-type (WT) controls and parent strains, suggesting that impairment in IL-1 signaling disturbs the balance between analgesic and hyperalgesic systems. The present study tested the hypothesis that IL-1 influences morphine-induced analgesia. We assessed the effects of morphine on pain sensitivity in IL-1rKO and WT mice, using the hot-plate test. A dose of 4mg/kg morphine was injected subcutaneously, and pain sensitivity was measured 30, 90, 150, 210, and 270 minutes later. Percent analgesia was calculated using the formula: [(after-injection latency - baseline latency)/(cutoff latency - baseline latency)] * 100. As expected, IL-1rKO mice displayed lower baseline pain sensitivity compared with WT mice (27.51 vs. 12.24 sec, respectively). Morphine displayed greater analgesia compared with WT mice treated with morphine (100%, 90%, 59.6%, 48.6%, 5% vs. 63.9%, 28.3%, 27.6%, 18.1%, 7.5%, at the different time points, respectively). These findings suggest that in addition to its involvement in basal pain sensitivity, IL-1-signaling also counteracts the algogenic effect of acute morphine.

Keywords: interleukin-1, morphine, pain sensitivity, analgesia

Characterization of the role of the C-terminus in the interaction of the voltage-gated K+ channels Kv2.1 and Kv2.2 with the proteins SNAP-25 and syntaxin

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Recently, we have shown that presynaptic Kv1.1 and Kv2.1 interact directly with SNAP-25 and syntaxin in synaptosomes and neuroendocrine cells-PC12, respectively. Integrated electrophysiological and biochemical analyses in Xenopus oocytes reveal that the Kv2.1 biophysical parameters are similar to its interactions with those proteins. These studies indicate that the C-terminus might have an important role in the interaction of the channel with the proteins. To further characterize the role of the C-terminus we study the interaction of the synaptic proteins with the Kv2.2 channel which is the most closely related to Kv2.1. The Nt- terminal cytoplasmic domains and the hydrophobic cores of both channels, which contain six transmembrane segments, have a 84.2% amino acid identity. Because the remaining COOH-terminal cytoplasmic portion starting at threonine 535 in Kv2.1 displays only 21% of identity. Our results point out to a substantial similarity between the effects of syntaxin and SNAP-25 on the two channels, however, with some differences. Kv2.1 and Kv2.2 are differentially localized in the brain and in various peripheral tissues, e.g. the greatest density of Kv2.2 mRNA is detected in the olfactory bulb, followed by the hippocampus, cerebellum and cortex whereas Kv2.1 mRNA levels are highest in the cerebral cortex, followed by the hippocampus, cerebellum and olfactory bulb (Hwang et al, (1992) Neuron Vol. 9:473-481). The differences may help us understand if the two channels may serve similar functional roles in different organs or they may mediate distinct functions.

Keywords: Kv2.1, Kv2.2, C-terminus, synaptic proteins

Neuropharmacogenetic changes in the depressive brain: Studies in an animal model of depression

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Understanding the abnormalities manifested in neurochemical pathways during depressive disorders and the dynamic effects of these abnormalities on the onset of action and efficacy of pharmacological treatments is crucial for the development of effective antidepressant drugs and therapeutic strategies. Over the last 40 years, eight classes of antidepressants have become available. Nonetheless, none of them improve the efficacy, speed the response or demonstrate long-term effects. In addition, studies have suggested many neurotransmitters and neuromodulators as candidate systems involved in depression. Neurontransmission changes in the brain should be monitored dynamically and the cross-talk between these neuronal systems should be at multiparametrical levels: neurophysiological, neurochemical and neurogenetic. The present review will discusses recent findings alterations in the neurobiology of the brain that accompany depressive behavior and effective antidepressant treatment in a unique animal model of depression (Flinders Sensitive Line of rats).

Keywords: depressive behavior, neurogenetics, neurochemistry, neuropharmacology

Working memory for multiple stimuli in monkeys

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We studied how monkeys hold several familiar stimuli in working memory. Two Macaque monkeys were trained on a modified delayed-match-to-sample task with multiple samples. Monkeys viewed a sequence of stimuli on a computer monitor and released a lever when any one of the same stimulus in the sequence was presented for a second time within the trial - i.e. a "match" stimulus. Trials had 2-8 stimuli with 1, 2 or 3 inter-stimulus intervals. Two type of errors occurred:

1. "Misses" when the monkey failed to respond to a "match" presentation. Distribution of these errors depended on the position of the "cue" (first presentation of the stimulus which was later matched). The greater the cue-match separation, the poorer the performance. This finding indicates that working memory of a given item erodes with time and/or the number of intervening stimuli. However, for a given cue-match separation, performance improved with sequence length, i.e. with number of irrelevant stimuli preceding the cue. This may reflect the rising expectation of reward.

2. "False positives" when the monkey erroneously responded to an image which was not a repetition of a previously presented stimulus within the trial (a "non-match" stimulus). Distribution of these errors depended on the time and number of stimulus since the beginning of the trial.

Taken together, these two findings suggest that noise, associated with the neural dynamics, spontaneously erases items from working memory and induces ghost memories of familiar, unseen stimuli. A detailed model has corroborated this picture.

Keywords: working memory
Erythropoietin (EPO) is neuroprotective in mice and rats and markedly reduces neuronal apoptosis in an experiment of head injury. Active fraction of EPO have deleterious effects on inflammatory and anti-apoptotic modalities ameliorate CHI outcome. Erythropoietin (EPO), a kidney-derived cytokine regulates hematopoiesis, acts as a growth factor and apoptotic inhibitor. Cultured brain cells produce EPO in response to oxidative stress, and EPO receptor is present on neuronal and brain capillary endothelial cells. This study examines the protective effects of EPO in rats and mice undergoing controlled CHI. Methods: CHI was induced using a weight-drop device. Clinical status was evaluated by Neurological Severity Score (NSS), which tests 10 or 17 tasks for mice and rats respectively. Animals were treated with 2 doses of i.p. 5,000 units/kg/dose (1 ml) of HuEPO, or vehicle 1h and 24h after CHI. NSS was evaluated at 1, 3 and 7d post CHI, and compared using a two-tailed student t-test. Brains were analyzed for a retraction at 7d. CHI was of similar severity, both rat groups. Recovery was facilitated in the treated mice starting at 24h and reaching statistical significance at 7d post CHI (NSS 8 vs 6.625 in treated and control mice, respectively, p=0.037). Similar recovery pattern was found in CHI mice. While a considerable neuronal apoptosis and minimal apoptosis of the leukocytes of the inflammatory infiltrates was found at the injury site in control animals, a markedly reduced neuronal apoptosis with increased inflammatory cell apoptosis were found after EPO treatment. We propose that EPO-treated animals recovered faster than controls with less neuronal and more inflammatory cell apoptosis compared to controls, probably through a cell-specific anti-apoptotic mechanism.

Keywords: traumatic brain injury, erythropoietin, apoptosis, neuroprotection

Tomosyn is involved in the last stages of the synaptic vesicle cycle. Yizhar O., Hagalili Y., Molamed R., Matti U., Retig J. and Ashery U.

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The process of neurotransmitter release is coordinated by a large number of synaptic proteins and depends on proper protein-protein interactions. Tomosyn (130kD protein that was identified as a novel binding partner for Syntaxin1). Tomosyn possesses a Synaptobrevin-homology domain and is capable of dissociating Munc18 from Syntaxin1. To investigate the role of Tomosyn in catecholamine secretion, we overexpressed Tomosyn in adrenal chromaffin cells. Using photolysis of caged-calcium, we studied the effect of Tomosyn on the different kinetic components of exocytosis with membrane capacitance measurements. While overall secretion under overexpression of Tomosyn was not different than control, we observed a pronounced difference in the kinetics of secretion. The exocytotic burst was significantly reduced in Tomosyn-overexpressing cells, indicating a decrease in the number of release-competent vesicles. Furthermore, a detailed examination of secretion rates revealed that under Tomosyn overexpression, higher [Ca2+]i is required to achieve normal secretion rates. To elucidate the function of the Synaptobrevin-homology domain, we overexpressed a mutated form of Tomosyn (TomosynACCC) which lacks this activity (TomosynACC). Under steady-state conditions, deletion of the coiled coil domain slightly reduced the inhibitory effect observed with wild-type Tomosyn. However, following elevation of [Ca2+]i, the inhibitory effect was abolished. The results suggest that Tomosyn acts at the priming step of the synaptic vesicle cycle. Tomosyn activity might reduce the number of fusion-competent vesicles through its interaction with Syntaxin, by interfering with the formation of fusion-competent SNARE complexes.

Keywords: tomosyn, syntaxin, chromaffin cell, exocytosis

Implications of APP processing, PKC and MARCKS-phosphorylation by rasagiline

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Erythropoietin (EPO), a kidney-derived cytokine regulates hematopoiesis, acts as a growth factor and apoptotic inhibitor. Cultured brain cells produce EPO in response to oxidative stress, and EPO receptor is present on neuronal and brain capillary endothelial cells. This study examines the protective effects of EPO in rats and mice undergoing controlled CHI. Methods: CHI was induced using a weight-drop device. Clinical status was evaluated by Neurological Severity Score (NSS), which tests 10 or 17 tasks for mice and rats respectively. Animals were treated with 2 doses of i.p. 5,000 units/kg/dose (1 ml) of HuEPO, or vehicle 1h and 24h after CHI. NSS was evaluated at 1, 3 and 7d post CHI, and compared using a two-tailed student t-test. Brains were analyzed for apoptosis at 7d. CHI was of similar severity, both rat groups. Recovery was facilitated in the treated mice starting at 24h and reaching statistical significance at 7d post CHI (NSS 8 vs 6.625 in treated and control mice, respectively, p=0.037). Similar recovery pattern was found in CHI mice. While a considerable neuronal apoptosis and minimal apoptosis of the leukocytes of the inflammatory infiltrates was found at the injury site in control animals, a markedly reduced neuronal apoptosis with increased inflammatory cell apoptosis were found after EPO treatment. We propose that EPO-treated animals recovered faster than controls with less neuronal and more inflammatory cell apoptosis compared to controls, probably through a cell-specific anti-apoptotic mechanism.

Keywords: traumatic brain injury, erythropoietin, apoptosis, neuroprotection

Tomosyn is involved in the last stages of the synaptic vesicle cycle.
Neuroprotective action of rasagiline is dependent on activation of Bcl-2, PKC and pro tease complex and inhibition of mitochondrial permeability transition (PT)

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Our cell culture (PC-12, SH-SY5Y) studies have established that the anti-Parkinson drug, rasagiline and its optical isomer TVP1022 have relatively potent neuroprotective-antiapoptotic action against several oxygen-sensitive neurons (N-methyl-D-aspartate and SIN-1). These actions are not dependent on monoamine oxidase (MAO) inhibition, since TVP1022 and other derivatives of rasagiline, which do not inhibit MAO, have similar actions. Some of the events in the mitochondrial protective action include suppression of the decline in mitochondrial membrane potential, activation of caspase 3 and nuclear translocation of BAX. We have now confirmed their ability to stabilize mitochondrial membrane potential in isolated mitochondria and prevent mitochondrial swelling. This would suggest that rasagiline and its derivatives directly affect the mitochondria, since they inhibit mitochondrial permeability transition (PT), a phenomenon similarly observed in SH-SY5Y cells with over expressed Bcl-2. Rasagiline (0.1 μM) increases antiapoptotic Bcl-2 gene expression in SH-SY5Y cells. The mRNA and protein levels of Bcl-2 and Bcl-xL are also increased significantly, with out affecting those of Bax and Bad. Furthermore, rasagiline, similar to Bcl-2 over expression, activates PKCα and prevents neurotoxin-induced inhibition of proteasome complex, which in turn results in inhibition of cytochrome c release. In addition, rasagiline increases the gene expression and protein level of glicocerebrosidase, which derived neuroprotective action in this model. Our studies have shown that the mechanism of neuroprotective-antiapoptotic action of rasagiline depends on inhibition of mitochondrial PT, increased expression of antiapoptotic-cyto-protective (Bcl-2, Bcl-xL and PKC) and neurotrophic genes.

Keywords: rasagiline, neuroprotection, mitochondrial permeability transition, Bcl-2 and PKC gene expression

Unilateral pentobarbital microinjection into the rat MPTA induces signs of general anesthesia that are bilaterally symmetrical

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We recently discovered a small area within the rat mesopontine tegmentum (MPTA) at which bilaterally symmetrical microinjection of minute quantities of pentobarbital induces a transient, reversible anesthesia with non-responsiveness to noxious stimuli, flaccid posture, and absence of the righting reflex. The behavioral suppression is accompanied by slow-wave EEG and, presumably, loss of consciousness (Devor and Zalkind, Pain 94:161-120 [2001]). Here we ask whether microinjection into the MPTA enables the rat to survive without the induction of behavioral signs of general anesthesia on both sides of the body. Using 22 rats that showed anesthesia on bilateral microinjection of pentobarbital, we microinjected the drug, in doses of 100 μg in 0.5 μl or 200 μg in 1 μl into MPTA on one side (46 and 44 experiments, respectively). Behavioral results were quantified on motor (posture and righting reflex), and sensory scales (responses to pinching the left and right foot, and the tail). Microinjection sites were located histologically. There were two principal observations. First, anesthesia was induced by unilateral microinjection (44 trials). The behavioral suppression was bilateral MPTA, indistinguishable from anesthesia induced by bilateral MPTA or by systemic pentobarbital administration. Second, anesthesia duration, recovery time and mean score following bilateral 100 μg pentobarbital were statistically indistinguishable from those resulting from microinjection of 200 μg unilaterally, but greater than 100 μg unilaterally.

Conclusion: MPTA on either side of the brainstem controls both ascending and descending pathways, and both motor functions and pain sensation, bilaterally.

Keywords: intracerebral, general anesthesia, unconsciousness, barbiturates, ataxia, analgesia, unilateral microinjections

Biophysical and bioinformatic analysis of the cytoplasmic portion of the neuronal cell adhesion molecule, gliotactin (Gli-cyt)

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In the past decade, a new class of single-span transmembrane neuronal cell adhesion molecules (N-CAMs) has been discovered and characterized. The sequences of the extracellular domains of these molecules show a high sequence similarity to acetylcholinesterase (ACHE), and they were thus designated ChE-like adhesion molecules (CLAMs). Their intracellular domains do not show sequence similarity to any known protein, but in the case of one CLAM, mammalian, this domain was shown to be important for adhesive function. No physicochemical or structural information on any of these molecules has so far been available. Here we report on the cytoplasmic sequence of one CLAM, gliotactin (Auld et al, Cell 81:757-67 [1995]), which is at its C-terminus (Gli-cyt), in milligram amounts in E. coli, purified it, and subjected it to biophysical analysis. Hydrodynamic measurements showed that Gli-cyt has a large Stokes radius (33 nm) relative to its molecular weight (23.5 KDa), indicative of low compactness. CD measurements, in the near UV, were devoid of ellipticity, indicating absence of tertiary structure, and measurements in the far-UV indicated that it has a "random coil" structure. NMR experiments conclusively confirmed that Gli-cyt has a wholly disordered conformation. The amino acid composition of Gli-cyt revealed that it contains both a low percentage of hydrophobic amino acids and a high overall net (positive) charge, as was shown to be the case for other proteins belonging to the class of "natively unfolded" proteins (Uversky et al, Proteins 41:415-427 [2000]).

Keywords: cholinesterase-like, natively unfolded, intrinsically disordered

Intrinsic bursting in hippocampal pyramidal neurons lacking apical dendrites

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The intrinsic firing modes of hippocampal CA1 pyramidal cells vary along a gradient of "burstiness" from regular firing to spontaneous bursting. The propensity to generate a somatic burst is positively related to the size of the spike afterdepolarization (ADP), generated by an inward current that activates during the spike and deactivates slowly thereafter. However, it is not known whether the ADP is locally generated by the somatic spike, is generated remotely at the apical dendrite by the backpropagating spike, or both. To differentiate between these alternatives, we have severed the apical dendrites of CA1 pyramidal cells near the soma and examined the firing modes of the amputated neurons in different experimental conditions. Their spectrum of firing modes was similar to that of intact neurons (mostly regular firing with a small proportion of bursts). Blocking the muscarinic-sensitive K+ current (IM) with linopidine markedly enhanced the propensity of amputated neurons to generate bursts, whereas blocking the Ca2+-activated K+ currents with intracellular (BAPTA) did not. The propensity to generate bursts was also augmented by raising extracellular [K+] or by removing extracellular Ca2+. In all conditions, bursting was readily suppressed by pentanin or by PKC activation. Activation was more sensitive to N2+ (1 mM), implicating the persistent Na+ current (INaP) in its generation. Thus, somatic spike backpropagation into apical dendrite is not required for somatic bursting in hippocampal pyramidal cells. Rather, large spike ADPs that trigger somatic bursts are generated at or near the soma once INaP is sufficiently larger than IM.

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Keywords: bursting, persistent Na+ current, M-current, dendrites, hippocampal pyramidal cells

olfactory learning is accompanied by reduction in post burst AHP in CA1 hippocampal neurons

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Rule learning of an olfactory discrimination task in rats is accompanied by reduction in the post-burst after-hyperpolarization (AHP) in piriform cortex pyramidal neurons (Saar et al, EJN 10:1518-1523, 1998). Here we examine whether such reduction also occurs in hippocampal neurons. Water deprived rats were trained in a 4-arm maze to discriminate positive cues in pairs of odors for a water reward.
AHP amplitude in CA1 hippocampal neurons was recorded in brain slices at different time intervals after the beginning of training. Neurons were depolarized to holding potential of -60 mV, and AHP amplitude was measured following a 100 ms depolarizing current step that generated 6 action potentials. Olfactory learning-induced reduction in AHP was observed in CA1 neurons as early as 3 days after the rats began their training (i.e. during the time in which they are learning the rule). The averaged AHP amplitude (in mV) was 2.88±1.14, n=23, in neurons from trained rats, 3.54±1.38, n=18 in neurons from naive and 3.95±1.51, n=36 in neurons from pseudo trained rats (p<0.05, one way ANOVA). Similar results were obtained one day after rule learning. Notably, three days after rule learning, the averaged AHP value in CA1 neurons from trained rats (4.52±1.64 mV, n=6) did not differ from that observed in controls, suggesting that the time course of post-burst AHP reduction and its subsequent return to control values differs between piriform cortex and hippocampal neurons.

We suggest that olfactory learning-related post-burst AHP reduction in CA1 neurons may represent a mechanism that enables olfactory rule learning.

Keywords: olfactory-learning, hippocampus, CA1, AHP

Solving the EEG inverse problem using genetic algorithms
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Background: The EEG Inverse problem takes an important role in cognitive neuroscience research as well as in clinical applications (e.g. Locating epileptic activities). Several methods have been studied in an attempt to solve this problem (e.g. MNE, BESA, LORETA, Pascual-Marqui, Int’l J. Bioelectromagnetism, 1(1):75-86 [1999]). None of the methods currently present a satisfying solution.

Objective: Solving the EEG inverse problem using a new approach based on genetic algorithms.

Methods: Genetic algorithms (Davis, Handbook of Genetic Algorithms, Van Nostrand Reinhold [1991]) are based on the concept of evolution found in nature. A set of population groups of solutions evolve over time to create better solutions according to a set of given genetic rules such as creating mutations, selecting good solutions and combining several good solutions to create better solutions. In our work we used a source model based on dipoles. Every member of a population group is a triple of coordinates that represent the location of the dipole source and its current potential. Mutations change either the location of the dipole or its potential; the selection process is based on a given error threshold between the forward computation of the population groups and the actual EEG measurements. The forward computation is based on the laws of electromagnetism and Maxwell’s equations. The initial population set is generated randomly. Previous work using genetic algorithms did not exceed a 2D brain model (Aguiar et al, Proceedings of the 2000 ACM symposium on Applied computing, p.80-84 [2000]) and thus could not be used in real applications.

Results: We tested our algorithm using simulated EEG data. The dipoles where simulated by averaging 20 cognitive evoked potential trials and the simulated EEG data was generated by using the forward equations on a homogeneous spherical head model. We tested data sets with 1-3 simultaneous independent dipole sources. The results showed a relatively small error of up to 10 mm, which is similar to the results, obtained by the conventional methods mentioned above.

Keywords: EEG, inverse problem, genetic algorithms