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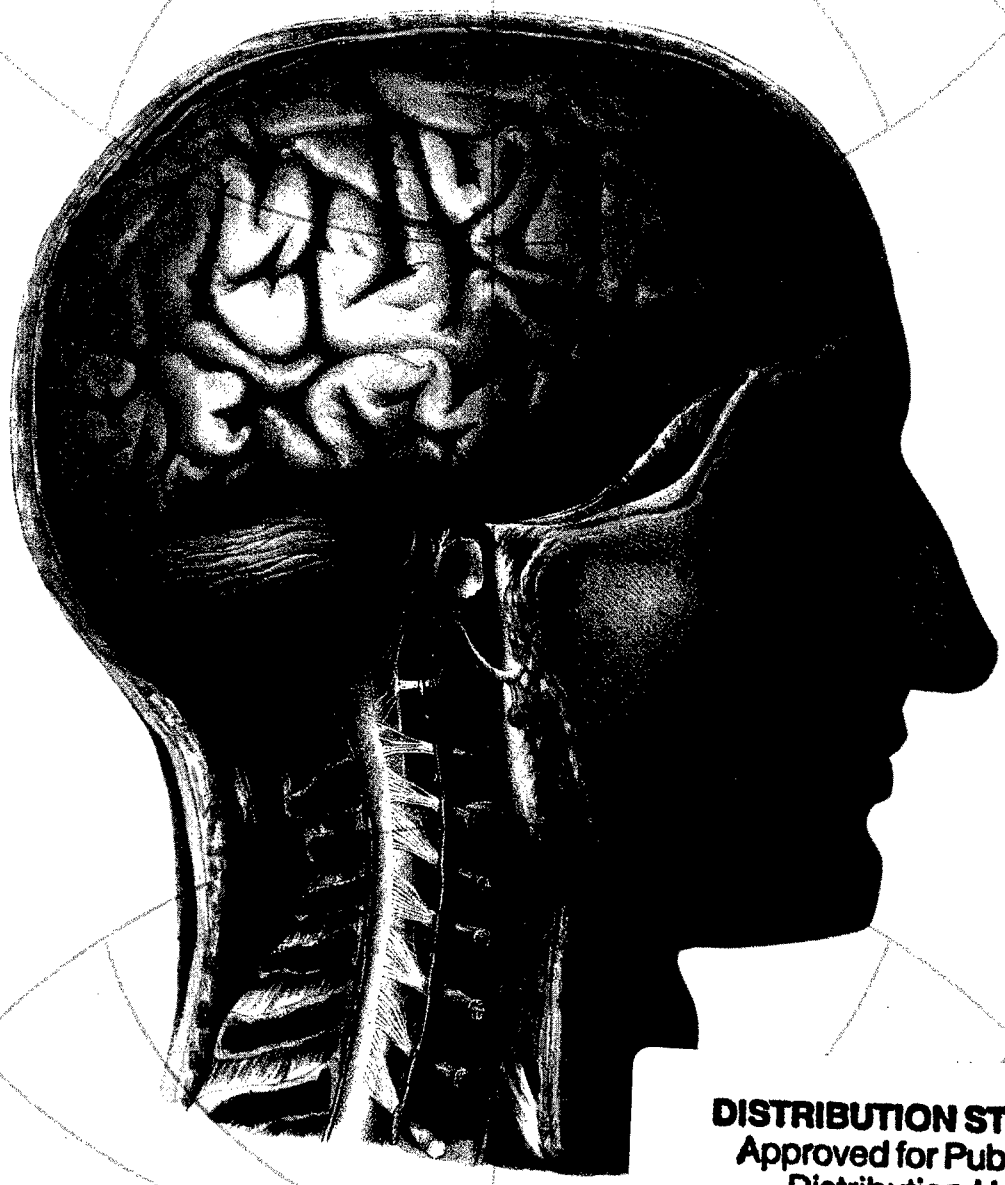

Abstracts

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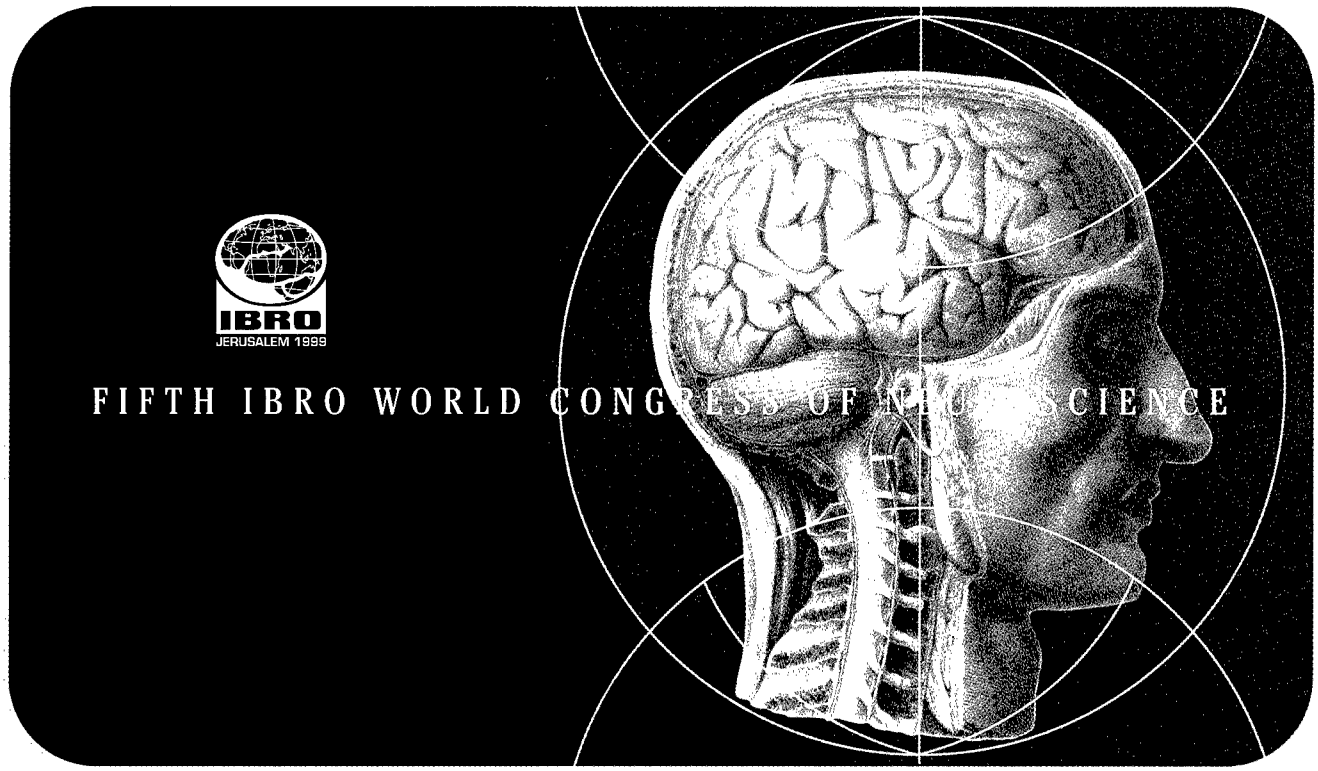

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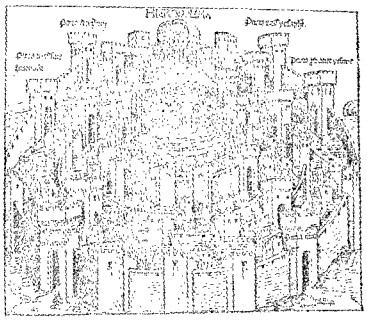

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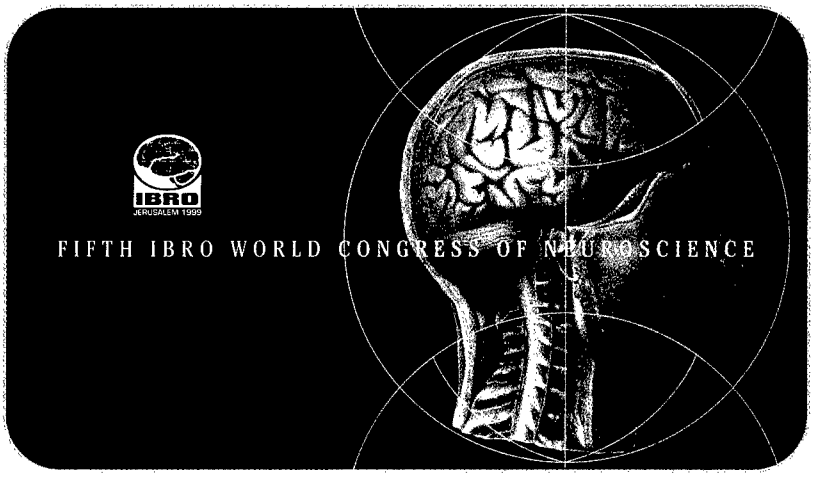

Abstracts

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STRUCTURE AND DEVELOPMENT OF FUNCTIONAL MAPS IN CAT VISUAL CORTEX.

T. Bonhoeffer, Max-Planck-Institute of Neurobiology, 82152 Munich-Martinsried, Germany.

Optical imaging of intrinsic signals is a technique which allows to study cortical maps *in vivo* with unprecedented ease and resolution. Using this method we have previously shown that orientation preference of cortical neurons in cat primary visual cortex is organized such that "pinwheels" surround numerous "orientation centers" in the orientation maps. Combined imaging and tetrode-recording experiments have now enabled us to assess the response properties of single cells at these pinwheel centers more precisely. These data show that neurons in pinwheel centers do not differ from cells in other regions of primary visual cortex, only the range of preferred orientations of neighboring cells is much larger than elsewhere in the cortex.

We have also investigated spatial frequency maps and their relationship to other maps like ocular dominance and orientation in the visual cortex. We could show that spatial frequency maps are most likely, at least in part, caused by segregation of the terminals from the X- and Y-cells in the lateral geniculate nucleus, indicating that, similar to the primate, also in cat primary visual cortex there might be a segregation into separate processing streams. These data also showed that the geometrical relationship between the different feature maps (spatial frequency, orientation and ocular dominance) is such that contour lines of all three tend to intersect at right angles.

Optical imaging also allows a detailed look at the postnatal development of cortical maps during the first few weeks of life and the influence of visual experience. While new data show that there is a certain capacity for plastic changes, in particular in a modified visual environment, overall the data suggest that the geometry of these maps in cat visual cortex is remarkably stable even during the peak of the critical period.

Supported by the Max-Planck-Gesellschaft.

FUNCTIONAL MAPS AND PATTERNS OF CONNECTIVITY IN VISUAL CORTEX.

D. Fitzpatrick, Dept. of Neurobiology, Duke University Medical Center, Durham NC USA.

We have used a combination of intrinsic signal optical imaging techniques and anatomical tracing techniques to examine the rules that relate horizontal connections and callosal connections to maps of orientation preference and visual space in tree shrew visual cortex. Small injections of anterograde or retrograde tracers into sites of known orientation preference in layer 2/3 of area V1 give rise to an elongated patchy distribution of connections that extends for distances up to 4 mm across the surface of the cortex. Within 500 um from the injection site, these connections are distributed in a radial fashion exhibiting little specificity for the orientation preference of the injection site. Beyond this region, the connections exhibit both modular and axial specificity, linking together sites that share similar preferred orientations and whose receptive fields lie along the axis of preferred orientation in the map of visual space. This collinear rule for horizontal connections is consistent with the enhanced responsiveness of layer 2/3 neurons to elongated arrays of contour elements that have the appropriate orientation and axial distribution. Callosal connections, by contrast, are relatively restricted in their extent (generally less than 1 mm) and exhibit a uniform, radial distribution with no sign of the elongation or patchiness that characterizes horizontal connections. By using optical imaging techniques to visualize the maps of visual space in both hemispheres simultaneously, it was possible to show that these connections link together visuotopically matching sites in the two hemispheres. This matching occurs for sites that are activated by stimuli that lie up to 15 degrees from the center of gaze, due to the presence of a substantial ipsilateral visual field representation in V1. These results suggest that horizontal and callosal connections make distinct contributions to the response properties of layer 2/3 neurons.

Supported by EY06821 and The McKnight Foundation.

TOPOGRAPHY OF CONTEXTUAL MODULATIONS RESULTING FROM SHORT-RANGE INTERACTIONS IN PRIMARY VISUAL CORTEX

Aniruddha Das and Charles D. Gilbert (presenting author: Aniruddha Das)

The response of a neuron in primary visual cortex (V1) to a simple visual element embedded within a complex image is generally very different from the neuron's response to the same element in isolation. This difference, a specific modulation by surrounding elements in the complex image, is mediated by short- and long-range connections within V1 as well as by feedback from other areas. Here we study the role of short-range connections in this process, and relate it to the layout of local inhomogeneities in the cortical maps of orientation and space. By using the measure of cross-correlation strength combined with the optical imaging of orientation columns we show, first, that the strength of local connections between cells is a graded function of lateral separation across cortex, largely radially symmetric and relatively independent of orientation preferences. We show, next, that in the cortical processing of complex visual stimuli the contextual influence of flanking visual elements on the responses of a neuron varies systematically with the position of the neuron within the orientation map on cortex. The strength of this contextual influence on a neuron can be predicted from a model of local connections based on simple overlap with particular features of the orientation map. This suggests that local intracortical circuitry could endow neurons with a graded specialization for processing angular visual features such as corners and T junctions in visual space, and this specialization could have its own functional map over cortex, linked with the map of orientation.

OPTICAL IMAGING OF CORTICAL ARCHITECTURE AND THE EXPLORATION OF THE SIGNALS UNDERLYING FUNCTIONAL BRAIN IMAGING TECHNIQUES

Amiram Grinvald, Ivo Vanzetta, Amir Shmuel, Doron Shoham, Amos Arieli
Dept of Neurobiology, the Weizmann Institute of Science, Rehovot, Israel

Optical imaging based on intrinsic signals utilizes changes in optical properties of active brain tissue to obtain high resolution functional maps. This method is relatively non-invasive, although the investigated cortical region must be exposed. This imaging technique permitted the high-resolution (~ 50 microns) imaging of elements of the functional architecture of the visual cortex in the living brain of cats and monkeys. In addition activity maps were also obtained through the intact dura and thinned bone. Furthermore, this technique was successfully applied to the imaging of functional architecture in the awake monkey as well as used to delineate function borders in the human cortex during neurosurgery. We also clarified of the relationships between cortical electrical activity and the responses of the microcirculation and determined the sequence of metabolic related events including oxygen delivery, blood flow and volume changes following the onset of electrical activity. These results settled the controversy regarding anaerobic brain metabolism and should assist in the interpretation of PET and f-MRI measurements as well as the improvement of the spatial resolution of f-MRI. The dynamics of cortical activity in the millisecond time domain can be visualized by another optical imaging technique based on the use of fast voltage sensitive dyes, rather than the slow intrinsic signals. It allows the investigator to image the flow of neuronal activity from one cortical site to another, in real time. The combination of real-time optical imaging with single unit electrical recordings facilitated the visualization of spatio temporal activity patterns of coherent neuronal assemblies.

FEATURE-BASED REPRESENTATION OF OBJECTS IN MACAQUE AREA TE REVEALED BY INTRINSIC OPTICAL IMAGING

M. Tanifuji* and K. Tsunoda. Lab. Integrative Neural Systems, Brain Science Institute, RIKEN, Saitama, Japan

Looking at an object in the visual field, our brain constructs a representation of the object image suitable for visual perception and recognition. To identify such internal representations of objects, we have studied spatial patterns of neural activation in monkey visual association area TE by using intrinsic optical imaging. Optical imaging with complex objects used as visual stimuli revealed that different complex objects activate different sets of multiple discrete spots with little overlap if these objects did not share common geometrical features. This observation is consistent with the concept that an object image is represented by a combination of partial features which are components of the object image. We confirmed this possibility by systematically comparing activation patterns by a complex object image and by partial features derived from the original object.

Furthermore, we found there were spots where their activities are deactivated by presenting some other geometrical features together. This result was confirmed by extensive unit recordings from identified spots by preceding optical imaging. Taken together, we proposed that combinations of partial features are used to represent complex object images in area TE not only in an additive way (A+B+C), but also in additive plus subtractive way (A+B-C or A-B+C), where A, B and C represent object features. Supported by the Japanese Science and Technology Agency.

BEHAVIORAL BIOLOGY OF SONG LEARNING

Peter Marler, Section of Neurobiology, Physiology and Behavior, University of California, Davis, CA 95616

Four themes of current interest in behavioral neurobiology are: (1) memory systems, (2) critical periods for development, (3) responsiveness to specific enabling stimuli, and (4) the roles of instruction and selection in development. Avian vocal learning provides insights into all four issues. The memorization of auditory stimuli occurs in two distinct contexts. One applies to stimulation by others, the other to self stimulation. Individually-distinctive songs are memorized and used in social behavior as a basis for personal and group recognition. The capacity for such "recognition" learning, perhaps seasonally modulated, probably persists throughout life. In "production" learning, possibly a separate process, songs are memorized and applied to development of a bird's own produced vocal repertoire. Tutor songs are memorized, retrieved later, and used as a basis for imitations, embellished by creative processes of improvisation and invention. "Production" learning is restricted to critical developmental periods. These may occur once in life (age-limited learners) or repeatedly (open-ended learners). Species and population differences contribute to the number and timing of critical periods for production learning, and ontogeny of song motor patterns. During critical periods songs of great complexity can be memorized with rapidity and precision. At such times birds are attuned to respond selectively to certain patterns of auditory and visual stimulation. Species-specific and population-specific rules underlie the attunement process. As the ontogenetic program of the species unfolds, experience of specified stimulus patterns in turn enables other pre-specified developmental events to occur, including changes in hormonal state, patterns of growth, stimulus responsiveness, and behavior. Developmental stages may be delayed, hastened, or canalized by certain classes of environmental stimuli. The underlying neural and behavioral mechanisms operate both by instruction and by selection. The overproduction of diverse motor patterns and associated circuitry is followed by selective winnowing, dependent in turn on social feedback from others. It has been theorized that neuroselective processes also contribute to the memorization of songs for production. In all of these areas, song learning has been a rich source of insights into principles of behavioral development, and the underlying neural mechanisms.

The role of auditory feedback in development and maintenance of birdsong

Masakazu Konishi, Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

Of the many animals groups that use sounds for communication, few of them need to learn their acoustic signals. Vocal learning requires control of voice by auditory feedback. The neural substrates for this process appear to be in the forebrain components of the song system. These brain areas are present only in bird species that can imitate song and absent in those that do not copy song. Many neurons of these areas respond to sound stimuli, although we do not know whether the same neurons serve both motor and auditory functions. Some of these cells respond exclusively to the individual bird's own song. This stimulus-selectivity emerges during the sensorimotor phase of song learning in which the bird controls voice by auditory feedback. The process of song developing into a stable form is called crystallization. Some birds (age-limited learners) maintain the crystallized song for life, whereas other birds (open-ended learners) change it annually.

However, the ability and inability to change song do not appear to be correlated with the presence and absence of brain plasticity. Recent experiments show that auditory feedback is necessary for the maintenance of adult song even in age-limited learners. Perturbation of auditory feedback in adult zebra finches causes dramatic changes in both spectral and temporal structure of their song. Remarkably, birds can restore their original song, when the perturbation is removed. These findings indicate that the brain of adult age-limited learners retains plasticity for vocal control.

Before truth and logic slip in

Fernando Nottebohm, The Rockefeller University, Field Research Center, Millbrook, New York, NY 12545, USA

The high vocal center (HVC) of oscine songbirds is the last relay of the classical ascending auditory pathway. It gives rise to two other pathways: a posterior one necessary for acquisition and production and an anterior one necessary for acquisition, but not for production of learned song. HVC cells that project to the anterior pathway are born before birds hatch; those that project to the posterior pathway are born after hatching and while song is learned. The latter cells, but not the former, are replaced throughout life by other cells of the same kind. In seasonally breeding song birds, such as the canary, peak replacement of HVC neurons occurs after the end of breeding, when blood testosterone levels are low, birds sing little and the quality of song changes. Low testosterone levels promote the death of HVC's replaceable neurons and high testosterone levels promote their survival. However, part of this effect may be indirect. Testosterone induces singing and singing induces a rise in the expression of brain derived neurotrophic factor (BDNF) in HVC. An increase in the level of BDNF protein in HVC promotes the survival of the neurons replaced in adulthood. What to make of this? In some systems the whole neuron may be the unit of learning and changes brought about by experience may be akin to irreversible cell differentiation. If so, then neuronal replacement may be a strategy to slough off old memories and make room for new ones. Intriguingly, this replacement of neurons occurs, too, in songbirds such as the zebra finch and song sparrow that do not add to their song repertoire in adulthood. Other evidence suggests that these birds use auditory feedback to maintain the song they already know. In them, relearning may train the new neurons and thus cancel the forgetting induced by neurons lost. All brains may have at their core neurons that, replaceable or not, learn only once and thus determine the bias and limits of what an individual can do -- or so at least I imagine before truth and logic slip in through a rear window and take up residence.

A VOYAGE ALONG THE TRANSLOCATION PATH OF THE ($Na^+ + K^+$)-COUPLED GLUTAMATE TRANSPORTER GLT-1

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Glutamate transporters prevent neurotoxicity by maintaining low synaptic concentrations of the transmitter and limit receptor activation. They achieve this by an electrogenic process where the transmitter is cotransported with three sodium ions' followed by countertransport of a potassium ion. Recently we have made a number of advances toward our understanding of the structural basis of transporter function. Two adjacent amino acid residues, tyrosine-403 and glutamate-404, appear to be involved in binding of the coupling ions, and tyrosine-403 behaves as if it is alternately accessible to either side of the membrane. They are located right in the middle of a stretch of 76 amino acids which contains at least part of the binding site of the non-transportable glutamate analogue dihydrokainate. Recently we have solved the topology of GLT-1 using a series of functional transporters containing single cysteines. Their topological disposition was determined using a biotinylated sulfhydryl reagent. The glutamate transporter has eight transmembrane domains long enough to span the membrane as *alpha*-helices. Strikingly, between the seventh and eighth domain a structure reminiscent of a pore-loop and an outward facing hydrophobic linker are positioned. The important residues, tyrosine-403 and glutamate-404, are located in the newly identified transmembrane domain 7.
Serine-440, which appears to be close to the binding site for glutamate is located in the ascending limb of the pore-loop-like structure. Strikingly, its mutation of S440 to glycine results in a transporter of broadened ion-specificity. This promiscuity is influenced by substitutions at another residue, serine-443, located at the outer edge of the pore-loop-like structure. Our findings suggest that at least part of this structure is crucial for the coupling of sodium and glutamate fluxes.

FUNCTIONS OF GLIAL GLUTAMATE TRANSPORTERS IN THE BRAIN

Koichi Tanaka, Tokyo Medical and Dental University, Tokyo, Japan

In the mammalian brain, two subtypes of astrocytic glutamate transporters, GLT1 and GLAST, have been identified. GLAST and GLT1 are highly concentrated in the telencephalon or the cerebellum, respectively. However, the roles of each transporter in synaptic transmission and neurotoxicity are still unclear, because of the absence of subtype specific inhibitor. In this study, we generated knockout mice deficient in GLT1 or GLAST and explored their functions in the brain.

Homozygous mice deficient in GLT1 show lethal spontaneous seizures and increased susceptiblity to acute cortical injury. Although glutamate remains elevated in the synaptic cleft for longer periods in mutant mice, there is no clear difference in the decay of EPSC in the hippocampus between the wild-type and mutant mice.

A deficiency of GLAST results in impairment of motor coordination, abnormal climbing fibre innervation of Purkinje cells and increased susceptibility to cerebellar injury. Moreover, synaptic transmission between photoreceptors and bipolar cells is impaired in GLAST-deficient mice, while the kinetics of climbing fibre- and parallel fibre-EPSCs are normal in the mutant Purkinje cells.

These results demonstrate that GLAST is essential for normal synaptic transmission at the photoreceptor synapse and protection of cerebellum from excitotoxic damage and that GLT1 plays an important role in preventing glutamate neurotoxicity in the cerebrum.

MOLECULAR CHARACTERIZATION OF THE GLYCINE TRANSPORTERS FROM CNS

Carmen Aragon
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Glycine, after being released from presynaptic terminals, is rapidly removed from the synaptic cleft by transporter proteins located in the plasma membrane of the presynaptic nerve terminals and the surrounding glial cells. Glycine is the major inhibitory neurotransmitter in the spinal cord and the brain stem of vertebrates. In addition, glycine potentate the action of glutamate, the main excitatory neurotransmitter in the brain, on postsynaptic N-methyl-D-aspartate receptors. The uptake of glycine is carried out by two different glycine transporters, named GLYT1 and GLYT2 encoded by two different genes, which belong to the sodium and chloride dependent neurotransmitter transporters family. GLYT1 has a wide distribution along the CNS and present three isoforms (named GLYT1a, GLYT1b and GLYT1c), which differ in their amino terminal sequences. Immunocytochemical studies performed in our laboratory demonstrate a tight association of GLYT2 (presenting two isoforms, GLYT2a and GLYT2b) with glycinergic neurones, being the responsible of the high cytoplasmic glycine content of these neurones. GLYT1 is also enriched in glycinergic areas, but it can be also found in areas devoid of inhibitory glycinergic neurotransmission, suggesting additional physiological roles. Studies performed on the structure-function relationship of these proteins, by using recombinant GLYT1 and GLYT2 stably expressed in HEK cells; GLYT2-GLY1 chimeras and site-directed mutagenesis of several residues of these transporters, reveal putative structural domains and positions involved in functional and pharmacological differences between GLUT1 and GLYT2.

LOCALIZATION OF GLUTAMATE TRANSPORTERS AND QUANTIFICATION IN ABSOLUTE TERMS

K.P. Lehre, F.A. Chaudhry, Y. Dehnes, K. Ullensvang, O.P. Ottersen, J. Storm-Mathisen, N.C. Danbolt University of Oslo, Norway

The extracellular glutamate concentration is kept low by transporter proteins localized in the plasma membranes of neurons and glial cells. We have determined the regional distributions and cellular localizations of glutamate transporter protein subtypes using light and electron microscopic immunocytochemistry, and the absolute concentrations of transporter proteins in the brain tissue and in the cell plasma membranes using quantitative immunoblotting and electron microscopic stereology. The quantitatively dominating transporters, GLAST and GLT are localized in astroglia. GLAST is concentrated in the cerebellar molecular layer (1.8 % of total tissue protein), while GLT is found at the highest concentrations in the forebrain (1.3 % in stratum radiatum of the hippocampus). GLAST and GLT immunoreactivities are higher in parts of the astroglial membranes facing neuropil than in membranes facing other astroglial cells, capillaries, pia, or large dendrites. At birth, GLT is not detectable, but GLAST is present at significant concentrations in the rat brain. The GLT concentration increases rapidly in the forebrain between 14 and 28 days, coinciding with synapse formation. The EAAT4 transporter subtype is localized in cerebellar Purkinje cell spines and dendrites (at 0.2 % in the cerebellar molecular layer). EAAT4 immunoreactivity is higher in Purkinje cell membranes facing astroglia (which express GLAST and GLT) than those facing neuronal membranes. In conclusion: the glutamate transporters in the brain are present at high concentrations and show highly differentiated localizations.

NEUROTRANSMITTER TRANSPORTERS DEFINE DISTINCT POPULATIONS OF SECRETORY VESICLES

C. Waites, D. Krantz, Y. Liu, R. Edwards
UCSF, SF, CA, USA

Synaptic transmission involves the regulated exocytosis of vesicles filled with neurotransmitter. In general, synaptic vesicles store and release classical transmitters whereas larger vesicles with a dense core contain neural peptides. However, the location of proteins that transport classical transmitters into secretory vesicles indicate the potential to store these transmitters in multiple vesicle populations, including dense core vesicles as well as synaptic vesicles. The vesicular transport proteins fall into two classes, one that includes the transporters for monoamines (VMATs) and acetylcholine (VAChT) and another that includes amino acid transporters such as the vesicular GABA transporter (VGAT). Using antibodies to the cloned sequences, we have found that VGAT co-localizes with typical synaptic vesicle markers such as synaptophysin. However, the VMATs appear at many other sites in the neuron, including dense core vesicles and tubulovesicular structures which may contribute to the well-characterized somato-dendritic release of dopamine. Although closely related to VMATs and also present in dense core vesicles, VAChT appears at higher levels in synaptic vesicles. We have identified two sequences responsible for the localization of VAChT and the neuronal VMAT2 on these vesicle populations that appear to operate through distinct mechanisms to influence sorting. Further, we have found that phosphorylation within these sites alters protein trafficking. Since synaptic vesicles and dense core vesicles reside at different sites in the cell, respond to different stimuli and release with different kinetics, the alterations in transporter localization have important implications for transmitter release.

MORPHOLOGICAL CORRELATES OF HIPPOCAMPAL LONG-TERM PLASTICITY.

F. Engert and T. Bonhoeffer, Max-Planck-Institute of Neurobiology, 82152 Munich-Martinsried, Germany.

The possible relationship between changes of synaptic strength and changes in the morphology of the respective subcellular compartments has generated considerable interest for a number of years. As easily as functional synaptic modifications are induced as difficult it has been to prove that these are accompanied or even caused by morphological changes, mainly because it is not easy to pin-point the location of the synapses which are expected to change. We have now tackled this problem by combining two-photon imaging with a local superfusion technique thereby confining the region on the postsynaptic dendrite where the synaptic changes could occur.

We were able to show that local LTP induction in such a restricted region of the dendrite reliably led to the appearance of new spines in this area. These novel spines remained stable in shape and position for the whole period of observation, which lasted up to 24 hours. We found furthermore that the disappearance of spines was not, as is the case in LTP and spine growth, controlled in a specific and activity-dependent manner but it rather occurred more or less randomly in time and space.

The most attractive explanation for the formation of additional spines is a concurrent emergence of new synapses on these structures. Although more experiments on the precise nature of these changes are necessary, our data provide strong evidence that in the mammalian hippocampus not only physiological but also structural changes play an important role when neurons change the efficacy of their connections.

Supported by the Max-Planck-Gesellschaft.

GENES, SYNAPSES, AND LONG-TERM MEMORY

Eric R. Kandel, M.D., Senior Investigator, Howard Hughes Medical Institute; University Professor, Columbia University College of Physicians & Surgeons, USA

Since the initial theoretical proposal by William James in 1890, it has become well established that there are at least two temporally distinct phases of memory storage: there is a short-term memory lasting minutes and a long-term memory lasting days or longer. These two phases of memory storage differ not only in their time course, but also in their molecular mechanisms: long term memory differ from short-term memory in requiring the synthesis of new protein. Recent studies in *Aplysia*, *Drosophila* and *mice*, have revealed that these temporally and mechanistically distinct phases in behavioral memory are reflected in temporally and mechanistically distinct phases of synaptic plasticity in the very cells that participate in storing that memory. These findings in turn suggest the interesting possibility that the distinction between short and long-term memory evident at the behavioral level results from this fundamental distinction at the cellular level. As a result, this behavioral distinction can now begin to be analyzed by molecular studies focused at single cells and their connections. In considering the molecular mechanism that contribute to long-term synaptic plasticity on the cellular level, I would like to divide my talk into two parts: First, I will briefly outline some of the recent studies in *Aplysia* by Dusan Bartsch, Andrea Casadio, Craig Bailey and Mary Chen that have led to the conclusion that the requirement for protein synthesis which characterizes long-term memory is reflected, on the cellular level, in the activation of a cascade of genes and *that this* cascade leads to the growth of new synaptic connections. I will then go on to consider in more detail the studies of Kelsey Martin and Andrea Casadio which have examined the cell biological consequences of having a long-term memory process that require gene transcription and synaptic growth.

NEURON-SILICON JUNCTIONS IN CELL CULTURE

Peter Fromherz
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We studied the electrical coupling of cultured neurons and silicon chips with the goal to fabricate hybrids of neural nets and microelectronics. We used neurons from segmental ganglia of *Hirudo medicinalis*, from pedal ganglia of *Lymnea stagnalis* and from the hippocampus of rat. Electrical activity was recorded by field-effect transistors and stimulated by capacitive patches.

We addressed the quality of signal transmission. (i) The width of the cleft between cells and chips was 30 - 100 *nm* as determined by fluorescence interferometry. The seal resistance was around 1 $M\Omega$ as measured by AC-techniques. (For lipid bilayers we found 1 *nm* and 100 $G\Omega$.) Due to the large distance and low resistance the electrical coupling of chips and neurons is weak. (ii) The attached membrane was depleted of ion conductances in some systems such that capacitive current alone was responsible for coupling. In other cases we observed a selective depletion and accumulation of sodium and potassium conductances which controlled recording and stimulation.

We made elementary neurochips. (i) We assembled a two-way interface where a leech neuron was stimulated by a capacitive patch, the resulting action potential was recorded by a transistor, the signal was transformed by the chip to a voltage pulse which was used again for stimulation. (ii) We cultured random nets of snail neurons on a chip. A stimulation patch excited an attached cell, the signal was transmitted through an electrical synapse to a second neuron where it elicited an action potential which was recorded by a transistor. (iii) We started to control the geometry of nets by anchoring the cells, by guiding outgrowth and bifurcation and by localizing synaptic contacts with the goal to create well defined hybrids with a sufficient yield.

EXPLORING THE LANGUAGE OF THE RETINA

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USA

The visual system has a delay of ~50 ms in processing flashes of light, largely arising in the retina. Such a delay poses severe problems for timing motor output with moving objects (for instance, hitting a tennis ball). To study how the retina represents a moving object, we used a planar array of 61 platinum electrodes to measure the spike trains from up to 80 ganglion cells, the output neurons of the retina. Spike waveforms on each electrode were bandpass filtered and clustered into signals arising from individual cells via their peak and width.

Using these techniques, we found that a moving bar elicits a travelling wave of ganglion cell activity that peaks at or even ahead of the bar's leading edge (Nature 398: 334). This anticipation of moving objects mirrors a recently reported visual illusion: observers perceive a moving bar to be ahead of a flash at the same location. The measured spatial extent and temporal dynamics of a ganglion cell receptive field cannot account for motion anticipation. However, a model that includes a contrast gain control mechanism is highly successful. In some sense, anticipation is a modest form of prediction that the eye makes about the near future.

TOWARDS A SUBRETINAL IMPLANTED VISUAL PROSTHESIS

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Current studies on retinal and cortical visual prostheses have aroused controversy whether it is actually possible to restore sight by electrical stimulation of specific neurones of the visual pathway. Two groups try to replace lost photoreceptor function with subretinal implants containing an array of microphotodiodes which converts incoming light in photocurrents and thereby excites the adjacent retinal network. Four groups are developing epiretinal implants which receive energy and pre-processed visual information via complex telemetric systems. Similar techniques are integrated in prostheses for stimulating the optic nerve or the visual cortex. An overview will be given on the present status of the work in this neuroprosthetic field.

The report focuses on the subretinal approach. During the last four years our consortium developed several prototypes of silicon chips and minimally invasive implantation techniques. Implanted chips were well tolerated by the tissue and retained their initial location as we found several months after subretinal implantation in rabbit (8 months), rats (20 months) and micropigs (14 months). In contrast to their biocompatibility, the chip materials (Si, SiO_2) are not biostable as revealed in long-term *in vivo* studies. In addition, retinal illuminance above naturally occurring levels is needed to generate subrathreshold photocurrents. *In vitro* multisite stimulation experiments with highly degenerated retinae from RCS rats exhibited local network excitability with charge threshold of about 1 nC per electrode.

Despite the sobering *in vivo* results and regarding the encouraging *in vitro* results we think that the subretinal approach is feasible with implants with an external power supply and improved encapsulation.

AUTOIMMUNITY AND THE AUTONOMIC NERVOUS SYSTEM

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Dysfunction of the autonomic nervous system commonly occurs in autoimmune diseases, often in association with dysfunction of other peripheral nerves. These syndromes may involve parasympathetic, sympathetic and enteric divisions of the autonomic nervous system or only one division, depending on the nature of the antigen targeted by the autoimmune response. Autoimmune diseases frequently follow infection (e.g. Guillain-Barré syndrome, Chagas' disease) or are associated with cancer and are paraneoplastic (e.g. Lambert-Eaton myasthenic syndrome, paraneoplastic autonomic neuropathy). Lambert-Eaton myasthenic syndrome is characterised by autonomic dysfunction and muscle weakness. Antibodies to the P/Q-subtype of voltage-gated calcium channel cause downregulation of the channels on autonomic nerve terminals, thereby inhibiting transmitter release. Since P/Q-type channels are normally required for the release of transmitters at a variety of autonomic synapses, the release of many different classical and non-classical transmitters is inhibited in the syndrome, and the symptoms are widespread, including: dry mouth, male impotence, constipation, difficulty emptying the bladder, blurred vision, reduced sweating and postural hypotension. Recent studies have demonstrated the presence of autoantibodies to metabotropic receptors in some autoimmune diseases affecting the autonomic nervous system. Antibodies to beta-adrenoceptors and M_2 muscarinic acetylcholine receptors are present in Chagas' disease, and are likely to cause the cardiomyopathy that is characteristic of the disease. Our recent studies indicate that previously unexplained symptoms such as bladder irritability and constipation in the connective-tissue disorder, Sjogren's syndrome, may be caused by antibodies to the M_3 muscarinic acetylcholine receptors. Future studies are likely to demonstrate other autonomic disorders caused by auto-antibodies to ion channels, neurotransmitters or other neuronal molecules.

MATERNAL AUTOANTIBODIES AND FETAL DEVELOPMENT

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There are a few conditions in which maternal antibodies are know to cross the placenta and cause neonatal disease, such as myasthenia gravis (MG) or thrombocytopenia, but the role of maternal antibodies in causing developmental disorders is not generally recognised.

Arthrogryposis multiplex congenita (AMC) is a syndrome in which multiple joint contractures, micrognathia, hypoplasia of the lungs and other abnormalities are caused by lack of fetal movement *in utero*. The condition is very heterogeneous and thought to be caused by different genetic or environmental factors. AMC occasionally occurs in babies born to women with myasthenia gravis (MG). MG is an autoimmune neurological disease in which antibodies to the muscle acetylcholine receptor (AChR) cause loss of AChR from the neuromuscular junction leading to muscle weakness. A small proportion of babies born to women with MG suffer from neonatal MG, and an even smaller number have AMC.

We found that a women with one healthy child and AMC recurring in each subsequent pregnancy had high levels of antibodies to AChR in her serum even though she had no symptoms of MG. The antibodies were specific for the fetal isoform of the AChR and dramatically inhibited its function in vitro (Lancet 1995). Antibodies inhibiting the function of fetal AChR have now been detected in 6 other women with similar histories (J Clin Invest 1996). Importantly, two women have given birth to healthy babies after immunotherapies during pregnancy (Huson, Newsom-Davis, unpublished observations).

We have established a mouse model of maternal antibody mediated AMC that reproduces the deformities in the fetuses (J Clin Invest 1999). We are now using this animal model to investigate the effects of serum antibodies from other women with AMC, and from mothers of children with other neurodevelopmental disorders and of monoclonal antibodies to specific neuronal antigens. Overall these studies should help to define the involvement of maternal antibodies or other factors in causing developmental problems, with the possibility of immunotherapy during subsequent pregnancies.

MULTIPLE SCLEROSIS: AN IMMUNOLOGIC ATTACK ON THE CENTRAL NERVOUS SYSTEM

Antel, Jack P. MD

Multiple sclerosis (MS) is considered an immune mediated disorder of the Central Nervous System characterized by multi-focal regions of demyelination with varying extent of associated axonal injury. The clinical disorder post vaccination encephalomyelitis and its animal counter part Experimental Autoimmune encephalomyelitis, illustrate the susceptibility of the CNS to an autoimmune response initiated by myelin antigen specific T lymphocytes. The initial phase of MS is likely initiated by migration into the CNS of pro-inflammatory neural antigen specific T cells, perhaps induced by host exposure to exogenous antigens (e.g., viruses) with structural homologies with myelin (molecular mimicry). Magnetic resonance imaging based studies confirm the association of new lesion formation with disruption of the blood-brain barrier. This initial nidus of inflammation then can attract a wide array of cellular and humoral immune constituents, with potential tissue injury capability. Relapses could reflect recurrent activation of lymphocytes through exposure to the initial or additional (determinant spreading) neural antigen either within the systemic compartment or within the CNS, the latter due to the antigen presenting capacity of resident cells of the CNS located either at the blood brain barrier or within the parenchyma. The subsequent progressive disease phase, which develops in up to 50% of untreated MS patients, is characterized by accumulating tissue injury and loss with or without concurrent new inflammatory lesion formation. Microglia and macrophages, activated either via their innate immune receptors or through CD40-CD40 ligand interactions with infiltrating T cells are the most prominent cellular participants in these lesions. The selective injury of myelin or its cell of origin, the oligodendrocyte, in MS could reflect properties of either the immune effectors (adaptive immune system components capable of specific target recognition)) or the target (selective response to non specific effectors consequent to surface receptors expressed (eg.TNF-R, fas) or intracellular signalling pathways induced). Therapeutic strategies for MS should thus be directed both at the effectors (immunotherapy) and at the target (neuroprotection).

CANCER, AUTOIMMUNITY AND THE NERVOUS SYSTEM

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Paraneoplastic syndromes affecting the nervous system are unique among immune mediated disorders in that the trigger of the immune response is known: tumor expression of proteins normally restricted to neurons (or other immunopriviledged sites, such as testis) but ectopically expressed in some cancers results in an immunological response characterized by high titers of antibodies targeting the "onconeuronal" antigen. A T-cell response is also elicited in some paraneoplastic syndromes and may be the cause of neuronal destruction. Several clinical syndromes are well recognized: the anti-Hu syndrome characterized by sensory neuronopathy and/or encephalomyelitis associated with small cell lung cancer, anti-Yo syndrome characterized by Purkinje cell destruction associated with ovarian and other gynecologic cancers, the anti-Ri syndrome characterized by opsoclonus/myoclonus or other eye movement disorders associated with a variety of cancer, and the anti-Ta syndrome characterized by limbic and brainstem encephalopathy associated with testicular cancer. In each of these instances, the antigens recognized by the autoantibodies have been identified, cloned and sequenced. Some of the proteins so identified are RNA binding proteins but their specific function has not been identified. Not all paraneoplastic syndromes are characterized by identifiable autoantibodies. Even similar syndromes associated with the same cancer may be antibody positive or antibody negative. An example is paraneoplastic cerebellar degeneration associated with small cell lung cancer. Anti-Hu positive patients are more likely to have widespread neurologic signs outside the cerebellum than anti-Hu negative patients. Moreover, the prognosis is worse in the antibody positive patients. Some individuals with cancer but no paraneoplastic syndromes, low titers of antibody can be identified in the serum. Low titers of anti-Hu antibody are associated with a better prognosis of the small cell lung cancer. Experimental animals immunized against the Hu antigen are partially protected against tumors that express the Hu antigen.

ANTIBODY-MEDIATED DISORDERS OF NEURONAL ION CHANNELS

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Ion channels are vulnerable to antibody-mediated autoimmune attack for several reasons. First, they have an extracellular domain that is accessible to circulating antibodies. Second, in the case of ion channels at the nerve-muscle junction, they lack the protection provided by the blood-nerve/blood-brain barrier. Third, ion channels can be anomalously expressed (for example in tumour cell membranes), provoking in some individuals an autoantibody attack that can cross-react with the corresponding neuronal channel, leading to neurological disease. Understanding the pathogenesis of these disorders has depended crucially on the availability of specific neurotoxins and on microelectrode physiology, as was evident in myasthenia gravis, the first ion channel disorder to be elucidated, in which the target is the muscle ligand-gated acetylcholine receptor. This talk will focus on two neuronal voltage-gated ion channel disorders. In the Lambert-Eaton myasthenic syndrome (LEMS), IgG antibodies target P/Q-type voltage-gated calcium channels (VGCCs) at peripheral motor nerve terminals, at post-ganglionic autonomic synapses, and potentially in cerebellar Purkinje cell and granule cell membranes. This leads respectively to skeletal muscle weakness, to autonomic dysfunction, and possibly to cerebellar ataxia. In some patients the antibodies are provoked by VGCCs expressed in the membrane of the associated small cell lung cancer. In Autoimmune Neuromyotonia, IgG antibodies appear to target voltage-gated potassium channels (VGKCs) of the *Shaker*-related family at motor nerve terminals and at the nodes of Ranvier of peripheral motor and sensory nerves. The former results in hyperexcitable motor nerves causing spontaneous motor unit activity, myokymia (muscle twitching), cramps and muscle hypertrophy. Associated paresthesias and central changes (hallucinations, insomnia) suggest that sensory nerves and CNS neurones may also sometimes be targets.

TOWARDS AN IN VIVO CELL BIOLOGY OF SCHIZOPHRENIA

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Evidence that schizophrenia involves neuronal pathology of dorsolateral prefrontal cortex (DLPFC) and hippocampus (HF) has emerged from many directions. Reports of reduced N-acetyl aspartate measured with in vivo proton spectroscopy, especially in these two cortical regions, suggest that neuronal pathology exists. DLPFC NAA measures have been shown to predict the physiological activity of a distributed intracortical network activated during working memory. The same DLPFC measure predicts baseline and stimulated mesostriatal dopamine activity inferred from PET imaging of available dopamine receptors. These associations suggest that DLPFC neurons, by virtue of specific connections, are effector neurons in core pathophysiological aspects of schizophrenic psychopathology. However, NAA measures in HF, which do not predict any of these relationships in ill subjects, is significantly reduced in unaffected siblings of patients with schizophrenia, and the relative risk of a reduced HF NAA phenotype is close to nine. These data suggest that abnormalities of hippocampal circuitry and presumably development reflect susceptibility genes associated with genetic risk for schizophrenia, i.e. a trait measure, in contrast to neuronal involvement of DLPFC, which appears to reflect a state measure of neuronal pathology. These assumptions have been tested in an animal model of developmentally disrupted hippocampal connectivity which impacts on the function of prefrontal cortex during adult life. The family data raise the possibility that a genetic variation affecting the plasticity of HF circuitry and connectivity is related to schizophrenic susceptibility.

ADVANCES IN THE MOLECULAR GENETICS OF SCHIZOPHRENIA

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Schizophrenia poses one of the greatest research challenges in clinical neuroscience. It has a worldwide prevalence of ~1% and leads to chronic disability in up to two thirds of cases. Decades of intensive research have yielded only fragmentary insights into the etiology of schizophrenia. Recent advances in molecular genetics provide a unique opportunity to uncover the molecular neuropathology of the disorder. There is compelling evidence from family, twin and adoption studies that genetic factors play a major role in vulnerability. The mode of inheritance is non-mendelian and is likely to involve multiple genes which interact with environmental triggers. As for other complex disorders, these genes may be sought by linkage and association methods. Linkage studies require the recruitment of large family samples made up, at minimum, of two affected siblings with known parental genotypes. Smaller sample sizes may be informative provided that families are recruited from ethnically homogeneous populations. An additional and complementary strategy is to conduct family- or population based association studies of candidate genes but this approach is limited by the paucity of known candidates. Our group is applying all three strategies in collaboration with Israeli and German researchers and clinicians. Our linkage studies on 72 families comprising >100 affected sib pairs, in the context of a whole genome scan with ~350 evenly spaced highly polymorphic markers, have yielded evidence supporting the localization of susceptibility genes for schizophrenia to chromosomes 6p, 22q, 5q, 10p and 18p. Our association studies have demonstrated a possible modifying role of the 5-HT2C receptor gene (HTR2C) on illness course and a role for the dopamine D3 receptor gene (DRD3) in vulnerability to tardive dyskinesia which may be a component of the schizophrenia phenotype. These findings will be presented and their implications for the further direction of the field, considered.

ROLE OF NERVE PATHOPHYSIOLOGY IN CHRONIC PAIN

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Injury to axons in the peripheral nervous system at least partially blocks the flow of afferent information from the periphery to the central nervous system. When this occurs, we expect there to be a loss, or at least a blunting, of sensation. Yet clinical experience shows that neural injury is frequently accompanied by amplified and distorted sensation in the form of allodynia, hyperalgesia, hyperpathia and ongoing neuropathic pain. At its most extreme, as in the case of phantom limb pain in amputees, deafferentation appears to create elaborate sensations from no input at all. Accumulating evidence points to abnormal afferent discharge originating at ectopic sites in injured primary sensory neurons as an essential substrate of neuropathic sensation, both spontaneous and stimulus-evoked. Sustained neuropathic discharge appears to be an outcome of intrinsic resonant properties of primary sensory neurons, augmented by axotomy. Recording from primary sensory neurons in excised rat dorsal root ganglia, we found that some cells show subthreshold sinusoidal oscillations in their membrane potential. Oscillations gave rise to action potentials when they reached threshold. Neurons without oscillations were incapable of sustained discharge even on deep depolarization. Prior nerve injury increased the proportion of neurons with subthreshold oscillations, and hence the proportion that generated ectopic spike discharge. Selective pharmacological suppression of subthreshold oscillations may offer a means of controlling neuropathic paraesthesias and pain without blocking afferent nerve conduction.

MEMBRANE CURRENTS INDUCED BY NOXIOUS HEAT IN DRG NEURONS

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Acute pain represents a warning signal to prevent tissue damage resulting in long lasting pain. Noxious heat induces in small DRG neurons in culture cationic membrane current (I_{heat}) (Cesare and McNaughton, 1996). We studied I_{heat} in more detail to understand better sensitization of nociceptors after slight burning. The criteria for classifying nociceptors were: small size (<25 μm), sensitivity to capsaicin and presence of cationic current induced by noxious heat. We recorded whole cell membrane currents induced by ramps of increasing temperature employing a multibarrel system for drug application that allows rapid heating of the superfusing solutions (Dittert et al. 1998). We found that in the noxious range 43-52°C, I_{heat} exhibits an unusually high Q_{10} (18 ± 2, S.D., n=41). This indicates that I_{heat} is different from membrane currents induced electrically or chemically that exhibit Q_{10} ~2. Maximum I_{heat} cannot be increased by elevation of the temperature above 52°C. A single temperature increase to 56°C irreversibly alters responses to noxious heat. Its maximum decreases, the threshold shifts to innocuous temperatures and Q_{10} decreases to 3-4. This indicates irreversible denaturation of heat sensing protein and explains sensitization of nociceptors after slight burning. I_{heat} can be observed in neurons that are sensitive to capsaicin. Capsaicin, acidic pH and bradykinin facilitate I_{heat}. This is explained by the effects of increased temperature on the membrane current induced by algogens and by activation of protein kinase C.

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Cesare, P. & McNaughton, P. (1996) *Proc Natl Acad Sci USA* 93, 15435-15439.

Dittert, I. et al. (1998) *J Neurosci Methods* 82, 195-201.

CENTRAL PAIN HYPERSENSITIVITY- AN EXPRESSION OF POST-TRANSLATIONAL AND TRANSCRIPTIONAL PLASTICITY

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Pain hypersensitivity is a key aspect of clinical pain and is the consequence of post-translational changes, both in the peripheral terminals of the nociceptor and in dorsal horn neurons, as well as transcription-dependent changes in effector genes, again in primary sensory and dorsal horn neurons. Two distinct aspects of sensory neuron function are modified as a result of these processes, basal sensitivity, or the capacity of peripheral stimuli to evoke pain, and stimulus-evoked hypersensitivity, the capacity of certain inputs to themselves generate prolonged alterations in the sensitivity of the system. Post-translational changes, which are quick and short-lasting, largely alter basal sensitivity and occur through phosphorylation of membrane bound proteins. Transcriptional changes, which are initiated both by activity and specific signal transduction pathways, both potentiate the system and alter neuronal phenotype and can persist for prolonged periods. Potentiation occurs as a result of the upregulation in the DRG of centrally acting neuromodulators, and simultaneously in the dorsal horn, of their receptors. This means that the response to subsequent inputs is augmented, particularly those that induce stimulus-induced hypersensitivity. Alterations in phenotype includes the acquisition by A fibers of neurochemical features typical of C-fibers, enabling these fibers to induce stimulus-evoked hypersensitivity, something only C-fiber inputs normally can do. Elucidation of the molecular mechanisms responsible for these processes provides new opportunities for therapeutic approaches to manage pain.

ADAPTIVE LESION-INDUCED RECEPTIVE FIELD PLASTICITY IN THE VISUAL CORTEX

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Reorganization of receptive field size and structure is observed within days to months in the primary visual cortex of the adult cat in the surrounding of focal cortical lesions (2-4 mm in diameter) induced by local heat coagulation or injections of ibotenic acid. With increasing distance from the border of small focal cortical lesions a narrow ring of suppressed activity is surrounded by a region of increased single cell excitability. The *in vivo* increased excitability is accompanied by an increase of NMDA receptor mediated excitatory responses, and a reduction of $GABA_A$ and $GABA_B$ receptor mediated inhibition in *ex vivo in vitro* preparations. In this region long-term potentiation is facilitated and NMDA-receptor gated channels are changed. The *in vivo* increased neuronal activity decreases with increasing post-lesion survival time. In the regions that are characterized by the above described phenomena, signs of neuronal reorganization such as shifts in topography of retinal representation and increase of receptive field size are observed. The receptive fields of some neurons at the border of the lesion showed a manifold enlargement two months after lesioning and covered the part of the visual field that was primarily affected by the lesion (Eysel and Schweigart, Cerebral Cortex, in press, 1999). The cells with enlarged receptive fields displayed orientation tuning and direction selectivity well comparable to normal cells. The increase of receptive field size was not observed within the first two days after lesioning without any visual experience. However, when the receptive fields at the border of the lesion were subjected to 1 hour of repetitive stimulation with 1 Hz on-off-stimuli covering the receptive field and its surrounding, the receptive field sizes increased significantly. This effect was quite comparable to the receptive field plasticity observed in normal adult cats with a the same experimental paradigm (Eysel et al., NeuroReport 9, 949-954, 1998).Receptive fields in the adult cat visual cortex display lesion-induced adaptive changes that are compatible with a reduction of the size of a cortical scotoma by up to 4° of visual angle in the central 5° of the visual field. The cellular events that accompany these long-term adaptive changes in the visual cortex suggest that increased NMDA-receptor mediated excitation, reduced GABAergic inhibition and the resulting increased excitability and hence facilitated long-term potentiation are important early mechanisms that promote local neuronal reorganization.

LEARNING-INDUCED RECEPTIVE FIELD (RF) PLASTICITY IN THE THALAMO-CORTICAL AUDITORY SYSTEM.

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Over the last decade a large number of studies have revealed the plasticity of sensory processing in adult animals. In the auditory system, RF plasticity of thalamo-cortical neurons was particularly described after behavioral training. Both in tonotopic and in non-tonotopic cortical areas, selective RF reorganizations were observed following (i) classical conditioning (ii) instrumental conditioning and (iii) frequency discrimination. These RF reorganizations involve increased evoked responses at the frequency of the significant stimulus and decreased evoked responses at other frequencies including the pre-training Best Frequency of the cell. These differential changes were often strong enough to re-tune the cell at the frequency which was significant during the behavioral training. The RF modifications were observed at least up to one hour after the end of the training situation. At the thalamic level, similar long-lasting RF reorganizations were observed in the non-tonotopic areas of the auditory thalamus (dorsal and medial divisions). In the ventral tonotopic division of the auditory thalamus, selective RF changes were observed when the significant frequency was very close (1/8 of an octave) from the pre-training Best Frequency, but the effects disappeared one hour after training. The potential mechanisms of these selective learning-induced RF reorganizations will be discussed, specially the involvement of the cholinergic and noradrenergic systems.

ORIGINS OF RESPONSE SPECIFICITY RECORDED IN AUDITORY AND SOMATOSENSORY CORTICAL NETWORKS

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A number of electrophysiological strategies conducted in in vivo and slice preparations, behavioral training/electrophysiological studies and computational modeling studies have been applied to define the rules governing the neural origins of -- and the plasticity of -- response specificity in the auditory and somatosensory cortex. Receptive fields can be easily reduced or enlarged in size under appropriate behavioral (or experimental electrophysiological) conditions. Cell assemblies cooperatively selecting and representing behaviorally important inputs can grow to several hundred times their normal neuronal memberships, or can shrink significantly in size under appropriate behavioral or experimental electrophysiological conditions. Most recorded changes are consistent with the predictions of a Hebbian network model. The rules governing these changes, and our state of understanding of underlying mechanisms shall be briefly summarized.

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CONTEXT, LEARNING AND ATTENTION DEPENDENT CHANGES IN EARLY VISUAL PROCESSING.

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The response properties of cells in primary visual cortex indicate a role in contour integration and surface segmentation. These responses are as dependent on the global characteristics of contours and surfaces extending beyond their receptive fields as they are on the attributes of features within their receptive fields. Features in the receptive field surround can influence the selectivity of cells for features within the receptive field, and can alter even the dimensions of the receptive field itself. These contextual influences show the strongest attentional modulation in primary visual cortex. Contextual influences are also modified by perceptual learning, and the influence of learning is manifest in a task-dependent fashion. The functional architecture of primary visual cortex of adult animals can be modified in an experience dependent fashion, the basis of which can involve alterations in intrinsic circuits within V1. The modulation of receptive fields and cortical functional architecture by experience, attention and behavioral task are likely to involve an interaction between long range connections within primary visual cortex and feedback connections from higher order cortical areas. Cells in V1 are capable of encoding more complex stimuli than previously believed, and this specificity is derived from the systematic relationship between short and long range cortical connections and cortical functional architecture. The emerging picture is that the properties of cells, even at early stages in visual processing, are dynamic, changing according to visual context, experience, attention and perceptual task.

SYNAPTIC PLASTICITY OF CORTICAL HORIZONTAL CONNECTIONS ASSOCIATED WITH MOTOR SKILL LEARNING

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Motor skill learning involves a reshaping of muscle activation patterns to achieve new actions. The primary motor cortex (MI) has been implicated in this form of learning because movement representation patterns undergo rapid reorganization. In addition, intracortical connections among MI neurons mediated by horizontal connections are capable of long term potentiation (LTP) and depression (LTD) as demonstrated in slice preparations using electrical stimulation. These findings suggest that MI horizontal connections form a substrate to restructure cortical representation patterns used in skill learning. We reasoned that learning-related synaptic changes would leave a trace in cortical circuitry, measured as a change in the strength of field potentials produced in a modified intracortical pathway.

To test this hypothesis, we trained naive rats to reach through an aperture for small food pellets and then examined the efficacy of layer II/III horizontal connections within MI in slice preparations. The amplitude of field potentials in this MI pathway increased in the region of the forelimb representation following five days of reach training, but not in the hindlimb area or in the the contralateral ('untrained') MI of the same slice1. Signs of modification occur with only a few days of training and persist in the 'trained' MI.

Potential mechanisms of this learning-related enhancement were evaluated by attempting to induce LTP and LTD following training. Subsequent to learning, the MI horizontal connections in slices showed smaller amounts of LTP and larger amounts of LTD, indicating that synaptic efficacy is near a functional ceiling within its dynamic operating range. The occlusion of LTP induction in horizontal connections following learning suggests that both processes engage the same mechanisms. Thus, using an LTP-like mechanism intracortical connections in MI modify in association with a novel experience; such modification could help to establish new spatiotemporal patterns of muscle activation used to acquire new motor skills. Supported by NS22517.

1Rioult-Pedotti, et al. (1998) Nat. Neur. 1:230 - 234

NEURONAL SPECIFIC GENE REGULATION

CO-ORDINATED REGULATION OF TRANSCRIPTION CONTROL IN ORGANOGENESIS
M. Geoff. Rosenfeld (UC San Diego)

TRANSCRIPTIONAL CONTROL IN BRAIN STEM CELLS
Ron McKay (Laboratory of Molecular Biology, NINDS)

GENETIC CONTROL OF MESENCEPHALON DEVELOPMENT
Wolfgang Wurst (GSF and Max Planck Inst.fur Psychiatrie, Munich)

INTERACTIONS BETWEEN PAX GENES CONTROL REGIONAL IDENTITY IN THE CNS
Peter Gruss (Max Planck Inst. For Biophysical Chemistry, Gottingen)

There is great progress in understanding the mechanisms that control the differentiation of specific cell types in the nervous system and in other tissues. One of the most important recent general advances in transcription is the identification of protein complexes where co-activators and co-repressors co-ordinate function. **Geoff Rosenfeld** will introduce the biochemistry of these protein machines and discuss their application to problems in development. The identification of stem cells in the central nervous is an advance that will have Important implications for our understanding of the signals that control the differentiation of neurons and glia. **Ron McKay** will discuss the role of members of the POU family of transcription factors in the specification of the stem cell state. The midbrain-hindbrain boundary is a key site of cellular interactions that establish the differentiation of specific neuron types including the clinically important dopaminergic neurons in the substantia nigra. **Wolfgang Wurst** will discuss a genetic approach to this problem using mutations in the Engrailed1 and 2, Orthodenticle1 and 2 (Otx1, Otx2), Pax2, 5, and 8, and other genes. The stem cells of the early nervous system are divided into domains that express distinct members of the Pax gene family. **Peter Gruss** will present a model suggesting that interactions between Pax proteins controls the regional identity of stem cells. Thus this group of talks will summarize progress in the transcriptional control systems that establish neuron specific patterns of gene expression.

THE CAUDAL LIMIT OF OTX2 EXPRESSION POSITIONS THE ISTHMIC ORGANIZER

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During embryonic development, the homeobox gene Otx2 is expressed in the prosencephalon and mesencephalon with a sharp border of expression at the mesencephalic-metencephalic (mes-met) junction. In contrast, the homeobox gene Gbx2 is expressed complementary in the metencephalon and spinal cord. Gene inactivation experiments of Otx2 and Gbx2 have shown that these genes are essential for the development of the pros- and mesencephalon and the metencephalon, respectively. To get insight whether the adjacent expression domains of Otx2/Gbx2 in the mes-met region are instrumental to position and form the mes-met organizer and, subsequently, to specify the mesencephalic and metencephalic territories, we ectopically expressed Otx2 in the presumptive anterior metencephalon driven by an En1 promoter using a knock-in strategy in murine embryonic stem (ES) cells. Transgenic offspring were immediately recognized by their altered motorbehavior. Morphological and histological studies of new born and adult brains revealed that in these transgenic animals the anterior vermis was missing and the inferior colliculi was enlarged. Studying the expression of mes-metencephalic marker genes such as Otx2, Gbx2 and Fgf8, Wnt1, Pax2 and Ephrin-A5 during early embryonic development in these transgenic mice, we could show that Otx2 and the mesencephalon-specific genes, Ephrin-A5 and Wnt1, are shifted caudally into the presumptive metencephalon territory. Furthermore, at the interface of Otx2 and Gbx2, Pax2 and Fgf8 are ectopically induced. Thus, our results demonstrate that the caudal limit of Otx2 expression is sufficient for patterning the mes-met organizer and encoding midbrain fate within the mid-/hindbrain domain.

OPTICAL STUDIES OF DENDRITIC SIGNALING PATHWAYS INVOLVED IN LONG-TERM SYNAPTIC DEPRESSION.

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Long-term synaptic depression (LTD) of the parallel fiber-Purkinje cell synapse involves a number of intracellular and extracellular signals. We have used local photolysis of "caged" compounds, in combination with confocal laser-scanning microscopy and whole-cell patch clamp recording, to identify these signals and their spatial range. Glutamate is released from parallel fibers (PFs) and depolarizes Purkinje cells by activating AMPA-type glutamate receptors. We have probed the function of AMPA receptors during LTD by localized (3-5 μm diameter) photolysis of PF activation double-caged glutamate. Our measurements reveal that LTD not only depresses AMPA receptors at the site of PF activity but also spreads to depress AMPA receptors more than 40 μm away. Calcium is produced by the climbing fibers that must be co-activated along with PFs to produce LTD. Localized photolysis of a new caged calcium compound (NPE-4) depresses PF synapses but this LTD spreads no more than 5 μm. PFs produce IP3 in Purkinje cells by activating metabotropic glutamate receptors. Localized photolysis of caged IP3 causes release of calcium from intracellular stores that spreads approximately 10 μm from the site of IP3 production. IP3 uncaging also causes LTD that spreads over a comparable distance. We conclude that pairing the activity of climbing fiber and PF synapses generates a signal that spreads to depress inactive PF synapses and that neither IP3 nor calcium are this signal.

HIGH-AFFINITY COOPERATIVE CA2+ BUFFERING AND ITS ROLE IN CEREBELLAR PURKINJ E CELLS.

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Ca2+ imaging of mouse cerebellar Purkinje cells in culture was performed wi th a low-affinity Ca2+ indicator, BTC (Kd=15 uM). We found a marked facilita tion in the increases in cytosolic Ca2+ concentrations ([Ca2+]i) during repe titive short depolarization (10-50 ms, 1-4 Hz), which eventually raised [Ca2 +]i greater than 50 uM in the dendrites. The facilitation was neither ascri bed to potentiation of Ca2+ influx nor induction of Ca2+ release from intrac ellular Ca2+ stores. The experiments using a high-affinity caged-Ca2+ compo und DMNPE-4 (Kd=14 nM) indicated that the facilitation was due to the satura tion of a large concentration (0.37 mM) of high-affinity Ca2+ buffers (Kd=0. 38 uM) with a Hill coefficient of 2. This is consistent with an abundance o f high-affinity Ca2+-binding proteins, such as calbindin, in this cell type. Our data suggest that neuronal high-affinity Ca2+ binding proteins play com putational role by prolonging temporal integration of Ca2+ responses at low [Ca2+]i and by triggering micromolar Ca2+ transients upon their saturation b y repetitive stimulation.

IMAGING OF INTRACELLULAR PROTEOLYTIC ACTIVITY USING MEMBRANE PERMEABLE SUBSTRATES AND PRODUCTS IN REGENERATING NEURONS

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The mechanisms underlying neuronal regrowth in relation to regeneration, development and some forms of learning is the subject of intense research. We have shown that transiently elevating the [Ca2+]i by either axotomy or the local application of ionomycin is a sufficient signal to induce the cascade of events leading to growth cone (GC) formation. Here we show by imaging of fluorescent endocytosis markers and of fluorogenic protease substrates, as well as by using immunolabeling, electron microscopy and capacitance measurements, that axotomy induces membrane cycling, accumulation of vesicles, local calpain activation and cytoskeletal remodeling.

Inhibition of the calpain activity by calpeptin abolishes GC formation and the restructuring of the cytoskeleton, demonstrating causal relations between proteolysis and growth related processes. The co-localization of calpain activity with large numbers of accumulated vesicles suggests that activated calpain is associated with the vesicular pool.

Calpain activity is monitored by measuring the increase in fluorescence caused when cleavage of peptide bonds in the fluorogenic substrate (bis(CBZ-Alanyl-Alanine amine)- Rhodamine 110) unquenches the fluorophore. Since both the substrate and the products are membrane-permeable, the fluorescent signal relates directly to proteolytic activity, thus enabling its spatiotemporal measurement.

Preliminary results using a different fluorogenic substrate (bis(CBZ-Arginine amine)- Rhodamin

EXPLORING DENDRITIC SIGNAL PROCESSING BY IN VIVO CALCIUM IMAGING

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In the fly visual system, there exists a group of large neurons (tangential cells) which are sensitive to the direction of motion. Each of them is indvidually identifiable and has a large dendrite on which it receives input from thousands of retinotopically organized elements. Located right underneath the rear surface of the brain the tangential cells can be imaged in vivo while being stimulated by their natural synaptic input from the eyes. We investigate the mechanisms underlying visually induced calcium accumulation and study dendritic signal processing using calcium as a reporter of pre- and postsynaptic activity.In the fly visual system, there exists a group of large neurons (tangential cells) which are sensitive to the direction of motion. Each of them is indvidually identifiable and has a large dendrite on which it receives input from thousands of retinotopically organized elements. Located right underneath the rear surface of the brain the tangential cells can be imaged in vivo while being stimulated by their natural synaptic input from the eyes. We investigate the mechanisms underlying visually induced calcium accumulation and study dendritic signal processing using calcium as a reporter of pre- and postsynaptic activity.

POTASSIUM CHANNELS AND DENDRITIC FUNCTION IN HIPPOCAMPAL PYRAMIDAL NEURONS.

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The dendrites of hippocampal pyramidal neurons possess a rich assortment of voltage-gated ion channels, although the types and densities of these channels can vary considerably across the soma-dendritic axis. We have been particularly interested in potassium channels in the apical dendrites of CA1 pyramidal neurons. Delayed rectifier type potassium channels appear to have a fairly uniform density in the soma and the dendrites, while transient, A-type potassium channels increase in density about 5-fold from the soma to about 350 um in the dendrites. These A-type potassium channels limit the amplitude of back-propagating action potentials and synaptic potentials and increase the threshold for the dendritic initiation of action potentials. Recently, we have been exploring the modulation of these channels by neurotransmitters and second messenger systems. Activation of cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) both decrease the activity of these channels by shifting their voltage range of activation to more positive potentials. Beta-adrenergic and muscarinic receptor activation, presumably acting through PKA and PKC, respectively, also reduce the activity of these channels resulting in an increase in the amplitude of back-propagating action potentials. Inhibition of mitogen activated protein kinase increases the activity of the channels by shifting the activation curve to more negative potentials. We have also recently explored the role of Ca-dependent potassium channels in the repolarization of action potentials. We found that the fast activating K(Ca) channel plays little or no role in action potential repolarization at dendritic sites more distal than about 150 um from the soma. The lack of a fast afterhyperpolarization in the dendrites results in a prolonged depolarization during back-propagating action potentials. The functional significance of dendritic K channels for signal propagation will be discussed.

INTERACTION OF ACTION POTENTIALS AND EPSPS: IMPLICATIONS FOR SYNAPTIC INTEGRATION

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Most neurons require the summation of many excitatory postsynaptic potentials (EPSPs) to sufficiently depolarise the membrane potential to threshold for action potential (AP) initiation. As APs are mediated by large conductances, and in many neuronal types propagate back into the dendritic tree, the interaction of APs with EPSPs is likely to influence synaptic integration. This issue was examined in neocortical layer 5 pyramidal neurons in brain slices using antidromic stimulation to evoke Aps at various times before and after EPSPs. APs caused substantial (up to ~80%) reductions in the peak and time integral of somatic EPSPs. By comparison, EPSPs in Purkinje neurons were only reduced by around 20%. This "EPSP shunting" was significantly less for EPSPs mediated by NMDA receptors than for those mediated by AMPA receptors. EPSP shunting by APs was greater when the AP occurred during the EPSP than when it preceded it, suggesting that conductances activated during the afterhyperpolarisation were less effective than those active during the AP itself. Consistent with this idea, EPSPs shunting was not blocked by 20 mM internal BAPTA and was greatest at the soma close to the site of AP initiation. These results are consistent with the idea that large conductances activated in the axon and soma during the AP can significantly shunt somatic EPSPs and thereby interrupt/reset ongoing synaptic integration. In the distal dendrites (> 400 µm from the soma) the interaction of EPSPs and APs could increase the amplitude of backpropagating APs by up to 4 fold. This amplification was dependent on the relative timing of EPSPs and APs, and was greatest around the time of EPSP peak, i.e. when EPSPs and APs were evoked approximately simultaneously. AP amplification was dependent on the initial amplitude of both the EPSP and the backpropagating AP, and was greater the smaller the initial amplitude of the backpropagating AP. Simulations indicated that AP amplification by EPSPs is spatially confined to the region of synaptic input and occurs as the dendritic depolarisation generated by EPSPs leads to increased activation of dendritic sodium channels by backpropagating APs. Amplification of backpropagating APs by EPSPs represents a form of local coincidence detection and may play a role in the induction of certain forms of Hebbian synaptic plasticity. Supported by the Wellcome Trust.

SPREAD OF SENSORY STIMULATION-EVOKED DENDRITIC EXCITATION IN PYRAMIDAL NEURONS IN BARREL CORTEX *IN VIVO*.

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Dendritic excitability depends on a variety of factors that are hard to reproduce in *in vitro* preparations. To study the properties of dendritic excitation *in vivo* we combined 2-photon imaging of dendritic [Ca^{2+}] dynamics with somatic and dendritic microelectrode membrane potential measurements. In dendrites of layer 2/3 neurons we find that whisker deflection produces action potential-evoked [Ca^{2+}] transients, but these transients are limited to regions close to the soma. Intradendritic membrane potential measurements show that Na^+ action potentials attenuate and broaden rapidly with distance from the soma. These results demonstrate that the restricted pattern of dendritic [Ca^{2+}] transients we observe is due to a failure of Na^+ action potential propagation into layer 2/3 dendrites. In dendrites of deep pyramidal neurons whisker deflection produced large calcium transients close to the apical nexus, sometimes as much as ~ 1 mm from the soma. These transients were associated with slow depolarizations in the dendrites and soma, suggestive of calcium spikes. These results demonstrate the importance of dendritic calcium channels in for amplification of synaptic potentials in deep pyramidal neurons. Supported by Lucent Technologies; NIH, Klingenstein and Whitaker Foundations (KS); Max Planck Society (FH); Swartz Foundation (ES).

THE MOLECULAR MECHANISMS OF THE ORGANELLE TRANSPORTS IN NEURONS; IDENTIFICATION AND CHARACTERIZATION OF NEW MOTOR PROTEINS, KIFS

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The neuron is a polarized cell composed of branching dendrites, cell body and a long axon along which nerve impulses are propagated. Because of the lack of protein synthesis machinery in the axon of the neuron most of proteins necessary in the axon and synapses ought to be transported down the axon after synthesis in the cell body. Thus, organelle transport is fundamental for neuronal morphogenesis and function. In this sense neuronal axon is also an excellent model system to study the mechanism of organelle transport in the cells. Recently we have succeeded in visualizing real dynamics and transport of membranous organelles carrying important proteins such as synaptophysin, GAP43, SNAP25, TrkA and TGN38 in living neurons using GFP technology. In order to understand this mechanism we searched new molecular motors and identified ten new members of microtubule based molecular motors (KIF1A, KIF1B, KIF2, KIF3A, KIF3B, KIF4, KIF5, KIFC1, KIFC2, KIFC3). Using multiple molecular cell biological approaches and gene targeting we have characterized these new members. KIF1A is the fastest (1.5µm/sec) anterograde monomeric motor for transport of precursor of synaptic vesicles and very important for neuronal function and survival while KIF1B is a unique monomeric anterograde motor (0.5 µm/sec) for transport of mitochondria. KIF3A and KIF3B, expressed ubiquitously, form a heterodimer associated with a protein, KAP3 (kinesin superfamily associated protein 3) and work as a new anterograde transporter for membranous organelles different from synaptic vesicle precursors fundamental for neurite outgrowth. Our recent gene targeting study revealed that KIF3 is essential for left-right deteremination of the body through intraciliary transportation of protein complexes for ciliogenesis of motile cilia that could produce a gradient of putative morphogen in the extraembryonis fluid along left-right axis in the node of embryos.KIFC2 is a neuron specific C-terminal motor domain type KIF which transports mutlivesicular body-like organelles to dendrites. Very recently we have identified more than 20 new KIFs. Thus, our recent studies revealed that transport of various kinds of membrane organelles and protein complexes are accomplished very precisely by these new motor molecules in addition to conventional kinesin and brain dynein.

ASSEMBLING THE PRESYNAPTIC JUNCTION (PSJ) OF VERTEBRATE CNS SYNAPSES

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Synapses are highly specialized cellular junctions designed for rapid and regulated signaling between nerve cells and their targets. A highly organized cytoskeletal matrix found associated with the cytoplasmic face of the synaptic junction is thought to act in concert with adhesion molecules and components of the extracellular matrix to hold the site of neurotransmitter release in register with the postsynaptic reception apparatus. In nerve terminals, this cytoskeletal matrix is also thought to play a central organizational role in clustering of synaptic vesicles (SVs) in the reserve and readily releasable pools and in defining the active zone as the site of neurotransmitter release. At present, the molecular components that compose the cytoskeleton of the presynaptic nerve terminal remain largely unknown. Furthermore, little is known concerning the cellular mechanisms which direct the trafficking and assembly of synaptic junctional proteins. We have recently identified two novel presynaptic proteins, Piccolo and Bassoon, that are associated with the cytomatrix underlying the active zone. Our studies reveal that both are structurally related multidomain proteins likely to perform a scaffold function in nerve terminals. For example, Piccolo contains two double-zinc fingers, multiple proline rich regions and several coiled-coil domains, features shared with Bassoon. In addition, in the COOH terminus of Piccolo is a PDZ and C2 domain, a unique feature shared with Rim, another zinc finger protein of the PSJ. In the present study, we have examined whether the zinc fingers in Piccolo and Bassoon act, as in Rim , to regulate the mobilization of SVs from the reserve to the readily releasable pool by interacting with Rab3A in a GTP dependent manner. In addition we have examined a potential role of Piccolo and Bassoon in synaptogenesis by examining their expression in differentiating hippocampal neurons. We find that both are expressed at early stages of neuronal differentiating prior to synapse formation. Moreover, both are found transported to newly forming synapses in association with vesicles. These vesicles are distinct from SVs and appear to represent a PSJ precursor particle.

CYTOSKELETAL INTERACTIONS IN THE POSTSYNAPTIC DENSITY OF EXCITATORY SYNAPSES

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Postsynaptic ionotropic glutamate receptors interact via their cytoplasmic C-terminal tails with specific PDZ domain containing proteins (NMDA receptors with PSD-95/SAP90, and AMPA receptors with GRIP/ABP). These PDZ proteins are believed to act as scaffolds for the assembly of a complex of signaling and cytoskeletal proteins associated with their specific transmembrane receptors. We have identified and characterized a family of postsynaptic density proteins (termed Shanks) that bind to GKAP, a PSD-95-associated protein. Shanks are multidomain proteins (containing ankyrin repeats, SH3, PDZ, proline-rich and SAM domains) with the potential to link the PSD-95 complex to other postsynaptic molecules. In particular, Shank binds to GKAP with its PDZ domain, and to Homer and cortactin with its proline rich region. Cortactin is an actin binding protein and a substrate for Src family tyrosine kinases, and has been implicated in the regulation of the actin cytoskeleton. In developing neurons, cortactin colocalizes with Shank in neuronal growth cones. In mature neurons that have formed synapses, cortactin translocates to dendritic spines (where Shank is localized) in an activity-dependent manner. A regulated interaction between cortactin and Shank may be a mechanism for linking NMDA receptor activity to remodeling of the postsynaptic actin cytoskeleton.

THE ROLE OF NEUROFILAMENTS IN MOTOR NEURON DISEASE

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Transgenic mouse approaches have been used to investigate the role of neurofilament proteins in neuronal function and in motor neuron disease. Our analysis of mice knockout for each of the three neurofilament genes confirmed a key role for neurofilaments in the control of axonal caliber. Surprisingly, the NF-M rather than NF-H subunit is required for optimum assembly of NF-L and for the radial growth of large myelinated axons. The absence of both NF-M and NF-H subunits resulted in the disruption of neurofilament network. Abnormal perikaryal accumulations of neurofilaments resembling those found in amyotrophic lateral sclerosis (ALS) can be produced by overexpression of human NF-H transgenes in mice. Remarkably, the motor neuronopathy in hNF-H transgenic mice was rescued by the overexpression of human NF-L subunits demonstrating the importance of subunit stoichiometry for correct neurofilament transport. The overexpression of transgenes ccoding for wild-type peripherin, a type III intermediate filament (IF) associated with IF inclusions in ALS, caused the selective death of motor neurons in aged mice. The peripherin-mediated disease was precipitated by a deficiency of NF-L made in the NF-L null background. Mice with deregulated levels of neurofilament proteins have also been used to investigate the contribution of neurofilaments in motor neuron disease caused by ALS-linked mutations in superoxide dismutase. While results demonstrate that axonal neurofilaments are not required for SOD1-mediated disease, the overexpression of human NF-H and human NF-L transgenes extended the life span of $SOD1^{G37R}$ mice by 65% and 15%, respectively. The combined results suggest protective effects of perikaryal neurofilament proteins in SOD1-mediated disease. From these transgenic mouse studies, it is concluded that neurofilaments can play both detrimental and beneficial effects in disease pathogenesis.

VISUALIZATION OF CORTICAL DYNAMICS BY OPTICAL IMAGING

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Recent progress in studies of cortical dynamics will be reviewed including the combination of real time optical imaging based on voltage sensitive dyes, single and multi- unit recordings, LFP and intracellular recordings. To image the flow of neuronal activity from one cortical site to the next, in real time, we use optical imaging based on new voltage sensitive dyes and the Fuji 128x 128 fast camera. We confirmed that the voltage sensitive dye signal indeed reflects membrane potential changes in populations of neurons by showing that the time course of the intracellular activity recorded intracellularly from a single neuron was highly correlated in many cases with the optical signal from a small patch of cortex recorded nearby. We found that the degree of cortical synchronization as reflected from the relationship between the membrane potential changes in individual neurons and the population activity was large. We showed that the dynamics of coherent activity in neuronal assemblies can be visualized and found the instantaneous cortical activity is the sum of a reproducible stimulus response component and the on-going network dynamics. We have also investigated the dynamics of shape perception by exploring the development of orientation selectivity in the millisecond time domain determined the dynamics of orientation tuning as a function of the previous luminance change. Chronic optical imaging, over a long period of times, was successfully applied also to the study of similar questions in the behaving macaque monkey. [Supported by grants from GIF, Minerva. The Wolfson Foundation, and BMBF]

PRECISION AND RESOLUTION OF THE FMRI SIGNAL IN MONKEYS

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Purpose: High-field imaging studies in humans showed that the early response in fMRI reflects signal changes that arise from the tissue's capillary bed rather than from large or medium size veins. Here we studied the localization precision and the spatial resolution of fMRI in monkeys, by mapping these early hemodynamic responses to pulse stimuli. **Methods**: In our studies a 4.7T Biospec (Bruker inc.) system with a 40cm bore, vertical magnet was used. The animal sat in a specially constructed cylindrical primate chair and visual stimulation was accomplished by means of an optic-fiber system projecting an LCD-image to the animal's eyes. Experiments with both anesthetized and alert monkeys were performed. **Results**: Statistically significant initial signal decreases, which are due to an increase of deoxyhemoglobin that follows the stimulus-induced local activation, were detected in the primary and extrastriate visual cortices. Such responses were absent in areas around large vessels, such as the sagittal sinus. Principal component analysis of the time series of voxels in the occipital lobe show that the response-type containing the early negativity is one of the first three PCs explaining more than 90% of the signal variance. Functional maps corresponding to the early response showed improved localization and resolution. **Conclusions**: The results confirm the existence of the initial negative change in the MR signal (Hu et al, 1997), which arises from an increase in the concentration of deoxyhemoglobin (Malonek & Grinvald, 96). *Supported by the Max Planck Society*

MEG FUNCTIONAL IMAGING AND INTRACRANIAL RECORDINGS

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Magnetoencephalography (MEG) maps brain electric currents, and allows estimation of the brain areas activated by sensory stimuli or producing spontaneous brain activity. The source areas can, in favourable conditions, be located accurately, and their activation sequence can be presented in milliseconds. Interesting MEG results about cortical reorganization after lesions, of speech- and reading- related cortical activities, and relations of cortical somatomotor spontaneous activity to muscle activity have emerged during last years. MEG source localization is hampered by non-uniqueness of the incverse problem, calculation of the activated area from the magnetic field outside the head. The estimated sources of MEG activity can be overlaid on MR images of individual subjects. As a part of presurgical evaluation, we have generated functional landmarks for somatosensory, motor and speech-related cortical areas by MEG, and compared them with active sites detected by electric simulation and evoked potential recordings during awake craniotomy. The sources of somatosensory evoked fields and of spontaneous activity correlating maximally with EMG signal match the intraoperative localization of somatosensory and motor cortex. The match of the sources of speech- or reading-related activations with stimulation data was less accurate. Probably synchronous activity in a small cortical region in somatosensory and motor activation is modelled more adequately by a single dipole than more widespread source structures.

EEG FUNCTIONAL IMAGING AND COMBINED EEG, MEG AND FMRT

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EEG and MEG signals reflect the spatio-temporal overlap of the brain activities involved in performing a specific task. A discrete, reductionist model can be generated by assigning an equivalent source to each active region or area. The lead field matrix describing the amount of electric or magnetic activity at each electrode or sensor produced by each source can be estimated using a volume conductor model of the head. The inverse of this matrix represents a spatial filter that deconvolutes the overlap and estimates source waveforms for each area. Locations and orientations of equivalent sources can be found by spatio-temporal multiple source analysis (MSA) using various iterative or scanning strategies. Alternatively, fMRI bold clusters can be used as seeds for sources to 'probe' active brain areas for their contribution to the surface EEG or MEG. Simultaneous EEG (32 channels) and MEG (Neuromag-122) of a visual flowfield experiment in normals and of a patient with myoclonic epilepsy were compared with fMRI data. MSA revealed specific enhancement related to visual motion in the source waveforms around 180ms after stimulus onset. The equivalent sources localized in the occipito-temporal motion complex in close proximity to the fMRI BOLD clusters. In the patient, unilateral jerks in hand muscles were preceded by a cascade of activities involving the contralateral motor and premotor cortex, postcentral gyrus and the descending pathway. Somatosensory stimulation triggered a dynamic network involving contralateral somatosensory, motor and premotor cortex, followed within a few milliseconds by ipsilateral motor cortex. In combination, fMRI validated MSA or supplemented spatial information. EEG/MEG provided the essential information on the temporal dynamics.

IMAGING NEURONAL ACTIVITY AND NEUROCHEMISTRY USING HIGH FIELD MAGNETIC RESONANCE

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Understanding the unique capabilities of the brain requires methods that can detect its activity non-invasively, over the entire brain, and with high spatial resolution. Recent developments introduced the ability to measure and image functional, physiological, and metabolic parameters in the human brain using magnetic resonance imaging (MRI) methods. MRI based functional maps in the brain can be generated based on deoxyhemoglobin or perfusion alterations originating from secondary metabolic and hemodynamic responses to increased neuronal activity. These methods are ultimately limited by signal-to-noise ratio and specificity of the MRI approaches to vascular dimensions. High magnetic fields, however, alleviate these limitation and provide numerous advantages as demonstrated by the ability to obtain high resolution functional maps at the columnar level in animal and human brain single shot temporally resolved maps, and images based on early deoxyhemoglobin changes following the onset of neuronal activity. Equally important are spectroscopy studies, which, at high magnetic fields, provide for the first time the opportunity to measure local metabolic correlates of human brain function and neurotransmission rates. Together, these MR methods provide a complementary set of approaches for probing important aspects of the nervous system.

LEARNING AND PERFORMING MOTOR SEQUENCES IN HUMANS

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Utilizing studies of regional cerebral blood flow (rCBF) with PET, we have investigated brain activation with different finger tapping sequences of different length. In all conditions the contralateral primary sensorimotor and premotor cortex, supplementary motor area and ipsilateral cerebellar cortex were activated. These areas showed a large increase in activation from rest to simple repetitive movement, and a further increase with the shortest sequence, suggesting an executive role in running sequences. The ipsilateral premotor area, bilateral posterior parietal areas and precuneus showed an increase in rCBF related only to the length of the sequences. These areas may function in the storage of motor sequences.

We have also examined the dynamic involvement of different brain regions in implicit and explicit motor sequence learning using PET with the serial reaction time task. Test sessions consisted of 10 cycles of the same 10-item sequence. Explicit learning, shown as a positive correlation of rCBF with the correct recall of the sequence, was associated with increased activity in the posterior parietal cortex, precuneus and premotor cortex bilaterally, also in the supplementary motor area predominantly in the left anterior part, left thalamus, and right dorsolateral prefrontal cortex. During the implicit learning phase, improvement of the reaction time was associated with increased activity only in the contralateral primary sensorimotor cortex. During the explicit learning phase, the reaction time was significantly correlated with activity in a part of the frontoparietal network.

Thus the two separate networks responsible for sequence production have different roles during the learning of sequences.

PARALLEL NEURAL CIRCUITS FOR SEQUENTIAL PROCEDURAL LEARNING

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Recent studies have shown that multiple brain areas contribute to different stages and aspects of procedural learning. Studies in our laboratory using the 2x5 task have shown that different regions in the cerebral cortex and the basal ganglia contribute either to new learning or to long-term storage of visuo-motor sequences. The results are summarized as follows. In the early stage of visuo-motor sequence learning, as in our 2x5 task, the prefrontal cortex and pre-SMA together with the anterior part of the basal ganglia would act to initiate learning. In the intermediate stage, the sequence information would then be stored in the parietal cortex (perhaps as a visuo-motor sequence). After a long-term practice, the motor sequence is now produced by the neural mechanism including the cerebellar dentate nucleus and the posterior part of the basal ganglia, so that the performance becomes automatic. Based on these results, we propose a hypothetical scheme in which a sequential procedure is acquired independently by two cortical systems, one using spatial coordinates and the other using motor coordinates. They are active preferentially in the early and late stages of learning, respectively. Both of the two systems are supported by loop circuits formed with the basal ganglia and the cerebellum: the signals going through the basal ganglia are evaluated by reward information, while the signals going through the cerebellum are processed by timing information. The proposed neural architecture would operate in a flexible manner to acquire and execute multiple sequential procedures.

ANTERIOR CINGULATE SULCUS NEURONS AND PROBLEM SOLVING IN THE MONKEY.

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Functional imaging studies in humans have shown that the anterior cingulate cortex is involved in the processes of attention for action, free selection of movement, and monitoring of behavioral outcomes specific to non-routine situations (learning, problem solving,...). In an attempt to study the neural correlates of these processes in animals, rhesus monkeys were trained in a series of spatial problem-solving tasks by trial and error. These animals were required to discover the sequence for touching three fixed spatial targets, and to repeat the solution three times. Then the order was changed and the animals had to initialize a new search within the same set of targets. Up to 60 searches could be initialized during a single recording session. Neural data collection was made in the anterior cingulate sulcus. The rank (first, second or third) of a target acquisition or target-fixation had the strongest effect on the activity of the task-related neurons. The spatial position of the corresponding targets was less relevant. An interpretation is that the cingulate neurons code for the 'distance' from the current action to the reward. A population of neurons displayed differential activity during the search and repetition periods. The search-related activity of the anterior cingulate cortex seems to be involved in monitoring action when the situation requires flexibility of the behavioral responses, evaluation of the outcomes, and short-term memory of prior movements. The repetition-related activity might correspond to a regime of memory-based motor performance in which errors are less frequent and attention to action less necessary. The transition between the two periods is observed at neuronal level as soon as the animal, having acquired enough information during the trial and error process, could predict or anticipate the solution and the reward.

HOW DO NEURONS IN THE SUPPLEMENTARY AND PRESUPPLEMENTARY MOTOR AREAS PROCESS INFORMATION FOR THE PERFORMANCE OF MULTIPLE MOVEMENTS IN A CORRECT TEMPORAL ORDER?

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Many lines of evidence have established that the two areas in the medial frontal cortex, the supplementary motor area (SMA) and presupplementary motor area (pre-SMA), are separate entities. We have recently found that both areas are crucially involved in organizing multiple movements in a correct temporal sequence. We now report similarities and differences in neuronal activity in the two areas in connection with sequencing of multiple movements in a behavioral condition where the spatial factor in motor control is minimized. Monkeys were trained to perform three different arm movements (push, pull or turn a manipulandum) in different orders. The three movements were separated by waiting periods of a few seconds. Each movement was initiated in response to an auditory trigger signal. Initially, a correct order was informed with visual signals. Thereafter, the order had to be memorized, and the animals had to produce a sequence of motor events in response to the trigger signal, without the visual information. Two types of long-lasting activity were of interest. First, a group of SMA or pre-SMA neurons were preferentially active in relation to a particular sequence of forthcoming movements guided by memory. They were active during a waiting period before the first movement-trigger signal, only if the sequence is a specific one. The second type of activity was found preferentially during the interval between two specific movements. It was also found that pre-SMA neurons exhibited more complex relation to the motor task than SMA neurons. Neurons specifically related to the rank order of multiple movements were found more in the pre-SMA than in the SMA. A separate type of pre-SMA neurons were active when the subject were asked to abandon a particular sequence and acquire a new (but previously learned) sequence. This neuronal activity, involved in updating the correct sequence, was not frequent in the SMA. Other differences in activity properties were found among neurons in the two areas.

GENOME ANALYSIS OF OLFACTORY RECEPTOR STRUCTURE AND EVOLUTION

Tzachi Pilpel, Israel

The human olfactory subgenome represents several hundred olfactory receptor (OR) genes in a dozen or more clusters on several chromosomes. Functional hypotheses suggested that in order to cope with a huge array of odorant molecules, ORs must have evolved a hyper-variable ligand binding site. The accumulation of hundreds OR sequences allows to analyze the OR amino acid variability patterns in a predicted structural context. An amino acid variability analysis, combined with molecular modeling, demonstrated that a pronounced majority of the variable residues in the molecule are clustered in space, forming a pocket with a partial overlap to the ligand binding site in other G-protein coupled receptors. A set of 17 hypervariable residues, which constitute a putative odorant Complementarity Determining Regions, is proposed.

An additional mechanism of binding site diversification was discovered by comparison among orthologous and paralogous pairs of primate OR genes. We observed a multiplicity of gene conversion events, which lead to segment shuffling in the odorant binding site, an evolutionary process reminiscent of somatic combinatorial diversification in the immune system.

On the other hand, highly conserved olfactory receptor-specific sequence motifs, were found in the second and third intracellular loops, which may comprise the G-protein recognition epitope. OR proteins thus appear to be a structural interface between recognition-related variability and transduction-related constancy. In this respect, they are well-suited to subserve the convergence of multiple chemical cues into a single common transduction pathway.

A HIERARCHICAL MODEL OF AXON GUIDANCE IN THE OLFACTORY SYSTEM

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We have proposed a hierarchical model of axon guidance to explain axon targeting in the olfactory pathway. We believe that there are several key stages in axon growth along the olfactory nerve pathway and that different molecules are associated with each one of these stages. First, the initial pathway is established by migratory ensheathing cells that are attracted to the olfactory bulb via chemotopic molecules. The trajectory of the ensheathing cells is restricted by the expression of chemoinhibitory chondroitin sulfate proteoglycans in the surrounding mesenchyme. These ensheathing cells provide a conducive substrate for olfactory axon growth. The olfactory axons ubiquitously express cell adhesion molecules such as N-CAM, L1 and TAG-1 that are non-specificially involved in axon fasciculation along the olfactory nerve and in the olfactory nerve fibre layer. In the absence of N-CAM, olfactory axons fail to defasciculate and sort out in the nerve fibre layer. Second, molecules such as OCAM, that are expressed in sub-regions of the olfactory neuroepithelium, are involved in the partitioning of axons into large spatially-defined bundles (eg. ventrolateral vs ventromedial). Third, subpopulations of axons in these bundles express distinct cell surface carbohydrates. These carbohydrates provide a glycocode that mediates axon sorting, via interactions with carbohydrate-binding proteins such as galectins, into smaller, discrete fascicles in the nerve fibre layer. Fourth, axons exhibiting a particular glycocode are further subdivided into smaller subpopulations by expression of specific odorant receptors. Interactions between these odorant receptors and gradients of guidance molecules across the olfactory bulb direct axons to topographically-fixed glomeruli.

THE MACROMOLECULAR ARCHITECTURE OF ACTIVE ZONE MATERIAL AT THE NEUROMUSCULAR JUNCTION AND ITS ROLE IN SYNAPTIC TRANSMISSION.

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The active zone material that lines the internal surface of the presynaptic membrane at the frog's neuromuscular junction is arranged in elongate patches parallel to the membrane. Each patch is flanked by a row of docked synaptic vesicles apposed to the presynaptic membrane and overlies the region of the presynaptic membrane which contains a high concentration of Ca2+ and K+ channels. The electron dense material extends about 50nm into the axoplasm. We are using electron tomography to generate 3D reconstructions of individual thin sections of frog neuromuscular junctions with the aim of characterizing the structural associations of the active zone material to docked vesicles and presynaptic membrane [Harlow et al., J. Physiol. (Paris) 92:75-78, 1998]. By applying a novel segmentation scheme to examine in detail the active zone material within 15nm of the presynaptic membrane, we find that within this region the material is arranged in parallel bands lying in the plane of the membrane and orthogonal to the long axis of the patch. The bands contact the presynaptic membrane at points, and at one end they contact the membrane of docked synaptic vesicles near the site where the vesicles abut the presynaptic membrane. As many as 4 such bands contact a vesicle. Their length, packing density and relationship to the presynaptic membrane altogether raises the possibility that each band associated with a docked vesicle is also, at different points, associated with Ca2+ and/or K+ channels in the presynaptic membrane, helping to anchor vesicles and channels in positions relative to one another that are required for normal synaptic transmission to occur. The bands may also include Ca2+ sensitive proteins that mediate synaptic vesicle exocytosis triggered by Ca2+ influx through the channels during synaptic transmission.
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GENETIC ANALYSIS OF POSTSYNAPTIC DIFFERENTIATION AT THE NEUROMUSCULAR JUNCTION

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Motor axons organize assembly of an elaborate postsynaptic apparatus at the skeletal neuromuscular junction. We are analyzing "knockout" mutant mice and myotubes cultured from such mutants, to define the intercellular signals and intracellular signal transduction pathways responsible for postsynaptic differentiation. A critical nerve-derived signal is agrin, a heparan sulfate proteoglycan synthesized and secreted by motoneurons. Of multiple agrin isoforms, ones initiated from an upstream promoter and containing a C-terminal "Z" insert are required for differentiation; forms lacking the Z insert, containing a "Y" insert, or initiated from a downstream promoter are dispensable. Agrin appears to signal through two membrane-associated protein complexes. One contains the tyrosine kinase MuSK, a critical component of the signal transducing receptor. One segment of the MuSK ectodomain mediates interactions with agrin that activate the kinase, recruit shc-like proteins to the cytoplasmic domain, and lead to aggregation of acetylcholine receptors via a cytoplasmic receptor-associated crosslinking protein (rapsyn). A separate portion of the ectodomain mediates MuSK-rapsyn interactions that recruit the receptor cluster to a primary synaptic scaffold. A second protein complex, the dystrophin-glycoprotein complex, is dispensable for the formation of the postsynaptic but essential for its stability. The dystrophin-associated protein alpha-dystrobrevin is critical for this function: in its absence, receptor clusters disperse rapidly following removal of agrin. Together, these results define a genetic pathway in which agrin is a critical signal, MuSK is a component of the receptor, rapsyn is an effector molecule, and dystrobrevin is important for synaptic stability. Within this framework, we are now elucidating the biochemical mechanisms by which agrin signals.

PHOSPHORYLATION AND ACETYLCHOLINE RECEPTOR AGGREGATION AT THE VERTEBRATE NEUROMUSCULAR JUNCTION.

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Agrin, a protein synthesized in motor neurons and released by their axon terminals at vertebrate skeletal neuromuscular junctions, triggers the formation of the postsynaptic apparatus in developing and regenerating muscle fibers. When added to myotubes in cell culture agrin induces the formation of specializations at which a variety of extracellular matrix, membrane, and cytoplasmic components of the postsynaptic apparatus accumulate, including acetylcholine receptors (AChRs). Agrin also triggers an increase in protein phosphorylation, including tyrosine phosphorylation of the muscle specific receptor tyrosine kinase MuSK and of the AChR beta subunit. We are examining the role of AChRs and AChR phosphorylation in agrin-induced aggregation. We have found that immobilization of AChRs prevents formation of all agrin-induced specializations, suggesting an obligatory role for AChRs or associated proteins in the aggregation process. In addition, mutant acetylcholine receptors that lack any sites for agrin-induced tyrosine phosphorylation nevertheless can be recruited to agrin-induced specializations. Thus, postsynaptic specializations may form by a two-step process, creation of an initial scaffold by a mechanism that requires tyrosine phosphorylation, followed by recruitment of additional postsynaptic components by phosphorylation-independent mechanisms.

THE DYSTROPHIN COMPLEX – A SCAFFOLD FOR SIGNALING PROTEINS AT SYNAPTIC SPECIALIZATIONS

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Dystrophin is encoded by a large gene that when mutated causes Duchenne and Becker muscular dystrophies. Although dystrophin is expressed primarily in skeletal and cardiac muscle, several other dystrophin-related proteins are found in many different cell types, including neurons. Dystrophin provides a link, via the dystroglycans, between the cytoskeleton (cortical actin) and laminin or agrin in the basal lamina. In addition to its structural properties, the dystrophin complex may also have signaling capabilities. The syntrophins, which are bound directly to dystrophin and other members of the dystrophin protein family, may be particularly important in signaling. Four syntrophins are known and each is a PDZ-containing adapter protein. β2-syntrophin appears to be particularly important at membrane specializations: it is found at the neuromuscular junction, at glutamatergic synapses in the outer plexiform layer of the rodent retina and on the basal lateral membrane of polarized epithelial cells. Using primarily biochemical techniques, we have found that two signaling proteins important in nervous system function are associated with syntrophins. In skeletal muscle, voltage-activated sodium channels are bound via their carboxy-terminal tails to the syntrophin PDZ domain. Neuronal nitric oxide (nNOS) is also bound to syntrophin PDZ but this interaction involves heterodimerization with the PDZ domain of nNOS. We have tested the syntrophin-nNOS interaction in vivo using a transgenic approach. A form of syntrophin lacking the PDZ domain competes effectively with endogenous syntrophins in skeletal muscle, thus acting as a dominant-negative protein. nNOS fails to associated with the sarcolemma in these transgenic mice, confirming the importance of the PDZ interactions. According to our model of the dystrophin complex, two syntrophins are present. We hypothesize that syntrophins bind functionally-interdependent signaling proteins and bring them into close proximity with each other. In this manner, the complex may provide a specialized scaffold for rapid and efficient signaling. The identity of the signaling proteins may depend on the cell type and the exact protein composition of the dystrophin-related complex.

GLUTAMATE RECEPTOR TARGETING AND ASSEMBLY OF EXCITATORY SYNAPSES

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The subcellular targeting of neurotransmitter receptors is vital in controlling polarized information flow in the brain. We analysed mechanisms of axon/dendrite targeting of metabotropic glutamate receptors and synaptic clustering of ionotropic receptors. Expression of mGluR subtypes, chimeras, deletion, and insertion mutants from defective herpesvirus vectors in cultured hippocampal neurons revealed polarized targeting signals in the C-terminal cytoplasmic domains. The C-terminal 65 amino acids of mGluR7 functioned as a dominant axon targeting signal, whereas the C-terminal 53 amino acids of mGluR2 functioned as a weaker axon exclusion signal. In separate studies, we analysed mechanisms of assembly of excitatory postsynaptic specializations, including the roles of the actin cytoskeleton, contact by glutamatergic axons, and synaptic activity in localizing several postsynaptic components. One finding from these studies was a consistent difference in AMPA versus NMDA receptor localization. Synaptic AMPA receptors on pyramidal neurons were more readily extractable by detergent than NMDA receptors. Whereas NMDA receptors formed spontaneous clusters, AMPA receptors did not cluster in the absence of glutamatergic input. Activity blockade had no effect on AMPA receptors but enhanced synaptic clustering of NMDA receptors. These results indicate that different mechanisms regulate postsynaptic localization of NMDA versus AMPA type glutamate receptors. This differential regulation may be important during development and in many forms of synaptic plasticity.

INFLAMMATORY AND IMMUNE EVENTS IN INJURY AND REPAIR IN THE NERVOUS SYSTEM

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Injury to axons results in Wallerian degeneration (WD) distal to the lesion site, and to the loss of neural function. Repair depends on successful regeneration through WD regions. In turn, regeneration depends, amongst others, on the rapid removal by phagocytosis of myelin that inhibits axonal growth if it is not removed from the path through which axons regenerate. We view WD as an inflammatory response to injury and study its significance to myelin removal and future regeneration in three systems. In PNS of normal mice; WD is efficient (resident Schwann cells and recruited macrophages are activated), myelin removal by these scavenger cells is rapid and regeneration is rapid too. In PNS of Wld mice; WD is deficient (Schwann cell activation and macrophage recruitment are slow), myelin removal is slow and regeneration is delayed. In CNS of normal mice; WD is extremely deficient (microglia activation is deficient), myelin removal is extremely slow and regeneration fails. We further study the role of cytokines (e.g. IL-1α, IL-1β, TNFα, IL-6, GM-CSF, M-CSF, IL-10, IFNγ), the mediator molecules of inflammation and immunity, in WD. For example, the efficient inflammatory response is characterized by a rapid onset of NFkB production (< 15 min), followed by a rapid up-regulation of production of the inflammatory cytokine GM-CSF (< 5 hours), primarily by fibroblasts. In turn, GM-CSF activates resident Schwann cells and recruited macrophages to phagocytose and degrade all myelin within 8 to 12 days. The cytokine IL-10 plays an anti-inflammatory role in WD by down regulating GM-CSF production. There are two phases to IL-10 production. The onset of the first is rapid but levels of production are low. High levels of IL-10 are produced during the second phase, primarily by recruited macrophages. Down regulation of the inflammatory events by IL-10 occurs after myelin has been removed. In contrast, low levels of both GM-CSF and IL-10 production throughout characterize the deficient inflammatory response is.

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REGENERATION, COMPENSATORY AXON GROWTH AND FUNCTIONAL RECOVERY IN THE ADULT CNS INDUCED BY ANTIBODIES AGAINST A MYELIN-ASSOCIATED NEURITE GROWTH INHIBITOR

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Following lesions to the brain and spinal cord axonal regeneration as well as compensatory growth from unlesioned fiber systems is very restricted in the adult CNS, but does occur to often large degrees during development. The loss of axonal growth capacity parallels myelin formation in many parts of the CNS, suggesting that the myelin-associated neurite growth inhibitory factors could be involved in down-regulating axon growth as maturation procedes. A very potent neurite growth inhibitory constituent of CNS myelin is the recently cloned membrane protein NOGO-A (formerly NI-250). Nogo is a novel gene with 3 major mRNA splice forms. NOGO-A is the predominant form in oligodendrocytes of the CNS but also occurs in other cell types especially during development. Recombinant (cellular or bacterial) NOGO-A inhibits neurite outgrowth as an in vitro substrate, an effect which is abolished by the antibody IN-1 raised against rat myelin derived NI-250. Antisera against NOGO-A sequences interm neutralize the inhibitory activity of CNS myelin protein extracts. mAB IN-1 or the recombinant, partially humanized IN-1 Fab applied to spinal cord or brainstem lesioned rats induce enhanced sprouting and long-distance regeneration of transected axons as well as sprouting and compensatory growth of unlesioned fiber systems. Functional recovery in sensory and motor tasks are observed in the same IN-1 antibody treated animals, but not in control antibody treated controls. – These results suggest that myelin and its specific constituent NOGO-A are involved in the restriction of fiber growth in the adult CNS. If growth is allowed, however, sprouting and regenerating fibers seem to be able to integrate into functionally meaningful circuits.

TISSUE PLASMINOGEN ACTIVATOR AND NEUROSERPIN IN MICROGLIAL ACTIVATION AFTER FOCAL ISCHEMIC STROKE

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Recent observations obtained with tissue-type plasminogen activator (tPA) indicate that tPA contributes to the development of tissue damage in an animal model of excitotoxicity-induced neuronal cell death. In brief, the damage of cerebral tissue after kainate-induced seizures was reported to be significantly smaller in $tPA^{-/-}$ mice, as compared with wild-type mice. This observation indicates that tPA enhances excitotoxicity-induced degenerative processes. We have recently cloned and characterized neuroserpin, a neuronally expressed and axonally secreted inhibitor of tPA. Because neuroserpin is coexpressed with tPA in several CNS areas it could be a promising candidate for an endogenous antagonist of tPA in the CNS. Therefore, we studied the role of neuroserpin in focal ischemic stroke. The results indicate that neuroserpin expression is enhanced after a focal ischemic stroke in the neurons of the ipsilateral hemisphere. In experiments with transgenic mice overexpressing neuroserpin in CNS neurons, we found a marked attenuation of the microglial activation in the reactive zone. The reduced microglial activation was accompanied by a reduced production of microglial tPA. Whether the attenuation of the microglial response has a neuroprotective effect is currently under investigation.

WHAT COMBINATION OF CELLS AND FACTORS WILL PROMOTE REPAIR OF INJURED ADULT MAMMALIAN SPINAL CORD?

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Our goal is to improve outcome after spinal cord injury by combining cell transplantation and administration of neuroprotective and neurotrophic factors. We have developed a model of Schwann cell (SC) transplantation into a complete transection gap in adult rat thoracic spinal cord. Whereas SC grafts provide bridges onto which fibers from both stumps grow, there is little response from supraspinal neurons and regenerated fibers do not exit the graft to enter the cord. However, brainstem neurons extend axons into the graft (despite the thoracic location), and also a modest number of axons leave the graft when methylprednisolone is administered at the time of transplantation. When neurotrophins, BDNF and NT-3, are infused around the SC graft, brainstem neurons but not cortical neurons regenerate axons into the graft. When the SCs have been genetically modified to produce human BDNF, axons from brainstem neurons regrow across the transection site and into a trail of the SCs extending 5 mm into the distal cord. Olfactory ensheathing glia (OEG), when transplanted into the cord stump next to the SC graft, promote egress of fibers from graft into cord and also long distance axonal regeneration. When SCs genetically modified to secrete BDNF and NT-3 are placed inside collagen Type IV tubes transplanted into thoracic cord and combined with FGF1-fibrin glue, compressive vertebral wiring, and methylprednisolone treatment, axonal ingrowth and myelination in the grafts and axonal re-entry into the distal cord are enhanced. These studies emphasize the importance of a multidisciplinary approach in the search for repair strategies in spinal cord injury.
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THE USE OF STEM CELLS IN BRAIN REPAIR

Ron McKay

Stem cells are multipotential cells that generate the different cell types of the nervous system. They can be isolated from the developing brain and expanded in culture to generate homogeneous precursors that differentiate into neurons and glial cells. An important general question is whether stem cells make functionally competent products after expansion in vitro. Data will be presented showing that functional glutamatergic, GABAergic and dopaminergic neurons can be derived from stem cells. In addition, stem cells can generate both functional astrocytes and myelinating oligodendrocytes. Human CNS stem cells have been isolated and shown to have similar properties to their rodent counterparts. These data suggest that stem cell biology will be important in the development of new cell therapies and drug discovery to promote neuroregeneration.

TRENDS AND ISSUES IN NEUROSCIENCE GRADUATE EDUCATION IN NORTH AMERICA

MIZE, R.R. Co-President, Association of Neuroscience Departments and Programs and Department of Cell Biology and Anatomy and the Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112 USA

Graduate education in the field of neuroscience has grown dramatically in North America during the past decade. A recent survey by the Association of Neuroscience Departments and Programs (ANDP) reveals the following trends: 1) there has been a three-fold increase in the number of neuroscience departments or programs since 1992; 2) the number of students applying and accepted to neuroscience related programs has also increased; 3) the number of PhDs awarded in the field of neuroscience has increased; 4) the time required to obtain the degree has remained constant (~5.5 years); 5) the percentage of PhDs moving to postdoctoral positions has increased while those moving immediately to tenure track faculty positions has declined; 6) the unemployment rate for neuroscience PhDs remains low; 7) The number of neuroscience related PhDs employed outside of academia (pharmaceutical, biotechnology, etc.) has increased; 8) Traditional basic science departments are training fewer neuroscientists.

Neuroscience graduate education is undergoing significant change. Both molecular biology/genetics and cognitive neuroscience have expanded the scope of the discipline. There is a trend to establish umbrella admissions programs and core curricula, which may diminish the identity of neuroscience as a discipline. The National Research Council has recommended restraint in the rate of growth in number of students in the life sciences, which may reduce graduate applications in the future. There is also increased oversight of postdoctoral fellowship positions, including salary and benefits, and future employment prospects. Neuroscience will experience additional change in the next century, but will likely continue to evolve as a lively discipline. Supported by IBRO and ANDP.

ELECTRONIC PUBLISHING AND EDUCATIONAL RESOURCES IN THE NEUROSCIENCES

THOMAS S. MERRIWEATHER AND PAUL M. CARTON, Neuroscience Group, Elsevier Science, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, U.K.

The electronic medium is providing the academic publisher with the means to both improve traditional publication processes and to offer entirely new types of products and services. For example, by utilizing the WWW, the speed of all stages of the journal peer-review process is being increased, from initial submission to an Editor through to release to the academic community. CD-ROMs, DVD and the like also have their place, as they provide sufficient storage capacity to enable the development of sophisticated interactive resources for e.g. the laboratory and lecture theatre.

This presentation will provide an exposition of the driving forces behind, and results of, one publisher's initial forays into electronic publishing for the neuroscience community, with particular emphasis on resources, which will be of interest to those teaching and learning the subject. Demonstrations of online journals, community websites, encyclopedia and CAL packages will be included.

NEW APPROACHES TO THE DESIGN AND CONTENT OF NEUROSCIENCE TEXTBOOKS.

GEORGE AUGUSTINE AND DAVID FITZPATRICK, Department of Neurobiology, Duke University Medical Center, Durham, NC.

The goal of our presentation is to describe our experience in producing the textbook Neuroscience (Sinauer Press). After a brief presentation on the overall structure of the book, we will field questions on how the textbook evolved from a collection of notes for a medical school course to a mature, fully illustrated publication. Issues to be discussed will include how to successfully integrate basic and clinical science, effective design and use of color graphics, and how digital materials are incorporated into the course as taught at Duke University. We also plan to show the new interactive program Sylvius that is being distributed with the latest edition of the textbook and which provides much of the basic neuroanatomy that is taught in the laboratory portions of the course.

HOW TO INCREASE NEUROSCIENCE AWARENESS IN THE SCHOOLS AND WITH THE PUBLIC

NORBERT R. MYSLINSKI, Department of Biological Sciences, University of Maryland Dental School, Baltimore, Maryland, USA

This decade has seen great strides in our understanding of the brain. It is critical that we transfer this new knowledge to the general public, and thus improve their daily lives, enhance their understanding of brain disorders, and increase their appreciation of brain research. To this end, the DANA Alliance for Brain Initiatives and the Society for Neuroscience have spearheaded International Brain Awareness Week (BAW) which involves neuroscientists reaching out to the public in the form of special exhibits, public lectures, classroom visits, newspaper articles, competitions, television and radio programs and other activities. This year more than 100 Neuroscience Departments and Programs, backed by nearly 800 partners, sponsored BAW activities in 27 countries. If you would like to participate in the March, 2000 BAW activities contact the following WEB sites: www.sfn.org/BAW and www.dana.org/brainweek. A new BAW activity initiated this year is the International Brain Bee, which is a question and answer competition about the brain for students from 14 to 18 years of age. This year neuroscientists in 20 American and Canadian cities conducted regional brain bees during January and February. The winner of each Regional was then invited to compete in the Nationals at the University of Maryland during BAW in March. The winners receive prizes and are honored by neuroscientists and statesmen during a ceremony in Washington, D.C. The Brain Bee is an attempt to motivate our youth to read about the brain, capture their imagination, and inspire them to pursue biomedical careers. Those interested in organizing a regional brain bee in their country or city should E-mail Dr. Norbert Myslinski at nrm001@dental.umaryland.edu.

ASSOCIATION OF NEUROSCIENCE DEPARTMENTS AND PROGRAMS: SHOULD THERE BE AN INTERNATIONAL ASSOCIATION?

MIZE, R. RANNEY Co-President of the Association of Neuroscience Departments and Programs, and RAMI RAHAMIMOFF, Co-President, Fifth IBRO World Congress of Neuroscience

The Association of Neuroscience Departments and Programs (ANDP) is a North American organization dedicated to the advance of education and scientific research in departments and programs that study the nervous system. ANDP currently has over 250 membership programs at graduate and undergraduate institutions in the United States, Canada, and Mexico. It holds an Annual Spring meeting in Washington D.C. This meeting focuses upon neuroscience education and curriculum issues and upon federal and other sources of funding for biomedical research and research training. It presents an annual Education Award at its Fall meeting banquet and sponsors workshops and forums on graduate education at the Society for Neuroscience meeting. It maintains a web site (www.andp.org) that provides a variety of information to the neuroscience community, including a complete listing of member programs, information about the graduate school experience, and sources of funding for research training. Every five years it conducts a Neuroscience Training Survey to determine the number of students trained and prospects for employment in the field.

ANDP has recently created an International Affiliates initiative to promote greater collaboration between programs within North America and the rest of the world. Plans include the development of a database of all neuroscience training programs on the planet, a search engine for identifying available training and employment opportunities for recent graduates, and educational materials for teaching neuroscience, all to be posted on the internet.

The prospects for creating an international Association of Neuroscience Departments and Programs will be discussed during the International Brain Research Organization meeting. Issues to be covered include benefits of membership, administrative structure, cost sharing, and the desirability of sponsoring a bi-annual meeting.

SYNAPTIC PLASTICITY IN THE MESOLIMBIC DOPAMINE SYSTEM

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Behavioral sensitization in response to administration of psychomotor stimulants such as cocaine and amphetamine is used as an animal model for the changes that occur during the development of certain forms of addiction. It is blocked by antagonists of excitatory amino acid receptors suggesting that, like many other forms of behavioral plasticity, psychomotor stimulant-induced behavioral sensitization involves long-lasting activity-dependent modifications of synaptic strength at critical sites within the neural circuitry that mediates the behavioral changes. Because of the potential importance of synaptic plasticity in mediating behavioral sensitization and therefore aspects of addiction, I will present an update on the latest developments in our understanding of the mechanisms underlying various forms of long-term potentiation (LTD) and long-term depression (LTD) that have been extensively studied in the hippocampus. Evidence will also be presented that excitatory synapses in the nucleus accumbens and ventral tegmental area, two structures implicated in the behavioral responses to drugs of abuse, can express LTP and LTD with mechanisms that may be analogous, but not necessarily identical, to the mechanisms underlying plasticity at hippocampal synapses.

MOLECULAR MECHANISMS OF DRUG ADDICTION

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A key challenge in addiction research is to identify neuroadaptations that underlie the relatively stable behavioral changes that characterize addiction. One mechanism that could play a major role in such stable neuroadaptations is the regulation of gene expression. This talk will focus on one particular transcription factor, *FosB, which appears to be part of the molecular mechanisms that underlie addiction.

*FosB, a product of the fosB gene, is a member of the Fos family of transcription factors. Members of this family are induced rapidly and transiently in specific brain regions in response to diverse acute stimuli. However, in contrast to other Fos family members, biochemically modified isoforms of *FosB accumulate in a region-specific manner in brain uniquely in response to many types of chronic perturbations. Prominent among these are drugs of abuse, which after repeated but not acute administration induce the *FosB isoforms in the nucleus accumbens (NAc) and related striatal regions. These brain regions are known to mediate many behavioral effects of these drugs. Importantly, once induced, the *FosB isoforms persist in brain for relatively long periods of time due to their extraordinary stability.

We have studied the functional role played by *FosB in addiction by generating transgenic mice in which *FosB can be induced selectively within the same subset of NAc and striatal neuron in which drugs of abuse normally induce the protein. Expression of *FosB dramatically increases the animal's sensitivity to the rewarding and locomotor- activating effects of cocaine. Some of these effects of *FosB appear to be mediated via altered expression of specific glutamate receptor subunits. Expression of GluR2 (an AMPA receptor subunit) but not of other glutamate receptor subunits is increased in the NAc upon expression of *FosB. Moreover, overexpression of GluR2 in the NAc by viral-mediated gene transfer increases an animal's behavioral responsiveness to cocaine, thereby mimicking the effect seen in the *FosB expressing mice.

Together, this work supports a scheme in which *FosB functions as a sustained "molecular switch" that gradually converts acute responses into relatively stable adaptations that contribute to drug addiction.

THE REGULATION OF ADENYLYL CYCLASE ISOZYMES BY OPIATES AND AGONISTS OF OTHER $G_{i/o}$-COUPLED RECEPTORS

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Acute stimulation of opiate receptors leads to adenylyl cyclase (AC) inhibition, while chronic activation leads to a progressive increase in AC activity. This phenomenon is referred to as AC superactivation and has been proposed to play a role in the mechanism underlying opiate addiction. We reconstituted the ability of opiates to inhibit AC upon acute exposure and to induce superactivation upon chronic exposure using CHO and COS cells transfected with mu-, delta- or kappa-opioid receptors. We found that AC superactivation is not dependent upon protein synthesis and is mediated via $G_{i/o}$ proteins, as it was inhibited by pertussis toxin pretreatment. Nine AC isozymes have recently been cloned. Transfecting these isozymes into COS cells, we found that acute activation of the mu receptor inhibited AC-I, V, VI and VIII, while AC-II, IV and VII were stimulated and AC-III was not significantly affected. Chronic opioid receptor activation led to superactivation of AC-I, V, VI and VIII, but not of AC-II, III, IV, or VII, demonstrating that superactivation is isozyme-specific. These results suggest that tolerance and withdrawal may involve specific AC isozymes. A similar pattern of AC isozyme regulation was observed with other $G_{i/o}$-coupled receptors (m_2- and m_4-muscarinic, D_2-dopaminergic, CB1-cannabinoid), demonstrating the generality of the phenomenon of AC superactivation. Moreover, we found that AC types V/VI interact with (beta)(gamma) dimers of $G_{i/o}$ proteins, and that these dimers have a role in the superactivation process, as transfection with scavengers to $G_{(beta)(gamma)}$ or with $G_{(gamma)2}C68S$ (which translocates the $G_{(beta)(gamma)}$ dimers into the cytosol) attenuated or abolished AC superactivation. *Supported by NIDA (DA6265), the German-Israeli Foundation for Research and Development, and the Forschheimer Center for Genetics.*

CORTICOSTEROIDS AND NEURONAL FATE IN THE HIPPOCAMPUS

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Of all hippocampal subfields, the dentate gyrus (DG) is the most sensitive to corticosteroids (CS) with respect to proliferation/survival and degeneration (apoptosis). Granule cell proliferation is stimulated by CS removal (adrenalectomy, ADX), but at the same time, ADX leads to profound apoptosis of granule cells. It now appears that CS with activity at the mineralocorticoid receptor are necessary for granule cell survival. Activation of glucocorticoid receptors leads to apoptosis in the granule cell layer; interestingly, this effect can be abolished if prior occupation of mineralocorticoid receptors has taken place. Further, the neurodegenerative effects of ADX can be attenuated by treatment with the neurosteroid tetrahydroprogesterone (THP) which allosterically interacts with the $GABA_A$ receptor. Sensitivity to manipulations of the CS environment is apparently dependent upon subject age. Thus, older rats are more susceptible to DEX-induced apoptosis, whereas younger subjects display greater cell losses in response to ADX. We have found that the structural changes in the DG resulting from altered CS levels result from changes in the expression of members of the *bcl-2* gene family: Bcl-2 and Bcl-X_L are survival-promoting proteins, whereas Bax is a death-promoting protein. Both ADX and DEX result in an increase in the ratio of *bax*:*bcl-2* and *bax*:*bcl-X_L* gene expression, whereas the protective effects of corticosterone result from a reduction in these ratios. Further, the apoptotic effects of DEX were found to be abolished in *bax* knockout mice, indicating an important role for Bax in mediating glucocorticoid actions on neuronal death. A role for the tumor-suppressor protein p53 was indicated by the observation that stimulation of GR, but not MR, resulted in increased levels of the phosphorylated form of this protein in the hippocampus. It is thus concluded that apoptosis and survival of hippocampal granule cells depends on which of the two corticosteroid receptors (mineralocorticoid *vs.* glucocorticoid) is activated; neuronal fate appears to be ultimately determined by subtle changes in the pattern of expression of death and survival genes.

CORTICOSTEROID EFFECTS ON CALCIUM HOMEOSTASIS IN HIPPOCAMPAL NEURONS.

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Principal neurons in the rat hippocampal CA1 area and dentate gyrus contain both mineralocorticoid and glucocorticoid receptors (i.e. MRs and GRs). Activation of these receptors leads to altered gene expression, potentially affecting membrane properties that are important for signal transduction. Previous work has shown that in particular calcium (Ca) currents through voltage gated Ca channels are sensitive to corticosteroid receptor activation. In CA1 neurons it was found that predominant MR activation is associated with small Ca-current amplitudes, while simultaneous MR and GR activation leads to large Ca-currents. In the absence of corticosteroids, i.e. after adrenalectomy (ADX) Ca-currents were also large, pointing to a U-shaped steroid dose-dependency. The increased Ca-influx with GR activation appears to play an important role in the neuropathology of the hippocampus. For instance, high levels of corticosterone during early phases of kindling epileptogenesis accelerates the onset of epileptic seizures and persistently increases Ca-currents, even when corticosteroid levels have returned to the control level again. Likewise, high corticosteroid levels during an ischemic-hypoxic insult exacerbate neurodegeneration and loss in synaptic function of the CA1 area 24 hours later; conversely, when GRs are not extensively activated during the insult, protection against loss in cells and function is observed.
While increased corticosterone levels, resulting in extensive GR activation, thus form a threat to the viability of CA1 neurons -supposedly through an increased Ca-influx- total absence of corticosterone is also risk factor for neurodegeneration. Thus, 3 days after ADX part of the granule cells in the dentate gyrus display signs of apoptosis. We recently investigated Ca-current properties at various moments after ADX. We observed that one day before morphological signs of apoptosis, i.e. 2 days after ADX, Ca-currents through voltage gated Ca-channels are increased; 3 and 7 days after ADX the increase was no longer apparent. By analyzing the RNA of the recorded neurons we are presently investigating whether the increased Ca-currents are associated with an enhanced expression for Ca-channel subunits.
We conclude that predominant MR activation results in moderate Ca-exposure of hippocampal cells, thus protecting against neurodegenerative changes. When MRs are unoccupied or when GRs are activated in addition to MRs neurons can become exposed to high intracellular Ca-levels, which may form a risk factor for the cellular integrity.

RAPID ACTIONS OF STEROID HORMONES MAY REVEAL NOVEL MECHANISMS

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The way steroid hormones exert their effects has been studied for decades and the actions on gene expression are known to a considerable extent. New developments come by recognizing new receptors, new hormone response elements, and new modes of action. Physiological studies of various steroid-induced changes point to the existence of mechanisms for which molecular mechanisms are not yet delineated. Many of the rapid steroid actions, including the behavioural actions have no well-established mechanistic explanations. Challenges of different kind (e.g. social contact with an unfamiliar individual, exposure to the open field, exposure to the plus-maze, etc) induce a rapid elevation of plasma corticosterone (CS). The quick rise in plasma CS feeds back into the brain, and affects the behavioral response to the challenge. This process consist a yet poorly known mechanism of behavior control. The process is biologically relevant if it is 1) fast enough to affect ongoing behavior; 2) the effects manifest at physiological concentrations; 3) has some behavioral specificity. As a challenge may induce a rise in plasma CS within 5 min, the short time lag of CS action means that a challenge-induced behavioral reaction is expressed under the effects of B. Thus, release and effects are fast enough to affect ongoing behavior. The effects of physiological CS changes both in the resident-intruder paradigm and the open field tests were studied. In both cases, CS showed a behavioral effect at stress-compatible plasma levels (1000-1500 nmol/l). Appearence of an intruder causes a rapid Rise in plasma CS and a gradually emerging aggressive behavior. The rise correlated significantly with the behavior of the intruder (it was lower when the intruder submitted early) but not with the amount of fights. Thus, it appears that CS rise was linked to a goal (the defeat of the intruder) and not to the mere physical stress effects of fights. Exogenous CS induced a very significant increase in fighting behavior. Some behaviors were strongly affected by corticosterone, others were not affected. However, in this paradigm the effect depended largely on the social experience of subjects. While in animals naive to dyadic aggressive encounters a marked increaase in environment-directed exploration and a reduction in resting occurred, fight-experienced animals responded to corticosterone by a marked increase in attack frequency. One can hypothesize that the effects of CS are behaviorally specific, and depend largely on the context and/or on experiential factors. Our data suggest that a challenge-induced CS rise rapidly modulates the behavioral response.

ESTROGEN RECEPTOR-α AND β IN THE CENTRAL NERVOUS SYSTEM: FROM GENE TO FUNCTION

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In 1996, Kuiper et al. (PNAS 93:5925, 1996) reported the cloning of a novel nuclear estrogen receptor (ER), designated ER-β. ER-β is highly homologous with the classical ER (now called ER-α) and exhibits a specific binding affinity for natural estrogens and is capable of activating the transcription of an estrogen response element/reporter gene construct. Recent studies have shown that ligand-bound ERs (both homo- and heterodimers) mediate gene transcription not only from an estrogen response element but an AP-1 enhancer site on DNA. The distribution of the two ERs has been studied at both the mRNA and protein level. In the adult rat brain, both receptors are present in the hypothalamic preoptic area, arcuate nucleus (AN), bed nucleus of stria terminalis, medial and cortical amygdaloid nuclei, and several brainstem nuclei, although the expression of each ER is region-specific. While ER-β is exclusively expressed in the rat hypothalamic paraventricular (PVN) and supraoptic nuclei, the cerebral cortex, CA_1-CA_3 regions of hippocampus and cerebellum, others, e.g., the hypothalamic ventromedial nucleus (VMN), contains primarily ER-α. The presence of a new nuclear ER suggests that estrogen, among other mechanisms, may exert its tissue- and cell-specific action via ER-α and/or ER-β or both, depending on their expression in certain estrogen targets. Our in vivo binding studies with ER-α knockout (ERαKO) mice have shown that the ER-β mRNA is translated into a biologically active protein. These studies found binding in areas of the ERαKO brain that exclusively express ER-β (e.g., PVN), while little or no binding was detected in the VMN which expresses primarily ER-α. Competition with an ER-α-selective ligand prevented binding in the VMN of wild type animals without affecting binding in the PVN. Although immunocytochemical experiments have demonstrated the presence of ER-β protein in these brain regions, the in vivo binding studies provide clear evidence for the functionality of this protein. Recent *in vivo* binding studies in ERβKO mice confirmed previous observations about the distribution of ER-β. The presence of two ERs provides an opportunity to develop ER-α and ER-β-specific ligands for the treatment of several neurological diseases.

CORTICOSTEROID RECEPTORS IN THE BRAIN: NOVEL MODES OF THEIR REGULATION AND ACTION

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The brain mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) are glucocorticoid-dependent transcription factors which play a critical role in the regulation of the hypothalamic- pituitary-adrenocortical (HPA) axis both at baseline and after stress. MRs are mainly localized in extrahypothalamic limbic regions such as the hippocampus, whereas GRs are ubiquitously distributed. However, it is currently unknown whether the concentration and function of these receptors is regulated by acute stressful challenges. Therefore, we investigated the effect of an acute stressor (i.e. 15 min forced swimming) on MR and GR levels in various rat brain regions. Immunohistochemical and receptor binding studies revealed that forced swim stress evokes an upregulation of MR levels within 24 h in the hippocampus, frontal cortex and amygdala, but not in the hypothalamus. GR levels were not affected in any of the brain regions tested. No changes were found in MR and GR binding affinities. Importantly, it was found that the effect of stress on MR levels was largely mediated by an action of corticotropin-releasing hormone within the brain. Use of the MR antagonist RU 28318 in a neuroendocrine challenge test revealed that the stress-induced rise in MR at 24 h was accompanied by an increased tonic inhibitory influence of these receptors on HPA activity. These data strongly suggest that stress via CRH enhances MR synthesis in distinct regions of the brain; a phenomenon which is associated with altered HPA regulation. This is a novel mechanism presenting an important role of MR in the organization of HPA axis control following stress.

LIPID MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR IN HEALTH AND PATHOLOGICAL CONDITIONS.

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Ion channels underlie a great variety of cellular functions; correspondingly they can be affected by a variety of pathologies. The peripheral nicotinic acetylcholine receptor (AChR), the best studied ligand-gated ion channel, is known to be the target of inherited and acquired diseases. We have recently studied normal and mutated muscle AChRs, some of them mimicking human myopathies with abnormal channel properties (Bouzat and Barrantes, 1996; *J. Biol. Chem. 271*, 25835-25841), mainly of kinetic nature, which I have termed "channel dyskinesias" (Barrantes, 1998: *The Nicotinic Acetylcholine Receptor: Current Trends and Strategies*, Springer Verlag, Berlin, 226 pp.). We found that steroids modify the kinetic properties of these pathological channels. Other conditions, not necessarily matched by human diseases, are man-tailored channel alterations found in AChR carrying point mutations at the purported lipid-facing M4 transmembrane segment. Single-channel recordings of these mutants, together with in vitro fluorescence spectroscopy studies (Antollini & Barrantes, 1998), support the existence of specific sites, moderately voltage-dependent, different from those of open channel blockers like QX-222, and probably located at the lipid-protein interface.

MULTIPLE ROLES FOR THE ELECTROSTATIC PROPERTIES OF CHOLINESTERASES I: MOLECULAR RECOGNITION

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In this first study we analyze the role of he electrostatic properties of cholinesterases (ChEs) in molecular recognition and cell-adhesion. Theoretical calculations of the surface potentials of ChEs reveal a negative external surface potential in an "annular" area around the entrance to the active-site gorge that becomes more negative as one approaches the rim of the gorge. These potentials are highly correlated among the structures examined, down to a sequence identity as low as 35%, indicating that they are a conserved property of the cholinesterase family. Homology modeling and a quantitative analysis of the surface electric potential of the ChE-like extracellular domains of gliotactin (GLI), neurotactin (NRT), and neuroligin-1 (NL-1) show that GLI, NRT and NL are all characterized by an electrostatic motif in the "annular" region very similar to that of acetylcholinesterase (AChE). The same analysis performed on lipases with a ChE-like fold does not reveal the presence of such a conserved electrostatic motif. These findings, taken together with the evidence for the involvement of ChEs in cell adhesion independent of their catalytic activity, suggests that the ChE-like domain of GLI, NRT and NL-1 may share a common recognition mechanism or a similar ligand in common with ChEs. Further evidence comes from the recent results of a study in which chimeric constructs of the C-terminal transmembrane domain of NRT fused to the extracellular cholinesterase-like domains of *Dm*AChE or *Tc*AChE, were shown to be endowed with the same heterophilic adhesion properties as wild type NRT. On the basis of these findings, we consider GLI, NRT, NL-1 and AChE to be members of a class of adhesion proteins which we have named "electrotactins". We hypothesize that a chimeric construct of the cytoplasmic domain of NRT with a mutant form of AChE, in which seven negative residues have been neutralized to abolish the electrostatic motif (7-hAChE), will not display the same adhesive properties as a construct built with wild-type AChE. This chimeric construct has been synthesized, along with other ones in which the extracellular domain of NRT has been substituted by other ChEs, such as human AChE (hAChE) and human butyrylcholinesterase (hBChE). We also have synthesized a chimeric construct of the cytoplasmic domain of NRT and the extracellular domain of GLI. We are currently in the process of establishing expression of these chimeric proteins in *Drosophila* S2 cells, in order to evaluate their cell-adhesive properties. Efforts are also underway for the large-scale production and purification of NRT and GLI, in the hope of crystallizing these neural cell-adhesion molecules and subsequently solve their structure by X-ray crystallography.

STRUCTURAL BASIS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR'S ALLOSTERISM

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The heteropentameric nicotinic acetylcholine receptor from *Torpedo* electric tissue and from skeletal muscle is an allosteric protein: It exists in a dynamic equilibrium of functional states, and it binds its agonists and opens its ion channel cooperatively, thereby preserving its axis of (pseudo-)symmetry. Noncompetitive inhibitors (NCIs) exist which promote agonist binding and facillitate interconversion from the resting (R) to the desensitized (D) states, which both are channel closed states. NCIs have been described as allosteric inhibitors. Binding sites for agonists/competitive antagonists, NCIs and the channel entrance have been localised by photoaffinity labeling experiments in close proximty near the surface of the plasma membrane's lipid bilayer. Together they form an active zone a special feature of which is its formation by several receptor subunits. Especially the agonist binding sites are located at the interface between subunits. By definition, allosterism requires a quaternary structure and protein-protein interaction between subunits. We proved the importance of the subunit interfaces for the receptor's allosteric activities by cross-linking experiments. By chosing the appropriate cross-linkers we were able to freeze the receptor in various allosteric states (1). These fixed states enabled us to investigate the ligand binding properties without interfering with the dynamic equilibrium.

(1) Watty et al. (1997) Proc.Natl.Acad.Sci. USA 94, 8202-8207.

STRUCTURAL REARRANGEMENTS UNDERLYING ACTIVATION GATING IN THE *Streptomyces* K^+ CHANNEL

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We have pursued a systematic exploration of the structural properties of KcsA using site-directed spin labeling and EPR spectroscopy, looking to detect and characterize conformational changes associated with channel opening. Initial functional studies have shown that lowering the external pH promotes channel opening (Cuello et al. Biochemistry 37:3229), and that a large conformational change consistent with an opening of the internal vestibule of the channel can be detected at selected positions of the internal transmembrane helix TM2 (Perozo et al., Nature Struct. Biol. 5:457). Here, we extended these results by comparing the EPR signal from spin-labeled KcsA cysteine mutants obtained at neutral or acidic pH. Three separate portions of the channel were studied: TM1 (residues 26-49), TM2 (residues 90-120), and residues flanking the selectivity filter (external residues 81-83, internal residues 72-74). The mutants were labeled with an MTS spin label and X-band CW EPR spectra were obtained from liposome-reconstituted channels at room temperature, using a loop-gap resonator. Secondary structure information was obtained from Fourier-transform periodicity analysis of position-specific environmental properties: probe mobility (Ho^{-1}) and O_2 and NiEdda accessibility parameters (O_2 and NiEdda). For each spin-labeled mutant, data were obtained at pH 7 (closed state) and pH 3.5 (open state). Large conformational changes were detected on TM2 (particularly its C-terminal end) and to a lesser extent in TM1, while the pore regions show little change in its extracellular portion. Overall, these results are consistent with both rotational and translational movements in the transmembrane helices of the channel, and point to the cytoplasmic face of the channel as the site of the main open-closed conformation.

VISUAL OBJECT RECOGNITION: COMPUTATIONAL STUDIES AND BIOLOGICAL IMPLICATIONS

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For biological visual systems, visual object recognition is a spontaneous, natural activity. In contrast, the recognition of common objects is still way beyond the capabilities of current computer vision systems. The major difficulty in performing recognition comes from the fact that the same three-dimensional object can have many different views, depending on such factors as the viewing direction, illumination conditions, and partial occlusion by other objects.

This talk will describe recent computational approaches to these problems and their biological implications.

OBJECT RECOGNITION IN MONKEYS

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The notion of the temporal lobe being involved in object recognition – as it emerged from clinical and lesion studies - has received strong support from previous electrophysiological experiments in monkeys. The results of these experiments showed that neurons in the inferior temporal cortex (IT) respond to a variety of complex two-dimensional patterns, including figures of animate objects, such as faces, hands and body parts. To better understand the role of this area in object recognition, we set out to determine whether the configurational selectivity found for IT neurons is specific for faces or body parts, or whether it can be generated for any novel object as a result of extensive training. Monkeys were trained to become "experts" at identifying exemplars of novel, computer- generated object classes. Critically, these objects had never been experienced by the monkeys, nor did they possess any inherent biological relevance. Nonetheless, after training, the animals learned to discriminate individual objects from a set of highly similar distractors, a task not unlike that of identifying a specific face or a particular bird species. Because all of the objects used in testing were composed of the same basic parts, good performance in this task had most likely to rely upon using holistic configurational information, and upon the detection of subtle shape differences. Physiological recordings from individual neurons in IT revealed a subpopulation of cells that were activated selectively by views of these previously unfamiliar objects. Many neurons fired selectively for a small set of views of spheroidal or wire objects that the monkey had learned to recognize from all viewpoints. The cells were most active when the target was presented from one particular view, and their activity declined as the object was rotated in depth. Remarkably, these cells could not be consistently activated by any other tested object, including numerous visually similar distractors and views of the target more than about 45 deg away from the preferred view. Attempts to simplify the objects lead, in most cases, to a significant reduction of the neurons' responses, a finding suggesting that some neurons in IT show the type of specificity to complex configurations, which was previously described for faces or other animate objects. Testing IT neurons with stimuli that can be perceived more than one way, showed that these cells discharge for their effective stimulus only when this stimulus is perceived. Phenomenal suppression of the preferred pattern caused almost invariably a profound suppression of the cells' activity. IT thus seems to be very closely related not only to shape representation but also to the conscious perception of a visual object.

COMMON CATEGORY-RELATED ACTIVATIONS ELICITED BY PICTURES AND WORDS

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We investigated object category-related activations in extrastriate cortex with functional magnetic resonance imaging (fMRI). Gradient echo echoplanar images from 18, 5-mm coronal slices were obtained while subjects (N=26) performed different tasks with pictures of animals and tools (viewing, naming, and delayed match-to-sample) and with the written names of animals and tools (word reading tasks). Data were analyzed with multiple regression to identify regions that responded differently to each object category.

Tasks that required subjects to process pictures of animals and tools consistently elicited bilateral activations in the posterior temporal cortex. Specifically, the lateral aspects of the fusiform gyrus and the superior temporal sulcus (right > left) showed a greater response to pictures of animals while the medial aspects of the fusiform gyrus and the middle temporal sulcus (left > right) showed a greater response to pictures of tools. Direct comparison of activations associated with reading and naming revealed common patterns of category-related activity in the posterior temporal cortex. Reading the names of animals and naming pictures of animals both activated the right lateral fusiform gyrus; possibly reflecting stored information about category-related differences in object form. In contrast, reading the names of tools and naming pictures of tools both activated the left middle temporal gyrus; possibly reflecting stored information about object motion. Taken together, these data suggest a single semantic system for objects and words.

TACTO-VISUAL MATCHING AND STIMULUS-REWARD ASSOCIATION ON OBJECTS

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Area TE of the monkey inferotemporal cortex represents the final pure visual stage of the occipitotemporal pathway, which is essential for visual object recognition. We had previously found that cells in TE respond to moderately complex visual features, that cells responding to similar features cluster in a columnar region, and that columns representing different but related features are located at neighboring positions with partial overlapping. To investigate the roles of these functional structures in object recognition, we have recently conducted two projects with behaving monkeys. In one project, single-cell activities were recorded from TE of one monkey performing a tacto-visual matching task. The monkey grasped one of 18 differently shaped objects under an opaque plate, and then matched visual objects presented on a display with the grasped object. About 1/5 cells recorded from the dorsolateral part of TE showed stimulus-selective discharges to the grasped objects before the start of the visual image presentation. Tacto-visual matching may occur either 1) in the polymodal areas that receive converging inputs from the visual and tactile pathways, or 2) in the interaction between the modality-specific pathways. The results suggest that the latter is at least partially working. In the second project, a visually cued GO/NOGO task with reversals was trained on monkeys, and single-cell recordings were made from the ventral regions of the prefrontal cortex, to which TE projects. One of 2 visual patterns was presented as a cue, and after a delay the monkey had to perform either GO or NO-GO motor response depending on the cue. Rewards were asymmetrically provided after either correct GO or correct NOGO responses. The monkeys were trained to quickly learn the stimulus-motor correspondences with daily introduced new visual patterns. They also had to switch or maintain the stimulus-motor correspondences when the motor requirement or the reward condition was reversed. For cells that showed differential responses to the visual patterns, we determined which of the three aspects of stimuli, i.e., 1) the visual features, 2) associated motor responses, or 3) associated rewards, was essential for the responses by testing their activities across both motor and reward reversals. We found that most cells in the orbitofrontal regions represent associated rewards, but not the other two aspects. Although we have not localized the site of stimulus-motor association, the results suggest that the stimulus-motor and stimulus-reward associations separately occur at different brain sites.

INTRACELLULAR SIGNALING PATHWAYS THAT INFLUENCE GROWTH CONE COLLAPSE

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The rate and direction of axonal extension is determined by an appropriate balance in expression of adhesive and anti-adhesive components at the cell surface. The guidance receptor Neuropilin-1 was originally identified as an adhesion molecule. However, the binding of a soluble ligand called collapsin-1/semaphorin 3 to neuropilin-1 induces a rapid collapse in the growth cone morphology by an as yet not fully understood mechanism. We have used the experimental paradigm of the growth cone collapse induced by collapsin-1 to identify signal transduction cascades that influence this response. In addition to using conventional pharmacological reagents, we have developed cell permeable peptides that can either inhibit or activate signaling pathways by binding to SH2/PTB domains of known adaptor and effector molecules. We will present results that implicate a number of signaling molecules in the growth cone collapse response.

GLIAL-DERIVED EXTRACELLULAR MATRIX IN THE CONTROL OF AXON GROWTH AND GUIDANCE

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Glycoproteins and proteoglycans of the extracellular matrix (ECM) mediate astroglial functions during neurohistogenesis and regeneration. Tenascin-C glycoproteins (TN-C) are transiently expressed by astrocytes during CNS development and contribute to neuron-binding, the control of neuron migration and neurite outgrowth, and the repulsion of neurons, their processes and growth cones, which seem all encoded by separate domains and hence mediated by distinct complementary receptor systems [1]. In some regions, TN-C co-localizes with the glial-derived chondroitin sulfate proteoglycan (CSPG) DSD-1-PG which has recently been identified as the mouse homologue of rat phosphacan [2]. The CSPG carries the DSD-1-epitope, a carbohydrate modification which is specifically recognized by mAb 473HD. Recent observations support the notion that the particular CS-carbohydrate DSD-1 is enriched in CSD, depends on sulfation and is by itself sufficient to stimulate neurite outgrowth [3]. Functionally, DSD-1-PG/phosphacan exerts opposite effects on neurite outgrowth in dependence of the neuronal cell type. In an in vitro model using astroglial-derived cell lines withe adverse effects on neurite outgrowth, the proteoglycan fraction derived from the cell line Neu7 inhibits neurite outgrowth. Current investigations aim at identifying CSPGs contained in this fraction and to evaluate their potential contribution to the formation of astroglial scars.

INTERACTIONS OF NR-CAM ARE CRITICAL FOR CLUSTERING OF ANKYRIN AND SODIUM CHANNELS AT THE NODE OF RANVIER.

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High concentrations of voltage-gated sodium channels are specifically clustered at nodes of Ranvier, allowing saltatory conduction of action potentials from node to node down the axon. Although the mechanisms involved in concentrating the sodium channel at the node are unclear, its interaction with the cytoskeletal protein ankyrin-G, which is also clustered at the node, appears to be crucial for node formation. Interactions of ankyrin-G with neurofascin and Nr-CAM, two members of the L1 family of cell adhesion molecules (CAMs), suggest that the early expression and localization of these proteins at the node of Ranvier may be a critical step in regulating node formation. We tested the effects of Nr-Fc, a chimera of the ectodomain of Nr-CAM with the constant region of human Ig, in node formation in vitro using myelinating co-cultures of rat Schwann cells and DRG neurons. Nr-Fc inhibited clustering of sodium channels and ankyrin whereas control treatments with human Ig and L1-Fc had no effect on node formation. Nr-Fc perturbation of nodal clustering was specific in that it did not affect myelination as detected by immunoreactivity for myelin basic protein, or Schwann cell proliferation measured by incorporation of BrdU in the co-cultures. Our data suggests that Nr-CAM interactions may be important in the development of the node of Ranvier. Supported by grants from NIH and National Multiple Sclerosis Society.

L1-TYPE NEURAL CELL ADHESION MOLECULES AND THEIR ADHESION-DEPENDENT INTERACTION WITH THE MEMBRANE SKELETON

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Sites of cell adhesion are important points of cytoskeleton interaction with the plasma membrane. Cell adhesion molecules (CAMs), as well as cytoskeletal elements are often segregated into specialized regions of neuronal cells. The neural CAMs Drosophila neuroglian and human L1-CAM, both members of the L1 gene family, directly interact with Drosophila ankyrin and recruit the assembly of the ankyrin-associated membrane skeleton in Drosophila S2 cells. The neuroglian/L1-CAM interaction with ankyrin appears to be activated by its homophilic extracellular adhesive activity, so that ankyrin selectively associates at sites of cell contact and not at other regions of the cell surface. These results indicate that L1 family members can transmit positional information directly to the ankyrin-spectrin matrix and thereby polarize the distribution of the membrane skeleton. This L1-induced polarization of the cytoskeleton may play an important role in the induction of physiological responses to cell adhesion, e.g. by recruiting signaling molecules to sites of cell adhesion. We have identified a second Drosophila ankyrin gene (Dank2) that also interacts with the neuroglian cytoplasmic domain. In contrast to the previously reported Drosophila ankyrin (Dank1), the expression of this new ankyrin is developmentally regulated and specific to neuronal cells. We propose, that in the developing fly nervous system neuroglian-mediated cell adhesion recruits Dank2 protein to axonal tracts.

SIGNALLING BETWEEN NEURAL CELLS VIA GPI-ANCHORED MOLECULES OF THE IMMUNOGLOBULIN SUPERFAMILY.

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F3, a glycosyl-phosphatidylinositol anchored molecule of the immunoglobulin superfamily can undergo multiple interactions via its modular immunoglobulin-like domains. We prepared a Fc chimeric molecule (F3IgFc) to identify a) the phenotype of cells bearing F3Ig receptors, b) the molecules interacting with these domains and, c) to characterize in *in vitro* models the functional impact of the interactions. In cerebellum primary cultures we observed a strong binding of F3IgFc coated fluorospheres to growth cones of granular neurons and to astrocytes. *In vitro* functional characterization indicated that F3IgFc coated as a substrate exerts a growth inhibitory effect on axons. This effect resulted from an heterophilic interaction with neuronal NrCAM, an adhesion molecule of the Ig superfamily which contains an ankyrin-binding site in its intracytoplasmic domain. On glial cells F3IgFc is able to bind tenascin-R, tenascin-C and isoforms of the proteoglycan-type protein tyrosine phosphatases z/ß . We showed that that the interaction of F3 expressed on neurons with the astrocyte-expressed PTPz/RPTPß stimulated neurite outgrowth by cerebellar neurons. In addition, interaction of preclustered F3IgFc with the astrocyte-expressed transmembrane PTPz/RPTPß specifically modified the distribution and intensity of phosphotyrosine labeling in the glial cells. In cerebellar explants, inhibition of the tenascin-R interaction with neuronal F3 prevented defasciculation of neurites which normally display a defasciculated outgrowth on a growth permissive substrate. Thus F3 has the ability to recognize multiple environmental cues expressed by different cell types. The interactions with NrCAM, RTPz/RPTPß and tenascin-R are potentially at the basis of reciprocal exchanges of information between neurons themselves and between glia and neurons.

SIGNALLING CASCADES INVOLVING NEURAL CELL ADHESION MOLECULES DURING NEURONAL GROWTH

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At least three cell adhesion molecules (NCAM, N-cadherin and L1) stimulate axonal growth via a mechanism that involves activation of the fibroblast growth factor receptor (FGFR). Evidence for this includes that observation that dominant negative FGF receptors inhibit the CAM responses, and that soluble versions of the CAMs can activate FGF receptors. The FGFR signal transduction cascade mediating the axonal growth response has also been dissected in considerable detail. A number of studies have shown that it requires PLCg activity to generate DAG, the conversion of DAG to AA by DAG lipase, and an AMMA dependent stimulation of calcium influx into growth cones.

More recently, calcium influx into growth cones has been shown to stimulate GAP-43 phosphorylation, and this provides a potential mechanism for modulating actin polymerisation. A similar response can be stimulated by soluble CAMs, and this involves activation of the same signalling cascade that has been implicated in the axonal growth response. Studies with "knock-out" mice have confirmed that GAP-43 function is essential for CAM stimulated axonal growth responses

MORPHOLOGICAL STUDY OF THE NEOCORTEX, ENTORHINAL CORTEX, HIPPOCAMPAL FORMATION, BASAL GANGLIA, THALAMUS, AND BRAIN STEM IN RETT SYNDROME PATIENTS

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Neocortex, entorhinal cortex (EC), fascia dentata (FD), hippocampus (HP), basal ganglia (BG), thalamus, and brain stem were studied in Rett syndrome (RS) compared with control brains. Nissl and Golgi methods were used. In RS most of the areas of EC, HP and FD showed severe cell hypochromia. Mild abnormalities of neurons in neocortex, thalamus and brain stem were found in RS compared to control cases. All layer II cells in the EC and most of them in layer III were in the state of total chromatolysis or "ghost" cells; the cells of layers V, VI were preserved and moderately hyperchromic. In FD and HP severely hypochromic was the majority of the granular cells and cells of CA3 and CA4 fields; whereas in the CA1 field most cells were normal or slightly hypercaryochromic. In BG mostly mild or moderate aberration from normal cell structure was observed: in striatum - mild hypercaryochromia of small neurons and more expressive hyperchromia of large neurons; in pallidum - mild or moderate hypercaryochromia, till severe hyperchromia in pallidum internum. In pallidum degeneration of thick myelinated fibers was evident. These data allow to surmise the cause of the main symptoms of the RS. The motor disorders, including specific stereotyped movements, could be related to the enhanced activity of BG cells due to their deafferentation from the side of the neocortex and due to supposed hyperactivity of the EC-striatal pathway; the mental retardation and epileptic seizures - due to FD-HP involvement.

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THE OLFACTORY NEURON AS A MODEL FOR RETT SYNDROME

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Rett Syndrome (RS) is a severe neurodevelopmental disorder which is clinically diagnosed by a period of relatively normal development, followed by a neurological and behavioral regression, microcephaly, and seizures. Pathological increased neuronal cell packing density, reduced neuronal size, and decreased dendritic arborization are reported. The olfactory receptor neuron (ORN) offers a unique model in which to test the hypothesis that RS results from a disruption in neuronal development. ORNs reside in the nasal cavity, and therefore are accessible to low-risk biopsies. In this study, olfactory neuroepithelial biopsies were obtained from control (Ctl) and RS patients. ORNs are unique in that they are replaced throughout life from a maintained population of basal precursor cells. Understanding their process of renewal and arrest in RS patients could provide insight into the defects in neuronal development in RS.

Olfactory biopsies were obtained, fixed, sectioned, and analyzed by immunohistochemistry for olfactory marker protein (OMP), which labels mature ORNs, and antibodies to neuron specific tubulin (NST), which labels precursor and immature neurons. Control biopsies showed a consistent and low ratio of precursors to mature ORNs, and normal ORN morphology as described in the literature. In contrast, RS biopsies showed a highly increased number of ORN precursors, while few mature ORNs were seen. In addition, the ORNs in RS biopsies were dismorphic, with dendritic and axonal abnormalities. Therefore, the peripheral olfactory system may display many changes seen in the CNS in neurodevelopmental disorders, and may be a model system for further investigations.

GENETICS IN RETT SYNDROME

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Rett syndrome (RS) is a neurological development disease. The first girl to be diagnosed was in 1966. Its biological and genetic basis remains still obscure. RS is commonly thought of as an X linked dominant disorder lethal in males. Few familial cases exist even though very rare they are extensively investigated in order to identify a genetic locus behind RS. We have performed a whole genome screen, candidate gene sequencing and *in situ* hybridization without obtaining any definite answers.
Results from theses studies will be discussed with a focus on the X chromosom and the distal region, Xq28.

COMBINING DECONVOLUTION AND NOISE ANALYSIS FOR THE ESTIMATION OF TRANSMITTER RELEASE RATES AT THE CALYX OF OF HELD SYNAPSE

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Synaptic transmission at the Calyx of Held can be studied with excellent precision since both the pre- and postsynaptic compartments can be voltage clamped (Forsythe (1994), J. Physiol. 479, 387; Borst et al. (1995), J. Physiol. 489, 825). This situation calls for a quantitative method for estimating transmitter release rates from the postsynaptic current time course. Unfortunately, deconvolution (Cohen et al. (1981), Brain Res. 223, 185; Aumann and Parnas (1991), Bull. Math.Biol. 53, 537) is not straightforward, since it appears that for strong stimuli the postsynaptic current contains a component resulting from glutamate accumulation in the synaptic cleft. Ensemble noise analysis allows to estimate release rates. However, it is accurate only, when many repetitions are given under very stationary conditions. We developped a new kind of deconvolution analysis which uses information from noise analysis to estimate the contribution due to glutamate accumulation. We show that for short pulses of Ca^{++} influx transmitter release rate is a power function of current magnitude, which is enhanced during second and third stimuli in a train of pulses. We expect that this approach allows a quantitative analysis of facilitation and depression.

INTRACELLULAR Ca2+ STORES: HETEROGENEITY AND ROLE IN TRANSMITTER RELEASE

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Studies carried out at synapses and neurosecretory cells by a variety of experimental approaches have documented the heterogeneity of the rapidly exchanging, ER-located intracellular Ca2+ stores, sustained by the non-parallel distribution of their molecular components: channels, SERCA pumps and Ca2+ binding proteins. Although direct evidence of the function of these heterogeneous stores is still not available, it is reasonable to expect those expressing high density of channels to be involved in pacemaker activity and in prompt responses, while those rich in Ca2+ binding proteins could sustain prolonged release. Identification of the role of those various stores in transmitter release is problematical also in view of the well known structural complexity of the process, especially at synapses. Studies have been carried out in pheochromocytoma PC12 cells by the electron energy loss spectroscopy imaging technique (in collaboration with F. Grohovaz), to reveal the distribution of total calcium; and by the parallel investigation in PC12 cells of surface extension (capacitance measurements, in collaboration with H. Kasai) and ultrastructure (of quick frozen-freeze dried cells). These results have revealed the fast kinetics of ER and mitochondrial Ca2+ uptake and the differential exocytic discharge of clear and dense vesicles in relation also to their resting distribution with respect to the plasmalemma. In addition, the existence of a third regulated exocytic pathway has been demonstrated in PC12 cells. The overall information thus obtained goes beyond the models presently available and emphasizes the multiplicity and complexity of the exocytic process in individual cellular systems.

VOLTAGE-GATED CALCIUM CHANNELS, THEIR EXPRESSION AND MODULATION BY G PROTEINS

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Voltage-gated calcium channel (VGCC) $\alpha 1$ subunits, when expressed in cell lines, reach the plasma membrane only to a small extent unless expressed with an accessory β subunit. In neurons these channels show a highly polarised phenotype with $\alpha 1A$ (P/Q type) and $\alpha 1B$ (N type) functional at presynaptic terminals and the $\alpha 1C/D$ (L type) channels present on cell bodies. We have investigated the mechanism of trafficking of VGCCs, using a polarised epithelial cell line as a model, and have shown that there is polarised trafficking of different $\alpha 1/\beta$ combinations, with the $\alpha 1$ subunit providing the main influence for targeting, although in the case of the $\alpha 1A$ subunit, β subunits modify targeting (1). Evidence will be presented concerning the signals involved in the polarised trafficking of $\alpha 1$ subunits to different membranes.

Concerning G protein modulation, we have compared the strongly G protein modulated $\alpha 1B$ with $\alpha 1C$ which is not modulated. We have shown previously that part of the N terminus of $\alpha 1B$ is essential for its G protein modulation (2). The N terminus of $\alpha 1C$, when substituted into $\alpha 1B$ prevents any G protein modulation. We have found a number of residues in the N terminus of $\alpha 1B$ that are absolutely required for G protein modulation. Furthermore, the I-II linker of $\alpha 1B$, which binds β subunits and $G\beta\gamma$ subunits, although important for G protein modulation, is not essential, as some modulation was observed when it was substituted by that of $\alpha 1C$. Further experiments will be described which approach the mechanism of G protein inhibition of $\alpha 1B$ and identify the essential amino acids in the N terminal sequence.

1. Brice, N.L. and Dolphin, A.C. (1999) J. Physiology **515** 685-694
2. Page, K.M, Stephens, G.J., Canti, C., Berrow, N.S. and Dolphin, A.C. (1998). *J. Neurosci.* **18,** 4815-24.

GATING OF Ca^{2+} INFLUX BY Ca^{2+} RELEASE CHANNELS

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Ca^{2+} content in internal stores, a subcompartment of the endoplasmic reticulum (ER), gates store-operated Ca^{2+} influx channel (SOCs) in the plasma membrane. Release of Ca^{2+} from the stores is obligatory for activation of SOCs. Until recently, the mechanism by which Ca^{2+} content of the stores gates SOCs remains elusive. Work to be presented will support a model in which Ca^{2+} release channels in the ER, the IP_3 receptors (IP_3R) and ryanodine receptors (RyR), regulate SOCs by conformational coupling. Store-operated hTrp3 channels, a homologues of the *drosophila* trp channel, were used as model systems to study gating of SOCs by Ca^{2+} release channels. Activation of IP_3R by IP_3 and of the RyR by caffeine or cADPR results in activation of hTrp3 in intact cells as revealed by recording hTrp3 activity in the cell attached mode. hTrp3 in the same excised patches was activated by IP_3 or caffeine/cADPR. Washing the cytoplasmic surface of patches resulted in loss of gating by the second messengers. Gating by IP_3 or caffeine/cADPR was restored by reconstituting the patches with native or recombinant IP_3R or RyR, respectively. Domain analysis of the IP_3R suggests that the N-terminal domain, which includes the IP_3 binding site, functions as a gate of SOCs. These results suggest that gating by conformational coupling is the molecular mechanism of capacitative Ca^{2+} influx and it provides a new mode of communication between the plasma and intracellular membranes.

MODULATION OF K^+ CHANNELS BY G PROTEIN SUBUNITS AND SODIUM.

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G protein-activated K^+ channels (GIRKs) are activated by Na^+ ions and by direct binding of $G_{\beta\gamma}$ subunits derived from pertussis toxin (PTX)-sensitive G proteins. It is not clear what are the factors governing the specificity of GIRK activation by different receptors and G proteins. We observed inhibition of GIRK in excised membrane patches by certain activated G_α subunits, especially $G_{\alpha i1}$ and $G_{\alpha s}$, but not $G_{\alpha i2}$ or $G_{\alpha i3}$, and proposed that $G_{\beta\gamma}$ released from heterotrimers containing $G_{\alpha i1}$ and $G_{\alpha s}$ will be inefficient, whereas $G_{\beta\gamma}$ associated with $G_{\alpha i2}$ and $G_{\alpha i3}$ will activate well. However, activation of $G_{\alpha s}$ in atrial cells or in intact oocytes via β-adrenergic receptors did not inhibit the channel but rather enhanced its activation. This enhancement was blocked by protein kinase A (PKA) inhibitors, suggesting the involvement of a cAMP-mediated phosphorylation. Specific activation of GIRK via chimeric constructs containing the muscarinic m2 receptor tethered to various subtypes of $G_{\alpha i}$ did not reveal substantial differences between activation via $G_{\alpha i1}$, $G_{\alpha i2}$, or $G_{\alpha i3}$. Thus, the data in whole cells do not confirm the involvement of $G_{\alpha i1}$- or $G_{\alpha s}$-induced inhibition in determining the specificity of GIRK activation. Specificity may be governed by colocalization of the relevant components of signal transduction pathways and/or by additional factors.

Na^+ was proposed to activate GIRK by a direct binding to a C-terminal site. We observed that, in excised patches, this activation is eliminated after full activation by $G_{\beta\gamma}$, and reduced after chelation of $G_{\beta\gamma}$. In intact oocytes, the channels also lost its sensitivity to Na^+ after chelation of $G_{\beta\gamma}$ or after full activation by coexpressed $G_{\beta\gamma}$. Thus, the Na^+ gate of GIRK seems to depend on the presence of free $G_{\beta\gamma}$; the latter is the main factor inducing both basal and transmitter-evoked GIRK activity.

SIGNALING PROTEINS INTIMATELY ASSOCIATED WITH NEURONAL POTASSIUM CHANNELS

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Ion channels can be modulated by a variety of molecular mechanisms. We have been exploring the possibility that some ion channels may be intimately associated with protein kinases, phosphatases or other signaling proteins that are involved in channel modulation. A co-immunoprecipitation/Western blot strategy demonstrates that the *Drosophila* Slowpoke calcium-dependent potassium channel (dSlo) can bind simultaneously to two protein kinases, one the Src tyrosine kinase, and the other the catalytic subunit of PKA. A yeast two-hybrid screen, using the extended carboxyl terminal domain of dSlo as bait, was used to search for novel dSlo binding partners. A previously unknown protein was identified in this way and named Slob (Slo-binding). Slob co-immunoprecipitates with dSlo from *Drosophila* heads as well as from transfected cells, and can modulate dSlo channel activity. Another two-hybrid screen, this time using Slob as bait, identified the ubiquitous signaling protein 14-3-3 as a Slob-binding protein. 14-3-3, acting via Slob, can also modulate dSlo channel activity dramatically by shifting its voltage sensitivity. The binding of 14-3-3 to Slob requires phosphorylation of Slob by CaMKII, and the interaction (and hence the modulation of dSlo) is subject to dynamic regulation by phosphorylation in flies as well as in transfected cells. The results are consistent with the idea that dSlo is a participant in a modulatory complex with multiple signaling proteins. This complex may respond to different cellular signals by modulating dSlo channel activity in different ways, and thereby will contribute to the regulation of neuronal activity and synaptic transmission.

MOLECULAR FORMS AND FUNCTIONAL ANCHORING OF ACETYLCHOLINESTERASE

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The classical function of acetylcholinesterase (AChE) is the hydrolysis of acetylcholine at cholinergic synapses. This symposium will examine some of the experimental evidence suggesting that both AChE and butyrylcholinesterase (BChE) are also involved in other functions. Non classical functions of cholinesterases may be of several types: a) hydrolysis of acetylcholine in nonsynaptic contexts; b) other catalytic activities; c) noncatalytic roles, based on protein-protein interactions, e.g. in cell adhesion, recognition and morphogenesis. The possible involvement of cholinesterases in protein-protein interactions is supported by the existence of homologous adhesive proteins such as neurotactin and neuroligin. Like cholinesterases, these heterophilic proteins possess an electrostatic dipole, and have been called electrotactins (Botti et al., 1998). The mammalian AChE gene produces two main types of subunits, by alternative splicing (Massoulié et al., 1993, 1998). These subunits possess the same catalytic domain, and differ only by short C-terminal peptides, which define the post-translational maturation and anchoring of the enzymes. The H peptide contains a signal for addition of a glycophosphatidylinositol (GPI) anchor; in adult mammals, it is mainly expressed in blood cells. The T peptide is sufficient for association with a proline-rich attachment domain (PRAD) of collagen ColQ (Bon et al., 1997): for this reason it is named the tryptophan amphiphilic tetramerization domain, or WAT (Simon et al., 1998). AChE subunits of type T are expressed in muscles and nervous tissue. The collagen-tailed molecules constitute the functional enzyme species at neuromuscular junctions, as recently demonstrated by inactivation of the ColQ gene in mice (Feng et al., 1999). In the brain, the major form of AChE is anchored in cell membranes by a hydrophobic protein, P, forming hydrophobic-tailed molecules. The C-terminal peptides condition the fate and the positioning of cholinesterases within supramolecular structures: they are clearly essential for cholinergic functions, but also for possible noncholinergic roles. The presence of the WAT domain, may in fact be responsible for some of the reported morphogenetic effects of AChE of type T (Karpel et al., 1996), since this domain behaves as an autonomous element in protein-protein interactions. This possibility should be kept in mind while considering nonclassical, in particular noncatalytic, roles of cholinesterases.

ACETYLCHOLINESTERASE PROMOTES AMYLOID FIBRIL FORMATION

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Alzheimer's disease (AD) is a neurodegenerative disorder whose hallmark is the presence of senile plaques. The core of such plaques is composed of amyloid β-peptide (Aβ) fibrils and acetylcholinesterase (AChE). We have previously shown that AChE promotes amyloid formation. Our aim is to determine the molecular domain(s) and the mechanism(s) involved in the interaction of AChE with the Aβ peptide, as well as cellular changes triggered upon neuronal exposure to Aβ complexes. (1) biochemical and molecular modeling studies suggests that the 4 kDa hydrophobic segment exposed on the surface of AChE -which contains W279 belonging to the peripheral anionic site (PAS)- is the AChE-Aβ-interacting domain. (2) using a solid-phase binding assay, we demonstrate that AChE interacts with similar high affinity with both, Aβ monomers or amyloid fibrils (Kd = 3 nM), and that the dye Congo Red inhibits the binding of AChE to Aβ monomers (IC_{50} = 18.9 μM), but not to amyloid fibrils. (3) AChE decreases the Aβ peptide critical concentration ca. 4-fold required for the Aβ aggregation process. Finally, we will present evidence showing that AChE-Aβ complexes are more toxic to neuronal cell cultures than Aβ fibrils grown alone, and that estrogen and lithium partially blocked this neurotoxic effect.

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CHOLINESTERASES IN NEURITOGENESIS AND GLIAL CELL REGULATION

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The close sequential expression of BChE, followed by AChE and neurite growth, as resolved at a high 3D-spatial resolution, showed that basically all long-projecting neurones in the chick embryo express AChE shortly before neurite growth is initiated (Weikert et al., 1990). Using elaborate in vitro assays, we could show pharmacologically that AChE regulates neurite growth, and remarkably, that a non-enzymatic mechanism was involved (Layer et al., 1993). Meanwhile, these early findings were supported and much extended by independent studies from several groups. Recently, we have focussed on genetic manipulation of AChE and BChE expression in different cell systems, thereby either overexpressing AChE, or inhibiting BChE expression by transfection approaches. Since BChE is associated with proliferative cells, and, noticeably, since BChE is a glial cell marker with unknown function, the possible action spectrum of BChE could be biomedically most relevant. Mesulam and Geula (1994) have pointed to the fact that BChE in neuritic plaques of AD patients resembles glia-derived BChE. In order to understand the role of BChE in glial cell systems, we have studied the rat OLN-93 cell line, which are highly proliferative cells and at the same time express markers of differentiated oligodendrocytes, but not of astrocytes (Richter-Landsberg and Heinrich, 1996). After successful transfection of OLN-93 cells with an antisense-BChE cDNA expression vector by calcium phosphate precipitation (Robitzki et al., 1997), we have found that a lower BChE expression diminishes proliferation and alters the expression patterns of major proteins of the cell surface and of the cytoskeleton. Our findings indicate that antisense BChE-transfected OLN-93 cells have shifted towards a more astrocytic phenotype. This hints to a major role of BChE in glia lineage and cell regulation, possibly playing a role in dementias and CNS nerve regeneration.
References: Weikert, T. et al. (1990) J. Neurobiol. 21, 482-498; Layer, P.G. et al. (1993) Cell Tissue Res. 273, 219-226; Mesulam, M.M. and Geula, C. (1994) Ann. Neurol. 36, 722-727; Richter-Landsberg, C. and Heinrich, M. (1996) J. Neurosci. Res. 45, 161-173; Robitzki, A. et al. (1997) Eur. J. Neurosci. 9, 2394-2405. Acknowledgements: This work was supported by HCM, G.I.F. (German-Israeli-Foundation) I 0512-206, and DFG SFB 296/A2 and B3.

MORPHOGENIC ROLE FOR AChE IN NEURAL DEVELOPMENT

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AChE has been shown to have a direct neuritogenic role that is independent of its catalytic ability. To further explore this role, we employ two in vitro model systems in which AChE expression is manipulated. First, AChE expression in rat dorsal root ganglion (DRG) neurons is altered by infection with adenoviral vectors containing the full length rat AChE-T sequence in either sense (Ad-S) or antisense (Ad-AS) orientations. Ad-S treatment results in increased AChE expression that is accompanied by increased neurite outgrowth and branching. Conversely, infection with Ad-AS reduces AChE expression to nearly undetectable levels and is accompanied by a significant reduction in process outgrowth as compared to controls. It has been proposed that the neuritogenic role of AChE may be mediated through an adhesive mechanism. Therefore, in the second system, we tested adhesive properties of neuroblastoma cells that are engineered for altered AChE expression (Koenigsberger, et al., 1997). Cell adhesion experiments reveal a positive correlation between AChE expression and the percentage of adherent cells on plastic, poly-lysine, and collagen types I and IV. Furthermore, anti-AchE antibodies and the AChE specific inhibitor, BW284c51, blocked the enhanced adhesion of the AChE over-expressing cells. These findings suggest that AChE expression can affect adhesive properties of these cells. Interestingly, AChE expression does not correlate with adhesion on laminin. The bidirectional regulation of AChE in these model systems reveals a correspondence between AChE expression and neuritogenesis as well as cell adhesion, supporting the hypothesis that the morphogenic role of AChE may be mediated, at least in part, by an underlying cell adhesive function.

MORPHOGENIC AND TOXICOLOGIC IMPLICATIONS OF DEVELOPMENTALLY REGULATED CHOLINESTERASE EXPRESSION IN THE RAT NERVOUS SYSTEM

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Regulated changes in expression of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are well documented during embryogenesis and the early postnatal period in a number of tissues including brain and spinal cord. The possibility is now being actively explored that upregulated expression of AChE, and reciprocally downregulated expression of BChE, may have morphogenic consequences for the developing nervous system. In particular, accumulating data suggest that AChE acts in some way to promote axonal elongation, possibly by modulating neural cell adhesion. In postnatal rats we have shown that transient upregulation of AChE expression at mRNA and protein levels is characteristic of certain brain areas (especially thalamic sensory relay nuclei). Sustained upregulation, in contrast, occurs in cholinergic brain areas (e.g., nucleus basalis) and in dorsal root ganglia. Several investigators, including Layer, Robertson, and Bigbee, have used similar findings to argue for a morphogenic role of AChE in nervous tissue. Consistent with such a role are our observations with neuroblastoma cells engineered for over-expression and under-expression of this protein: these cells demonstrate, respectively, greater and less than normal ability to extend neurites in tissue culture. More recent work by Soreq's group shows that similar results can be obtained even with mutant forms of AChE lacking enzymatic activity. Developmental changes in cholinesterase expression may also have neurotoxicological implications, especially as regards vulnerability to anticholinesterase agents, which may have dramatically different effects on organisms at different stages of life. For example, newborn rats are typically more sensitive to acute challenge with high dose organophosphorus anticholinesterases, but they show less AChE inhibition than adults when treated chronically at lower dosage. Immunoprecipitation experiments suggest that such effects do not represent variations in intrinsic sensitivity of AChE itself. Instead, the abundance of other enzyme targets, the activity of metabolic disposition pathways, and the rate of AChE synthesis are probably more important determinants of response to anticholinesterase challenge.

ALTERNATIVE VARIANTS OF ACETYLCHOLINESTERASE DISPLAY DISTINCT STRUCTURAL ROLES

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Apart from its catalytic function in hydrolysing acetylcholine, acetylcholinesterase (AChE) affects cell proliferation, differentiation and responses to various stress insults. These functions are at least in part specified by the three C-terminal AChE variants, produced by alternative splicing of the single ACHE gene. **"Synaptic" AChE-S multimers** constitutes the principal enzyme of brain and muscle. AChE-S causes process extension from cultured amphibian and mammalian neurons and glia in a manner that is C-terminus dependent, refractory to engineered inactivation of catalytic ability and, in certain cases, redundant to the neurogenic function of AChE-like proteins such as neuroligin. **Soluble, monomeric "readthrough" AChE-R** appears in embryonic and tumor cells and is induced under psychological, toxic and physical stress. AChE-R and its C-terminal peptide further exert proliferative and differentiation effects on mammalian hematopoietic stem cells. Finally, **glypiated dimers of erythrocytic AChE-E** associate with red blood cell membranes, where their as yet unexplained function is most likely structural and not catalytic. We postulate that the developmental functions of AChE variants reflect their homology to cell adhesion proteins such as gliotactin, glutactin, neuroligin and neurotactin. Competition between an AChE variant and a homolog for interaction with the homolog's binding partner (e.g. a neurexin) would inevitably modify the cell signalling exerted through the cytoplasmic domains of either the AChE homolog or its partner. Thus, the presence of AChE in amyloid plaques in the degenerating human brain and the progressive cognitive and neuromotor deficiencies observed in AChE-transgenic animal models most likely reflect the combined contributions of catalytic and structural roles of AChE.

NEUROTROPHIN SIGNALLING DEATH IN NERVE CELLS

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In mouse and human, neurotrophins form a 4-member family of growth factors that profoundly affect the survival, as well as the functional properties of a multitude of neurons[1]. These secreted, homodimeric proteins are best known for their trophic actions on cells of the nervous system, including the prevention of programmed cell death. This property can be accounted for by their ability to activate a subset of tyrosine kinase receptors. However, it has recently become apparent that the founding member of the family, nerve growth factor (NGF), also participates in regressive events, as a result of the activation of the neurotrophin receptor p75 ($p75^{NTR}$). The killing action of NGF is an integral part of normal development, as observed in the CNS of avian or rodent embryos, using function-blocking antibodies to NGF or to p75, or animals carrying mutations in the corresponding genes. P75 is a glycoprotein with characteristic cysteine repeats in its extracellular domain, a structural hallmark of a large family of receptors including the TNF receptors and CD95 (FAS/APO-1). Like all members of this family, p75 is not directly catalytic and its activity necessitates the recruitment of cytoplasmic proteins. Some of these interactors have been identified and help to explain the diversity of actions mediated by the neurotrophins in the nervous system.

1. **Barde, Y.-A.** (1999) Biological Roles of Neurotrophins. In: Neurotrophic Factors, Handbook of Experimentlal Pharmacology **134**. (F. Hefti, ed.) pp. 1-31, Springer, Berlin-Heidelberg.

SELECTIVE ROLE OF NF-κB ACTIVATION IN MEDIATING THE SURVIVAL RESPONSE OF DEVELOPING SENSORY NEURONS TO NEUROTROPHIC FACTORS

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We have investigated whether the transcription factor NF-κB plays a role in regulating neuronal survival by manipulating NF-κB activation in sensory neurons that are supported by either NGF or CNTF *in vitro*. Overexpression of the p65 NF-κB subunit resulted in NF-κB activation and promoted neuronal survival as effectively as NGF or CNTF. Expression of a super-repressor IκB-α protein prevented NF-κB activation in p65-overexpressing neurons and caused the neurons to die as rapidly as neurotrophic factor-deprived neurons. NGF and CNTF treatment activated NF-κB and preventing activation with super-repressor IκB-α reduced the number of neurons surviving with CNTF and NGF. Antibodies that block binding of NGF to the p75 receptor prevented NGF-induced NF-κB activation and reduced the NGF survival response to the same extent as super-repressor IκB-α. Neurons cultured from $p65^{-/-}$ embryos showed a reduced survival response to CNTF and NGF, and there was increased apoptosis in the sensory ganglia of $p65^{-/-}$ embryos *in vivo*. However, as with p75-deficient sensory neurons, p65-deficient sensory neurons showed a normal survival response to BDNF. These results reveal a role for NF-κB in regulating neuronal survival in response to certain neurotrophic factors.

NEUROTROPHIN-INDUCED SYNAPTIC POTENTIATION: DEPENDENCE ON SYNAPTIC ACTIVITY AND CAMP

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Recent evidence indicates that neurotrophins may also be involved in activity-induced modification of synaptic efficacy and connectivity. The expression of neurotrophins is regulated by neuronal activity, and the secretion of neurotrophins can be triggered by membrane depolarization or synaptic activity. In addition to its effect on the expression and secretion of neurotrophins, electrical activity also facilitates the action of neurotrophins in potentiating synaptic transmission. A brief depolarization of the presynaptic neuron followed by exposure to a low concentration of brain-derived-neurotrophic factor (BDNF) results in a marked potentiation of spontaneous and evoked transmitter secretion at developing neuromuscular synapses, while the same treatment of either depolarization or the BDNF alone had no effect. The synaptic potentiation requires the activation of specific TrkB receptors and results from an increased probability of transmitter secretion from the presynaptic nerve terminal. In a separate series of studies, we found that synaptic potentiation induced by BDNF, but not neurotrophin-3 (NT-3), was prevented by blockers of cAMP signaling. Activators of cAMP signaling by themselves were ineffective in modifying synaptic efficacy, but greatly enhanced the potentiation effect of BDNF. Blocking cAMP signaling also abolished the facilitation of BDNF-induced potentiation by presynaptic activity. Thus the synaptic action of BDNF is gated by cAMP and coincident signals that modulate neuronal cAMP levels. During activity-dependent refinement of nerve connections, synapses made by active nerve terminals may be selectively strengthened by secreted neurotrophins due to an elevated level of cAMP triggered by the presynaptic activity.

MOLECULAR SIGNALS CONTROLLING THE ONSET AND PROGRESSION OF NEURAL CREST MIGRATION

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Although the neural crest is a discrete structure and exists transiently in the early embryo, it embodies most of the crucial issues of developmental biology. Its highly pluripotent component cells yield an astonishing variety of cell types, from neurons and glia of the peripheral nervous system, bones, tendons, connective and adipose tissues, to melanocytes and endocrine cells. The neural crest forms according to a rostrocaudal gradient along the body axis and releases free moving mesenchymal-like cells that follow definite migration routes at precise times of development, finally reaching target embryonic sites where they settle and differentiate. What triggers this migratory behavior? Which signals do these cells require to move along these definite pathways and what causes them to stop and accumulate? These are among the most puzzling problems raised by morphogenesis, questions that go beyond developmental biology and concern the stability of the histiotypic state and its disruption in metastasis. In the first talk I will deal with the mechanisms that trigger the onset of neural crest cell migration. We have evidence that graded concentrations of BMP4, created in the dorsal neural tube by an interplay between BMP4 and its inhibitor noggin, selectively trigger the epithelial-mesenchymal conversion of specified neural crest cells. Following mesenchymalization, these progenitors migrate through stereotyped pathways determined by their microenvironment. The timing and cellular pathways of migration have been fully elucidated, yet the controlling mechanisms remain obscure. Hence, I will address the identity and mode of action of molecules that regulate crest migration. We have found that somite-derived F-spondin, a novel ECM protein, serves as an inhibitory signal important for patterning directional movement of the neural progenitors.

Dimensionality reduction in the basal ganglia of normal and MPTP primates

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The basal ganglia are part of a loop connecting the entire cortex to the frontal cortex. Despite a large body of clinical and experimental data, the processing they perform remains obscure. Our previous physiological studies revealed uncorrelated spiking activity of basal ganglia neurons in contrast with the more correlated activity of neurons in the frontal cortex. Analysis of rate covariance (±10 seconds) of pairs of neurons in the input stage (cortex) and output stage (pallidum) of the basal ganglia revealed a drastic decrease in correlations. Finally, cross-correlation of the pauses in firing of neurons in the external segments of the globus pallidus also revealed no correlation between the pauses.

Following MPTP treatment, the spiking activity of many pallidal neurons became oscillatory and highly correlated. Oscillatory auto-correlation functions tended to be dominated by lower frequencies (~6Hz), and cross-correlograms by higher frequencies (~13Hz).

These results contradict available anatomical data showing the existence of extensive convergence and lateral inhibitory connections. We therefore propose that the basal ganglia reduce the dimensionality of cortical information using optimal information extraction methods.

Simulations implementing key aspects of the cortico-striato-pallidal circuitry predict that during learning the efficacy of the lateral synapses diminishes and neural activity becomes uncorrelated. The efficient extraction of cortical information, which may be used by the frontal cortex for planning upcoming action, is lost in Parkinson's disease-like states.

PATHOPHYSIOLOGY OF BASAL GANGLIA DISORDERS

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Movement disorders of basal ganglia origin constitute a spectrum of hypo- and hyperkinetic disorders. Circuit models of these have been elaborated based on newer anatomic, physiologic and imaging data. Imbalances between intrinsic striatal "direct" and "indirect" output pathways are believed to account for the different signs and symptoms. In parkinsonism, striatal dopamine depletion results in increased and disordered discharge and synchronization in motor areas of the subthalamic nucleus (STN) internal pallidum (GPi). In addition, abnormal activity in one or more of several basal ganglia feedback loops may contribute to the development of parkinsonism. Individual parkinsonian motor signs may be caused by distinct abnormalities in basal ganglia discharge or by involvement of specific subcircuits related to distinct cortical targets. By contrast, hyperkinetic disorders show a common feature of decreased pallidal output. Differences in the balance between direct and indirect pathways and in the degree of synchronization of discharge in the basal ganglia output nuclei must be invoked to explain the striking clinical differences between such different hyperkinetic disorders as chorea and dystonia. A critical analysis of the effects of pallidal and thalamic lesions in hypo- and hyperkinetic disorders strongly suggests that the main features accounting for the different signs of movement disorders are the appearance of altered discharge patterns, changes in the degree of synchronization of discharge, altered processing of proprioceptive feedback, and the appearance of "noise" in basal ganglia output signals.

The current models of basal ganglia pathophysiology should be taken as a first draft of basal ganglia dysfunction in the different disease states. Most pertinently, changes in phasic discharge patterns, and new anatomical connections need to be better incorporated into any new concept of basal ganglia function and a greater emphasis placed on the manner in which thalamic, brainstem and cortical neurons utilize basal ganglia output.

REWARD-PREDICTIVE NATURE OF STRIATE NEURON ACTIVITY DEPENDS ON THE THALAMIC AND DOPAMINERGIC INPUTS

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Primate striate neurons acquire responses to reward-predictive stimuli through behavioral conditioning, and the acquired responses persisted for long time without additional conditioning. Our recent studies have suggested that the reward-predictive properties of activity of a class of striate neurons, tonically active neurons (TANs), depend on inputs coming from (1) CM and Pf nucleus in the thalamus, and (2) dopamine-containing neurons in the midbrain. Inputs from CM and Pf thalamus responded not only to various sensory events but also movements appeared in the behavioral tasks. Inactivation of activity of CM and Pf neurons by local infusion of muscimol almost completely abolished responses of striatal TANs to reward-predictive stimuli. Discharge level of CM and Pf neurons during task performance increased about twice as much as that during quiet wakefulness. The thalamic input to the striatum, thus, seems to convey vigilance- or arousal-related information, but is not sensitive to the reward predictability. On the other hand, Schultz and his group have shown that the dopamine-containing neurons respond to external events and that its activity is highly dependent on the predictability of reward. We have recently found that the dopamine-containing neurons exhibit significant modulation of their activity during early phase of learning sequential motor tasks. Responses of two-thirds TANs recorded in the striatum to reward-predictive stimuli almost disappeared when a specific antagonist of D2-class dopamine receptors, (-)-sulpiride, was iontophoretically applied. One-third of TANs were sensitive to D1-class antagonist.

These findings suggest a new view about the functional scheme of the basal ganglia in which thalamostriate, nigrostriate and corticostriate systems play specific roles in acquisition and retention of behavioral acts.

NEURONAL ORGANIZATION OF THE PRIMATE GLOBUS PALLIDUS

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The aim of the study was to analyze the intrinsic organization of the globus pallidus at the level of individual neurons. Macaques (*Macaca fascicularis*) and green monkeys (*Cercopithecus aethiops*) received restricted stereotactic injections of biotine dextran amine (BDA) into various parts of the basal ganglia, resulting in the labeling of individual dendritic and axonal arborizations. Labeled neurons were reconstructed from serial sections through the camera lucida (Leitz Orthoplan microscope, x40 and x100 oil objectives) and digitized in the three dimensions using a specific computer program. The dopaminergic innervation of the globus pallidus was investigated using tyrosine hydroxylase (TH) immunoreactivity and a quantitative image analysis system (VisioScan, Biocom, France). Pallidal neurons consist of large fusiform or polygonal cell bodies which give rise to fewly branched dendrites forming large disk-shaped dendritic arborizations. The latter are oriented parallel to the lateral pallidal boundaries and to the longitudinal bands formed by the thin intrapallidal collaterals of striatal axons. Each striato-pallidal axon bears a small number of varicosities (one per 10 µm length) suggesting that the remaining boutons that pallidal dendrites receive (one per μm^2) come from remote striatal regions. 80% of the axons of pallidal neurons give off an intranuclear collateral arborization which is smaller than the dendritic arborization of the parent neuron and does not overlap it. Axons coming from mesencephalic dopaminergic areas give rise to terminal arborizations of variable size, shape and density, suggesting that the dopaminergic innervation of the globus pallidus does not follow a single topographic pattern. A significant decrease of TH-positive fibers was observed in the globus pallidus of MPTP-treated green monkeys. In conclusion, the anatomical organization of the afferent inputs and of the intrinsic circuitry of the globus pallidus does not favor the notion that the basal ganglia consist of completely segregated channels. Conversely, convergence, divergence and local integration of afferent inputs are likely to be an anatomo-functional characteristic of information processing in the globus pallidus.

SEROTONIN 5-HT_{2C} RECEPTORS AND REGULATION OF FEEDING BEHAVIOUR

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The serotonergic (5-HT) system has been extensively implicated in the control of feeding behaviour. However, due to the absence of selective ligands, the role of 5-HT receptor subtypes in the control of feeding has remained unclear. Recently, mutant mice lacking functional 5-HT_{2C} receptors have been generated which exhibit an obese phenotypes. In addition, compounds that are claimed to act selectively as agonists (Ro 60-0175) and antagonists (SB 242084) at 5-HT_{2C} receptors have recently been described. We have investigated the role of 5-HT_{2C} receptors in the control of feeding using these selective 5-HT_{2C} receptor ligands and by examining the effect of the appetite suppressant *d*-fenfluramine on food intake and postprandial feeding behaviour in mice lacking functional 5-HT_{2C} receptors. *d*-fenfluramine significantly decreased food consumption in wild-type mice but produced only a marginal decrease in feeding in 5-HT_{2C} knockout mice. After vehicle or *d*-fenfluramine, all animals exhibited a behavioural sequence consistent with the enhancement of satiety. However, mutant mice were less sensitive to the satiating effects of *d*-fenfluramine. These results suggest that *d*-fenfluramine enhances satiety in the mouse by a 5-HT_{2C} receptor-dependent mechanism. These findings are consistent with studies in rats in which we observed that the hypophagia induced by *d*-fenfluramine was dose dependently attenuated by the selective 5-HT_{2C} receptor antagonist SB 242084.
The selective 5-HT_{2C} receptor agonist Ro 60-0175 dose-dependently inhibited food consumption in free feeding rats and this effect was largely attributable to a decrease in meal size. In a subsequent study, the effect of Ro 60-0175 on the behavioural satiety sequence was assessed. Ro 60-0175-treated rats consumed a reduced amount of palatable wet mash and, in addition, exhibited a behavioural sequence consistent with enhancement of satiety. Thus, Ro 60-0175 advanced the offset of feeding and onset of resting whilst preserving the qualitative pattern of behaviour characteristic of the behavioural satiety sequence. The decrease in food intake induced by Ro 60-0175 was prevented by the selective 5-HT_{2C} receptor antagonist SB 242084.
Interestingly, the effects of Ro 60-0175 on meal size and the behavioural satiety sequence were similar to those observed after administration of *d*-fenfluramine.
These data suggest that selective 5-HT_{2C} receptor activation leads to a reduction in meal size that is consistent with enhancement of satiety.

NEUROPEPTIDE Y ANTAGONISTS: POTENTIAL FOR THE TREATMENT OF OBESITY

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Neuropeptide Y (NPY) is a 36 amino acid amidated peptide found in high concentrations in the brain. When administered into the rat hypothalamus, NPY produces a sustained increase in food consumption. Subchronic administration results in weight gain, elevated blood glucose, elevated corticosterone and insulin resistance. NPY interacts with at least six different receptors. The Y1 and Y5 receptor subtypes have a pharmacology most consistent with the feeding response. To understand the role of the Y1 receptor in NPY-induced feeding we have developed specific nonpeptide antagonists. These benzimidazole compounds exhibited subnanomolar affinity for the Y1 receptor with no detectable inhibition of [^{125}I]-PYY binding to the Y2, Y4 and Y5 receptors at concentrations less than 1 µM. In vivo potency was evaluated using ex vivo binding of [125I]-Leu31-Pro34-PYY to mouse brain after s.c. administration of the compounds. Several examples within the series exhibited dose dependent inhibition of binding in these studies which persisted for at least 8 hours after administration. In feeding studies, a dose-dependent reduction in food intake was observed with chronic administration to ob/ob mice. After seven days of administration, these mice exhibited significant reductions in body weight. These results are consistent with a role for the Y1 receptor in experimental obesity.

STRESS-RELATED NEUROPEPTIDES AND INGESTIVE BEHAVIOR.

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Participation of the hypothalamo-pituitary-adrenocortical axis, and its primary brain trigger, corticotropin-releasing factor (CRF) in the control of ingestive behavior can be inferred from data suggesting that CRF and its homologue urocortin act in brain to limit appetite following administration in rodents. Moreover, levels of endogenous CRF, CRF1 and CRF2 receptors and CRF-binding protein, which sequesters CRF and urocortin, are altered by changes in nutritional status brought about by food restriction/repletion. Mediation of the anorexic effects of CRF and urocortin appear not to privilege CRF1 receptors unlike the anxiogenic-effects of CRF which are primarily a consequence of CRF1 receptor activation. Such fear-like consequences of CRF system activation constitute a non-specific mechanism whereby the emergence of behaviors incompatible with food intake may appear to suppress appetite without affecting hunger, per se. However, enhanced appetite following administration of CRF receptor antagonists and the involvement of CRF systems in sexual appetite and drug seeking behavior all suggest a role for CRF in ingestive behavior. In particular, available evidence suggests that physiologically relevant suppression of appetite may accompany CRF system activation occurring as a consequence of stressor exposure induced by nutrient imbalance, for example, or under conditions of excessive intake or consumption of unfamiliar foodstuffs.

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ENDOGENOUS CANNABINOIDS AND APPETITE: PRELIMINARY INVESTIGATIONS.

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The discovery of cannabinoid receptors and their putative ligands, anandamide and 2-arachidonyl glycerol, has spurred research into the behavioural role of central endogenous cannabinoids (CB). The reported ability of the exogenous cannabinoid, D9-THC, to increase food intake in people, coupled with the localization of CB receptors within appetite-related brain regions, suggest that central CB systems may play a role in the normal control of eating. We have begun to characterize the feeding effects of exogenous and endogenous cannabinoids, using animal models. The following studies were conducted with adult, male Lister Hooded rats, during the nocturnal phase of the daily cycle.

In satiated rats, THC (0.5-2.0 mg/kg, po) produced substantial hyperphagia, with a maximal 4-fold increase in intake. Smaller, but more persistent effects were obtained with anandamide (0.5-10.0 mg/kg, sc). Anandamide and THC hyperphagia were dose-dependently attenuated by the CB1 antagonist, SR141716 (0.05-1.0 mg/kg, sc), but not the CB2 antagonist SR144528, indicating mediation by central CB1 receptors. Additionally, hyperphagia was induced by injection of anandamide and its more stable analogue, methanandamide, (2.5-5 µg) into the nucleus accumbens shell.

To examine possible interactions of CB with food reward processes, we tested the ability of SR141716 (1.0-10 mg/kg, sc) to attenuate sucrose sham feeding in gastric-fistulated rats. High baseline intakes of 0.4 M sucrose (~80 ml/hr) were unaffected by the antagonist, implying that CB activity is not essential to the orosensory reward maintaining ingestion. However, in intact rats, THC hyperphagia was attenuated by sub-anorectic doses of the general opioid receptor antagonist, naloxone (0.05-1.0 mg/kg, sc). These data suggest some indirect influence of CB over opioidergic incentive processes. In line with this notion, detailed observational analyses reveal that, under open-field conditions where control intakes are negligible, hyperphagic doses of THC and anandamide greatly enhance the salience of food; promoting an early approach to food, dramatically reducing the latency to eat and inducing substantial increases in the number of feeding bouts.

Overall, our data provide support for a role of CB systems in the physiological regulation of eating, and indicate the possibility of new cannabinoid-based therapies for the treatment of disorders of appetite and body weight.

NMDA RECEPTOR NR2B SUBTYPE SELECTIVE ANTAGONISTS AS NEUROPROTECTIVE AGENTS IN BRAIN ISCHAEMIA

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One approach to the problem of preventing pathological NMDA receptor over activation whilst permitting sufficient normal glutamatergic function to avoid unacceptable side effects has been the development of NMDA receptor subunit-selective compounds. In the forebrain the predominant NR2 subunits are NR2A and NR2B. We have shown that the prototypic NMDA NR2B-selective receptor antagonist, ifenprodil, exhibits a state-dependent block of NMDA receptors such that it binds with a higher affinity to activated and desensitised states of the receptor relative to the agonist unbound, resting state. Studies with the more potent NR2B selective antagonists Ro 25-6981 and Ro 63-1908 suggested that these compounds also produce a state-dependent block. It is predicted that such compounds will block preferentially NMDA receptors that are continuously activated by sustained high glutamate levels in ischaemic brain areas whilst leaving those physiologically activated in normal brain areas relatively unaffected. In agreement, NMDA receptor-dependent hippocampal LTP and learning and memory in the Morris water maze are unaffected by neuroprotective doses of Ro 25-6981. This profile, together with their subtype selectivity, makes this class of compounds an attractive therapy for the treatment of stroke and acute brain trauma. Ro 63-1908 inhibited ^{3}H-MK-801 binding to rat brain membranes in a biphasic manner with IC_{50}'s for the high and low affinity components of 2 nM and 97 µM, respectively. In Xenopus oocytes, it's selectivity for NR2B vs. NR2A – containing receptors was >20,000 fold. In DBA/2 mice, Ro 63-1908 was active against sound-induced seizures with an ED_{50} = 4.50 mg/kg, i.p., and there was no motor impairment at doses required to produce a full anticonvulsant effect. The neuroprotective effect of Ro 63-1908 was determined using the rat permanent middle cerebral artery (MCA) occlusion model. Ro 63-1908 or vehicle was administered within 5-7 min after MCA occlusion as a bolus dose, over 2 min, followed by an infusion over 5 h. Ro 63-1908 produced a dose-related decrease in the volume of cortical damage with maximum protection of 42% without marked CNS side-effects and with no effect on cardiovascular parameters (MAP and heart rate). This tolerability profile is in marked contrast to non-selective NMDA blockers such as MK-801 and CGP 19755, which caused marked ataxia, muscle hypotonia, increased blood pressure and increased heart rate at maximally protective doses in this model of focal ischaemia.

NMDA ANTAGONISTS IN BRAIN ISCHEMIA: REVISITING THE STRATEGY

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Despite strong experimental evidence suggesting that NMDA antagonists can reduce brain neuronal vulnerability to hypoxic-ischemic insults, efforts to administer these drugs to patients presenting acutely with ischemic stroke have so far been disappointing. One possible explanation for this lack of observed efficacy lies in the category of therapeutic index: since motor disturbances and psychotomimetic side effects are likely mechanism-based, they may become problematic in man at lower levels of pan-receptor blockade than required for the induction of neuroprotection. If this is the case, it may be possible in the future to achieve higher levels of neuroprotective NMDA receptor blockade by harnessing improvements in drug selectivity at the level of receptor subtypes or spatial localization.An alternative possibility, not mutually exclusive, is that mechanisms other than NMDA receptor overactivation and consequent neuronal cellular calcium overload, play a more important role in human stroke than in animal models of focal ischemic brain damage. In particular, three mechanisms of ischemic brain damage, if weighted differently in humans vs. animals, could limit the effectiveness of NMDA receptor blockade by itself as a therapeutic strategy: 1) AMPA receptor-mediated damage to neurons and oligodendrocytes; 2) zinc neurotoxicity, mediated by the excessive translocation of synaptic zinc into vulnerable postsynaptic neurons via routes such as voltage-gated calcium channels; 3) a prominent contribution of ischemic programmed cell death, that might even be enhanced by NMDA antagonist drugs due to intracellular calcium starvation. However, if countermeasures are implemented to prevent calcium starvation from occurring, then NMDA receptor blockade may have specific value in helping to attenuate neuronal potassium efflux, another event that may promote apoptosis in the ischemic brain.

ENDOGENOUS ANTIOXIDANTS AS NEUROPROTECTIVE AGENTS IN TRAUMATIC BRAIN INJURY

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The brain is highly vulnerable to the harmful effects of reactive oxygen (ROS) species, and endogenous antioxidants (enzymes and low molecular weight antioxidants, LMWA) neutralize ROS after brain injury. In a model of closed head injury (CHI), we studied the post-injury changes in total LMWA. We assumed that a decrease in LMWA is due to their consumption by overproduced ROS. We tested the hypotheses that clinical outcome after CHI depends upon the brain's ability to neutralize ROS, and animals under chronic oxidative stress display greater damage after CHI. Control rats or mice were subjected to CHI, using a weight-drop device. Their functional recovery was assessed using a series of reflexes and motor tasks, referred to as Neurological Severity Score (NSS). Heat acclimated rats (30d in climatic chamber of 34^{0}C), diabetic rats (30d after injection of Streptozotocin), Apolipoprotein-E deficient (ApoE knockout) mice, and rats fed on iron-deficient diet were subjected to CHI (along with respective controls). Edema (at 24 h), NSS (24h-30d) or axonal degeneration were assessed. In addition, brain LMWA levels were determined (using cyclic voltammetry) as a measure of the tissue total reducing capacity. In controls, a rapid decline in LMWA levels followed by return to normal at 1-4 h. and another decrease at 24 h, suggesting two waves of overproduction of ROS. In the heat acclimated rats increases in LMWA were noted at 5 min and 24 h, along with a facilitated clinical recovery. In the ApoE ko a more dramatic decrease in LMWA and slower clinical recovery were displayed. The diabetic rats, which were under chronic oxidative stress showed slower absorption of edema and retarded recovery of motor function, along with lower levels of brain LMWA as compared to normoglycemic controls. The iron-deprived rats showed less axonal degeneration. We conclude that higher damage and slower recovery were achieved in those animals that 1.were under chronic oxidative stress, and 2. responded to CHI by lower elevation of the endogenous LMWA.

Post-traumatic CNS maintenance and repair by autoimmune T cells.

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The loss of function after axonal injury in the mammalian central nervous system (CNS) results from primary degradation, poor regeneration, lack of neurogenesis, and spread of damage to neurons that escaped initial injury. The immune system is known to contribute to tissue healing, maintenance and repair in cases of pathogen-associated damage. We show that an adaptive immune response, in the form of autoimmune T cells against a myelin-associated CNS antigen, significantly promotes CNS post-traumatic maintenance by reducing secondary degeneration and rescuing spared neurons. In our rat models of partial CNS axotomy (optic nerve partial crush injury and spinal cord contusion), injury was accompanied by a transient, non-selective accumulation of T cells. In addition, there was a reduction of injury-induced degradation that was associated only with the T cells recognizing myelin basic protein (MBP). Morphometric and electrophysiological measurements after systemic administration of the latter T cells, regardless of their virulence, revealed 2- to 3-fold more functioning optic nerve neurons than in controls. Similar treatment of the spinal cord following contusions of differing severity resulted in an increase of up to 30% in spared neural tissue, as shown by behavioral, morphological and imaging (diffusion MRI) criteria. Autoimmunity that does not cause autoimmune disease might therefore be a benign physiological immune response needed for post-traumatic maintenance of the injured CNS. It seems, however, that the CNS does not normally gain optimal benefit from this physiological response, probably because of its immune-privileged status. Potential therapies should be aimed at boosting the autoimmune response while avoiding an autoimmune disease.

IMAGING, ABLATIONS AND BEHAVIOR: OPTICAL STUDIES OF NEURONAL CIRCUITS IN ZEBRAFISH.

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One of the key problems in neurobiology is to monitor activity in single neurons non-invasively during behavior, so that the pattern of active cells can be correlated with the behavior. We have taken advantage of the transparency of larval zebrafish and used calcium imaging and confocal microscopy to study which neurons are active during escape behaviors. We have also developed approaches for using lasers to kill individual neurons in intact fish so that we could study the behavioral consequences of these ablations. Our work has focused on descending reticulospinal neurons that interact with spinal circuits to produce the rapid escape movements fish use to avoid predators. The reticulospinal neurons we studied include the Mauthner cell, MiD2cm and MiD3cm, which form a serially repeated set of neurons in hindbrain segments 4, 5 and 6. Our functional imaging and ablation experiments support the hypothesis that high performance escape movements are produced by this segmentally repeated set of hindbrain neurons. Many hindbrain neurons are segmentally arranged, so it is likely that there are other segmentally repeated functional groups. The approaches we have used to study hindbrain cells can be applied to studies of the behavioral roles of neurons throughout the brain and spinal cord of both normal and mutant lines of zebrafish.

PURINERGIC CONTROL OF MOTOR PATTERN GENERATION IN *XENOPUS* EMBRYOS

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Many rhythmic motor patterns undergo temporal run-down. Following a triggering sensory stimulus, their frequency and strength gradually decline until motor activity spontaneously terminates. This sequence, which occurs in the absence of sensory feedback, is intrinsic to the motor circuitry. In the frog embryo, run-down is mediated by the opposing actions of the purinergic transmitters, ATP and adenosine, on voltage-gated ion channels. A key element of the proposed control mechanism is that production of adenosine should be delayed with respect to the release of ATP so that the ratio of the two transmitters varies with time. By utilising a novel adenosine sensor, I have shown that adenosine levels rise slowly during swimming and reach a peak that can be delayed by up to a minute with respect to the cessation of motor activity. A realistic computational model suggests that the delayed appearance of adenosine can be explained if: 1) ATP is converted to adenosine in the extracellular space; and 2) there is feed-forward inhibition by ADP of the ecto-5'-nucleotidase that converts AMP to adenosine. By studying directly the breakdown of ATP by the isolated spinal cord, I have shown that adenosine can indeed be produced from ATP via a sequential pathway through the action of ecto-enzymes. Furthermore formation of adenosine from AMP is inhibited by ADP. Computer simulations suggest that feed-forward inhibition of the ecto-5'-nucleotidase can: determine the rate of run-down; explain why run-down may be paradoxically slower when motor circuits are more strongly activated; provide flexibility by allowing resetting of run-down. The kinetic properties of an extracellular enzyme cascade therefore appear to hold the key to the intrinsic temporal modulation of motor activity.

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CELLULAR AND NETWORK PROPERTIES UNDERLYING LOCOMOTOR ACTIVITY IN THE NEONATAL RAT SPINAL CORD

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Neuronal networks in the spinal cord are capable of producing rhythmic movements, like walking and swimming, when the spinal cord itself is isolated from the brain and sensory inputs. These spinal networks, also called Central Pattern Generators or CPGs, serve as relatively simple model systems for our understanding of brain functions. In this talk I will concentrate on spinal CPGs controlling hindlimb movements in the newborn rat. I will discuss the use of lesions to isolate the rhythm and pattern-generating parts of the CPG network (Kjaerulff and Kiehn 1996; Kiehn and Kjaerulff 1998) and how spike activity in interneurons in rostral and caudal segments of the spinal segments reflect an observed excitability gradient in rhythmogenic potential (Tresch and Kiehn 1999). These findings will be incorporated into a general scheme of spike coding in the neonatal rat (Raastad and Kiehn 1999). I will also present studies that investigate the interactions between circuit and cellular properties (Kiehn et al. 1996; Raastad et al. 1998), in particular the use of conductance clamp to evaluate the contribution of a hyperpolarization-activated inward current, I_h, to the rhythmic output of motor neurons (Kiehn et al.). The research is supported by the Lundbeck and Novo foundations and the Danish MRC.

PRELIMINARY IDENTIFICATION OF INTERNEURONES MEDIATING GROUP I DISYNAPTIC EXCITATION OF EXTENSORS DURING FICTIVE LOCOMOTION IN THE CAT.

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During locomotion the reflex actions of group I afferents on extensor motoneurones are profoundly altered. The 'non-reciprocal' inhibition of extensors evoked at rest from extensor group Ia muscle spindle and Ib tendon organ afferents is suppressed and replaced by a disynaptic excitation. The emergence of this disynaptic excitation is due to the recruitment of a group of previously unknown population of spinal interneurones. Along with Drs. E. Jankowska and M. Angel, we have begun to investigate these interneurones during fictive locomotion evoked by midbrain (MLR) stimulation in decerebrate cats. Based on the characteristics of disynaptic reflexes recorded in motoneurones, we searched for interneurones with axonal projections to lumbar extensor motor nuclei that were not excited by group I afferents at rest and that were readily recruited at monosynaptic latencies during the extension phase of locomotion. Cells fulfilling these criteria were located in the intermediate laminae of the spinal cord and rostral-caudally near their target motoneurones. A striking finding was that in the absence of peripheral nerve stimulation, most cells were rhythmically active during the extension phase of locomotion. If these cells are indeed excitatory to extensor motoneurones, they must also be a part of the network distributing excitatory output from the CPG. It is hoped that investigations of interneurones responsible for group I reflex excitation during locomotion will also reveal part of the organization of the mammalian CPG. To this end we (Quevedo, Fedirchuk, Gosgnach) have recently described a similarly organized set of locomotor dependent, group I reflexes to flexor motoneurones. Supported by the MRC of Canada.

MECHANISMS OF SPONTANEOUS ACTIVITY IN DEVELOPING SPINAL NETWORKS.

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Mammalian and avian embryos move spontaneously throughout their development. We have studied the neural mechanisms responsible for these movements in an isolated preparation of the chick lumbosacral spinal cord, between embryonic days (E) 7 to 12, using electrical recording and calcium imaging of spinal neuron activity. The activity comprises recurrent episodes, in which motoneurons and interneurons are activated rhythmically for up to a minute, separated by periods of quiescence. We have proposed that the episodic nature of this activity occurs because of an episode-induced depression of network excitability that recovers slowly in the interepisode interval. The depression is complex and affects several systems. After an episode, synaptic efficacy in active pathways is depressed, spinal neurons are hyperpolarized and the amplitude of both miniature and evoked synaptic currents decreases. The mechanisms responsible for these changes in network excitability are not understood but appear to involve a combination of both pre- and postsynaptic factors. One of the post-synaptic factors is a reduction in the driving force of GABA-mediated conductances. Post-episode synaptic depression, present in all active pathways examined, is also seen at the monosynaptic connections between motoneurons and Renshaw-like interneurons. One consequence of this activity-dependent network depression is that the network output exhibits a homeostatic-like behavior following pharmacological blockade that may be important in stabilizing network output during development.

NICOTINIC RECEPTORS AND NEURONAL NETWORKS: A PARALLEL BETWEEN HUMAN AND RAT BRAIN.

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Acetylcholine (ACh) interacts with different nicotinic receptor (nAChR) subtypes to mediate synaptic transmission or to control synaptic transmission mediated by glutamate or (-aminobutyric acid (GABA). Here, we discuss the physiological and clinical implications of the findings that nAChRs can participate in inhibitory and disinhibitory mechanisms in the brain. The patch-clamp technique was used to record responses triggered by the non-selective agonist ACh and the ∀7-nAChR-selective agonist choline to interneurons visualized by means of infrared-assisted videomicroscopy in human cerebral cortical and rat hippocampal slices. In both human cerebral cortical and rat CA1 hippocampal interneurons, choline induced an inward current that decayed to the baseline prior to termination of the agonist pulse and that was sensitive to the ∀7 nAChR-selective antagonist methyllycaconitine (MLA, 50 nM). In contrast, ACh induced slowly decaying whole-cell currents that were sensitive to the ∀4∃2-nAChR-selective antagonist dihydro-∃-erythroidine (DH∃E). Application of ACh to interneurons of both species also triggered GABA-mediated postsynaptic currents (PSCs), which were the result of GABA release evoked by nAChR activation in interneurons synapsing onto the neuron under study. ACh-evoked PSCs were insensitive to MLA (50 nM) and were reversibly inhibited by DH∃E (10 μM). Thus, ACh-induced GABA release from human cerebral cortical interneurons is mediated by ∀4∃2-like nAChRs. Evidence is provided that the nAChRs whose activation resulted in GABA release are located in preterminal axon segments of the interneurons. In the CA1 field of rat hippocampal slices, ∀7-like nAChRs were also seen to control the release of GABA. By modulating the release of GABA from interneurons that synapse directly onto pyramidal neurons, neuronal nAChRs could function as filtering devices that enhance the signal-to-noise ratio in neuronal circuitries and could shunt excitatory inputs to the dendrites of pyramidal neurons. Conversely, by modulating GABA release from interneurons, which synapse onto other interneurons that, in turn, synapse onto pyramidal neurons, nAChRs could reduce GABAergic inhibition of the latter, thereby producing synaptic strengthening similar to that seen in long-term potentiation. These mechanisms, which appear to be retained across species, can explain the involvement of nAChRs in cognitive functions and in such neurological disorders as Alzheimer's disease and schizophrenia. (Support: USPHS grant NS25296; U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-95-C-5063; PRONEX from Brazil)

NEURONAL NICOTINIC RECEPTORS AND EFFECTS OF CHRONIC EXPOSURE TO NICOTINE

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Neuronal nicotinic receptors (AChRs) are formed from subunits termed α2-9 and β2–β4. AChRs are formed from five subunits arranged around a central cation channel. There are many potential AChR subtypes, but a few prominent ones. One major subtype which binds αbungarotoxin is formed only from α7 subunits. Another major subtype which accounts for about 90% of brain high affinity nicotine binding is thought to be formed from two kinds of subunits organized around the channel in the order α4β2α4β2β2. A third group of AChR subtypes containing α3 subunits is thought to be largely responsible for ganglionic transmission in the autonomic nervous system and to be expressed in smaller amounts in the brain. α3 and α7 AChRs are often expressed in ganglionic neurons. α3 AChRs may have subunit compositions as simple as $(\alpha3)_2(\beta2)_3$ or $(\alpha3)_2(\beta4)_3$ or as complex as $(\alpha3)_2\beta2\beta4\alpha5$. α5 can also associate with α4β2, α3β2, or α3β4 AChRs. ACh binding sites are thought to form at specific interfaces between α7 subunits, between α3 or α4 and β2 or β4 subunits, but not between α5 and other subunits. AChR subtypes differ in their responses to nicotine. Nicotine is an agonist when acutely applied to most AChR subtypes. Chronic exposure results first in reversible desensitization and later in permanent inactivation. α4 AChRs and α7 AChRs are much more susceptible to inactivation than are α3 AChRs. Chronic exposure to nicotine also causes an increase in the amount of AChRs. α4β2 AChRs are more sensitive to upregulation than are α7 AChRs. α3β2 AChRs, but not α3β4 AChRs, appear to be upregulated by chronic exposure to nicotine. The extent and mechanism of upregulation may depend on both the AChR subtype and on the cell type in which the AChRs are expressed. Inactivation and upregulation of AChRs by chronic exposure to agonists are relevant to understanding effects of nicotine including the development of tolerance. These effects may also apply to potential nicotinic agonist drugs

EXOCYTOSIS, ENDOCYTOSIS AND KISS-AND-RUN

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Exocytosis and endocytosis are coupled. It is, however, uncertain the degree of coupling between these two processes. The classical view of membrane recycling proposes that internalization of the excess of membrane occurs at a distant location from the active zone, therefore, indicating a loose coupling between exo and endocytosis. Recycling is not immediate and the internalized membrane has little resemblance to the membrane that originally fused to the plasma membrane. On the other hand, a higher degree of coupling between exocytosis and endocytosis would occur faster and the internalized membrane may maintain the same components as the vesicular membrane. An extreme of this mechanism, would be to consider that exocytosis and endocytosis are just the reflection of the open or close state of the water channel connecting the lumen of a secretory vesicle with the extracellular space, the so-called fusion pore. This model of transmitter is termed the "kiss-and-run" hypothesis. This mechanism would be the tightest mechanism for exo-endocytosis coupling and from a functional point of view is fast and economical to the cell. Our current view is that both coupling mechanisms can coexist into the same cell, and that the relevance of one over the other depends on the degree of stimulation of a given cell. We have used patch clamp techniques in combination with electrochemical detection of transmitters to monitor the life cycle of single secretory vesicles.

EXO/ENDO CYCLING VESICLE POOL AND SYNAPTIC TRANSMISSION AT THE LARVAL *DROSOPHILA* NEUROMUSCULAR JUNCTION

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Synaptic vesicles are recycled after fusion to the terminal membrane and release of transmitter. This process can be conveniently studied by the use of a fluorescent dye, FM1-43. We used this technique with electrophysiological measurements of synaptic transmission in larval *Drosophila* neuromuscular junctions. When the nerve is stimulated in the presence of dye in the external medium the dye is taken up into synaptic vesicles and the presynaptic boutons are stained. In a temperature sensitive mutant, shibire^{ts1}, endocytosis is blocked at non-permissive temperature (>29 ° C) but normal at room temperature. Using this mutant we found that there were two distinct pools of synaptic vesicles, termed exo/endo cycling and reserve pools. With high K^+ stimulation the dye is incorporated into the exo/endo pool and released completely after a second challenge with high K^+ saline. Staining was observed primarily in the periphery of boutons, resulting in ring-shapes. When mutant larvae were stimulated at non-permissive temperature, all vesicles were released because of a defect in endocytosis. At room temperature endocytosis resumed and the boutons were filled with the dye. When these terminals were stimulated again with high K^+ saline, fluorescence in the periphery of boutons disappeared leaving the core still stained. The remaining vesicles in the core constituted the reserve pool. Treatments with various drugs affected the relative size of these pools. Cyclosporin A, a protein phosphatase inhibitor, increased the total uptake of dye without changing the size of exo/endo pool. Thus, the reserve pool must have increased. In contrast, forskolin, an activator of adenylate cyclase, increased the size of exo/endo pool. The quantal content of nerve-evoked synaptic potentials also changed by these treatments in parallel with the size of exo/endo pool. It appears that the size of these pools is dynamically regulated, affecting the efficacy of synaptic transmission.

ENDOCYTOSIS AND VESICLE RECYCLING AT THE SYNAPSE OF A RETINAL NEURON

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We have investigated vesicle cycling in the giant synaptic terminal of depolarizing bipolar cells isolated fom the retina of goldfish. Two techniques were used: capacitance measurements of changes in membrane surface area and fluorescence measurements of vesicle staining with the dye FM1-43. After short depolarizations that caused a rapid burst of exocytosis, the membrane capacitance recovered with a rate-constant of about 0.8 s^{-1}. But after longer depolarizations the fall in membrane area occurred along a double exponential time-course with rate-constants that averaged 0.8 s^{-1} and 0.1 s^{-1}. Increasing the duration of the depolarizing stimulus increased the fraction of membrane recycled by the slower mechanism. Once endocytosed, vesicles mixed in to a pool of about 700,000 vesicles with a delay less than one minute. The conversion of these vesicles into a state ready for rapid release was accelerated by calcium. Calcium had two actions that could be differentiated by the introduction of the calcium chelator EGTA: one action stimulated refilling of the rapidly-releasable pool of vesicles from a reserve pool, and a second action stimulated recruitment to the reserve pool. The capacity of the reserve pool was about 3500 vesicles, which is similar to the number that can attach to ribbon structures associated with the active zones in this terminal. These properties of endocytosis and vesicle recycling will act to maintain continuous high rates of exocytosis that occur in response to maintained depolarization.

MOLECULAR MECHANISM OF CLATHRIN-MEDIATED ENDOCYTOSIS

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To understand the molecular mechanism of clathrin-mediated endocytosis we must begin to see how the many proteins identified fit into the pathway. To this end our laboratory has taken a structural and biochemical approach. We have provided evidence that in nerve terminals, endocytosis has a distinct calcium trigger from exocytosis, the calcium dependent phosphatase calcineurin. This catalyses the dephosphorylation of endocytosis proteins (including amphiphysin and dynamin) on nerve stimulation. Amphiphysin recruits dynamin to sites of endocytosis by binding to the adaptor protein complex AP-2, which in turn binds to membrane proteins to be endocytosed. We have solved the structures of domains from the adaptin proteins and amphiphysin to understand the specificity and adaptations of these proteins in endocytosis. Furthermore we have investigated the function of dynamin in vesicle fission using electron microscopy. We find that dynamin only associates with PIP2 containing lipids and have identified critical residues for lipid binding in its PH domain. On multimerisation around the neck of a vesicle, dynamin's GTPase activity is activated. We observe that on GTP hydrolysis dynamin spirals undergo a lengthwise extension- which we believe drives the vesicle away from the membrane causing lipid fission.

COMPOSITIONALITY OF NEURAL ACTIVITY

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Compositionality is our ability to combine a limited number of primitives in a variety of ways to generate plethora of complex mental items. Examples are concatenation of phonemes to words, line elements to figures, or simple strokes to writing.

We hypothesize, that elementary primitives are represented by sequences of synchronous volleys of cortical neurons, and compositionality is achieved by concatenating such sequences. As suggested by Bienenstock, a simple neural network model that may have the adequate properties to exhibit compositionality is the synfire chain.

We showed, by way of simulations and numerical analysis, that synfire chains can indeed be dynamically combined to form a whole hierarchy of composite representations. The space of parameters at which this behavior is feasible was studied by further abstracting the synfire chain model into a model of waves, having only two degrees of freedom (the wave's position and velocity).

Experimental verification of these concepts were achieved by recording several single units simultaneously in behaving monkeys. We hypothesized that whenever the same mental process occur, the same sequence of neuronal firing will occur. When simultaneously recording from a number of single units (5-15 in out setup) we expect to see repeating patterns of firing among some of the neurons. Such repeated patterns were found in multi-electrode recordings, both by us and by others.

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RESPONSE SYNCHRONIZATION AND VISUAL PROCESSING

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The question is addressed whether precise temporal relations among the discharges of distributed neurons matter for subsequent processing or whether neuronal networks are solely sensitive to variations in discharge rate. An answer to this question is important for the design of experiments in search of neuronal codes: If information is contained in precise temporal relations it can only be retrieved by correlation analysis of simultaneously recorded responses. The reason is that specific temporal relations among discharges are often generated by internal neuronal interactions and therefore cannot be deduced from stimulus or movement triggered histograms of successively recorded neuronal responses. Evidence will be reviewed which indicates that the nervous system is sensitive to variations in the precise temporal relations among the discharges of distributed neurons and that it interprets the synchronicity of discharges as a signature of relatedness. It is proposed that synchronized discharge patterns are processed preferentially and jointly because they have a stronger impact on subsequent processing stages than temporally dispersed discharges. This conjecture is supported by multi-site recordings which provide direct evidence for an enhanced impact of synchronized responses. Experiments combining multi-stable perception with multielectrode recordings from visual cortex reveal a close correlation between the probability and strength of internally generated response synchronization on the one hand and selection and perceptual grouping of the responses on the other. Because stimulus-induced variations of the discharge rates of individual cells failed to exhibit such correlations it is proposed that precise temporal relations among distributed discharges matter in neuronal processing, synchronization serving as a mechanism for response selection and grouping.

RAPID AND SPECIFIC EXPERIENCE-DEPENDENT SHORT-TERM CHANGES IN OLFACTORY NETWORK DYNAMICS ENABLE MORE PRECISE ODOR REPRESENTATION

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Neural assemblies display self-organized, synchronized oscillations in response to sensory stimuli in a variety of brain areas and animal species. In the olfactory system of insects, odor-evoked oscillatory synchronization of antennal lobe projection neurons (PNs) is superimposed on slower and stimulus-specific temporal activity patterns. Hence, each odor activates a specific and dynamic PN assembly whose evolution during a stimulus is locked to the oscillation clock. Here we examine the changes in population dynamics over repeated odor stimulations, as would occur when an animal first detects and then repeatedly samples an odor for identification or localization. We find that the responses of PN assemblies undergo a rapid decrease in intensity, concomitant with a dramatic increase in spike time precision and inter-neuronal oscillatory coherence. Once established, this enhanced precision in the representation endures for several minutes. This change is stimulus-specific, and depends on events within the antennal lobe circuits, independent of olfactory receptor adaptation. It thus constitutes a form of short term memory.

Our results suggest that this progressive change in olfactory network dynamics serves to converge, over repeated odor samplings, on a more precise and readily classifiable odor representation.

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ANALYSIS OF TRANSGENIC MODELS OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is one of an increasing number of inherited neurodegenerative disorders that are caused by a CAG/polyglutamine (polyQ) repeat expansion. The mechanism by which a polyQ expansion causes neuronal dysfunction and cell death and the factors that govern the selective neuronal vulnerability between these diseases are unknown.

We have generated lines of mice transgenic for exon 1 of the human HD gene under the control of the HD promoter which develop a progressive neurological phenotype. PolyQ aggregates have been detected in the brain in the form of nuclear inclusions and neuropil aggregates which accumulate progressively from before onset of symptoms. Inclusions have also been detected in some post-mitotic non-CNS cell types, in which case they are always nuclear. Despite an extensive search, cell death has only been detected in a very selective subset of neurons in mice at the very end stage of the disease. There is increasing evidence to support a neurodysfunctional rather then neurodegenerative basis to the observed phenotype.

The striking correlation between the length of polyQ tract that causes the exon 1 huntingtin protein to aggregate and the HD pathogenic repeat threshold suggests that polyQ aggregation is the primary molecular event in the pathogenicity of HD.

NEUROBIOLOGY OF DEFENSIVE RAGE AND DEFENSIVE TEMPERAMENT: INROADS INTO UNDERSTANDING THE ROLE OF NEUROPLASTICITY IN STRESS PRECIPITATED CHANGE IN AFFECT

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Harm avoidance appears as a temperamental trait in a variety of species. Early investigations of electrically and naturally evoked activity in limbic circuits modulating defensive rage in more and less harm avoidant cats suggested the trait is expressed as an amplification of amygdala efferent neural transmission. Subsequent work revealed that the harm-avoidant behavioral pattern may be produced experimentally by inducing long term potentiation (LTP) in amygdala efferents by epileptic (partial kindling) and pharmacological means (administration of FG-7142). Comparison of behavioral and LTP effects of kindling and FG-7142 has focused attention on LTP in right amygdala efferents as a candidate mechanism for lasting increases in harm avoidance. The benzodiazepine receptor (BZR) blocker, Flumazenil, reverses behavioral effects of partial kindling and FG-7142. In cats given FG-7142. Flumazenil selectively reverses LTP in right amygdala efferent transmission to the periaqueductal gray (PAG) in a drug dependent manner. A similar dependence of kindling induced behavioral change on LTP in right amygdala efferents to PAG has recently been shown using low frequency depotentiation methods to reverse LTP selectively. Subsequent work has shown behavioral and LTP effects of administration of FG-7142 are NMDA receptor dependent. The effects of FG-7142 are of interest clinically, because this inverse BZR agonist mimics many of the effects of severe stress. Therefore, the changes in behavior and brain function described may provide insights into the neural changes underlying the lasting effects of traumatic stress on affect in humans. Inspired by the work in felines, studies of the behavioral and neural response to more natural stressors have been undertaken in rodents. Lasting effects of a brief, unprotected, exposure to a cat (predator stress) have been found. Behavioral changes include enhanced acoustic startability, increased open arm avoidance in the plus maze, and reduced risk assessment. Behavioral effects are blocked by systemic administration of NMDA receptor blockers prior to predator stress. Subsequent work identified critical sites of NMDA receptor blockade to be in the amygdala. The work also suggests separable neural substrates in the amygdala mediate the different behavioral effects of predator stress. In sum the work suggests lasting negative affective (harm avoidant) behavioral biases involve lasting potentiation of amygdala function. While is unclear if harm avoidant temperament comes into being via some NMDA dependent process, the data indicate that harm avoidance change is closely tied to NMDA dependent amygdala function change.

CORTICOSTEROIDS AND THE ESCALATION OF VIOLENCE IN HYPOTHALAMIC AND TERRITORIAL AGGRESSION IN MALE RATS

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An important question in the behavioural neurosciences, is why so many behavioural mechanisms connecting frontal areas involved in appraisal of behavioural relevance to brain stem mechanisms pass through the hypothalamus. The classical view is that the hypothalamus subserves some kind of behavioural-neuroendocrine integration, but the mechanisms of this process is poorly known. This contribution reports on a mutual facilitation of a brain mechanism involved in aggression and a mechanism regulating stress hormones. Direct activation of the "aggressive area" in the hypothalamus of a rat rapidly raises circulating corticosterone to high stress levels, even in the absence of fighting. Mimicking such an increase in rats without adrenals, by i.p. injections, dramatically facilitates aggression evoked by stimulation in the "aggressive" area. The facilitation is apparent within 10 minutes following injection and disappears at 60 minutes when corticosteroid levels have returned to baseline. Corticosterone also facilitates the elicitation of aggressive behaviour by hypothalamic stimulation in rats without any prior fighting experience. Moreover, corticosterone also has a role in the development of territorial aggression. Adrenalectomized rats do not develop the species-typical aggressive behaviour in defense of a territory when confronted by an intruder into their territory, but display an anxiety-like pattern instead. Corticosterone injections 10 minutes before introduction of an intruder in the home cage of adrenalectomized rats re-establishes the normal territorial aggressive pattern. Rapid increases in circulating corticosteroids commonly accompany the social challenges prior to violent conflicts in animals and man. Corticosteroids readily enter the brain. The observed corticosteroid-dependent facilitation is fast enough to the decrease central thresholds for aggression within the time frame of a single conflict. Our findings show that the adrenocortical stress response does not merely prepare the organism for fight and flight. The adrenocortical stress response apparently also directly controls the type and threshold of aggressive responses. The rapid, mutual, positive feedback between the aggressive mechanism activated in the hypothalamus and the adrenocortical stress mechanism may very well contribute to the well-known escalation of violence under stressors.

CORTICAL SEROTONIN AND 5-HT1A AND 1B RECEPTORS, AND IEG EXPRESSION IN HEIGHTENED AGGRESSION

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Activity in the ascending projections of serotonin-containing neurons and at 5-HT receptor subtypes may be significant in violent individuals. (1) The current inquiry began with ethologi-cal analyses of adaptive patterns of aggressive behavior as frame of reference for studying excessively heightened or ex-aggerated aggression. (2) The appli-cation of in vivo microdialysis method-ology allows continuous moni-toring of extracellular concentrations of 5-HT in limbic and cortical regions of individual animals that have a history of repeated aggressive behavior and are provoked to initiate an ag-gressive con-frontation and then engage in a burst of aggressive acts. In the prefrontal cortex of aggressive rats a de-cline in 5-HT is detected after, rather than be-fore, the aggressive confronta-tion. These data point to a po-tential role of cortical 5-HT in stopping an aggressive burst. (3) New op-tions for pharmacotherapeutic mana-gement of aggressive behavior stems from recent developments in 5-HT receptor pharmacology. Pharmacological agonists at 5-HT1A and 5-HT1B receptors decrease aggressive behavior in laboratory animals effectively and in an antagonist-reversible manner, the latter with notable behavioral speci-ficity. While moderate doses of alcohol have often no reliable effects on aggressive behavior, they can engender very large in-creases in aggressive behavior in a subgroup of resident rodents that con-front an intruder. Very low doses of 5-HT1A and 5-HT1B receptor agonists are sufficient to attenuate exaggerated high levels of aggressive behavior due to alcohol treatment or due to social provocation. How to ac-com-modate these re-sults with the "serotonin deficit" hypothesis? (4) Epi-sodes of fighting them-selves are sufficient to induce immediate early gene expression in discrete neural cells, including those in raphe n. and LC that appears related to the enduring sensitization at the behavioral and neural level. Is heightened aggressive behavior based on 5-HT1B modulation of sensitized mesocorticolimbic DA activity?

NEURAL MECHANISMS MEDIATING AGGRESSION AND RAGE IN THE CAT

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Studies conducted in our laboratory have provided evidence of the importance of the roles of substance P (SP) and GABA in the hypothalamus. Two models of aggression used for these studies included defensive rage (DR),which is elicited by electrical stimulation of the medial hypothalamus (MH) or dorsolateral aspect of the midbrain periaqueductal gray (PAG) and predatory attack (PA), which is elicited from lateral hypothalamus (LH). DR is characterized by marked pupillary dilatation, increased heart rate and blood pressure, hissing, arching of the back, retraction of the ears and a paw strike at any moving object in its visual field and PA is characterized by the stalking of an anesthetized rat followed by the biting of the back of its neck. Anatomical studies, which have utilized receptor autoradiography, in situ hybridization, immunocytochemistry and retrograde neuronal tracing with Fluoro-Gold, have demonstrated that there exists moderate to dense quantities of mRNA for SP-neurokinin-1 (NK1) that are largely limited to the anterior medial hypothalamus (AH) as well as dense quantities of SP-immunopositive labeled axons and axon preterminals in this region that arise principally from the medial amygdala (ME) and septal area. The functional importance of NK1 receptors in the AH was revealed by a series of experiments which showed that either medial amygdaloid or septal area facilitation of DR and suppression of PA was eliminated by NK1 receptor blockade in AH and that he suppressive effects of ME are mediated through a second, short GABAergic neuron from MH to LH. However, GABAergic neurons also project from LH to MH, which provides the basis by which LH can suppress MH-elicited DR, thus indicating that the functional relationships between MH and LH are governed by reciprocal inhibitory pathways.

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TESTOSTERONE-SEROTONIN INTERACTIONS IN AGGRESSION

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Our understanding of the neurochemical and neuroendocrine systems regulating the display of offensive intermale aggression has progressed substantially over the past twenty years. A compelling argument now can be made that serotonin, via its action at $5HT_{1A}$(1A) and/or $5HT_{1B}$(1B) receptor sites, modulates the display of intermale aggressive behavior and that its effects serve to decrease behavioral expression. At the same time, male-typical aggression is testosterone-dependent and studies of genetic effects, metabolic function, and steroid receptor binding have demonstrated that neuroendocrine regulation can occur via independent androgen-sensitive or estrogen-sensitive pathways. Our efforts have been directed toward characterizing the interaction between these systems, with a primary focus on hormonal modulation of serotonin function at $5HT_{1A}$ and $5HT_{1B}$ receptor sites. These investigations have shown that the androgenic and estrogenic metabolites of testosterone differentially modulate the ability of 1A and 1B agonists to decrease offensive aggression and that there are partially distinct neuroanatomical substrates mediating these effects. The combined findings should contribute to the development of an integrative neurobiology of offensive intermale aggression.

CIRCADIAN CLOCKS

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Daily biological rhythms are universal phenomena, encompassing a range of activities as diverse as nitrogen fixation in cyanobacteria and sleep-wake cycles in humans. Underlying these rhythms is an endogenous clock mechanism that recognizes local time and measures its passage. At the heart of the clock is a self-sustaining, light-entrainable pacemaker that oscillates with a circadian period when it is maintained in a constant environment free of external timing cues. In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus is the site of such a pacemaker; the body of evidence for this interpretation is so compelling and multi-disciplinary in nature that the strength of this functional localization is unsurpassed by that of any other structure in the central nervous system. This symposium highlights the remarkable advances that are being made in understanding the biology of mammalian circadian timekeeping. We now know that the oscillatory mechanism in the SCN is intracellular, and Okamura (Japan) reviews the emerging roles for gene transcription and protein synthesis. Multiple lines of evidence suggest that glutamate is responsible for the pacemaker's responsiveness to ambient light, and Gillette (U.S.A.) examines the signal transduction pathways that might mediate photic entrainment to environmental light-dark cycles. Local intercellular interactions within the SCN must coordinate the circadian oscillations of individual cells into a coherent rhythm with stable period and amplitude, and Dudek (U.S.A.) and Yarom (Israel) discuss a role for GABA. Finally, output pathways must convert the SCN's oscillation into a temporal program of rhythms exhibiting a range of waveforms and phases, and Zisapel (Israel) shows how melatonin rhythmicity may act as a neuroendocrine transducer of the pacemaker's timing signal. All the speakers demonstrate why the study of biological timekeeping is now at such a fertile and exciting stage, involving diverse methodologies and uniting investigators from a wide array of disciplines.

CLOCK OSCILLATION BY MAMMALIAN PERIOD GENES

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Mammalian period homologues *mPer1*, *mPer2* and *mPer3* are speculated to be oscillatory genes in mammalian circadian pacemaking system. Indeed, all three *mPers* showed a robust rhythmic expression in the mammalian circadian center, the suprachiasmatic nucleus (SCN). The pattern of their expression was characterized by a day time peak and night time trough in constant dark conditions (DD). Among these, *mPer1* and *mPer2* draw very sharp curve, but *mPer3* showed a weak and broad curve. Differing responses to a light stimulus were demonstrated in these *mPer* genes: *mPer1* and *mPer2* were rapidly induced by short duration exposure to light at levels sufficient to reset the clock, but *mPer3* was not induced by exposing animals to light during all phases of circadian clock, thus distinguishing *mPer3* from both *mPer1* and *mPer2*. A product of *mTim*, a mammalian homologue of *timeless*, binded to mPER1 inside the nucleus, but did not show clear circadian rhythm in the SCN. In *Drosophila*, the process of nuclear translocation of PERIOD protein is a key step of the negative feedback loop for the rhythm generation. In mammalian cell lines, we found that the dimerization accelerated the nuclear entry of mPER proteins. Studies of the regulatory mechanisms of these genes in mammalian cells *in vivo* and *in vitro* are likely to further elucidate the nature of the circadian feedback loop at the molecular level and deepen our understanding of mammalian circadian biology.

CLOCK-CONTROLLED GATING OF DISTINCT GLUTAMATERGIC SIGNALING PATHWAYS: CELLULAR AND MOLECULAR MECHANISMS WITHIN THE SCN

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The circadian clock in the suprachiasmatic nucleus (SCN) restricts its own sensitivity to resetting signals across the 24-hour cycle. Nocturnal light is the dominant regulator of clock phasing. Photic information is transmitted via glutamate (GLU) released from direct retinal projections to SCN. Light-induced clock resetting requires GLU-mediated membrane depolarization, activation of NMDA receptors, rapid Ca^{2+} influx and nitric oxide (NO) production. This results in formation of the transcriptional activator, P-CREB. GLU induces diametrically different responses, depending upon the clock's temporal state. We sought to understand the signaling mechanisms that mediate differential clock sensitivity and phase adjustment. During late night, NO stimulates cGMP production and PKG activation; this advances clock phase. In contrast, in early night, NO induces intracellular Ca^{2+} ($Ca^{2+}i$) release through neuronal ryanodine receptors (RyRs), mediators of coupled Ca^{2+} signaling; this results in phase delay. These stimuli are without effect in subjective daytime. Imaging of Ca^{2+}-Green fluorescence using 2-photon microscopy in adult brain slices from rat revealed distinct spatial and temporal patterns of $Ca^{2+}i$ fluxes in early night. Although the cellular targets of pathways activated by Ca^{2+} movement are unknown, GLU-induced phase delay correlates with induction of *mtimeless*, a putative clock gene. These studies provide insights into cellular mechanisms mediating integrative signal processing within the SCN. They underlie decision-making events wherein the clock evaluates signals relaying states of the environment, brain and time-of-day to generate appropriate phase adjustments. These gating mechanisms profoundly shape adaptive behaviors and invest them with temporal relevance.

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GABA-MEDIATED CIRCUITS AND ACTIONS IN THE SCN

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Most suprachiasmatic nucleus (SCN) neurons use the neurotransmitter γ-aminobutyric acid (GABA), and anatomical studies indicate that many GABAergic synapses and local axon collaterals are present within the nucleus. However, physiological evidence for synaptic communication between SCN neurons has been indirect. With G.J. Strecker and J.P. Wuarin, we have used whole-cell recording in hypothalamic slices to provide evidence for local GABAergic synapses in the SCN. In particular, focal photolysis of caged glutamate, a method that selectively activates SCN neurons independent of fibers of passage, caused an increase in GABA-mediated postsynaptic currents. We have also collaborated with two other groups to test the hypothesis that GABA is excitatory during subjective day. Three independent electrophysiological approaches (multiple-unit recordings with R.L. Pieschl, T.A. Wisialowski and V.K. Gribkoff; cell-attached recordings with G.J. Strecker and W.K. Park; and, gramicidin perforated-patch recordings with M.T.G. de Jeu and C.M.A. Pennartz) in hypothalamic slices provided evidence that GABA is predominantly inhibitory during subjective day. Further studies are required to understand the physiological role of GABA and its system of local neuronal circuits in the SCN.

METABOTROPIC GABA$_B$ RECEPTORS

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Recent evidence has indicated that $GABA_B$ receptors in mammalian brain exist as heterodimers with the components R1 and R2 linked via their C-terminals. Both R1 and R2 have seven transmembrane spanning domains but whether both act as receptors remains to be seen. No agonist binding site has been demonstrated on R2, but the presence of R2 is obligatory for the expression of R1 in the plasma membrane and R2 alone has been expressed as a functional receptor. The R1-R2 receptor is G-protein coupled and on activation inhibits adenylate cyclase to decrease the formation of cAMP. $GABA_B$ receptors have been implicated both physiologically and pathologically in brain function, eg.modulation of primary afferent output and generation of absence epilepsy, respectively, and changes in receptor densities in diseased brain have been noted. Thus, in Alzheimer's disease a 50% loss of agonist binding to $GABA_B$ sites in human cerebral cortex has been reported. However, antagonist binding to the same tissue shows no difference from control. This suggests that there is not a loss of receptors but instead perhaps uncoupling from the G-protein has occurred. In spinal cord obtained from rats with a chronic neuropathic or monoarthritic lesion a down-regulation in agonist but not antagonist binding to $GABA_B$ sites has been noted. Perhaps uncoupling is provoked under these conditions as well. Studies to determine this are in progress. There are numerous pharmacological effects associated with $GABA_B$ receptor activation. Whether uncoupling can arise in all cases and whether this can be differentially influenced by separate receptor ligands remains to be seen.

METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) AS PUTATIVE THERAPEUTIC DRUG TARGETS

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Eight mGluR subtypes have been identified. They can be subdivided into 3 groups based on their sequence similarities and transduction mechanisms.
With the Ca^{2+}-sensing receptors, mGluRs comprise some vomeronasal receptors and GABA-B receptors, family 3 of GPCRs. Family 3 GPCRs possess a very large N-terminal domain (NTD) which constitutes the binding site and a 7 transmembrane core (7 TMC) which interacts, via its intracellular loops, with G proteins. The i2 loop is involved in the specificity of G protein recognition and i3 in G protein activation. Since binding of glutamate is at the NTD level and activation at TMC level, it was expected, and indeed found, that drugs acting at both sites can be localised. Thus CPCCOEt act at the TMC level. Inactivating and activating mutations of Ca^{2+}-sensing receptors are responsible for pathologies. An inactivating mutation found in the i3 loop of the Ca^{2+}-sensing receptor, at a conserved residue (R), can be experimentally reproduced in mGluR1.
A clear implication of mGluR1 in nociceptive transmission both at the spinal and thalamic levels, has been demonstrated. Reduced epileptic events may also be expected with mGluR1 antagonists.
Groups II and III inhibit glutamate but also GABA release via several mechanisms. Using electrophysiological and a direct visualization method, we have demonstrated a direct effect on release machinery. Groups II and III agonists can be useful in excitotoxic pathologies such as ischemia and in apoptotic-induced neurodegenerative diseases. However, *in vitro*, we have shown that group III agonists may be neuroprotective on glutamate neurons and neurotoxic on GABA neurons. Recently, LY354740 has been used to demonstrate the potential therapeutic utility of group II agonists in treating anxiety, Parkinson's disease, schizophrenia and drug addiction.

5-HT RECEPTOR DIVERSITY IN HEALTH AND DISEASE.

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Serotonin (5-hydroxytryptamine, 5-HT) receptors belong to both the G-protein coupled and ligand-gated ion channel receptor super-families; they are divided into seven classes according to structure, transduction and pharmacology. The 5-HT_1 class has 5 receptors which share 41-63% sequence identity and couple to $G_{i/o}$. 5-HT_{1A} ligands have been developed for depression, anxiety and hypertension, 5-$HT_{1B/1D}$ ligands (and 5-HT_{1F}) for migraine (DHE and various "triptans"). The 5-HT_2 class comprises 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors which exhibit 46-50% sequence identity and couple to $G_{q/11}$. 5-HT_2 antagonists have been targeted on depression and schizophrenia (5-$HT_{2A/2C}$), whereas 5-HT_{2B} ligands may be useful in the treatment of migraine. The 5-HT_3 class belongs to the ligand-gated cation super-family: 5-HT_3 antagonists are used in cancer chemotherapy related emesis and other GIT indications. 5-HT_4, 5-ht_6 and 5-HT_7 receptors couple to Gs, but are classified as distinct families as they exhibit <40% overall sequence identity and rather different profiles. 5-HT_4 agonists are efficient gastro-prokinetics and improve memory. Ligands for 5-ht_6 and 5-HT_7 receptor have only recently been described, and their function remains enigmatic; 5-HT_7 receptors correspond to previously named "5-$HT_{1\text{-like}}$" receptors which have been characterised in the cardiovascular system but could not be equated with known receptors. Two 5-ht_5 receptors (5-ht_{5a} and 5-ht_{5b}), sharing 70% sequence identity have been cloned from rodents, (only 5-ht_{5A} in humans); coupling, function and selective ligands remain to be identified. Splice and/or editing variants exist for several 5-HT receptors whose functional consequences need further investigation. A relationship between human 5-HT receptor polymorphism and disease (e.g. schizophrenia) is suggested.

A Decision-Theoretic Analysis of Parietal Cortex

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Until recently, studies of the nervous system have focused almost exclusively on either the sensory, motor, or memory systems. Very little work has been devoted to an examination, in a more general sense, of how the nervous system flexibly employs sensory data and stored information to match motor outputs to environmental circumstances. Indeed, few neurobiological theories have seriously considered any aspect of the decision-making processes which must link the sensory, memory and motor systems. Behavioral studies of choice and decision-making, however, describe a rich mathematical framework for understanding these processes. For this reason, we have begun to examine the possibility that neurons known to lie between purely sensory and purely motor areas of the brain can be described as participants in a mathematically definable decision-process. The framework of decision-theory provides a methodology for describing the entire sensory-motor process formally, and an experimental tool for localizing individual neural processes within a sensory-decisional-motor circuit. In a series of experiments conducted in awake-behaving monkeys, we have shown that neurons in parietal cortex often described as "sensory-attentional" or "pre-motor", carry signals which can be disassociated from the properties of *both* sensory stimuli and future movements, signals which carry information about the magnitude and probability of reward associated with target/movement combinations. Signals like these have not been predicted by existing neurobiological theories but are essential components of any decision-theoretic analysis of the brain. These data and others suggest that decision-theory may provide a powerful tool for describing the previously ambiguous neurobiological processes that connect sensation and action.

WHERE THE ACTION IS: VISUOMOTOR FUNCTIONS OF THE HUMAN POSTERIOR PARIETAL CORTEX

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Evidence from neurological patients and from neuroimaging in healthy volunteers has shown that the visual control of skilled actions is mediated by a set of dedicated visuomotor networks in the posterior parietal cortex, with different actions depending on different networks. The action systems in the human parietal lobe appear to be homologous with the monkey dorsal stream – and are quite distinct from the more visuoperceptual networks in occipitotemporal areas, which are homologous with the monkey ventral stream. The networks in the ventral stream transform visual input into perceptual representations that embody the enduring characteristics of objects and their relations. Such representations enable us to identify objects, to attach meaning and significance to them, and to establish their causal relations – cognitive operations that are essential for accumulating knowledge about the world. In contrast, the transformations carried out by the action systems in the dorsal stream deal with moment-to-moment information about the location and disposition of objects with respect to the effector being used and mediate the visual control of skilled movements directed at those objects. Both streams work together in the production of adaptive behaviour. The selection of appropriate goal objects and the action to be performed depends on the perceptual machinery of the ventral stream, but the programming and execution of a goal-directed action is carried out by dedicated on-line control systems in the dorsal stream.
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ON OBJECTS, HANDS, AND MOVEMENTS

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In my talk I will describe first the processes that transform the object's intrinsic properties into a pattern of distal movements. In monkeys, this transformation takes place in a circuit that is formed by the parietal area AIP and premotor area F5. Neurons in both these areas code size, shape and orientation of objects, and specific types of grip necessary to grasp them. Recent fMRI studies showed that a similar circuit exist in humans and includes part of the cortex buried in the intraparietal sulcus and Broca's area.

I will then show that some F5 neurons that discharge during object manipulation discharge also when the monkey observes another individual making a similar action. Analogous properties are present in the parietal area PF, an area anatomically connected with area F5. In this area, however, many neurons respond to biological actions without having, as in F5, a motor counterpart.

I will conclude discussing the role of the fronto-parietal connections. I will propose that: a) they give salience to certain stimulus configuration congruent to the intended action, b) they select stimulus descriptions for future action on the basis of motivation and external contingencies, and finally c) by matching knowledge of the external reality derived from motor experience with sensory data, they play an important role in giving a meaning to the observed actions and, possibly, intervene in object semantics.

ASSEMBLY OF THE GLYCINERGIC POSTSYNAPTIC MEMBRANE

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The postsynaptic inhibitory glycine receptor (GlyR), a pentameric chloride channel protein, copurifies with the tubulin-binding peripheral membrane protein, gephyrin. Gephyrin is a 93 kDa polypeptide whose N-and C-terminal domains display significant homologies to bacterial, invertebrate and plant proteins involved in molybdenum cofactor (moco) biosynthesis. In spinal cord, gephyrin colocalizes with GlyRs at postsynaptic membrane specializations. During development, this colocalization involves: i) the regulated expression of different GlyR isoforms; ii) formation of gephyrin clusters at presumptive postsynaptic sites; and iii) accumulation of GlyRs at these gephyrin-rich membrane domains. The steps ii) and iii) require GlyR-triggered membrane depolarization and Ca^{2+} influx. Disruption of the gephyrin gene results in early postnatal death, loss of GlyR accumulation at synapses and a strong neuromotor phenotype. In addition, gephyrin-deficient mice lack enzyme activities dependent on the molybdenum cofactor, indicating that gephyrin has dual functions *in vivo*. Evidence that gephyrin is also involved in the synaptic localization of $GABA_A$ receptors will be discussed. Our data establish gephyrin as an essential organizer molecule at inhibitory postsynaptic membrane specializations.

MODULATION OF NEUROMUSCULAR SYNAPTIC TRANSMISSION IN *DROSOPHILA* MUTANTS

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The larval *Drosophila* neuromuscular synapse has a unique advantage in studies of synaptic transmission as many established mutants defective in various aspects of synaptic function are available. By examining the altered synaptic functions in detail we might be able to dissect the sequence of events for transmitter release. Neuronal synaptobrevin (n-syb) is a major vesicle protein and a target of tetanus toxin. In the absence of functional n-syb, presynaptic nerve stimulation does not result in synaptic transmission. However, spontaneous release of transmitter remains. We examined miniature synaptic currents (mSCs) in mutant larvae lacking n-syb. The frequency of mSCs increased with external Ca^{2+} concentrations in high K^+ saline, suggesting that the Ca^{2+} sensing mechanism is operating in this mutant. Tetanic nerve stimulation also increased the frequency of mSCs. The effect of repetitive nerve stimulation was not observed in the absence of external Ca^{2+}, suggesting that influx of Ca^{2+} is involved and that voltage-gated Ca^{2+} channels are functional in these mutants. In contrast, these mutants did not respond to an elevation of cAMP, which in wild-type synapses causes a marked increase in the mSC frequency even in the absence of external Ca^{2+}. These findings suggest models that invoke a special role for n-syb in coupling fusion to the transient, local changes in Ca^{2+}. We have shown recently that an activation of metabotropic glutamate receptors (mGluRs) in the presynaptic terminal results in facilitation of synaptic transmission. To test if this effect is mediated through the cAMP cascade we used another mutant, *rutabaga* , which has defective adenylate cyclase. In this mutant the effect of mGluR activation was drastically reduced, suggesting that mGluRs are positively coupled with adenylate cyclase and their activation leads to elevation of cAMP level in the presynaptic terminal. These mutants are useful in elucidation of the synaptic mechanism.

MODIFICATION OF DEVELOPING SYNAPSES BY CORRELATED ACTIVITY: HEBB'S POSTULATE REVISITED

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To account for temporal specificity underlying associative learning, Donald Hebb postulated that correlated pre- and postsynaptic activity strengthenes the synaptic connection. Hebb's rule and its derivatives have become a corner stone in many theories of activity-dependent refinement of developing neural circuits and in the description of synaptic plasticity underlying learning and memory. However, rigorous examination of the role of temporal relation in the pre- and postsynaptic spiking activity in the induction of synaptic modifications has only recently been carried out. I will summarize our efforts in studying the role of spike timing in activity-induced long-term potentiation (LTP) and long-term depression (LTD) in artificial networks of cultured rat hippocampal neurons and in an intact developing frog tectum. In both systems, we found that for repetitive spiking activities to induce LTP and LTD, they must appear in the pre- and postsynaptic cells within a critical time window. Synaptic inputs that are repetitively activated within 20 ms before the spiking of the postsynaptic neuron become potentiated, while inputs activated within 20 ms after the postsynaptic spiking become depressed. Both potentiation and depression depend on activation of NMDA-subtype of glutamate receptors and can be readily reversed by subsequent spiking activity of appropriate patterns. Thus correlated pre- and postsynaptic spiking is required for both strengthening and weakening of the synapse, but with an opposite temporal sequence of spiking. Our results argue that a more precise and quantitative formulation of the Hebb's rule must now incorporate features that reflect the narrow and asymmetric time window for the induction of synaptic modifications.

EMERY DREIFUSS MUSCULAR DYSTROPHY: A NOVEL GROUP OF MUSCLE DISORDERS CAUSED BY NUCLEAR ENVELOPE ALTERATIONS

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Emery-Dreifuss Muscular Dystrophy (EMD) is a disorder characterized by early contractures of elbows and Achilles tendons, slowly progressive muscle wasting and weakness, and a cardiomiopathy with conduction block. Two modes of inheritance exist, X-linked (X-EMD) and autosomal dominant (AD-EMD).The two forms of the disease are clinically identical. In 1994, mutations in the STA gene, encoding a protein localized to the nuclear envelope, emerin, were found in patients affected with the X-linked form of the disease. Following the identification of the X linked gene, patients could be selected who were likely to be affected with AD-EMD and the locus for AD-EMD was mapped to a 8-cM interval on the chromosomal region 1q11-23. The locus was first mapped in a large French family, but other smaller families included in the study were potentially linked to the same locus. Among many other genes and ESTs, the locus contained the Lamin A/C gene, one of the components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane which is thought to provide a framework for the nuclear envelope. Lamins have the same cellular localization as emerin. This suggested LMNA as a candidate for AD-EMD. By sequence analysis, mutations in the LMNA coding region were found in the five selected families as well as in additional patients presenting the symptoms of EMD. Co-localization of emerin and lamin A/C to the inner nuclear membrane and the almost identical phenotype caused by mutations in the two proteins suggest that there is a common pathogenic mechanism for mutations in emerin and lamin A/C and underscore the potential importance of nuclear envelop components in the pathogenesis of neuromuscular disorders.

THE SPECTRUM OF DYSTROPHINOPATHIES: FROM DUCHENNE MUSCULAR DYSTROPHY TO HYPERCKEMIA

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Duchenne and Becker (DMD and BMD) muscular dystrophies are allelic neuromuscular disorders due to the deficiency of dystrophin, a protein expressed at the sarcolemma of all muscle types. Dystrophin is also expressed in the central nervous system and in the T-tubules in the cardiac muscle. Cardiac involvement is very frequently found in these patients; while in DMD there is a correlation between the severity of the cardiac and skeletal muscle involvement, in BMD a severe cardiomyopathy can be present in patients with mild weakness; in addition, various groups have shown that mutations in the dystrophin gene can result in an isolated cardiomyopathy (XLDCM). From a genetic point of view patients with DMD almost invariably carry a deleterious mutation leading to loss of the open reading frame (ORF); in BMD the mutation affects either the quantity of the protein produced or its quality (or both) but the ORF is maintained. In XLDCM usually the mutation induces a loss of the ORF exclusively in the heart, with absent protein expression only in this tissue; while in the muscle there is a residual expression of dystrophin. More recently intragenic deletions of the dystrophin gene have been reported in families with isolated elevation of the serum CK. Finally, mental retardation can be present in both DMD and BMD indicating an important role for dystrophin in the brain.

THE DMD GENE: STRUCTURE, EVOLUTION, EXPRESSION AND FUNCTION OF PRODUCTS

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Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are caused by mutations in the dystrophin gene, the largest known gene. The gene encodes three full length dystrophins which are regulated in a tissue specific manner by three promoters. It also encodes four smaller products which are regulated by promoters located in introns of the huge gene. In muscle dystrophin is a part of a large complex which links the actin cytoskeleton with the extracellular matrix and stabilizes the membrane of the myofibrile during contractions. The most abundant and ubiquitous non-muscle product of the DMD gene is Dp71 which consists of the cysteine-rich and C-terminal domains of dystrophin. The function of Dp71 as well as that of the other nonmuscle products of the DMD gene is not known.

We have specifically inactivated Dp71 expression in mice by replacing its first and unique exon with a b-gal reporter gene. The expression of other DMD gene products in these mice was not affected. The absence of Dp71 resulted, in selected tissues, in some changes in the level of other DMD gene products, utrophin and dystrophin-associated proteins. However, we did not detect pathological effects in the Dp71 knock-out mice. X-gal staining of Dp71 null embryos revealed a very specific and interesting pattern of staining which includes parts of the nervous system, eyes, limb buds, lungs, blood vessels, vibrissa and hair follicles. High activity of Dp71 promoter often seemed to be associated with morphogenic events and terminal differentiation. In some tissues the activity greatly increased towards birth.

To gain an insight into the evolution of the huge and complex DMD gene and its function in lower organisms, we have recently cloned the sea urchin homologue of dystrophin/utrophin genes. The sea urchin gene already has a complex structure characteristic of the DMD gene. It encodes at least two products: a 448 kd protein in which all the domains of dystrophin are highly conserved, and a 98 kd protein which is the evolutionary homologue of Dp116. Interestingly, both proteins are expressed in eggs and early sea urchin embryos, even before muscle development.

CALCIUM-DEPENDENT MECHANISMS UNDERLYING SHORT-TERM MODULATION AT THE CALYX OF HELD

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Presynaptic modulation of transmitter release may be achieved through modulation of the presynaptic potassium channels (thus influencing action potential duration), the calcium channels triggering exocytosis or directly by an action on one or more components of the exocytotic machinery. Our focus has been to study the calcium channel subtypes at an identified synapse (calyx of Held) and then to examine how activity at the synapse may influence the calcium current and therefore transmitter release. The calyx of Held arises from bushy cells of the anterioventral cochlear nucleus. Patch-clamp recording from the presynaptic terminal and the bushy cells indicates that there is a distinct polarization in the localization of voltage-gated calcium channel subtypes. T-type channels are present in the cell body but are excluded from the terminal, while P/Q-type channels contribute little to somatic currents but predominate at the synaptic terminal, where P-type channels are responsible for triggering transmitter release. The intrinsic properties of these presynaptic P-type calcium currents have an important influence on synaptic transmission. The presynaptic calcium channels inactivate in a calcium- dependent manner and this inactivation can accumulate, so that transmission is depressed following a tetanic stimulus. Interestingly, a short-term facilitation of the presynaptic calcium current is also present. Using paired-pulse protocols we estimate that this facilitation lasts for around 100ms and find that it is also dependent on calcium influx. Thus during a stimulus train the presynaptic calcium current undergoes an initial facilitation followed by a reduction as inactivation accumulates. This short term modulation of the presynaptic calcium current, apparently by the permeant ion itself, will complement vesicle recycling and residual calcium phenomena.

MODULATION OF PRE- AND POST-SYNAPTIC POTASSIUM CHANNELS IN AUDITORY NEURONS.

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Our laboratory has identified several potassium channel genes that are expressed at high levels in auditory brainstem nuclei. These include i) Slack, a calcium-regulated channel, ii) rTOK, a member of the two pore channel family and iii) Kv3.1 a voltage-dependent potassium channel this is specifically expressed in neurons that fire at high frequencies. The presentation will focus on the regulation of Kv3.1, which is expressed at very high levels in bushy cells of the cochlear nucleus and their postsynaptic target, neurons in the medial nucleus of the trapezoid body (MNTB). In particular Kv3.1 is present both in the giant presynaptic endings in the MNTB and in the postsynaptic membranes of the MNTB neurons. The unique biophysical properties of this channel allow this synaptic pathway to follow stimulation at high frequencies and with temporal precision. The Kv3.1 gene gives rise to two different splice variants Kv3.1a and Kv3.1b, which differ in their response to activation of protein kinase C. The amplitude of Kv3.1 currents, which influences the ability of the synapse to follow high frequency inputs, can be regulated in the short term by activation of protein kinase C, and, in the long-term, by calcium-dependent regulation of Kv3.1 gene transcription. Modulation of Kv3.1 channels may be particularly important in the presynaptic endings, where changes in the amplitude of Kv3.1 current alter the rate of recovery from synaptic depression. Our findings suggest that, by modulation of Kv3.1 currents, the auditory system makes a trade off between high fidelity firing and the ability to respond to auditory stimuli for prolonged periods of time.

KINETICS AND MODULATION OF FAST ENDOCYTOSIS AT HIPPOCAMPAL SYNAPSES.

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Optical and electrophysiological measurements in hippocampal neurons suggested that a vesicle can become available to release transmitter within ~40 s or less after a previous round of exocytosis. Surprisingly, the time constant for endocytosis has been estimated as ~20-30 s – at least half of the total recycling time and far slower than endocytosis in other secretory systems. Here we report evidence for fast endocytosis (τ~5 s) in hippocampal nerve terminals, derived from fluorescence measurements with a series of FM dyes varying by 10-fold in their departitioning kinetics. The time constant of destaining upon stimulation by either local application of high K+ solution or short current pulses correlated strongly with dye kinetics, as expected if exocytosis were followed by departitioning at different rates. Strikingly, however, the amplitude of the early phase was also significantly different, being largest with the most rapidly departitioning dye. We suggest that the loss of fluorescence reflects the balance between exocytosis and a hitherto overlooked process of fast endocytosis. The fast endocytosis effectively competes with dye departitioning, leading to its retrapping. The use of three different dyes with known departitioning rates allowed us to estimate the rate constants for exocytosis, endocytosis, and repriming in a simple model. This rapid mode of vesicular retrieval appears to be greatly speeded by exposure to staurosporine or elevated $[Ca^{2+}]_o$. These results suggest that hippocampal synapses can capitalize on efficient mechanisms for endocytosis, of advantage for preserving synaptic transmission during repetitive firing. Supported by grants from B.I.F.(J.K.), the AHA (E.T.K.), and NIMH (R.W.T.).

VESICLE DEPLETION AS A MECHANISM OF SHORT TERM MODULATION AT THE CALYX OF HELD SYNAPSE

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The Calyx of Held shows complete (>95%) depression, when stimulated by a maximal Ca^{++} inflow for periods of several milliseconds. The depletion of a pool of readily releasable vesicle is believed to play a major role in this depression. We used four approaches to test this assumption and to estimate the size of such a pool. First, the potentiation of EPSC amplitudes upon elevation of extracellular [Ca^{++}] was used to extrapolate the quantal content at saturating presynaptic Ca^{++} influx. Second, trains of stimuli at 100 Hz, which induced rapid and strong depression, were used to calculate cumulative EPSC-amplitudes for short time intervals (smaller or equal to 60 ms). In a third approach we combined presynaptic electrical stimulation with flash-photolysis of caged Ca^{++}. While each of these stimuli by itself elicited the release of an estimated average of ~ 500 quanta, the combination of the two stimuli at short time intervals (10 ms) led to a strong (~ 90%) cross-inhibition of postsynaptic responses. Cyclothiazide, a blocker of glutamate receptor desensitization, did not reverse this cross-inhibition, indicating that vesicle pool depletion might be primarily responsible. In a fourth approach we estimated the time course of the release rate by a combination of deconvolution and fluctuation analysis. The results from these approaches all indicated that there is a surprisingly large pool of readily-releasable transmitter quanta (range 300 to 800), of which about 25 %, or a larger fraction in some cells, is released during a single presynaptic action potential at 2 mM extracellular [Ca^{++}]. These results make a transient decrease in the number of readily-releasable transmitter quanta a probable mechanism for the induction of short-term depression at the Calyx of Held.

EXOCYTOSIS IN CHROMAFFIN CELLS IS MODULATED BY A CALCIUM-PERMEABLE CURRENT ACTIVATED BY STORE DEPLETION.

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In neurons and neuroendocrine cells, exocytosis is triggered by large-amplitude calcium (Ca) influx through voltage-gated Ca channels. In some cells, exocytosis is also triggered by stimulation of G-protein coupled receptors that activate Ca release from internal stores. The resulting rise in intracellular [Ca] is biphasic: an initial transient peak is followed by a prolonged plateau that requires extracellular Ca. In non-excitable cells, the plateau phase can be supported by Ca-permeable conductances activated by store depletion (store-operated currents; SOC). We investigated whether excitable cells possess an SOC and if this pathway can influence exocytosis. Isolated bovine adrenal chromaffin cells were recorded with perforated-patch voltage-clamp methods and exocytosis was assayed with capacitance detection techniques as changes in cell surface area.

Depletion of internal Ca stores with thapsigargin (Tg), a SERCA pump inhibitor, activated a small inward current at negative potentials ("I_{Tg}"). I_{Tg} was carried by both Ca and Na ions. A similar current was activated when high concentrations of a Ca chelator were included in the pipette solution in ruptured patch recordings. I_{Tg} activated and inactivated over several minutes and was accompanied by a concurrent rise and fall in intracellular Ca as measured with Ca-sensitive fluorescent dyes. Activation of I_{Tg} triggered an increase in cell-surface area in the absence of depolarization that began with a clear delay, proceeded at a slow rate, but was extensive. In addition to triggering depolarization-independent exocytosis, I_{Tg} caused a strong facilitation of depolarization-evoked exocytosis. Thus in bovine chromaffin cells, SOC can support sufficient Ca influx to both trigger exocytosis at negative potentials and facilitate exocytosis evoked by voltage-gated Ca entry.

INTERACTIONS BETWEEN PRESYNAPTIC CALCIUM CHANNELS AND THE MOLECULAR MACHINERY OF EXOCYTOSIS.

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Synaptic release of neurotransmitters requires voltage-gated calcium entry. The short latency (0.2ms) between calcium influx and exocytosis suggests a close association between channels and release sites. The plasma membrane proteins syntaxin and SNAP-25 form a stable trimeric SNARE complex with the synaptic vesicle protein VAMP (synaptobrevin), which is thought to assemble at the vesicle / plasma membrane interface and may drive membrane fusion. Synaptotagmins, which are vesicle proteins that possess C2 domains, interact with this trimer to form the 7S complex, and may confer calcium-dependent regulation. Co-immunoprecipitation and binding assays with recombinant proteins indicated that P/Q type calcium channels are linked to the 7S complex via residues 780-969 located in the intracellular loop connecting homologous domains II and III of the channel alpha 1A (BI) subunit. Synaptotagmin binding to the pore-forming subunit of the P/Q-type channel may optimally locate the calcium-binding sites that initiate exocytosis within a zone of voltage-gated calcium entry. Immunofluorescent confocal microscopy at the frog neuromuscular junction indicated that these proteins are co-localized at active zones where transmitter release occurs. Induction of transmitter release with alpha-latrotoxin has been used to examine SNARE protein redistribution during recovery from massive exocytosis.

K+ CHANNELS IN THE BRAIN: FROM HYPEREXCITABILITY TO EXCITEMENT

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Voltage-gated potassium (Kv) channels of the Shaker related family are heterooligomeric complexes being assembled from membrane-spanning Kvα and auxiliary Kvß subunits. We have cloned the human genes for six Kvα subunits and for three Kvß subunits. Three of the Kvα subunits (KCNA1, 6, 5) are clustered at chromosome 12p13. Mutations in the KCNA1 open reading frame may be correlated with one form of ataxia, mutations in the KCNA5 gene with an acquired LQT syndrome. Kvß subunits may increase the surface expression of *Shaker* related Kv channels. In addition, Kvß1 and Kvß3 subunits may confer rapid inactivation on certain Kv channels. Thus, the activity of certain rapidly inactivating A-type Kv channels depends on the presence of Kvß1/Kvß3 subunits. We show by analyzing Kvß1 k.o. mutants that Kvß1-dependent A-type Kv channels contribute to action potential repolarization and frequency-dependent action potential broadening. Loss of Kvß1 function may lead to a decreased slow afterhyperpolarisation (sAHP) amplitude, probably because of an altered Ca^{2+}-homeostasis. The observed changes in learning and memory abilities of the Kvß1 k.o. mice may be correlated with the observed alterations in Ca^{2+}-homeostasis and sAHP-amplitude.

MOLECULAR IDENTIFICATION OF PACEMAKER ION CHANNELS

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Rhythmic activity of neurons and heart cells is endowed by pacemaker channels that are activated by hyperpolarization and directly regulated by cyclic nucleotides (termed HCN channels). These channels constitute a multigene family which belongs to the superfamily of voltage-gated potassium channels and cyclic nucleotide gated channels. The members of this channel family differ functionally in their activation kinetics and voltage range of activation and it is assumed that the properties of each member are adjusted to fit its particular function in the cell in which it resides. Here we report the molecular and functional characterization of a human subtype hHCN4. hHCN4 transcripts are expressed in brain but also in heart, and testis. Within the brain, the thalamus is the predominant area of hHCN4 expression. Heterologous expression of hHCN4 produces channels of unusually slow kinetics of activation and inactivation. The mean potential of half-maximal activation was $V_{1/2}$ = -75 mV. cAMP shifted $V_{1/2}$ by 11 mV to more positive values. The hHCN4 gene was mapped to chromosome band 15q24-q25. The characteristic expression pattern and the sluggish gating suggest that hHCN4 controls the rhythmic activity in both thalamocortical neurons and pacemaker cells of the heart.

SHAPING THE ACTIVITY OF A K^+ CHANNEL: MOLECULAR MECHANISMS AND THE INVOLVEMENT OF CYTOSKELETON

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Modulation of A-type voltage-gated K^+ channels can produce plastic changes in neuronal signaling. It was shown that the delayed-rectifier (noninactivating) Kv1.1 channel can be converted to A-type (fast inactivating) upon association with Kvβ1.1 subunits. We have identified processes leading to phosphorylation or dephosphorylation of Ser 446 catalyzed by protein kinase A or protein kinase C which modulate the extent of A-type conversion of Kv1.1/Kvβ1.1 channels. Further, we identified interactions of the channels with microfilaments that modulate the extent of A-type conversion; part of these interactions were determined to be via a post synaptic density (PSD-95) - like protein and are modulated by phosphorylation of Ser 446. Recently, modulation of Kv1.1/Kvβ1.1 inactivation by direct interaction of G protein βγ subunits with the channel protein has been identified by us. The mechanism suggested is that Gβ1γ2 directly affect the interaction between Kv1.1 and Kvβ1.1 during channel assembly which , in turn, disrupts the ability of the channel to interact with microfilaments, resulting in increased extent of A-type conversion. Underlying the partial nature of A-type inactivation of Kv1.1/Kvβ1.1 channels is a bimodal gating of these channels consisting of a noninactivating (delayed-rectifier -type) mode and an inactivating (A-type) mode. We argued that the modulation of the extent of macroscopic inactivation by the various factors described above is achieved by influencing the equilibrium between the two modes, and succeeded to demonstrate it with regard to phosphorylation.

2P DOMAIN K^+ CHANNELS : STRUCTURE, PHYSIOLOGICAL FUNCTIONS, PHARMACOLOGY AND THERAPEUTIC IMPLICATIONS

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We recently have cloned and expressed a new family of K^+channels comprising 4 transmembrane domains and 2P regions. These channels are non voltage-dependent background channels and are expressed in different tissues but are usually very abundant in the brain.

Two types of channels have a particular interest with respect to CNS function. The first one is TASK, a channel that strictly follows the Goldman equation and which is highly regulated by external pH (active at a physiological pH of 7.3, inactive at pH < 6.9-7). Changes of this channel activity probably play an important role in situations such as ischemia. The second type is TREK-1, a K^+ channel that is activated by arachidonic acid and polyunsaturated fatty acids. This channel is also highly mechano-sensitive and inhibited by intracellular increases of cAMP and by PKC. The molecular mechanisms by which the regulations take place will be discussed.

This class of channels is the target of volatile anaesthetics and of drugs that provide potent neuroprotection.

SEQUENTIAL SIGNALING EVENTS DURING ZEBRAFISH MIDBRAIN-HINDBRAIN BOUNDARY DEVELOPMENT

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We have studied development of the primordium of the midbrain-hindbrain boundary (MHB) in wildtype and in zebrafish mutant embryos for *no isthmus* (*noi*), *acerebellar* (*ace*) and *spiel-ohne-grenzen* (*spg*). *noi* alleles affect the *pax2.1* gene, and *acerebellar* affects the *fgf8* gene. *noi* mutant embryos lack the MHB constriction, the cerebellum, optic tectum and much of the tegmentum. The defect in *ace* mutant embryos is more restricted: MHB and cerebellum are absent, but a tectum is formed, although it is disturbed in its polarity. Using markers that are expressed during MHB formation (e.g. the *eng* genes, *wnt1* and *pax2.1)*, we find that the MHB region is misspecified in *noi* mutant embryos already during late gastrulation, because it fails to initiate eng gene expression properly. In contrast, expression of *wnt1*, *fgf8* and other genes is initially unaffected. As a consequence, we observe expansion of posterior forebrain fates into the midbrain primordium over time. MHB gene expression in *acerebellar/fgf8* mutants only becomes defective during somitogenesis stages, suggesting that *fgf8* functions to maintain activity of the MHB organizer and ultimately thus contributes to polarization of the midbrain. The results of our mutant studies show that multiple and sequential signaling events act in induction and subsequent development of the MHB territory, and are consistent with fine-structure-mapping of the relative expression domains through development, and of misexpression experiments.

Establishment of forebrain identities in the zebrafish neural plate.

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The organisation of the neural plate depends upon a complex cascade of signals during gastrulation. It was classically thought that the organiser initially induces neural tissue with anterior or forebrain character and that other signals subsequently posteriorise neural tissue in the trunk. More recent data are suggesting that the embryo may also have an anterio-posterior pre-pattern preceding gastrulation events.

We have previously shown that development of the zebrafish forebrain additionally depends upon a small group of ectodermal cells located in the prospective head region (row1). Removal of these cells during gastrulation perturbs subsequent forebrain neural patterning and differentiation, resulting in widespread brain death. Transplantation of row1 cells shows that they can induce, in the gastrula, forebrain specific gene expression in posterior regions of the neural plate. These results indicated that an early step in forebrain patterning is the establishment of a small population of signalling cells within the most anterior region of the embryo.

We are beginning to explore the nature of the signal(s) emitted by row1 and will present some data on candidate players in such signal(s)..

We also recently began to address the mechanisms controlling the establishment of the signalling territory. We will first assess the link between anterior neural plate boundary and establishment and/or maintainance of the signal. We will then present new evidence showing that one of the important players in forming row1 signalling centre comes from the extraembryonic yolk syncitial layer (YSL). Indeed, YSL injections of sense and anti-sense RNA coding for homeobox genes such as *hex* or *otx* have dramatic effects on the fate of the anterior neural plate. The rescue of the injection phenotypes by transplantation of WT row1 suggests that the YSL plays a critical role in establishing and/or maintaining the signalling function of the anterior row1 population. This YSL function depends most likely on specific expression of an homeobox protein from the otx gene family or a close relative.

REGIONALIZATION AND DIFFERENTIATION IN THE TELENCEPHALON

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The telencephalon consists of a pair of forebrain vesicles that develop from the rostrolateral part of the neural plate. Its pallial subdivision gives rise primarily to cortical structures, whereas its subpallial subdivision gives rise to the basal ganglia. I will review evidence for the location of patterning centers that contribute to regional specification and morphogenesis of the telencephalon. Next, I will describe the role of the *Nkx2.1* homeobox gene in specifying the fate of the pallidal part of the basal ganglia. I will conclude by describing the roles of the *Dlx* homeobox genes in regulating differentiation of subpallial projection neurons and pallial interneurons. It appears that regionalization is coupled to cell-type specification, such that gabaergic and cholinergic neurons are primarily produced in the basal ganglia. Gabaergic projection neurons remain in the basal ganglia, whereas many types of gabaergic interneurons tangentially migrate via at least two pathways to all regions of the cerebral cortex.

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PATTERN FORMATION AND THE ROLE OF *HOXB1* IN HINDBRAIN DEVELOPMENT

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Transient segmentation of the head during development is a conserved feature of vertebrate evolution. The hindbrain is subdivided into rhombomeres, while tissues adjacent to the neural tube are subdivided into a series of branchial arches. Each arch is innervated by a branchiomotor nerve that emerges from a specific rhombomere, and each branchial arch/rhombomere unit develops differently according to its position in the rostrocaudal sequence. *Hox* genes are expressed in a nested array in both rhombomeres and branchial arches, and the patterning of both structures can be changed by perturbing *Hox* expression. However, it is not clear whether *Hox* genes initiate a repertoire of regionally-specific developmental pathways, or whether, as in *Drosophila*, they provide a set of positional values which are then *interpreted* by segmental developmental programmes within each rhombomere and branchiomere. To answer this central question, we generated a mismatch in *Hox* gene coding between branchial arches and hindbrain neural tube by a combination of overexpression of *Hoxb1* and microsurgical grafting. Branchiomotor axonal projections were used to gauge the effect of targeted changes in *Hox* gene expression. We show that overexpression of a single Hox gene within a single rhombomere can induce the homeotic transformation of a defined population of neurons. This demonstrates an equivalence between Hox gene function in vertebrates and flies, in conferring positional identity on single elements of a repetitive ground plan.

NOTCH AND APP PROTEOLYTIC PROCESSING CONTROLLED BY PRESENILIN 1

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Presenilin 1 (PS1) is a multitransmembrane protein located in the endoplasmatic reticulum. Missense mutations of PS1 are an important cause of familial Alzheimer's disease. In contrast, homozygote PS1-/- mice die late in embryogenesis and display a severe growth retardation, which is most outspoken in the caudal region. In the brain a collapse of the subventricular zone, hemorrhagies and cortical dysplasia featuring leptomeningeal fibrosis and overmigration of cortical plate neurons into the marginal zone is observed, resembling human type 2 lissencephaly. This neuronal migration disorder is associated with the disappearance of the majority of Cajal-Retzius pioneer neurons from the developing cortical anlage. The deficient somitogenesis together with aberrant Notch immunostaining in the brain, suggest that presenilin 1 modulates the Notch signaling pathway in agreement with previously documented genetic interactions between Notch and PS1 homologues in *C. elegans*. The Notch pathway is involved in crucial cell fate decisions during development and requires ligand-induced cleavage of the Notch receptor for signal transduction. We identify this cleavage step as the specific biochemical deficit caused by the absence of PS1. We have previously demonstrated that PS1 also facilitates a proteolytic activity called γ-secretase that cleaves the integral membrane domain of Amyloid Precursor Protein (APP). Since γ-secretase inhibitors block also Notch processing, we suggest that related protease activities are responsible for cleavage within the predicted transmembrane domains of Notch and APP. While the PS have been implicated in many cell biological processes, we would like to propose that their primordial function is the (control of) proteolytic processing of a subset of integral membrane proteins. Disturbance of this function could explain the multiple effects of PS1 deficiency or PS1 overexpression in cell culture systems. Targeting PS1 function to lower amyloid peptide production in the brain of Alzheimer's Disease patients may therefore cause severe side effects.

CLINICAL ASPECTS OF ALZHEIMER'S DISEAE (AD): EARLY DIAGNOSIS AND BIOLOGICAL MARKERS

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Alzheimer's disease (AD) is a chronic disease both in clinical and preclinical aspects. The duration of the clinical phase of the disease, characterised by progressive cognitive decline is approximately 9 years from the occurence of first signs until death. It is assumed that the clinical phase is preceded by a 15 - 30 years preclinical period of continuous deposition of amyloid plaques and neurofibrillary tangles.

The development of causal treatments which are targeted to interfere with the early mechanisms occurring in AD requires that, first, the diagnostic standard is improved both in terms of early detection and accuracy and, second, that surrogate markers are available which would indicate the success of intervention in disease progression or halt.

Candidate biochemical markers of AD are cerebrospinal fluid (CSF) levels of Aβ(1-42) and Tau protein. Preliminary longitudinal studies suggest that concentration changes in AD patients depend on the stage of the disease: CSF levels of Tau seem to increase from mild to moderate AD, while CSF levels of Aβ(1-42) seem to be decreased in mild stages of the disease and the levels stay low throughout the further process of cognitive deterioration. Other candidate markers include proteins associated with proteolytic processing, such as cystatin C, and with neurotrophic systems, such as NGF and NT-3.

Further studies focus on the differentiation of age-related changes from the beginning AD pathology (sensitivity), and the separation from other neurodegenerative, neurological and psychiatric disorders (e.g. major depression) (specificity).

GENETIC AND ENVIRONMENTAL CLUES TO THE ETIOLOGY OF ALZHEIMER'S DISEASE THROUGH THE STUDY OF THE AMYLOID PRECURSOR PROTEIN

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The biogenesis of Aβ amyloid from the amyloid precursor protein (APP) plays a critical role in the causation of Alzheimer's disease (AD). There are many pathways which may affect the rate at which Aβ amyloid accumulates in the aging brain, some of which include:

- Increased synthesis of APP: a five- to six-fold elevation in the rate of APP synthesis in Down's syndrome and in transgenic mice appears sufficient to cause the characteristic lesions of AD.
- Alterations of α, β, and γ secretase activities: pending full characterization of these proteolytic activities, *in vitro* studies clearly demonstrate the feasibility of targetting the secretases. γ-secretase activity, which determines long (42) vs short (40) forms, may be a prime target. The presenilins may interact at this level.
- Mutations of APP in the proximity of the Aβ domain: critical point mutations which affect APP processing have now been demonstrated to increase the ratios of Aβ 42:40. The fully penetrant, dominant nature of these mutations indicates that environmental factors may have minimal effects in affected pedigrees.
- Processing pathways of APP: mechanisms as diverse as cholinergic agonists and cytoplasmic domain interacting proteins may affect APP targetting and release, and thereby affect the rate of p3/Aβ formation.
- Alternate splice products of APP and the balance between APP/APLP1,2: preliminary evidence suggests that some isoforms of APP [exon 7 (KPI) and exon 15] may have a greater or lesser propensity to yield amyloidogenic products. The ratios of APP:APLP 1, 2 may also prove relevant.
- Aggregation and the toxicity of Aβ amyloid: metal ions (Zn, Cu), redox conditions, pH, the conformation of Aβ (α-helix; β-sheet) and interacting proteins (ApoE, α2M, ApoJ) may determine the physical state of Aβ in which it is capable of inducing synaptic dysfunction and neurodegeneration.

These pathways present multiple independent mechanisms through which genetic and environmental factors conspire to produce AD. So far, no major environmental risk factor for AD has been discerned. The hope is that a clearer definition of the Aβ/APP pathway will guide us in the right direction.

ACUPOINTS STIMULATION–INDUCED ANALGESIA
——It involved in segmental and systemic controls

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It has been generally accepted that the pain and the nociceptive responses are powerfully controlled by acupuncture. The aim of the present study was to investigate relation between the acupoints in different stimulation intensity and segmental or systemic inhibition of nociceptive reflex.

1. Pain- relieving effects induced by ipsi- and contralateral acupoints in the human

The nociceptive flexion reflex (RIII reflex) elicited by sural nerve stimulation at random were studied in healthy volunteers. Electrical needling in 0.8 times threshold of RIII reflex at ipsilateral Zusanli acupoint(St36, located below the capitulum fibulae and lateral to the tibia) could elicit inhibitory effects of RIII reflex. By contrast, these stimuli in the contralateral St36 were totally ineffective. Electrical stimulation in 1~3 times threshold at bilateral St36 could all inhibit RIII reflex.

2. C-fiber reflex modulated by ipsi- and contralateral acupoints in the rat.

Electrical stimuli were delivered to the right sural nerve territory through a pair of needle electrodes. Electromyographic A δ and C-fiber reflex response were recorded in the ipsilateral biceps femoris muscle. Ipsilateral St36 stimuli in 0.8 times threshold of A δ -fiber reflex could result in an obvious inhibition of the C-fiber reflex, but these stimuli in the contralateral St36 were totally ineffective. Threshold of the C-fiber reflex electrical stimuli in the ipsi- and contralateral St36 could all inhibite this C-fiber reflex response. Lesioning C-fiber in left sciatic nerve with capsaicin, stimulation from the limb of the lesion could not elicit obviously inhibitory effects.

These results suggest that painful RIII reflex in human and C-fiber reflex in the experimental animals can be depressed by stimulation of weaker intensity than C-fiber in the local acupoint, also by stimulation of stronger intensity than C-fiber in the heteropic acupoint.

NEUROBIOLOGICAL CORRELATES OF ACUPUNCTURE POINTS AND MERIDIANS

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Acupuncture point (AP) sites relative to non-AP regions contain a greater density of free nerve endings (Ciszik et al, 1985), a larger number of cutaneous nerve branches, and a higher probability of a nerve-vessel bundle penetrating the fascia (Heine, 1988). Additional correlations with APs include motor points (Gunn et al, 1976), trigger points (Melzack et al, 1977), mast cells and gap junctions (Zheng et al, 1996). APs can also be localized by their bioelectrical properties. The pioneering studies of Niboyet in France and Nakatani in Japan were elegantly confirmed by Becker et al (1976). A 36-electrode square grid produced "topographical maps" with APs as the high points of individually contoured conductivity fields, while a wheel electrode rolled along a traditional meridian on the skin produced conductivity profiles with peaks corresponding to AP sites. The theory that APs are functionally linked along linear meridians is likely to have developed as a result of the propagating sensations reported by sensitive subjects when needled at single APs (Xie et al, 1996). Demonstrations of electrical coupling of successive APs along a meridian (Reichmanis et al, 1977), and of vectorial spread of the isotope technicium-99m along meridian-like paths following its injection into an AP (De Vernejoul et al, 1985; Kovacs et al, 1992) comprise evidence of peripherally-based meridians. An alternate explanation is suggested from reports of amputees who experience acupuncture-induced sensations propagating along meridians into phantom limbs (Xue, 1986). As with phantom limb pain, these sensations are likely to reflect central connectivity patterns in the spinal cord and brain. In this scheme, needling at a single AP would trigger the sequential firing of ascending and descending chains of dorsal horn spinal neurons connected via primary afferent axon collaterals and interneurons. Because of synaptic delays, the impulse from each successive neuron in the chain would reach the brain at a slightly later time, giving rise to the propagated sensation (Kendall, 1989). The meridian concept has also been explored with medical imaging techniques. Functional MRI has revealed spatially and temporally-summated signals in the occipital lobe in response to needling APs UB 61-67, on the lateral side of the foot, a traditional Chinese medicine treatment for eye disorders (Cho et al, 1998).

COMPARISON BETWEEN ACUPUNCTURE TREATMENT OF PAIN AND OPIATE ADDICTION

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The scientific basis of acupuncture-induce analgesia has been extensively studied in recent decades, and a consensus has been reached that acupuncture stimulation accelerates the production and release of opioid peptides in CNS to achieve an analgesic effect. It was natural to reason that the same work system may be used for treating other diseases, such as opiate addiction. Experiments in rats (Drug Alcoh Dependence 1993; 31:169) revealed that naloxone precipitated morphine withdrawal syndrome could be suppressed by electroacupuncture (EA) of either 2 Hz or 100 Hz, which accelerates the release of endorphin/enkephalin or dynorphin, respectively. This was then applied in heroin addicts using Han's acupoint nerve stimulator (HANS, "acupuncture without a needle") instead of EA. A striking similarity was found between the analgesic effect of EA and the opiate-withdrawal suppressive effect produced by HANS. Thus, the time course of a decrease of heart rate (tachycardia during drug withdrawal) forms a mirror image of the increase in pain threshold. In addition, the optimal frequency of electrical stimulation for analgesia was very similar to that for suppression of withdrawal, i.e., the dense-and disperse (DD) mode (2 Hz alternating with 100 Hz, each lasting for 3 s) was more effective than 2 Hz or 100 Hz constant frequency mode. In one study, buprenorphine (BPN) was used in combination with HANS for heroin detoxification in an attempt to fully prevent the opiate abstinence syndrome. Mock HANS (applying skin electrodes without electric output) was used for control (n=14 in each group). BPN was needed only in the first 5 days in HANS group as compared to 14 days in control group, and the total dose of BPN needed was only 7.8% of that needed in control group ($p<0.001$). The relapse rate was significantly lower in HANS group compared to control, i.e., 71% versus 100% in 2 months, and 78% vs. 100% in 3 months of time after discharge from the detoxification center ($p<0.05$). It is concluded that acupuncture like stimulation is effective to reduce opiate withdrawal syndrome and to help lowering relapse rate, presumably via activation of the body's own endorphin system to combat the craving for exogenous opiates (supported by the Climbing Project, Ministry of Science and Technology)

THE DIFFERENCE BETWEEN ELECTROACUPUNCTURE(EA) ONLY AND EA WITH MANIPULATION ON ANALGESIA IN RATS

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Plain acupuncture uses manipulation (rotation or varying the depth of insertion of the needle) to increase its effect. However, in electroacupunture (EA), manipulation has not been used. This study was performed to investigate the possibility of an increase in analgesic effect by adding manipulation to EA. Tail-flick latency (TFL) was used as a pain index in the rat, which was lightly anesthetized with thiopental sodium (i.p.). Rotation and varying the depth of the needle (RN and VN) was employed using two different types of manipulation during each 20 min stimulation of EA. Each manipulation persisted for 1 min out of every 5 min (long - duration and long - interval : LDLI) or 12 sec every 1 min (short - duration and short - interval : SDSI). EA produced an increase in TFL ; peak value was 49.7 ± 12.2 % of the pre - EA and occured immediately after cessation of 20 min of stimulation EA. Performing RN or VN combined with EA also increased TFL more than EA and a greater peak increase in TFL was observed with a SDSI - RN and SDSI - VN as compared to a LDLI - RN and LDLI - VN (77.5 ± 13.8 %, 79.2 ± 19.8 % and 67.3 ± 14.0 %, 65.6 ± 23.7 % of the pre - EA respectively). These results indicate that manipulation combined with EA produces a more potent antinociception than EA only does.

VAGAL PATHWAYS TO THE HEART

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In all species vagal stimulation reduces both the heart rate and force of atrial contraction. In amphibians the pathway involves transmission through parasympathetic ganglia, located within the heart. At each ganglion cell one and occasionally two pre-ganglionic fibres form synapses. one of these release sufficient acetylcholine, ACh, to invariably initiate action potentials in the post-synaptic cell body. Individual post-ganglionic axons branch, giving rise to many terminal varicosities. These again release ACh which activates muscarinic receptors and slow the heart rate. It has been assumed that vagally released ACh, like added ACh, activates a potassium conductance, g_K, a response readily blocked by barium ions, Ba^{2+}. Together these observations suggest that parasympathetic pathways lack sophistication, the ganglia have little integrative function and ACh simply diffuses through the heart.
In the guinea-pig heart, cardiac ganglia are more complex. A proportion of ganglion cells receive a vagal input which invariably releases sufficient ACh to trigger an action potential in the post-ganglionic axon. However they also receive many sub-threshold inputs, some of which originate in the heart itself. Other ganglion cells fail to receive a detectable input from the vagus, rather they receive many inputs which also appear to arise from within the heart. Other cells are devoid of synaptic inputs, suggesting that they have a sensory function. In the cardiac pacemaker cells. ACh released by the post-ganglionic fibres activates muscarinic receptors which trigger inhibitory responses that are not blocked by Ba^{2+}.
Together these observations suggest that considerable integration occurs in this parasympathetic pathway and that post-ganglionic axons target restricted regions of the heart.

INTERACTIONS BETWEEN INTRINSIC PRIMARY AFFERENT NEURONS RESPONSIVE TO MUSCLE TENSION AND LUMENAL CHEMISTRY IN THE CONTROL OF INTESTINAL MOTILITY

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The network of neurons contained within the wall of the intestine, the enteric nervous system, contains all the neural elements needed for the control of the movements of this organ. These include intrinsic primary afferent neurons (IPANs), ascending and descending interneurons, excitatory and inhibitory motor neurons innervating the circular muscle and motor neurons that innervate the longitudinal muscle. Over the last 15 years, we have been able to identify the electrophysiological, neurochemical and morphological properties of all of these neuronal subtypes. We have recently shown that the myenteric plexus contains IPANs sensitive to the contractile state of the muscle and others that are sensitive to chemicals applied to the mucosa. In the guinea-pig small intestine, these neurons are all AH-neurons, with large cell bodies and several axons and most are immunoreactive for calbindin, choline acetyltransferase and substance P. These neurons interact via slow excitatory synaptic potentials mediated by NK3 tachykinin, and possibly also via NK1, receptors. This suggests that activation of the mucosal chemoreceptors can modify the activity of the stretch-sensitive IPANs that are known to mediate motility reflexes in the intestine. Furthermore, we have shown that mucosal mechanoreceptors can enhance reflexes evoked by distension. The interactions of the IPANs indicate that the composition of the lumenal content can modify intestinal motor patterns that are normally thought to depend solely on the volume of this content.

ENTERIC CIRCUITRY IN THE STOMACH

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The enteric nervous system (ENS) in the stomach plays a crucial role in regulating gastric functions. In the stomach enteric circuits innnervating various targets are located in the myenteric plexus. Here we describe their projections, neurochemical coding and neuropharmacology. Polarised projections consisting of ascending cholinergic and descending nitrergic pathways were found for circular and longitudinal muscle motor neurones (CMN and LMN), for mucosa neurones (MucN) and interneurones (IN); ascending pathways dominated. The coding was target specific in that neurones containing ENK and/or SP were muscle neurones. Interestingly, the detailed coding of LMN and CMN did not differ indicating that muscle neurones have similar coding. NPY/VIP containing cholinergic and nitrergic neurones and purely cholinergic neurones projected to the mucosa. Some interneurones contained 5-HT or calbindin. Additional target specific projection preferences were observed: LMN and IN had longitudinal, CMN had crcumferential and MucN rather radial projections. Independent of their targets ascending neurones are activated by 5-HT3 and descending neurones by 5-HT1p receptors. Noradrenaline inhibited acetylcholine release mainly at synapses activating excitatory neurones. Both inhibitory and excitatory neurones received nicotinic input from vagal fibers. The results suggest that the ENS in the stomach contains the necessary components to control muscle and mucosa functions. They consist of ascending and descending pathways with target specific neurochemical coding and projections. The pathways can be selectively activated through different serotonin receptors. (Support: DFG)

NEUROTRANSMISSION IN THE GALLBLADDER.

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The neurons in gallbladder ganglia play a critical role in the coordination of bile storage and bile flow. Although these neurons share a common ancestry with the neurons of the gut tube, the neurobiology of this organ is uniquely adapted to carry out its functions. Gallbladder ganglia consist of small clusters of neurons, located in the outer aspect of the muscularis. Unlike those in the gut tube, all gallbladder neurons are cholinergic, but subsets of these also express various complements of neuropeptides and nitric oxide synthase (NOS) in species-specific patterns. Gallbladder neurons appear to be driven by vagal preganglionic activity since they not spontaneously active, and they all receive nicotinic fast synaptic input from the vagal terminals. The vagal efferent terminals are an important target of regulatory input to the gallbladder since cholecystokinin (CCK) can facilitate nicotinic input at physiological concentrations, and norepinephrine as well as prostaglandin E_2 and histamine can suppress the fast synaptic events. Extrinsic sensory fibers can also modulate ganglionic transmission in the gallbladder by releasing tachykinins and calcitonin gene-related peptide which depolarize gallbladder neurons and increase their excitability. In conclusion, though not as complicated as their sibling ganglia in the bowel, gallbladder ganglia are sites of synaptic, neuroendocrine, and neuroimmune modulation.

PREJUNCTIONAL MODULATORY MECHANISMS AT CHOLINERGIC TERMINALS IN THE URINARY BLADDER

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In most tissues activation of prejunctional receptors (M_2 muscarinic and α_2 adrenergic) inhibits neurotransmitter release. However in the rat urinary bladder prejunctional muscarinic and α-adrenergic receptors facilitate the release of acetylcholine (ACh). The present study was undertaken to examine the mechanisms underlying prejunctional facilitation in the rat urinary bladder. The release of radiolabeled ACh in urinary bladder strips during electrical field stimulation was influenced by the frequency, pattern and duration of stimulation. Continuous stimulation (10-20 Hz, 5-70 shocks) elicited a 10-20 fold greater release than intermittent stimulation (10 shocks every 5 sec) at the same frequency. The facilitation of release was blocked by atropine or pirenzepine, an M_1 muscarinic antagonist. Eserine, an anticholinesterase agent, also facilitated release (4-10 fold increase). The facilitation was mimicked by application of phorbol dibutyrate (0.5 μM), an activator of protein kinase C (PKC) and was inhibited by H7 (50 μM), a PKC blocker. Nifedipine (1 μM), an L-type Ca^{+2} channel antagonist, blocked facilitated release but did not alter nonfacilitated release of ACh. Omega conotoxin (100 nM), an agent that blocks N-type Ca^{+2} channels did not alter the facilitation of release. Prejunctional muscarinic facilitation of ACh release was also detected in human bladder strips. Phenylephrine (1-10 μM), an α_1 adrenergic agonist, facilitated ACh release and the contractions of bladder strips evoked by electrical field stimulation. The facilitation was antagonized by selective α_1 adrenergic antagonists (5-methyl urapidil or trazosin). It is concluded that prejunctional facilitatory M_1 receptors on cholinergic terminals in the urinary bladder could be involved in homosynaptic as well as heterosynaptic modulation of transmitter release. The facilitatory M_1 muscarinic mechanism is dependent upon a PKC mediated second messenger pathway and influx of Ca^{+2} into the parasympathetic nerve terminals via L-type Ca^{+2} channels. The latter channels seem to be involved exclusively in facilitated release of ACh . Prejunctional facilitatory mechanisms represent new targets for pharmacological treatment of bladder hyperactivity.

PHANTOM PAIN: A PERIPHERAL DISORDER WITH CENTRAL MANIFESTATIONS

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Amputation is a radical example of a chronic neuropathic pain condition with complete peripheral denervation of a body part. Pain is a common sequelae to such denervation and is seen in approximately 70% of amputees irrespective of lesion site, body part, gender or age. Pain may also occur following amputation other than limbs such as breast, viscera, eyes and teeth. The phantom limb experience has all the elements of a cortical organised phenomenon: mechanical and temperature sensation, shape, movement, texture and size Recent experimental work in animals has suggested that cortical reorganization is a common consequence of the amputation of a body part. Evidence from neuromagnetic and neuroelectric source imaging shows that the extent of cortical reorganisation following amputation in humans is closely related to the amount of phantom limb pain experienced by these patients.
Despite the clear cortical manifestations there are also indications that these phenomena originate in the periphery: Severe phantom pain is related to severe stump pain; blockade of sensory input from the stump eliminates phantoms; percussion of the stump elicits phantoms; chemical stimulation of stumps elicits pain; sensibility of stumps is related to the presence of phantom pain; microneurograhic activity elicited from stumps correlates with experienced activity; wind-up like pain from the stump and experienced pain are related and blocked by NMDA antagonists.
Taken together the findings are compatible with the notion that phantom pain is a peripheral disorder with central manifestations.

COMPARING THE RETINOTOPY OF VISUAL LOSS IN MACAQUES AND HUMANS

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Early visual cortical areas in macaques and humans, e.g. V1, are precisely retinotopic, as shown by the mapping of physiological receptive fields in macaques and by fMRI mapping in humans. In macaques, retinotopy decreases in precision in extrastriate visual areas and then disappears completely in cortical areas MST and TE. The purpose of this study was to begin to describe, in macaques and humans, the extent to which visual loss is retinotopic following lesions of different cortical areas.

When lesions were placed in cortical areas V1, V2, or V4 of macaques, the nature of the loss ranged from blindness after V1 lesions to loss of only complex abilities after V4 lesions. However, in all cases, the loss was precisely retinotopic. More recent studies have examined the visual loss resulting from unilateral TEO lesions in macaques, and this loss is subtle, but still retinotopic. In humans, cortical lesions in area V1 also resulted in dense, retinotopic loss. A more anterior lesion in the fusiform gyrus caused only partial visual loss, but the loss remained retinotopic, affecting only one quadrant of the visual field. On the other hand, unilateral temporal lobectomies resulted in loss that was general across the visual field. Such comparisons of the retinotopy of visual loss in humans and macaques help clarify parallels in the organization of primate cortical visual streams.

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RAPID EVOLUTION OF SHAPE-SELECTIVE ACTIVATION IN HIGH-ORDER HUMAN VISUAL AREAS AND ITS CORRELATION WITH PERFORMANCE.

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Recent fMRI studies of human visual cortex revealed a region located on the lateral-ventral aspect of the occipital lobe (termed the LO complex, LOC) which is preferentially activated by images of complex natural objects compared to a variety of noise and texture stimuli. However, the temporal evolution of this shape selectivity is presently unknown. The fMRI signal has a time constant (4-6s) which is far too slow to directly approach this question. To circumvent this low temporal resolution we employed a backward masking paradigm in which pictures of objects (man-made, animals, faces) were briefly flashed (exposure duration: 40, 120, 500ms) followed by a noise masking pattern. Object selective activation was detected in the LOC extending ventrally along the fusiform gyrus and dorsally into the intra-parietal sulcus (IPS). The fMRI signal in LOC showed a highly non-linear relationship to the duration of object presentation. Thus, a four fold decrease in presentation time (from 500 to 120ms) produced only slight signal reduction (to 89±2% SEM), but shortening the presentation to 40ms resulted in a substantial decrement (to 37±3%). This non-linear function was correlated to recognition rate - i.e. the ability of subjects to correctly name the briefly presented objects. To explore more directly the correlation between fMRI signal and recognition, we modified the recognition rate while maintaining identical visual stimuli. This was accomplished by training subjects to recognize the same set of briefly (40ms) presented images. Training significantly improved subjects' recognition rate of the trained objects. fMRI scanning revealed that LOC showed a concomitant signal increase when the trained images were presented. These results indicate that: A. Human cortical shape selective activation evolves rapidly (in~40 ms). B. Training enhances this activation in a stimulus- specific manner. C. The fMRI signal in LOC is tightly correlated to conscious recognition rate.

STRATEGIES OF SERIAL MEMORY: BEHAVIORAL, NEURONAL AND COMPUTATIONAL PERSPECTIVES

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Behavioral aspects: Previous studies have shown that monkeys are able to recall the serial order of a list of learned items. To discern what are the non-verbal strategies used in such a memory test, we trained two macaque monkeys on a novel delayed sequence recall task. Thirty images, divided into 10 triplets, were presented in fixed temporal order. On each trial the monkeys viewed three sequentially presented visual "sample" stimuli, followed by a "test" stimulus, consisting of the same 3 images together with a distractor stimulus (chosen randomly from the remaining 27 images in the set).
The monkeys' task was to touch the three stimuli in the order of their prior presentation, without touching the distractor. Initially, choice errors occurred mainly when the distractor had the same ordinal number as the correct choice image. Thus, the monkeys' natural strategy for recalling ordinal number sequences was retrieval of ordinal categories from long term memory. Working memory and recall of associations between triplet members were utilized as additional secondary strategies at later stages.
Neuronal and computational aspects: A population of neurons in inferotemporal (IT) cortex maintains its stimulus selective response during the delay period between sample and test stimuli. This activity could act as a neural trace of working memory. When training is done with stimuli presented in fixed order, the set of visual stimuli that generate delay activity in the same IT neuron often includes a group of neighboring stimuli. This overlap may serve as a long-term memory association. We found that stimulus specific sustained activity following a specific test stimulus persists across the inter-trial-interval, until the next trial's sample stimulus. This could serve as the necessary link to generate associations between neighboring stimuli, by modification of synaptic structure between network members.

MEMORY FOR MOTION: THE ROLE OF CORTICAL AREAS MT/MST

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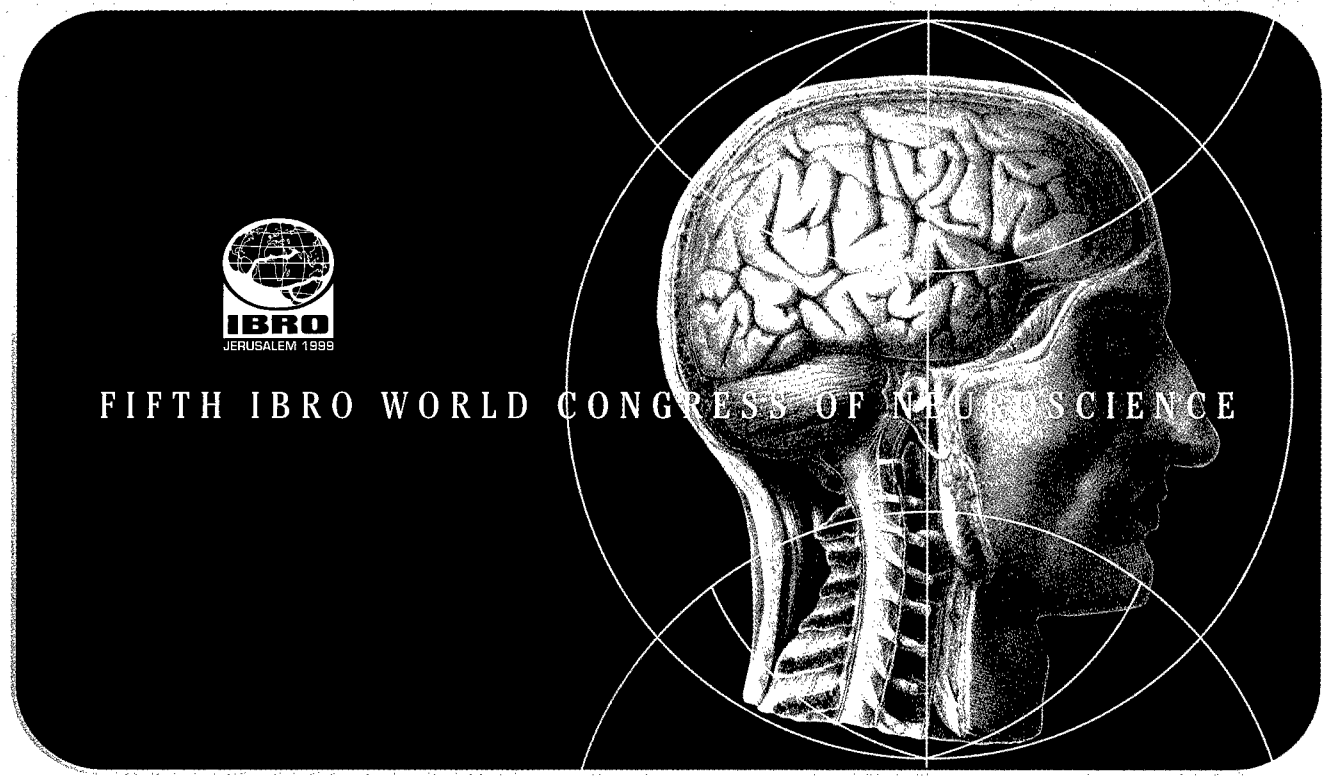
Match-to-sample tasks, commonly used to study short-term retention of visual information, consist of two comparison stimuli, sample and test, separated by a temporal delay. To examine the separate contribution of MT/MST to the main components of this task: encoding (sample), retention (delay) and retrieval/comparison (test), we separated the spatial location of sample and test stimuli during a single trial, placing one in the lesioned field and one in the intact field. Two macaques with unilateral lesions of MT/MST were tested with random-dots on two types of direction discrimination. In one, direction difference thresholds were measured with coherently moving random-dots. In the other, motion integration was measured by broadening the direction distribution in either the sample or the test (direction range thresholds). For the accuracy task, the deficits were found only when the test was in the lesioned field, irrespective of the location of the sample or the length of the delay. In contrast, the loss in direction range thresholds was present only when the non-coherent stimulus, sample or test, was in the lesioned field. This deficit only increased at the longer delay when the non-coherent stimulus was presented as the sample, and not when it was presented as the test. Our results show that encoding of motion direction from coherently moving dots can be performed without MT/MST, but these areas do play a role in integrating motion direction. Moreover, it appears that mechanisms involved in encoding direction of motion may also play a role in retaining that information. Finally, our results show that MT/MST may play a role in the comparison of directions when accurate discrimination is required. Thus, in some tasks, MT/MST is involved in encoding and retention of motion signals while in other tasks it plays a role in retrieval and/or comparison of recently stored directional information.



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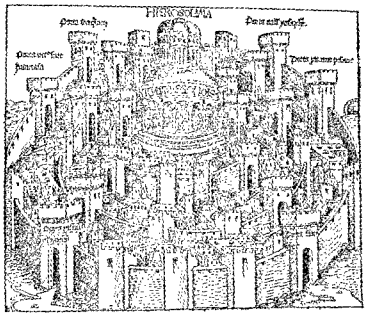

VASOPRESSIN- AND TYROSINE HYDROXYLASE-EXPRESSING MAGNOCELLULAR NEURONS UNDER CHRONIC OSMOTIC STIMULATION IN RATS

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This semi-quantitative immunocytochemical and in situ hybridization study has evaluated the dynamic of vasopressin (VP) and tyrosine hydroxylase (TH) synthesis in the VP cell bodies of the supraoptic nucleus (SON) as well as of the VP and TH turnover in the distal axons in the posterior lobe (PL) of adult Wistar rats drinking 2% NaCl for 1, 2 and 3 weeks. The number of VP neurons increased significantly over the 2nd week of osmotic stimulation, probably, due to the onset of VP synthesis by VP neurons keeping ¾silence¾ (non-synthetized VP) under normal conditions or by the oxytocin neurons. In contrast to VP neurons, there were only few TH neurons in intact rats, but their number increased permanently for three weeks of salt-loading. The synthesis of both VP and TH mRNAs highly enhanced over the first week of salt-loading remaining at the same level for two subsequent weeks. Nevertheless, the dynamic of the intracellular concentration of either peptide was quite different. The TH concentration in the cell bodies as well as in the large axon swellings, Herring bodies, increased gradually over the whole period of osmotic stimulation showing that the rate of TH synthesis prevailed that of TH degradation. In contrast to TH, the VP intracellular concentration decreased gradually in cell bodies and axons for three weeks of osmotic stimulation though there was a tendency to reach the dynamic equilibrium by the 3rd week of salt-loading. It means that the rate of VP release always prevailed that of VP synthesis except the third week of osmotic stimulation. Thus, the chronic activation of the VP system triggered a number of the adaptive mechanisms such as: the onset of the VP synthesis by the neurons which do not synthetize VP under normal conditions, the activation of TH synthesis and its intracellular accumulation, activation VP synthesis up to its dynamic equilibrium with the intensified VP release.

DOES THE HIPPOCAMPAL COMMISSURE PLAY A ROLE IN GUIDING THE CROSSING OF DEVELOPING CALLOSAL AXONS?

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The temporal sequence of effects of prenatal ^{60}Co irradiation on the development of the corpus callosum (CC) and cerebral cortex was studied in Swiss mice. Pregnant females on gestational day 16 were exposed to total doses of 2 or 3Gy. The offspring were analyzed at prenatal and postnatal ages (2Gy-irradiated=29 animals, 3Gy-irradiated=49 animals, normal=50 animals). One day after irradiation, a great number of pyknotic figures was seen in the cerebral wall, especially in the proliferative zones (PZ). At perinatal ages, the thickness of the PZ was reduced and the glial sling was never identified. From five days after birth onwards, we observed a severe shrinkage of neocortical layers II+III and IV. The majority of the irradiated mice was totally acallosal (particularly when the 3Gy dose was used), but some animals presented callosal remnants. These remnants were identified above the ventral hippocampal commissure (VHC), except for two animals in which a larger remnant extended from the columns of the fornix to the dorsal hippocampal commissure (DHC). Callosal remnants were observed in animals irradiated with 3Gy and analyzed at perinatal ages, but never in older animals. Callosal defects can be explained at least by three factors: 1) Death of layer III callosal neurons. This is supported by the presence of pyknosis in the cerebral wall and the neocortical hypotrophy. 2) Postnatal axonal elimination. 3) Absence of the glial sling. Callosal agenesis in the absence of the sling indicates that this structure may play a crucial role in guiding callosal axons. However, the presence of callosal remnants indicates that surviving callosal axons can use structures other than the sling to cross the midline. We suggest that axons of the middle portion of the CC cross the midline using the VHC as a guide. Additionally, the DHC may play a role in directing axons of the posterior part of the CC.

A POSSIBLE INTERACTION BETWEEN OF CHOLECYSTOKINERGIC AND GABAERGIC SYSTEM.

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Previous work from our laboratory demostrated that the aminoacidergic system was affect for the administration of CCK-8S (Cholecystokinin sulphated octapeptide) (Acosta, Gen Pharmacol 31(4):637-641.1998) and the magnitude of these effects were dose-dependent (Acosta and Rubio, APPTLA 47(4) Supp1:52. 1997).

The purpose of the present study was to evaluate the participation a selective CCK_B receptor antagonist on the endogenous levels of GABA in defined areas of the rat brain.

We used male Wistar rats (180-200 g) maintained in a 12-12 light dark cycle with free access to food and water. The animals were divided into four groups: 1- Control group was received vehicle i.p. 2- CCK treated group was received 10 nmol/kg i.p. of CCK-8S. 3- PD treated group was received PD 135,158 1mg/kg i.p. 4- CCK + PD group was injected with CCK-8S 10 nmol/kg i.p. plus PD 135,158 1mg/kg i.p. Thirty min after drug administration the animals were killed by decapitation. The brain was removed, the cerebral frontal cortex (CF), the corpus striatum (CE), the olfactory bulbs (OB), the hippocampus (HIC) and the hypothalamus (HYP) were dissected out. The endogenous GABA concentration were measured by the method of Lindgren et al (1982).

The administration of CCK increased the endogenous levels of GABA by 29% ($p< 0.05$) in the CE and 27% ($p<0.05$) in the OB.

While the selective CCK_B receptor antagonist alone did not affect the endogenous content of GABA, the administration of PD plus CCK prevented in both tissues the increment in the GABA levels induced by the agonist.

In the HYP, HIC and CF did not observe changes after the administration of CCK, PD or to the injection of CCK+PD.

These results shows that PD 135,158 did not have effect per se but prevented the increase induced by the agonist, indicate that the action of CCK may be mediated via selective action on CCK_B receptor subtype.

VISUALIZING LIVING GABAERGIC NEURONS EXPRESSING GREEN FLUORESCENT PROTEIN

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The goal of this study was to develop a reliable method to visualize GABAergic neurons in living brain slices, for electrophysiological and morphological studies. Our strategy was to transfect brain slices with a green fluorescent protein (GFP) gene driven by the glutamic acid decarboxylase (GAD) 67 promoter. Two DNA constructs, which contained 3.5 or 8.7 kb of the GAD67 promoter region and 1.7 kb from the 5' end of the transcript, were fused in-frame to the eGFP reporter gene (Clontech). Brain slices, 300 μm thick, were prepared from neonatal mice (newborn to 8 days old) and cultured on Millicell membranes as described (Stoppini et al, 1991). After 1-7 days in culture, the Biorad gene gun was used to bombard slices with gold particles coated with one of the DNA constructs. Slices were cultured for an additional 3-14 days and then imaged using a Zeiss LSM510 confocal microscope. Neurons expressing GFP were brightly fluorescent, and could often be visualized with their entire dendritic and axonal arbors. Fluorescent neurons were observed in a variety of brain structures, including neocortex, hippocampus, septal nuclei, thalamus, and cerebellum, and the great majority exhibited morphologies typical of GABAergic neurons of the same brain region. Further characterization of the GFP expressing neurons is under way.

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QUANTUM THEORY OF BRAIN AND NEW INTRACELLULAR METHOD OF TREATMENT OF SCHIZOPHRENIA AND OTHER MENTAL DISORDERS

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Theoretical research carried on for many years makes it possible to come out with a new concept, i.e. quantum theory of brain. Brain organization of psychical activity represents four-dimensional spatial and temporal continuum. In accordance with the principal laws of quantum mechanics the brain structure is subject to E.Schrodinger's wave equation "molecule=solid=crystal". Functional activity of the brain represents proton-electron system of conductivity. Proton-electron conductivity determines space configuration of each separate macromolecule and the brain as a whole. The brain is charachterized by discrete quantities of energy called energy levels. Transition from one condition to another, from one configuration to another is called a quantum jump. The probability of such jump is determined by an expectation time. Proceeding from the fundamentals of quantum theory of brain we've worked out special complex of preparations united by a single principle, i.e. intracellular influence on energy condition of a macromolecule and the brain as a whole. Aiming at approbation of the new intracellular method of energy influence, a group of mental patients (250 persons) at the age of 18 to 70 (165 men, 95 women) was selected. The first group (100 persons) consisted of mental patients suffering from schizophrenia, schizophrenia like psychoses, reactive states and neuroses. The second group (150 persons) consisted of patients suffering from psychotic and non-psychotic states after undergoing neurosurgical operations on the brain (tumours, injuries, bullet wounds). The results of treatment proved high positive effect. In the first group 78% of the patients were completely cured and 22% of the patients were cured with incomplete remission. In the second group 62% of the patients became practically healthy and 38% of the patients had residual effects after undergoing surgical operations. The catamnesis examination (average duration is 2.7 years) of the whole group of the patients proved positive result of the new method of treatment.

BASOLATERAL AMYGDALA AND UNDERWATER TRAUMA MODULATION OF HIPPOCAMPAL SYNAPTIC PLASTICITY

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In a previous work we found that a 30-sec underwater trauma (UWT), following 8 days of training for a spatial memory task in the water maze, resulted in poor performance in the spatial memory task at both 1 hour and 3 weeks after the trauma.
Here, we found that compared to naive animals and animals that were trained for the spatial learning task, but were not traumatized, the traumatized rats showed a reduced level of dentate gyrus (DG) long-term potentiation (LTP) 30 minutes post high-frequency stimulation (HFS) to the perforant path (PP). Thus, the UWT has short-term effects that resemble those observed in other models of stress.
In another experiment, We examined the effects of priming of the basolateral group of the amygdala (the BLA) on LTP in the DG. Compared to the control group, which received just HFS to the PP, priming the BLA resulted in an enhanced LTP in the DG 30, 90, 150 and 180 minutes post-HFS.
Since the amygdala mediates responses to aversive situations, we examined the effects of the UWT on the BLA priming ability to enhance the level of potentiation in the DG. We found that the UWT blocked the facilitatory effects of BLA priming on synaptic plasticity. These results suggest that the effects of the UWT on hippocampal synaptic plasticity are mediated via the amygdala.

UNSPECIFIC FUNCTIONAL COUPLING OF NEURONS DURING EPILEPTIC CONDITIONS (BUCCAL GANGLIA, HELIX POMATIA)

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The epileptogenic drug pentylenetetrazol induces synchronized paroxysmal depolarization shifts (PDS) in the small B3-network of neurons (buccal ganglia, Helix pomatia). With lasting epileptiform activity, neurons not coupled to the B3-network under physiologic conditions show depolarizations of augmenting amplitudes following each PDS in the B3-network. The nature of these augmenting responses (AR) is studied presently.
Neuronal activities were recorded simultaneously in several identified giant neurons with intracellular microelectrodes. 40 mM of pentylenetetrazol were applied. The effects of verapamil (40 µM), carbamazepin (10 mg/l) and variations of $[Ca^{2+}]_o$ on AR were tested.
PDS in the efferent neurons B3 (axons run to the kidney) induced AR in the efferent neurons B1, B2 and B4 (axons run to oesophagus, salivary glands and pharyngeal muscles, respectively). Latency of AR was: ca 5s, duration: ca. 1min, amplitude: up to 15mV. AR were triggered from the beginning of PDS and they were independent from duration or termination of PDS. Amplitude of AR increased during several hours of epileptiform activity and they could trigger PDS in neurons B1 and B2. Verapamil and carbamazepin and a reduction of $[Ca^{2+}]_o$ reduced amplitudes of AR.
AR represent a horizontal spread of activity that is thought not to result from spatial buffering of $[K^+]_o$ via glia but from unspecific neuronal secretion which is induced from the epileptic depolarization in combination with the increased intracellular $[Ca^{2+}]$.

THE ROLE OF THE FRONTAL AREAS OF THE LIMBIC CORTEX IN REGULATION OF ACTIVITY OF VISCERO-SENSORY NEURONS OF VAGO-SOLITARY COMPLEX.

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In cats anesthetized with mixture of chloralose and nembutal and immobilized with ditilin was studied the influence of different functional areas of frontal limbic cortex on activity of viscero-sensory neurons of solitary tract nucleus (NTS) identified by vagal nerve stimulation. It was shown that 68,3% of primary and secondary vago-sensitive neurons of NTS responded with evoked discharges to electrical stimulation of anterior dorsal area of limbic cortex. In case of stimulation of ventral area responded 50% of vagal-solitary neurons. High frequency stimulation of dorsal area suppressed spontaneous activity of the majority of investigated neurons, whereas similar stimulation of ventral field of anterior limbic cortex is less effective. Study of character of interaction of limbic and vagal signals to paired stimuli revealed that effect of conditioning downward discharge of dorsal field of anterior cingular gyrus in most cases suppressed vagal discharges during interstimuli interval of 10 - 500 ms, while conditioning stimuli of ventral area blocked the vagal test response in more short intervals (50 - 150 ms). Mechanisms of descending limbic cortical regulation of activity of bulbar vagal viscero-sensory neurons are discussed.

Identification of a silencer element in the regulatory region of glutamine synthetase that restricts gene induction by glucocorticoids to neural tissues

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Glutamine synthetase (GS) is a key enzyme in the recycling of the neurotransmitter glutamate. It is expressed in most cell types at a relatively low level, but in neural tissues the expression of GS is particularly high. Expression of GS is regulated by glucocorticoids which induce a high level of GS expression in neural tissues only. This is in spite of the fact that non-neuronal cells express functional glucocorticoid receptor molecules that can stimulate the transcription of other target genes. Sequencing and functional analyses of the regulatory region of the GS gene identified at a position upstream to the glucocorticoid response element (GRE) a 21bp element that can markedly repress the induction of gene transcription by glucocorticoids and can confer repression on a heterologous GRE promoter in a cell-type specific manner. Repression of gene induction in non-neuronal cells is several fold higher than in neuronal or glial cells. The repressive activity of the silencer element is orientation- and position- independent in respect to the transcriptional start site, but appears to require proximal location to GRE. Gel-shift assays revealed the presence of a GS-silencer element- binding activity in non-neuronal cell extracts but not in neuronal or glial cell extracts. This binding activity is also abundant in early embryonic retina and declines progressively with age. Our results suggest that this element, which shares a considerable functional and structural homology with the neural restrictive silencer element (NRSE), might be involved in repression of GS induction in non-neuronal tissues and in prevention of precocious expression of GS in early embryonic retina.

CHANGE OF EXCITABILITY OF HIPPOCAMPAL NEURONS UNDER IMPART OF AMYGDALOFUGAL CONNECTIONS

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So far not only the question of the role of the septum andintrahippocampal connections in hippocampal theta rhythm formation, but as well the pecularities of subcortical structures participation in this process remain unclear. In present warle in attempt to clarity the involvement of different hipothalamus nuclei in theta rhythm regulation mechanisms, the important role of amygdalo-hipothalamic connections in hippocampal functional state formation was revealed. The experiments showed that application both reversible (novocain) and irreversible (electrocoagulation) methods of damage of functional integrity of the dorsal amygdalofugal pathway, in contrast to ventral one, brought to disappearance of not only theta rhythm but the hippocampal EEG as a whole. The similar remits were accepted in pulse activity registration of CA3 area neurones. It was observed that in course of the amygdalofugal connections blockade with novocain the frequencies of neuronal pulse activity gradually decreased to complete disappearance of action potential generation. Testing of hippocampal activity by electrostimulation of septum or hippocampus itself did not lead to EEG recovery. The hippocampal activity recovered only under the influence of strychnine and carbocholine, while serotonin or noradrenaline were without any effect. It were supposed that under the damage of the dorsal amygdalofugal pathway the hippocampal total metabolism was disturbed that is probably related to the hypothalamo-hypophysal system activity.

MECHANISMS OF STRIATAL INHIBITION OF JAW OPEN REFLEX.

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In recent years some studies have suggest that striatum participates in pain processes. It is consistented with the fact that this nucleus receives nociceptive afferents from the orofacial and other areas of the body. In addition, it is the central nervous structure with the highest number of opiate receptors and endogenous opiodes content. Moreover, many Parkinson patients complain for pain attributed to dopamine deficiency in the striatum. The present experiments were designed to extend previous studies by exploring the role of the sensory nucleus of the trigeminal nerve in the mechanisms of analgesia induced by stimulation of the striatum. In rats anesthetized with urethane (1.2 g/kg, ip) painful responses were evoked by stimulation of the tooth pulp of lower incisors. Single unit activity was recorded by mean of glass microelectrode, in the nucleos caudal of the trigeminal nerve. Spontaneous and evoked activity to dental nociceptive stimulation was recorded, while the striatum was stimulated with a microinjection of glutamate (163 nmol/0.5 µl). Only neurons of the caudal nucleus responding to dental pulp were analyzed. The 43% (n=13) of neurons respond with 2 peak of excitation, while the 37% (n=11) with one excitation. The rest, include 4 neuron of the border between caudal nucleus and bulbar reticular formation and 2 within the nucleus, which responses are complex. This consists in inhibition followed by a long excitation or various peaks of activation. The 59% of the total were nociceptive specific and the 41% wide dynamic range.There was not significant difference in the latency to the first peak (20,2 ± 4.2 ms. vs. 33.2 ± 7.6 ms.) between groups, the same as durations (47 ± 7.3 ms. vs 63.2 ± 15 ms.). The second peak had a latency of 90 ± 6.6 ms. and a duration of 81.6 ± 14.7 ms. (n=13). The activity in the peaks increase significatly from the baseline of 1.89 ± 0.5 Hz to 28.03 ± 6.7 Hz ($P<0.001$, n=24) by noxious stimulation of dental pulp. The above results are consistented with the activation of Aδ, the first peak, and C fibers, the second peak.The chemical stimulation of the striatum in the sites in which electrical activation produce an inhibition of the jaw open reflex, inhibited all the neurons responses to dental stimulation in the caudal nucleus. The frequency activity of the neurons was decreased by 55.64 ± 3.49 % of control values (responses before striatal stimulation, $P<0.001$, n=24).This effect coincides with the inhibition of the jaw open reflex. Our results support the interpretation that the antinociceptive effect of the striatun on dental pain is due to inhibition of the second order neurons in the caudal nucleus of the trigeminal nerve.

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DYNAMIC LOCALIZATION OF EQUIVALENT CURRENT DIPOLE (ECD) OF ALPHA RHYTHM

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The aim of the study was testing of the scanning hypothesis: in experiments with 12 healthy subjects we tried to find a shift in localization of equivalent current dipole (ECD) of alpha rhythm in the visual cortex. Dynamic localization (with 2 ms step) of the ECD of alpha rhythm was studied using one-dipole 3-layerd spherical head model and MRI. Under flicker stimulation through closed lids with frequency of individual alpha rhythm all subjects perceived clear illusory visual objects (ring, spiral, grid). The probability of ring and spiral illusion was highest at dominant alpha frequency. Ten typical trajectories of the travelling alpha waves on scalp were revealed and interrelation between occipito-frontal trajectory and illusions of ring and spiral was found. The ECD was localized typically in *fissure Calcarina* and during single alpha wave shifted along it, while dipole's moment revealed fan-like rotation mainly in sagittal and horizontal planes. Changing localization of the ECD in the region corresponding to area 17 evidence that alpha wave reflects the spreading process, confirming the scanning hypothesis that is still under discussion.

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TRANSMISSION STUDY OF TWO EXONIC POLYMORPHISMS IN THE PERIPHERAL MYELIN P2 GENE

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Major protein zero (MPZ), peripheral myelin protein 22 (PMP22), connexin 32 (Cx32) and protein 2 (P2) are the major proteins of peripheral myelin. Mutations in MPZ, PMP22 or Cx32 genes have been involved in the most common inherited peripheral neuropathy: the Charcot-Marie-Tooth disease (CMT). One subtype of CMT: CMT4A has been linked to chromosome 8. Moreover, the human P2 gene has been mapped to 8q21. This result identifies P2 gene as a candidate for CMT. We demonstrated two polymorphisms in exons 2 and 4 (E2P and E4P) of P2 gene using the single strand conformation polymorphism technique. Sequencing revealed that E2P consisted in a GAA to GAG transition of the codon 61 and created a Bgl II restriction site. E4P consisted in a C to T transition at the twelfth base after stop codon. Presence of E2P was tested on 65 controls by PCR followed with Bgl II digestion: the wild allele showed a frequency of 0.82. E4P was screened by mismatch enhanced allele specific amplification where the penultimate base of the forward primer was deliberatly uncomplementary while the last base was complementary either to the wild or mutant sequence: the wild allele showed a frequency of 0.55. The polymorphism information content has respectively a value of 0.25 and 0.37 for E2P and E4P. Vertical transmission of the two polymorphisms was observed in French families demonstrating a classical mendelian inheritance. Because P2 gene is located on chromosome 8, the two described polymorphisms may help to use this gene as a candidate for familial CMT4A or sporadic CMT cases.

SEROTONINERGIC SYSTEM OF BRAIN AND MIGRAINE

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Lately an interest in serotonin concoeption of migraine has risen again in connection with the availability of a new class of antimigraine drugs - selective agonists of 5HT1 receptors.Having this in mind we attempted to study efficacy of amino-acid tryptophan - ptecursor of 5HT and methysergide - antagonist of 5HT on 42 patients with migraine.The patients received the drugs on appearance of precursors of the fit or at the beginning of the fit's commencement.Group I of 27 patients received tryptophan 6.0 g and group II of 15 patients were administered methysergide 4.5 mg.Mainly female patients had 10-12 years duration of the disease.In the majority of the patients the fits appeared for the first time at the age of 14-17 years.The frequency of the fits was 1 in 2-3 months and the average duration - up to 24-48 hrs. All of them underwent CT,MRI and ultrasound check-ups.Group I - in 19 patients the fits stopped 1-2 hrs after tryptophan had been given,this was followed by a considerable concentration of 5HT in the blood(3.01 ± 0.15 μmol/l,the norm - 0.81 ± 0.25 μmol/l;$P<0.001$) and 5-OIAA in the daily urine (39.8 ± 2.76 μmol/d,the norm - 26.15 ± 3.4 μmol/d; $P<0.001$).In group II only 3 patients had their fit stopped and then only 4-5 hrs after methysergide administration.The obtained data allow us to conclude that in arresting migraine fits it is not antagonist of serotonin to lower it but on the contrary it is the substance that promotes increase in serotoninergic mediation.

LAYER 4 NEURONS OF THE MOUSE BARREL CORTEX EXPRESS LOW-CONDUCTANCE NMDA CHANNELS WITH REDUCED SENSITIVITY TO Mg^{2+}

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We previously showed that excitatory interaction between spiny stellate (SS) neurons in Layer 4 of the adult mouse barrel cortex is largely mediated by NMDA receptors with distinctive voltage-dependence (Neuron 21: 1055-65, 1998). Here, we compared NMDA single channel properties and NMDA-R mediated synaptic currents in Layer 4 neurons (tangential slices) with those of Layer 5 neurons (coronal slices). Whole cell recording from SS cells in slices bathed in 2 mM Mg^{2+} revealed prominent DNQX-resistant, APV-sensitive, slowly decaying spontaneous EPSCs at –70 mV. Single NMDA channel currents induced by glutamate applied to outside-out patches excised from the same neurons were predominantly of the low-conductance class (main conductance, 30 pS; subconductance, 19 pS) and displayed reduced sensitivity to Mg^{2+}. Channels with these properties were also seen in cell-attached recordings from Layer 4 cells, when glutamate was included in the pipette solution. By contrast, in Layer 5 neurons, no activity was recorded at negative membrane potential in the presence of DNQX. Single NMDA channels in patches from these cells were of the high-conductance class (main conductance, 50 pS; subconductance, 40 pS) and showed "classical" Mg^{2+} sensitivity. These data are consistent with the hypothesis that the distinctive characteristics of NMDA-R mediated synaptic transmission in Layer 4 cells reflect their expression of the ε3 (NR2C) subunit. Supported by a grant from the French-Israeli Association for Scientific and Technological Research (AFIRST).

HIGH RESOLUTION TIME – FREQUENCY ANALYSIS OF EEG DURING VOLUNTARY MOVEMENT

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It has been shown that planning and execution of movement are connected with EEG desynchronization (ERD) in □ band occurring in motor area and synchronization in other brain areas, followed by synchronization in □ and □ bands after movement. The obstacle in better recognition of these phenomena was inadequate resolution of methods used so far: FFT and bandpass filtering. We applied a method based on Matching Pursuit algorithm, which may be considered a generalization of wavelet analysis. It relies on adaptive decomposition of the signal into waveforms from a very large dictionary of functions well localized in time and frequency. The method allows for description of the signal structures by means of: time localization, time span, frequency and amplitude. In Fig.1 the time-frequency evolution of the EEG energy is shown during voluntary finger movement. One can distinguish two □ components. Their existence was suggested earlier, but the methods applied so far did not allow for their separation. We have estimated for 10 electrodes, placed over motor area, the ERD and ERS time evolution for □1, □2 and □ band. The starting point of ERD for □2 was observed earlier in contralateral hemisphere. For □1 ERD occurred usually later and was more pronounced on ipsilateral side. ERS for □ band occurred earlier than for □. High resolution of the method opens new perspectives for brain – computer interface.
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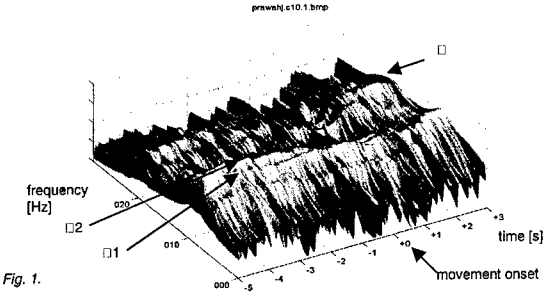

Fig. 1.

EFFECT OF NEW NOOTROPIC DIPEPTIDE ON TRANSCALLOSAL EVOKED POTENTIAL

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IIn the numerous electrophysiological studies stimulation of the point on the cortex was found to produce biphasic positive-negative potentials (transcallosal evoked potentials: TEP) at the symmetrical surface of the cortex of the opposite hemisphere. Origin of these waves is associated with the changes in synaptic functions at different cortex layers and synaptic process modifications are known to influence learning and memory. Thus, TEP are unique potentials for studying brain functions which are related to learning and memory imprint. Since nootropics are to improve these functions it is interesting to investigate the effect of new dipeptide with nootropic properties on TEP. Male white rats (300-400g) were anaesthetised with ether, electrodes for stimulation and recording were placed on dura mater in symmetrical holes in the skull over cortex precentral area of both hemispheres and indifferent electrodes were pricked in the head muscles. The TEP were recorded monopolarly after electrical stimulation 26-29 V, duration 0.1 msec. The averaged potentials (after 32 stimulations) were recorded every 30 min after the i.p. administration of the drugs. Piracetam (150-250 mg/kg) increased the total amplitude of TEP in 70% cases. The latency of this effect was about 60 min, maximum came in 90-120 min and after some time effect of the drug slowly decreased to normal level withing 3 h. Piracetam considerably increased the amplitude of the positive-wave but the negative-wave increasing was very small. New nootropic dipeptide GVS-111 (ethyl ester N-phenyl-acetyl-L-prolyl-glycine) in dose 0.5 mg/kg increased total TEP amplitude in 80% cases. The latency of this effect was 15-30 min, maximum came in 60 min and amplitude stayed at this level more than 4 h. GVS-111 considerably increased the amplitude of the both waves and moreover its influence on the negative-wave was larger than on positive one. Revealed advantages of GVS-111 effect on TEP compared to piracetam correlate with its more expressed action on learning and memory which have been obtained in behavioural tests. This correlation indicates that one of the possible brain mechanisms of GVS-111 cognitive enhancing effect is facilitation of interhemispheric transfer.

AUDITORY STREAM SEGREGRATION AND ATTENTIONAL FOCUSING IN SCHIZOPHRENICS

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Schizophrenics and Controls are compared in a procedure that estimates: 1) temporal discrimination, 2) ability to create, and focus attention on auditory streams. Tasks involve detection of a temporal irregularity in one of two isochronous tone sequences (forced choice between a regular and irregular sequence). To ensure equivalent difficulty among subjects, individual thresholds allowing easy detection of the irregularity are measured in 1) a simple sequence, 2) a complex sequence composed of three simultaneous subsequences (each subsequence has distinct tempo and frequency tone; one of the subsequence contains the temporal irregularity).
Step 1: Simple sequences at each one of five specific tempi and frequencies, are tested.
Step 2: Four complex sequences are tested. Attention is drawn towards the irregular subsequence by preceding the complex sequences by the target subsequence (cue).
Step 3: idem with no cue.
Thresholds are higher in schizophrenics for both simple and complex sequences.
Step 1: Schizophrenics' detections are lower than Controls' at all tested tempi.
Step 2: Detection is better at medium-range and slow tempi for Controls, not for Schizophrenics.
Step 3: At all except high tempi, cues improve detection for Controls, not for schizophrenics.
Thus, besides the alteration of temporal discrimination, the predicted dysfunction of top-down processes is observed. Stream segregation and attentional focusing are less efficient in Schizophrenics, illustrating impaired use of contextual information.

TEST OF DICHOTOMY LISTENING IN DYSLEXIC CHILDREN

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The estimate laterality of speech abilities by dichotic listening techniques has lately attracted attention of neuropychological studies. We used dichotic listening test to investigate auditive asymetry of dyslexic children and children who had no speech problems.

We tested 452 children who were of chronological age 8,9, 10 and 11 years old. We formed the experimental group consisting of 35 children (25 boys and 10 girls) who had reading problems the children from the control group in number sex and age. The control group consisted of children who had no speech problems. After the examination of hearing threshold (by tonal audiometry) the examines listened in two strages to the verbal stimulus material after the dichotomy model in the combination V/V and V/C, using KSAFA tehnical apparatus.

Statistical model ANOVA was used in the statistical processing of results.

The results point to the following :

1. On the test of dichotomy listening V/V :
   a. dyslexic children sow REA in 23%, LEAin 40% and NEA in 37%;
   b. children without speech-language disorders show REA in 69%, LEA in 29% and NEA in 3%.
2. On the test of dichotomy listening V/C :
   a. dyslexic children show REA in 9% LEA in 34% and NEA in 0%,

MATERNAL DEPRIVATION INDUCES CHANGES OF SPINE AND SHAFT SYNAPSES IN THE ANTERIOR CINGULATE CORTEX

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Maternal deprivation has been shown to result in alterations of behavior, learning capacities and brain metabolism. Deprived animals display bizarre stereotypies and asocial behavior, which bear some resemblance to affective disorders in humans. These similarities of behavioral symptoms may be associated with abnormalities of brain function, and thus animals that have experienced maternal separation may resemble psychotics behaviorally as well as neuroanatomically. We hypothesize that the emotional bond between the newborn and the mother is essential for the development of synaptic connections and the functional maturation of brain circuits. To test this hypothesis we studied the influence of maternal deprivation on the development of synaptic architecture of layer III pyramidal neurons in the anterior cingulate cortex (ACd) of the precocious rodent Octodon degus. Densities of spine synapses on the apical and basal dendrites of layer III pyramidal cells in the ACd were quantified light- and electron microscopically. Three experimental groups were compared at postnatal days 14 and 45: I) Pups which were repeatedly separated from their mothers (three times/day for one hour); II) pups which were handled accordingly but without separation and III) pups which remained undisturbed in their family. Spine densities on the basal and apical dendrites of the pyramidal cells in isolated pups were significantly higher compared to handled controls. On the electron microscopic level no difference of overall synaptic density was found between the three experimental conditions. However, when synaptic profiles were divided according to their postsynaptic target, dendritic shaft and spine, significant differences were found for both synaptic subtypes. Isolated animals displayed significantly more spine synapses, but less shaft synapses in comparison to the other two experimental groups. Our results confirm our hypothesis that early traumatic experience leads to abnormal synaptic connections of medial prefrontal cortex. Since the ACd is involved in family-related behavior such as nursing, audiovisual communication and play and appears to play a role in the initiation, motivation and goal-directed behavior, the deprivation induced synaptic changes may reflect alterations of socio-emotional affiliation and learning capabilities.

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MATERNAL DEPRIVATION INDUCES REDUCTION OF NADPH-DIAPHORASE REACTIVE NEURONS IN THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS

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We postulate that early stressful experience, such as maternal separation, represents a psychological trauma that leads to biological priming and neurological "scars" in limbic circuits, which underlie the behavioral and cognitive deficits that are found in adulthood. In order to evaluate a possible involvement of the presumptive retrograde transmitter nitric oxide (NO) in emotional learning, To test this hypothesis we studied the influence of early postnatal socio-emotional deprivation on the development of NADPH-diaphorase reactive neurons in prefrontal cortical areas and in subdivisions of the n. accumbens in the precocious rodent *Octodon degus*. 45 day old degus from two experimental groups were compared: 1) degus which were repeatedly separated from their mothers (three times/day for one hour from postnatal day 1 (P1) until P21, and thereafter reared in complete isolation; and 2) degus which were reared under normal social conditions. Socially deprived animals displayed a significant decrease of NADPH-diaphorase containing neurons in anterior cingulate cortex, the same tendency was observed in the infralimbic, precentral medial and prelimbic prefrontal areas. Similarly, the core region of nucleus accumbens expressed reduced NADPH-diaphorase-reactive neuron numbers in deprived animals, whereas the shell region remained unchanged. Since during normal postnatal development the number of NADPH-diaphorase reactive neurons gradually decreases in all prefrontal cortical and accumbal regions, the observed deprivation-induced changes may reflect either an excessive reduction of NADPH-diaphorase positive neurons or a downregulation of the enzyme in neurons that normally express it. Our results indicate a yet to determine link between early adverse socio-emotional experience and the maturation of NADPH-reactive neurons, which may be related to findings in schizophrenic patients, where significant alterations of NADPH-d-reactive neurons were reported (Akbarian et al., Arch. Gen. Psychiatry, **50,** 169-177, 1993).
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UPREGULATION OF D1-RECEPTORS IN THE LIMBIC SYSTEM AFTER BRIEF MATERNAL SEPARATION IS SUPPRESSED BY MATERNAL VOCALIZATIONS

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EXPERIENCE DURING EARLY PHASES OF LIFE, IN PARTICULAR TRAUMATIC EMOTIONAL EXPERIENCE SUCH AS MATERNAL SEPARATION, APPEARS TO BE IMPORTANT IN SHAPING AN INDIVIDUAL'S RESPONSIVENESS AND BEHAVIORAL STRATEGIES AT LATER STAGES OF LIFE. WE SPECULATE THAT SUCH TRAUMATIC EVENTS INTERFERE WITH THE STRUCTURAL AND FUNCTIONAL MATURATION OF LIMBIC BRAIN CIRCUITS. TO TEST THIS HYPOTHESIS WE INVESTIGATED I) CHANGES IN THE DENSITY OF DOPAMINERGIC D1-RECEPTORS AFTER REPEATED BRIEF MATERNAL SEPARATION AND EXPOSURE TO A NOVEL ENVIRONMENT AND II) THE INFLUENCE OF A LEARNED, POSITIVELY ASSOCIATED EMOTIONAL VOCAL SIGNAL, THE MATERNAL CALL (1,2), ON D1 RECEPTOR REGULATION. EXPLORATORY ACTIVITY WAS TESTED IN A) THE "CLASSICAL" OPEN FIELD TEST, I.E. WITHOUT PRESENTATION OF ANY STIMULI, AND B) "ENRICHED" OPEN FIELD, DURING WHICH MATERNAL CALLS WERE PRESENTED TO THE PUPS. D1 RECEPTOR DENSITY WAS ANALYZED IN DIFFERENT SUBREGIONS OF THE MEDIAL PREFRONTAL CORTEX, HIPPOCAMPUS AND AMYGDALA USING THE LIGAND [^{3}H]SCH23390. OUR RESULTS SHOW I) THAT REPEATED BRIEF MATERNAL SEPARATION AND EXPOSURE TO AN UNFAMILIAR ENVIRONMENT INDUCES AN UPREGULATION OF D1 RECEPTOR DENSITY IN THE HIPPOCAMPAL CA1 REGION, AMYGDALAR NUCLEI AND THE MEDIAL PREFRONTAL CORTEX, BUT NOT IN CA3 OF HIPPOCAMPUS, AND II) THAT THE PRESENTATION OF MATERNAL CALLS DURING MATERNAL SEPARATION COUNTERREGULATES THIS EFFECT. THE ACOUSTIC PRESENCE OF THE MOTHER REDUCED THE EXPLORATORY ACTIVITY DURING OPEN FIELD TESTS AND IT SIGNIFICANTLY SUPPRESSED D1 RECEPTOR UPREGULATION THE MEDIAL PREFRONTAL CORTEX, BUT NOT IN THE OTHER BRAIN AREAS. THESE RESULTS INDICATE THAT VOCAL COMMUNICATION, WHICH IS AN ESSENTIAL PART FOR THE ESTABLISHMENT AND MAINTENANCE OF THE INFANT-MOTHER ATTACHMENT IS USED TO PROVIDE THE NEWBORN WITH EMOTIONAL INPUT THAT REDUCES STRESS DURING EXPOSURE TO AN UNFAMILIAR ENVIRONMENT. FURTHERMORE, THE MERE ACOUSTIC PRESENCE OF THE MOTHER IS SUFFICIENT TO REGULATE EXPERIENCE-INDUCED CHANGES OF D1-RECEPTOR DENSITIES.
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(1) POEGGEL, G., BRAUN, K. (1996) BRAIN RES. 743, 162-170
(2) Braun S., Scheich H. (1997) J. Comp. Physiol. A 181, 697-709

AUDITORY AND SOMATOSENSORY ACTIVITY IN THE "VISUAL" CORTEX OF THE ANOPHTHALMIC MUTANT MOUSE

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We are interested in the factors involved in the development and specialization of cortical areas. The anophthalmic mutant mouse (ZRDCT-An) provides an excellent model for the study of the role of the sensory periphery on cortical development. In this mouse eyes and optic nerves fail to develop and therefore the lateral geniculate nucleus (LGN) never receives any retinal afferents. Incomplete penetrance of the mutation produces microphthalmic mice that may serve as controls because they develop eyes with small optic nerves, which reach LGN. We used combined electrophysiological recording and neuroanatomical tracing techniques in five anophthalmic and one microphthalmic adult mice to examine the functional organization and thalamic connectivity of the sensory cortex. Animals were anaesthetized and the neuronal activity was recorded in response to somatosensory, visual or acoustic stimulation at a number of closely spaced sites in the occipital, temporal and parietal cortices. Throughout the occipital cortex of the anophthalmic mice multiunit responses to auditory clicks could be recorded. In more medial parts of the occipital area, responses to somatosensory stimulation of the trunk were recorded in addition to the auditory ones. In the microphthalmic mouse, visual responses were recorded in the corresponding areas; auditory and somatosensory responses were limited to the temporal and parietal areas respectively. After we determined the stimulus preference for numerous neurons, fluorescent tracer (fluororuby) was injected into the occipital cortex at sites with known physiological properties. From serial coronal sections the distribution of back-labeled thalamic cells was reconstructed using *camera lucida*. The occipital injection in anophthalmic mice yielded back-labeled cells in the dorsal LGN. The microphthalmic mouse had a similar labeling pattern in LGN from corresponding sites of injections. These results suggest a subcortical component of the reorganization. Somatosensory input from the dorsal column nuclei to the dorsal LGN has been described previously (*Asanuma and Stanfield, Neurosci, 39:533, 1990*). In the anophthalmic mice we were able to trace auditory fibers within the dorsal LGN by injecting the inferior colliculus with the anterograde tracer dextran-biotin. We continue to explore the possible thalamocortical component of the functional re-arrangement of visual cortex by using single unit recording and small tracer injections into the occipital cortex. The anophthalmic mutant mouse seems to present a compensatory development of the non-visual sensory systems comparable to that found in the naturally blind mole rat (*Heil et al., Neuroreport, 2:735, '91; Doron and Wollberg, Neuroreport, 5:2697, '94*). Supported by Swiss N.S.F. 31-39184.93.

THE EFFECTS OF HIPPOCAMPAL LESIONS ON THE REVERSAL OF PERFORMANCE IN PROBABILISTIC CHOICE.

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The purpose of this experiment was to investigate the memory processes involved in the reversal of discrimination learning and the role of hippocampus in these processes. Fourteen rats were rewarded with food for pressing bars in several forms of "two-armed bandit" situation (Davis and Staddon, 1990). The animals were submitted to a choice situation in four conditions: L (only left responses rewarded), R (only right responses were rewarded), N (extinction) and F (forced-alternation). After the training eight animals were submitted to a neurotoxic lesion in dentate gyrus of hippocampus, by intra-dentate injections of colchicine and six rats were submitted to a sham lesion. After the recovery from the surgery, the animals were retrained as in the pre-lesion trials. The pre- and post-lesion data were compared. The statistical analysis revealed no difference between the experimental and the sham groups. The post-lesion trials did not show effects of the lesion in dentate gyrus of hippocampus on reversal performance of rats submitted to choice situations. The results did not support the dynamic choice models. Lesion in dentate gyrus did not affect the reversal performance of rats in probabilistic choice perhaps because the task used involves an egocentric strategy that does not require hippocampal function.
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THYROID HORMONE AND RETINOIDS ALTER PHENOTYPE OF SPINAL MOTONEURON AFTER SCIATIC NERVE CUT.

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Thyroid hormone and retinoids are ligand-responsive transcription factors capable to enhance or repress the expression of target genes. Their role in phenotype regulation of neurons during adult life is well established. We investigated influence of long time (5 weeks) hypo-(PTU, 10mg/kg ip, once, then 500mg/l in drinking water), hyperthyroidism (thyroxine, 0.2 mg/day) and treatment with all-trans retinol palmitate (2 mg/day) on choline acetyl-transferase (ChAT) mRNA and neurotrophin low-affinity receptor (p75) expression in spinal motoneuron. We also challenged experimental animals by sciatic nerve cut, and then we studied ChAT mRNA 10 days, and p75 10, 25 and 40 days after lesion. ChAT mRNA level is higher in retinoid-treated rats (grain counting, $p<0.05$). The up-regulation of p75 induced by peripheral nerve lesion is delayed in retinoid-treated rats (cell count, $p<0.05$). p75 immunostaining is much stronger in hypothyroid rats, also in neurons not affected by lesion (sacral plexus). Sciatic nerve cut induced a slight, but significant decrease in ChAT mRNA level in the lumbar motoneuron in the lesioned side in control rats 10 days after lesion (grain counting, $p<0.05$). This decrease is comparable in control, hypo- and retinoid-treated rats, but it is not observed in hyperthyroid rats. These results indicate that thyroid hormone and retinoid regulate motoneuron phenotype and its adjustment after lesion in adult animals.
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MOTONEURON PHENOTYPE IS ALTERED DURING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS.

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The main pathophysiological feature of multiple sclerosis (MS) is demyelination. Thus, MS has been interpreted as oligodendrocyte disease. More recently, the possibility of a neural damage has been raised. Experimental allergic encephalomyelitis (EAE) is the most widely used experimental model for MS. We investigated choline acetyl-transferase (ChAT) mRNA regulation in spinal motoneurons during EAE. EAE was induced in female Lewis rats, by injecting guinea pig spinal cord tissue in complete Freund's adjuvant (CFA) to which heat-inactivated Mycobacterium was added. Rats injected with CFA and uninjected rats were used as controls. Animals were killed 12 and 20 days after sensitization or CFA, corresponding to the peak (12 days) and the resolution (20 days) of force defects. ChAT mRNA was studied by in situ hybridization in the lumbar spinal cord and a computerized grain counting procedure was used for quantification. No differences in ChAT mRNA level were found between control and CFA-injected rats. ChAT mRNA level was strongly reduced in EAE 12 days after immunization ($p<0.01$) and then it partially recovered in non-symptomatic animals (20 days post-immunization, $p<0.05$). These data indicate that synthesis enzyme for the main transmitter in spinal cord neurons is down-regulated during EAE, supporting the possibility of motoneuron damages. Whether ChAT mRNA decline is fully or partially reversible remains to be investigated.
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BEHAVIORAL DIFFERENCES BETWEEN ADULTS AND NEONATES MICE IN THE TAIL SUSPENSION TEST

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We have previously adapted the tail suspension test (TST) in order to study the ontogenesis of behavioral laterality. Here, we examined behavioral differences between newborn and adult mice in the TST. In this setup, the animal is held by the tail and hangs free from the ceiling of a circular chamber, held by the tail. The tests were performed under red light for 6 minutes and were videotaped. Twenty mice at P2 (second postnatal day) and 50 adults were tested in the TST. From the counts of the right (R) or left (L) trunk contraction, we analyzed the number of reversals of direction (RD); the total activity (R+L) and the difference between preferred and non-preferred side (net trunk contractions). The adults displayed a pronouncedly smaller net and a larger RD than the newborn animals. This result indicates that, differently from adults, newborn mice are more lateralized and have a greater tendency to persevere toward a particular side. We conclude that, the adult behavioral pattern is acquired during postnatal development. Further analyses on other postnatal days will indicate the critical period for behavioral change.

PHENOTYPIC KNOCKOUT OF NERVE GROWTH FACTOR IN TRANSGENIC MICE BY THE NEUROANTIBODY APPROACH

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Nerve growth factor (NGF) is a member of the family of neurotrophins that influences the differentiation, maintenance and regeneration of the central and peripheral nervous system. The functional role of NGF has been recently investigated in transgenic mice by gene knockout. However, the letal phenotype observed only allows their investigation at very early postnatal ages. In this study, we generated transgenic mice that express the neutralizing recombinant antibody against NGF (αD11), under the transcriptional control of the early promoter of human cytomegalovirus. Transgenic lines with distinct expression levels of the antibody chains were obtained. This determined the generation of: (i) one line showing a phenotype similar to that observed by gene knockout; (ii) a line that survived until adulthood. In adult mice, the transgenic antibodies are effective in competing with the endogenous NGF, as shown by the reduction of ChAT expression in the basal forebrain and by the decrease of cell size in superior cervical ganglia. Transgenic mice also showed an atrophy of longitudinal dorsal muscles and an increased apoptosis in the zona marginalis of the spleen. We conclude that this phenotypic knockout approach may provide an alternative to gene knockout by homologous recombination, allowing to study the effects of NGF deprivation *in vivo* during adulthood.

PHARMACOLOGICAL CHARACTERIZATION OF PE2I AS A SELECTIVE AND POTENT INHIBITOR OF THE NEURONAL DOPAMINE TRANSPORTER

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The membrane dopamine transporter (DAT) has major physiological roles in regulating neurotransmission processes through rapid removal of DA from the synaptic cleft back into the presynaptic nerve endings. It also mediates the pharmacological effects of drugs such as cocaine and amphetamine, and is very involved in neurospychiatric disorders such as Parkinson's disease. Its exploration is therefore highly valuable for understanding the mechanisms of action of several drugs and for the diagnosis and follow-up of various cerebral diseases. For this, one reliable approach uses radioactive probes in vitro or in vivo for scintigraphic investigations. In order to obtain a radioiodinated tracer possessing high selectivity for the DAT, we have recently developed several new iodinated derivatives of cocaine. The pharmacological properties of the most interesting compound, the (*E*)-N-(3-iodoprop-2-enyl)-2β-carbomethoxy -3β-(4'-methylphenyl) nortropane (PE2I) were evaluated in vitro in the rat. Binding experiments on striatal membranes showed that PE2I recognised selectively the DAT according to a single binding site model with high affinity (Kd of 4 nM, Bmax of 12 pmol/mg protein). In the cortical membranes the binding of PE2I was also selectively associated with the DAT (IC_{50} GBR 12909 = 6 nM vs more than 1000 nM for paroxetine), with similar affinity to that of the striatum. Autoradiographic experiments on brain sections with [^{125}I]PE2I were in agreement with the localization of the DAT. In addition, PE2I was shown to be a potent inhibitor of dopamine uptake, with similar IC_{50} values to GBR 12909 and β-CIT (2 to 6 nM). All these findings combined with previously published data, support the use of PE2I as a selective and potent tool to study the DAT both in vivo and in vitro. Moreover, PE2I could be used not only in the striatum but also in the frontal cortex where the density in DAT is poor.

CONDITIONING IMMEDIATELY IN NEURONAL POPULATIONS OF THE CORTEX

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In our previous investigations it was been established that conditioning developed in neuronal populations of the cortex as result of pair combinations of stimulations of subcortical structures. The purpose of the present study was to consider conditioning in analogous model at cortical stimulations in neurons of the intact cortex and of its neuronal isolated part.

Experimental investigations were carried out in the sensorimotor cortex of 25 rabbits. One of hemispheres was intact (95 neurons), an another hemisphere had a neuronal isolated cortex part (97 neurons).

It was found, that pair combinations of stimulations of two cortical points produced conditioning in neuronal populations of the cortex. Phenomena consisted in intensification of evoked responses of neurones on first of the combined stimuli and appearance of rearrangements of neuronal activity during omissions of the reinforcing stimulus. These activity rearrangements included reproduction of responses on stimuli (20 - 40% neurones) and activity changes which differed from them on base of patterns and usually developed in later terms (25 - 60% neurones). In the intact cortex the former of rearrangements predominated. The latter prevailed in neuronal isolated cortical part.

Thus, conditions for development of both discovered kinds of the conditioned alterations exist immediately in neuronal populations of the cortex. Nevertheless connections of the cortex with subcortical structures modulate conditioned rearrangements in neuronal activity.

IMMUNOABSORPTION OF THE ANTI-NSA-ANTIBODIES IN THE COMPLEX THERAPY OF PATIENTS SUFFERING FROM HYPER-TOXIC SCHIZOPHRENIA

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The autoimmunoaggression of antibodies (AB) against neurospecific antigens (NSA) at blood-brain barrier (BBB) dysfunction in pathogenesis of some neuropsychiatric diseases was demonstrated earlier. Take into consideration scientific dates about the application of the immunoabsorption procedure for immunoaggressive disease treatment we developed high selective immunosorbents (IA) to remove AB to following NSA α_1-BG, α_2-BG, α_2-GP, GFA and NSE from blood of patients suffering from hypertoxic schizophrenia. For this purpose, the highly purified NSA were immobilized on macro pore co-polymers of styrene and divinylbenzene. IA were characterized by the compatibility, specificity and high capacity for compounds to be absorbed. The quality of NSA immobilization on solid phase makes procedure of immunoabsorption (IAS) possible in a wide range of blood speed and regenerated a multiple. IAS was used for complex therapy of patients suffering from hypertoxic schizophrenia whose cerebro-spinal fluid (CSF) contained the anti-NSA-AB. The immunoabsorption procedure gave the possibility to remove from the blood up to 2 mg of anti-NSA-AB, at this time we didn't observe the "rebound"-phenomenon. The restoration of the original level of AB lasted for 3-4 days.

So, there is the basis to recommend the AB IAS for brain defense from AB aggression in complex therapy of patients suffering from hypertoxic schizophrenia.

THE CORTICONUCLEAR PROJECTIONS OF THE CEREBELLUM OF RAT ARE ARRANGED IN A BIDIMENSIONAL PATTERN

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The possibility that single sagittal bands of the cerebellar cortex (CC), as depicted by the inferior olive (IO) projections, projected to various regions of the cerebellar nuclei (CN) was tested in this study. With this aim, a mixed solution of 1:1 of FR and BDA was iontophoretically injected in various areas of the CC of rat. The cell bodies labeled by the FR and the BDA were studied in the IO and the fiber terminals labeled by the BDA were studied in the CN. Since the injections were randomly distributed in the CC, the selection of the rats for the analysis was based on the segregated labeling of cell bodies in regions of the IO related to single cortical bands. In these rats selected for the analysis the corticonuclear projections showed a diverging pattern. In each rat, one contingent of corticonuclear projections, here termed primary projections, was clearly more abundant than the other contingents (secondary projections). Moreover, the projections from the CC to the CN were arranged according to a bidimensional pattern. The mediolateral dimension was shown by the primary projections. In fact, projections were traced to single nuclear targets from the entire extension of the various sagittal bands. The anteroposterior dimension was shown by the changeable set of nuclear targets reached by the secondary projections traced from areas dislocated at different anteroposterior levels of single sagittal bands. These findings suggest that the functional units of the CC are a mosaic of areas (in the anteroposterior dimension) of the sagittal bands, because of the intrinsic homogeneity of their nuclear targets.

DYNAMICS OF CEREBELLAR NEURONAL DISCHARGES AFTER LOCUS COERULEUS STIMULATION IN RAT

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It is established that norepinephrine (NE), drugs which facilitate NE or stimulation of locus coeruleus (LC) are involved in recovery of function following brain injury. Moreover, the NE neurotransmission changes in cerebellum modulates functional recovery. The aim of this study was to determine different oscillatory components of Purkinje neuronal discharges after intensification of NE input. The experiments were performed on adult Wistar male rats. Surgical procedure was done under Nembutal anesthesia (Sigma, 40 mg/kg, i.p). The activity of individual Purkinje cells, recognized by occurrence of complex and simplex spikes, was recorded extracellularly within the cerebellar vermis before and 10 s, 5 min, 10 min and 15 min after short lasting (10 s) stimulation of the locus coeruleus. Analog signals in sequences up to 64 s each, were sampled by A/D converter at the rate of 30000 samples/s. Spectral analysis of neuronal discharge rate (spikes/bin, bin duration varied from 0.0019 to 0.0156 s) was obtained by Fast Fourier Transformation. Sequential power spectra of neuronal discharge rate at the sampling rate of 512, 256, 128 or 64/s were derived from 8-16 consecutive epochs of 4 s or 8 s. There were changes in the mean total power spectra, particularly in the spectra of the low frequency band up to 1 Hz (dominant frequency was at most between 0.125 - 0.5 Hz) of Purkinje neuronal discharge rates 10 s and 5 min after LC stimulation, while 10 min thereafter, the changes were less prominent with tendency of reaching the prestimulus values 15 min after cessation of LC stimulation. The appearance of the slow oscillations of neuronal discharge rates were not always in relation with reversible suppression of unitary discharges evoked by LC stimulation. Therefore, the dynamics of discharge pattern of Purkinje cells could be an important indicator of LC effects and may suggest cerebellar role in modulation of various regulatory systems in physiological and pathophysiological states caused by NE.

DECLINE IN BRAINSTEM AUDITORY-EVOKED POTENTIALS COINCIDES WITH LOSS OF SPIRAL GANGLION CELLS IN ARYLSULFATASE A DEFICIENT MICE

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Deficiency of arylsulfatse A (ASA; EC 3.1.6.8) causes the autosomal recessively inherited lysosomal storage disease, metachromatic leukodystrophy (MLD). We have described ASA deficient knockout mice, presenting histopathological, electrographic and behavioural alterations, reminiscent of the early stages of MLD. Brainstem auditory evoked potentials (BAEP) were recorded in control and ASA deficient mice of 3, 6, 9 and 12 months. Responses were readily evoked in control mice of all age groups studied. BAEP responses were completely absent in all ASA (-/-) mice of 9 and 12 months. In 6-month-old ASA (-/-) mice, a delay in the wave pattern was noted. The decline of the response in ASA (-/-) mice was paralleled by a decrease in spiral ganglion cell numbers. Histological examination and morphometric analysis showed that young ASA (-/-) mice were equipped with a fairly normal number of spiral ganglion cells. After the first three months of life the density of neurones declined rapidly. By the end of the first year a low level (approximately 9% of the density in the first three months) was reached which continued throughout the second year of life. Decline or loss of BAEP responses has been noted in MLD patients as well, and has been considered an early sign of the disorder. The mechanism of this loss of spiral ganglion cells remains to be investigated, and may be related to early degenerative processes in MLD patients. The characterization of spiral ganglion cell loss and concomitant BAEP decline could provide useful outcome measures for therapeutical experiments with this model.

EARLY SOMATOSENSORY EVOKED POTENTIALS CHANGES AS A RESULT OF HUMAN BRAIN REACTIVITY MODIFICATION

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Simultaneously recorded cortical (from both hemispheres) and brain stem early somatosensory evoked potentials (SEPs), induced by electrical transcutaneous stimulation of median nerve in the wrist, in two groups of subjects with brain reactivity modification were compared with SEPs in controls (26 healthy volunteers). The first group: 12 patients with the forebrain somatosensory afferent pathways lesions (and no cortical lesions) as a result of acute stroke (with computer tomography scane conformation of clinical diagnosis in all cases), endured from 8 to 24 months before the study. The second group: 16 healthy volunteers who had been practicing transcendental meditation (TM) (the specific psychological training tecnique used in complex treatment of various neurologic diseases in some clinics) for 2 years. In this group SEPs were recorded before and during TM after 5 minutes from its beginning. In all patiens we observed statistically significant increase in the amplitude of cortical early SEPs components in intact hemisphere combined with abnormalities caused by afferent pathways lesions (absense of early SEPs or their significant reduction or increase of their peak latencies in damaged hemisphere after contralateral median nerve stimulation). Four of them also demonstrated statistically significant increase in the amplitude of several cortical early SEPs components and significant reduction of the rest in dameged hemisphere after contralateral median nerve stimulation. Brain stem SEPs amplitude didn't change in all patients. In healthy meditators cortical and brain stem SEPs, recorded before meditation were the same as in control group of healthy voluntears and their changes during meditation manifasted in parallel statistically significant increase in the amplitude of early cortical and brain stem SEPs from 1,6 to 1,7 times. An increase of early SEPs amplitude in this group can be explained by functional modification of cortical and dorsal column nuclei inhibitory interactions resulted in somatosensory afferent conduction facilitation. As we observed no changes in the patients' SEPs we concluded that their descending inhibitory pathways were not damaged. Therefore an increase in the amplitude of their cortical SEPs, reflecting compensatory processes in CNS, can be explaned by cortical mechanisms such as blocade of lateral interhemispheric inhibition and local increase in the exitability of cortical neurons.

HIPPOCAMPAL-SEPTAL EXCITABILITY IS INCREASED BY REPEATED ELEMENTAL CONDITIONING BUT NOT BY CONTEXTUAL CONDITIONING

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Recent data, using aversive classical conditioning, showed firstly, that re-exposure to conditional contextual cues (CCs) was associated with a decrease in hippocampal-lateral septal (HPC-LS) excitability. In contrast, this phenomenon did not occur when, during acquisition, a simple conditional stimulus (CS) was explicitly paired with the aversive (foot-shock) unconditional stimulus (US). We postulated that the observed CCs exposure-induced decrease in HPC-LS excitability would be linked to the processing of CCs, and that making these CCs less predictive for the occurrence of the US by a major simple CS-US association resulted in diminished processing of the CCs (i.e. context overshadowing). Using C57Bl/6 mice as subjects, the present experiment was designed to investigate whether repeated explicit CS-US associations would lead to an increase in HPC-LS excitability during re-exposure to the CCs. As compared to a group of subjects for which the CS and the US were unpaired, animals of the paired group exhibited a gradual increase in HPC-LS excitability when re-exposed to the CCs. Finally, five days following the end of the training, animals of both groups received high-frequency stimulation (HFS) of fimbrial fibers. Results showed that HFS induced a HPC-LS synaptic potentiation in the paired group, but not in the unpaired group. These results provide additional evidence for the role of HPC-LS synaptic neurotransmission in orienting the cognitive processing towards either CCs or a simple CS with respect to their respective predictibility upon the occurrence of an aversive event.

HETEROGENEITY AND STABILITY OF WAKING-SLEEPING RELATED LOCAL REDOX STATE POTENTIAL (E) VARIATIONS IN THE BRAIN CORTEX

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E shifts in living tissue are widely used as one of indicators in energy metabolism changes. The brain E variations were monitored with platinum electrodes implanted in the brain cortex of white rats. It has been found that near in half of the monitored brain sites transition from wakefulness (W) to slow wave sleep (SWS) and paradoxical sleep (PS) were accompanied by the brain E shifts (a decrease of the E during SWS and a rise of it during W and PS) and by changes in patterns of the brain E oscillations (quasisinusoidal ones with frequency 12-20 osc/min were typical for W unregular oscillations with higher amplitudes and slower frequencies 1.5- 12 ocs/min- for SWS). These variations in the brain E were stable in the same sites during 1- 1.5 months. We defined the sites with the E changes as "metabolically active" ones and the sites where the brain E variations were absent as "metabolically silent" ones. W-SWS-PS related EEG changes were observed in all monitored brain sites. The data obtained demonstrated only part of the brain cortex were involved in integrative function related to supporting routine behavior in a well know environment and that the distribution of metabolically active and silent sites remained a rather stable for a long time. To check if peculiarities of same electrodes or local brain damages in same sites were reasoned of metabolic inertness, KCN and Na NO_2, injection effects were studied and it tuned out that they could evoke significant shifts and well expressed E oscillations in the silent brain sites, i.e. these brain sites did not lose the possibility to metabolic changes.

INFLUENCE OF PRIMING ON THE DEVELOPMENT OF INNATE PREDISPOSITION AND *c-fos* GENE EXPRESSION IN THE CHICK BRAIN.

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Establishment of filial preference in young chicks is known to consist of two processes: imprinting and development of innate predisposition to approach conspecifics. While much is known about mechanisms of the former the neural bases of the latter remains poorly understood. Innate predisposition can be initiated experimentally by the treatment known as "priming". The aim of this work was to investigate the neural effects of different priming treatments on the chick brain using *c-fos* expression mapping. Development of predisposition in chicks was induced by injections of testosterone (5µ mg/chick), corticosterone (13ì mg/chick) or by motor activity in a running wheel (2x40 min with 45 min interval). These procedures lead to elevation of preference to conspecific object 24 hours later. Injection of antagonist of testosterone receptors - flutamide (1.3mg/chick) prevented development of predisposition caused by motor priming. Motor priming under dimmed light conditions induced substantial activation of c-fos expression in a number of brain areas including intermediate medial hyperstriatum ventrale (IMHV) and medial neostriatum/hyperstriatum ventrale (MNH) and the structures of the thalamofugal (hyperstriatum dorsale and nuclei dorsolateralis thalami) and tectofugal (ectostriatum and tectum opticum) visual systems. Expression of c-fos was lower in the structures of the tectofugal visual system compared to the thalamofugal visual system. It is concluded, that this difference may reflect the different involvement of these structures in development of natural predisposition.
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FEEDBACK CELLS IN MARMOSET VISUAL CORTEX: LAMINAR POSITION, MORPHOLOGY AND TOPOGRAPHY OF V1-PROJECTING PYRAMIDAL NEURONS.

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Recent studies in both Old World and New World monkeys have demonstrated that the morphology of pyramidal neurones differs markedly amongst visual areas. However, pyramidal neurones form the basis of different types of projections, and these subpopulations of cells have been shown to have differing morphologies. Therefore, we were interested to determine if similar variation also occurs within a given connectionally defined group of cells, amongst different areas. To accomplish this, we targeted specific feedback neurones for injection with Lucifer Yellow and processed the slices for a permanent DAB reaction product to reveal their morphology in fine detail. Animals were anaesthetised (ketamine, 50 mg.kg-1 and xylazine, 3 mg.kg-1), placed in a stereotaxic frame, and the skull overlying the occipital lobe was removed to allow multiple 1µl injections of the fluorescent tracer Fluoroemerald (7% in H2O; Molecular Probes D 1820) in area V1. The animals were recovered for a period of two weeks, then overdosed with pentobarbitone sodium (50 mg/kg) and perfused with 4% paraformaldehyde in phosphate buffer (0.1 mol/l). We found that 1) V1-projecting neurones were topographically organized, 2) the vast majority of V1-projecting feedback neurones were located in infragranular layers and 3) V1-projecting neurones in the second (V2), dorsolateral (DL), dorsomedial (DM), dorsointermediate (DI), dorsoanterior (DA), posterior parietal (PP), middle temporal (MT), middle temporal crescent (MTc) and inferotemporal (IT) areas each had differing morphologies. Neurones in IT were the largest, most complex and most spine dense of all neurones that project to V1. They were, however, relatively few in number. Neurones in DL were the next in rank order in terms of size, complexity and spine density, followed by neurones in the parietal areas (PP, DM, DA, DI, MT). Area V2 had the smallest and least complex cells that project to V1. By virtue of the differing morphologies, feedback neurones in the different visual areas are likely to integrate different numbers of inputs, both excitatory and inhibitory.

COMPLEX FEATURE DETECTION - COMPLEX CELL MORPHOLOGY.

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In primates, it is generally accepted that visual areas in the occipital lobe project to two separate, but interconnected, processing "streams" in the parietal and temporal lobes. Neurones in the parietal "stream" process object motion and spatial relationships, whilst neurones in the temporal "stream" are involved with analysis of shape and object recognition. Whereas it was originally thought that the differences in neuronal functional characteristics could be attributed solely to the difference in connections between cortical areas (Zeki, 1978), recent studies have shown marked differences in cellular morphology in the different visual areas (Lund et al., 1993; Elston and Rosa, 1997, 1998a,b; Peters et al., 1997). More-over, these morphological differences have been implicated in models of surround inhibition (Lund et al., 1993) and cortical integration (Elston and Rosa, 1997, 1998a,b). In the present study we detail the morphology of pyramidal neurones in two visual areas of the temporal lobe, cytoarchitectonic area TE and the superior temporal polysensory area (STP). Macaque monkeys were overdosed with pentobarbitone sodium (50 mg/kg) and perfused with 4% paraformaldehyde in phosphate buffer (0.1 mol/l). Neurones in fixed (4% paraformaldehyde) flattened cortical slices (250mm) were injected with Lucifer Yellow and processed for a permanent diaminobenzidine reaction product. We found that basal dendritic fields of neurones in STP are significantly larger, more complex and more spinous than those in TE. Moreover, the basal dendritic fields of neurones in STP and TE are markedly more spinous than cells in V1, V2, V4 and TEO. For example, neurones in STP have, on average, at least13 times more spines on their basal dendritic fields than do those in V1. Thus, pyramidal neurones in high-order areas are able to integrate a much larger number of inputs.
Elston GN and Rosa MGP (1997). Cereb Cortex 7, 432-452; Elston GN and Rosa MGP (1998a). Cereb Cortex 8, 278-294; Elston GN and Rosa MGP (1998b). NeuroReport 9, 127-131; Lund J et al., (1993). Cereb Cortex 3: 148-162; Peters A et al., (1997). Cereb Cortex 7: 405-421; Zeki SM (1978). J Physiol (Lond) 277: 273-290.

SPATIAL ORGANIZATION OF THE VESTIBULOSPINAL NEURONS IN THE FROG

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In experiments in the preparation of a frog perfused brain (Rana ridibunda) field and intracellular potentials of neurons of the vestibular nuclear complex following stimulation of the ipsilateral vestibular nerve as well as of different levels of the spinal cord were recorded. Stimulation of the vestibular nerve evoked mono- and polysynaptic excitatory postsynaptic potentials (EPSPs) and orthodromic action potentials. The vestibulospinal neurons were identified on the basis of antidromic activation following stimulation of the cervical (C-neurons) and lumbar (L-neurons) segments of the spinal cord. A rather high conduction velocity along vestibulospinal fibers (mean 15.5 m/s) was observed. Somatotopic arrangement of the vestibulospinal system was established in spite of extremely large overlapping of zones for the fore- and hindlimbs representation in the vestibular nuclear complex. The hindlimbs were represented more poorly than the forelimbs. C- and L-neurons were found in the medial and descending and with the greatest density in the lateral vestibular nuclei. Relative uniform distribution of C-neurons and more caudal location of L-neurons in the medial and descending vestibular nuclei were also noted. Computer-aided design of somatotopical organization of the frog vestibulospinal system was conducted on the basis of electrophysiological study of spatial distribution of the vestibulospinal neurons. Analysis of axonal conduction velocities of the vestibulospinal neurons revealed multiplicity of their origin in the frog vestibular nuclei.

TOWARDS ENGINEERED NEURAL NETWORKS

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In the study of neural function and development, current cell culture techniques complement in vivo studies by allowing direct observation and greater control over the extracellular environment. However, dissociated neuronal cultures lack the stereotyped synaptic connectivity observed in living organisms. An ideal way to artificially define connectivity in culture systems would be to isolate neurons on adhesive islands chemically patterned onto the substrate, then actively form synaptic connections across the intervening non-adhesive spaces. It may be possible to form these connections using the phenomenon of towed elongation. Towed elongation mimics developmental processes by using tension as a signal for the formation and elongation of neurites. Essentially, the tip of a micropipette is allowed to adhere to the membrane of a neuron, after which tension is applied by movement of the micropipette. Continued tension triggers the elongation of a neuritic process that exhibits vesicular transport, filopodial waving, and a growth cone-like structure at the distal tip. We have used this technique to form connections between pairs of neurons in dissociated embryonic chick forebrain cultures, and observed a 10% success rate. Failures occur when the micropipette does not adhere to a cell's membrane, an elongating neurite becomes a tether (a thinner membraneous process that shows no signs of normal neurite structure), or a towed growth cone fails to adhere to a target neuron. Experiments are under way to determine the factors influencing such failures, as well as to evaluate the formation of synapses after adhesive contacts are made.

REACHING MOVEMENTS: PROGRAMMING TIME COURSE IS INFLUENCED BY TARGETS' SPATIAL DISPERSION.

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We previously demonstrated that the time course of the process by which information from a visual target is used to accurately program reaching movements is not influenced by the number of possible targets, nor by the number of uncertain trajectory parameters. The effect of targets' spatial dispersion on the time course of response programming is now investigated.
Subjects moved a hand-held cursor on a digitizing tablet from a common start point; targets and a screen cursor were displayed on a computer monitor. Movements were initiated in synchrony with the last of a series of equally interspaced tones; targets appeared at unpredictable times before the last tone. Targets were all at the same distance, in one of four possible directions, in each of three standard sessions (directions: 45°, 75°, 105°, 135°; 30°, 60°, 120°, 150°; 15°, 45°, 135°, 165°, being 0° the right and 90° the vertical upwards on the screen).
Responses produced at short stimulus-response intervals were unimodally distributed around the center of the range (90°) in the first type of session, while in the third one they were directed to either the right or the left of 90°, showing a bimodal distribution. As time available for processing increased, responses became progressively better correlated to the targets. The optimal level of correct response programming was achieved more rapidly in the first session than in the second and third one (the slowest).
In conclusion, the processes responsible for programming movement direction differ when targets are widely separated (discrete planning) or close together (continuous planning). Continuous programming allows a faster specification. Thus, an effect of targets' spatial dispersion on programming time course is found: the wider the dispersion, the longer becomes the time course of the corect specification process.

SYNAPTIC CONNECTIONS BETWEEN EXCITATORY SPINY NEURONS IN LAYER 4 OF RAT BARREL CORTE

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Spiny layer 4 (L4) neurons are the main target of thalamic afferents and are thought to relay incoming excitation to superficial cortical layers. The first step of signal processing occurs at the level of layer 4 between synaptically coupled excitatory spiny neurons. Whole cell voltage recordings combined with biocytin fillings were made in acute slices of rat somatosensory cortex (P12-15). Synaptic connections were established between 131 pairs of identified spiny L4 neurons. A single AP in the presynaptic neuron evoked unitary excitatory postsynaptic potentials (EPSPs) were mediated by both AMPAR and NMDAR and had a mean peak amplitude of 1.6 ± 1.5 mV (range 0.3 - 9.6 mV). Peak amplitudes fluctuated randomly with a coefficient of variation (CV) of 0.36 ± 0.18. The percentage of failures of a single presynaptic action potential to evoke an EPSP was 5.3 ± 7.8%. CV and percentage of failures decreased with increasing amplitude of the unitary EPSP. The number of putative synaptic contacts established between projection and target neuron varied between one and five (mean 3.4±1.1, n=11); they were exclusively located in the barrel in which the cell pair was located at a mean geometrical distance from the soma of 68.8±37.4 µm. No significant correlation between number of synaptic contacts and mean amplitude of the unitary EPSP was found. Thus, spiny L4 neurons are well suited to relay and amplify incoming thalamic activity within a cortical column.

SOME REGULARITIES OF EEG SPECTRAL PATTERNS DYNAMICS DURING HUMAN COGNITIVE ACTIVITY

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The using of classical spectral analysis which is based on strongly averaged EEG spectral estimations within the limits of large temporal intervals leads to nonsynonymous data. If consider EEG as quasi-stationary signal, it may be get a set of different types of "elementary" spectra according to the number of available in EEG quasi-stationary segments instead of averaged spectrum at the same estimation interval.

In this case the peculiarities of portional participation of different spectral types in forming of current EEG may be more sensible characteristics of microstructural organisation of EEG, then the averaged spectra. Here the problem of spectral pattern typification is arised. This problem was solved by working out the methodology of adaptive classification of EEG spectral patterns.

Using this methodology during human cognitive activity we got unknown early data. Thus, the steady microdynamics of spectral transformations which is manifested in definite combinations of elememtary spectral patterns is hid in traditional EEG spectral descriptions, which were obtained by averaging the short-term spectra even in small temporal intervals (20-30sec.). It was shown that the sets of EEG spectral patterns and representation of each of such patterns in real EEG are specificaly depended on character and productivity of human cognitive activity, and on basic individual peculiarities of subject's EEG.

Spatial combinations of EEG spectral pattern and their dynamic characteristics are specific in respect to character of subject's cognitive activity. The most intensive modification of spectral patterns is typical for state of active remembering of matrix images.

OPERATIONAL SYNCHRONY OF HUMAN BRAIN ACTIVITY

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EEG "grammar", its internal construction remains still a secret. Understanding of this "grammar" would give in the researchers hands the "Rozett`s stone", which permits to describe information processes of the brain in the terms of EEG-phenomenology more adequate. A substantial generalization of this research direction was done by our author`s collective.

In these investigations the idea that dynamic consistency of EEG segments between different brain areas is basic mark (feature) of functional consolidation of appropriate cortex areas, at first got in "sheer state" theoretical foundation and experimental conformations.

We suggest that the participation of brain areas in the organization of a common functional act can be found not so much by the presence of a shared EEG rhythms in both cortical areas as by the frequency of coincidences of moments of switching between modes of EEG activity in different brain structures. In this connection we introduced the concept of "operational synchrony" of elementery brain operations [1].

New experimental data about discrete dynamic of brain potentials which were received in the frame of our investigations belong to total EEG which not undergo any spectral dissolution. Meanwhile exactly separate frequency components of EEG are the markers of "switching on/turning off" of individual morpho-functional brain systems. So, required logical continuation of this work is the study of operational synchrony of EEG basic rhythms in temporal and spatial aspects. Our data show that operational synchrony as new and unknown before type of different brain formations interaction together with already known frequency sinchronization of EEG rhythms are the necessary base of intercortex relations. This base appoints integrity of spatial-temporal cooperation of regional EEG-phenomena, which accompany realization of cognitive and psychological acts.

1. Kaplan A.Ya., Fingelkurts An.A., Fingelkurts Al.A., Darkhovsky B.S. Topological mapping of the sharp reorganization synchrony in the multichannel EEG. American Journal of Electroneurodiagnostic Technology, No. 37, P. 265-275, 1997.

VISUALIZATION OF SYNAPTIC VESICLE RECYCLING AT FROG MOTOR NERVE TERMINALS

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Using electron microscopy we studied the generation of recycling synaptic vesicles in frog motor nerve terminals by tracking FM1-43 photoconversion products. Cutaneous pectoris nerve-muscle preparations were bathed in 4 uM FM1-43 while the nerve was electrically stimulated (1000 pulses, 20 Hz). Some preparations were fixed immediately after stimulation, while others were left in FM1-43 for 15 minutes before fixation. FM1-43 in the terminals was photoconverted to an electron-dense according to the method described by Henkel et al, (Proc. Natl. Acad. Sci. USA 93: 1918) with some modifications (Harata et al., Soc. for Neurosci. Abs.: 77, 1998)). In terminals that were allowed recovering from stimulation for 15 minutes in the presence of FM1-43, approximately 40% of synaptic vesicles contained photoconversion product. However, in terminals that were fixed immediately after stimulation, few synaptic vesicles contained reaction product. Instead larger cisternal-like structures that were not present in rested terminals were labeled with reaction product. This technique can be used to follow the steps involved in synaptic vesicle recycling.

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UNILATERAL GLUR2 HIPPOCAMPAL KNOCKDOWN: A NOVEL PARTIAL SEIZURE MODEL IN THE DEVELOPING RAT

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Kainic acid (KA) induces status epilepticus in adult and young rats but with different consequences on pathology and gene expression. In adults, GluR2 AMPA subunit expression is markedly reduced in CA3 neurons prior to neurodegeneration. In pups, the GluR2 subunit is sustained possibly contributing to neuronal survival. Mechanisms underlying the reduced vulnerability of developing neurons to seizures was investigated by examining the effects of unilateral microinfusions of GluR2(B) antisense oligodeoxynucleotides (AS-ODNs) into the hippocampus of young rats in the presence or absence of a subconvulsive dose of KA. GluR2 AS-ODN infusions resulted in spontaneous seizure-like behavior, high stimulus intensity population spikes in the absence of long term potentiation (LTP), and neurodegeneration of CA3 neurons lateral to the infusion site. Electroencephalography (EEG) revealed high-rhythmical activity and high-frequency high-amplitude discharges associated with vigorous and continuous scratching, wild running, or bilateral jerking movements. Pups lacking phenotypic behavior exhibited high-rhythmic oscillations and status epilepticus by the dose of KA used. Radiolabeled AS-ODNs accumulated throughout the ipsilateral dorsal hippocampus. GluR2(B) but not GluR1(A) receptor protein was markedly reduced. Therefore transient increases in the hippocampal GluR1(A):GluR2(B) protein ratio unilaterally reduce the seizure threshold and survival of CA3 neurons in the immature hippocampus, possibly providing a novel partial seizure model in the developing rat.

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REDUCED GLUTAMATERGIC ACTIVITY IN CEREBRAL CORTEX IN SCHIZOPHRENIA

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Autopsy material from 13 schizophrenic patients and 12 controls was impregnated with a Golgi method. Spines were counted on dendrites of pyramidal neurons in temporal and frontal association cortex of which the soma was in layer III. Measurements were blind: diagnoses were only revealed after measurement was complete. There was lower ($p<0.001$) numerical density of spines in schizophrenia (276/mm in control temporal cortex, 112 in schizophrenics; 299 and 101 respectively in frontal cortex). Co-exisiting Alzheimer's disease, age at death and post-mortem interval did not affect the results.
We also studied neurons immunoreactive for the glutamate kainate receptor in orbitofrontal cortex (area 11) in 9 schizophrenic and 8 control patients. Sections were stained with cresyl violet to determine the total neuronal numerical density. There was significant reduction (about 20%) in numerical density of kainate neurons in schizophrenics (488 cells/mm^2) compared to controls (618 cells/mm^2). There was no correlation of reduced kainate cell number with age at death, post-mortem interval, or other neuropathology. There was no significant difference in total neuronal numerical density, but there was a tendency for an approximately 10% elevation in schizophrenics. This suggests that there are fewer kainate-positive neurons in schizophrenic orbitofrontal cortex.
Our results support the concept of reduced glutamatergic activity in cerebral cortex in schizophrenia, and a defect in the fine structure of dendrites of glutamatergic neurons. They may help to explain loss of cortical volume without loss of neurons in this condition.

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RECENT PHONOLOGIC DEVELOPMENT IN BABIES AND BAIES WITH PERINATAL BRAIN LESION

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In habilitation procedures performed on 30 babies, both sexes, with perinatal unilateral and bilateral brain lesions, showed supersingly an unexpected phenomenon of vocalization sequence. Namely, there is a scale of order in phoneme sequence appearance and this rule specific to the population of Slave languages was applied in logopaedic treatment for rehabilitation of developmental speach disorder in youngsters. Babies, who suffered from perinatal brain lesion were treated for vocalization habilitation and showed a 50% delay of efficacy compared to controls. This expected score due to injury consequences showed also, that the observed most critical phonems in phonologic appearance were "g, h and c", as a phenomenon different from the customary rule and indicates to respect developmental follow up to correct through evidence of changes recent valuable rules.

STUDY OF THE EFFECTS OF TWO SELECTIVE $GABA_B$ ANTAGONISTS AND THE NOOTROPIC DRUG OXIRACETAM ON LEARNING AND MEMORY PROCESSES IN RATS

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Some phosphinic analogues being a selective $GABA_B$ antagonists were studied as prospective drugs for treatment of epilepsy. We study the effects of two $GABA_B$ antagonists - CGP 76291 and CGP 76290A and the nootropic drug Oxiracetam on learning and memory processes in shuttle box active avoidance in rats. Standard programme for rats in automatic reflex conditioner (Ugo Basile, Italy) was appled. Six seconds light and buzzer stimulation (670 Hz, 70 dB) followed by foot shock 0.4mA were used. Five days learning (30 trainings per session) and 7 days later (12^{th} day) the memory retention was done. The automatically counted parameters were: the number of avoidances, escapes and intertrial crossings. Seven groups (n=8), injected 30 min before training with saline (control) or 0.1 or 0.01 mg/kg from respective substances were used. In learning session the rats treated with CGP 76291 in both doses did not changed the number of avoidances and escapes compared to the control group. The rats injected with CGP 76290A or Oxiracetam in both appled doses increased the number of avoidances and escapes compared to the control in learning session. On memory retention test $GABA_B$ antagonist CGP 76291 did not showed improving effect compared to the control. The $GABA_B$ antagonist CGP 76290A as well as with the nootropic drug Oxiracetam showed statistically significant increasing the number of avoidances and escapes compared to the controls on memory retention test. The $GABA_B$ antagonist CGP 76290A has improving effect on learning and memory retention compared with those of the nootropic drug Oxiracetam.

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NICERGOLINE INCREASES nNOS mRNA EXPRESSION IN THE BASAL FOREBRAIN IN OLD RATS.

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The ergot alkaloid nicergoline ameliorates regional cerebral blood flow and cerebral metabolism in rat. Nitric oxide (NO) is a crucial modulator in blood-brain perfusion, being responsible for activity-regulated regional vasodilatation in the brain. In order to investigate if NO could be involved in nicergoline regulation of brain perfusion, we analyzed NO synthesis enzyme nitric oxide synthase (NOS) mRNA in aged rats (male, Sprague-Dawley rats, 18 months old) receiving long-term treatment (3.5 months) with nicergoline added in the food-pellet (N=10,10mg/kg/animal/day). NOS mRNA expression was then investigated in brains regions including cerebral cortex, basal ganglia, septum, vertical diagonal band and horizontal diagonal band nuclei by means of in situ hybridization (quantitative analysis: grain counting on single cells). Chronic nicergoline restores reduced expression of nNOS mRNA in single neurons in caudato-putamen and basal forebrain in 24 month old rats and it also increases nNOS mRNA expression in other brain areas, like cerebral cortex, where nNOS level is not affected by aging. We then suggest that modulation of NOS mRNA level could be part of the mechanism of action of nicergoline, by potentiating efficiency of coupling brain activation and perfusion (i.e. in the cerebral cortex) or participating in other neuroprotective mechanism (i.e. in the basal forebrain, where nerve growth factor administration increases NOS expression, Holtzman et al., 1994).

EFFICACY OF THALAMOCORTICAL AND INTRACORTICAL SYNAPTIC CONNECTIONS: QUANTA, INNERVATION AND RELIABILITY.

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Thalamocortical (TC) synapses carry sensory information into the neocortex, but they are far outnumbered by excitatory intracortical (IC) synapses that recombine cortical information. We measured the basic synaptic properties that determine the efficacy of TC and IC axons converging onto spiny neurons of layer 4 in the mouse somatosensory cortex. The quantal size of TC and IC synapses was measured by replacing extracellular Ca^{2+} with Sr^{2+}, or by applying Cd^{2+} to the perfusing solution. The sizes and shapes of quantal events from TC and IC synapses were indistinguishable. However, minimal stimulation of single axons revealed that TC axons have, on average, about 3 times more release sites than IC axons. The release probability of the two tracts was compared by blocking IC- and TC-NMDAR-dependent currents with the NMDA open channel blocker MK-801. The mean release probability at TC synapses was about 1.5 times higher than that at IC synapses. We conclude that differences in innervation ratio and release probability make the average TC connection several times more effective than the average IC connection within layer 4. These specializations may allow the small numbers of TC synapses to dominate the activity of cortical layer 4 cells during sensory inflow.
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CONTINUOUS AND LURCHING PULSES IN ONE-DIMENSIONAL NETWORKS WITH DELAY.

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Evoked neuronal population discharges propagate over long distances in cortex when $GABA_A$ inhibition is blocked, with velocity of about 15 cm/s. Spindle-like discharges propagate in thalamic slices, with velocity which is about 100 times slower; excitatory and inhibitory interaction together with the post-inhibitory rebound mechanism in excitatory thalamocortical cells can be modeled as a network of excitatory neurons with delay. We have studied theoretically and computationally a one-dimensional network model of integrate-and-fire neurons, in which each neuron is allowed to fire only one spike. Synaptic EPSCs have a delay τ_d that does not depend on the distance between the pre- and post-synaptic neurons. Synaptic interaction between cells ("footprint length") decay with distance, either exponentially or as a step function. Continuous pulses (Fig.1A) propagate if the total synaptic input a cell receives, g_{syn}, is above a critical value; the velocity at criticality is finite and decreases with τ_d. At large enough τ_d, continuous pulses become unstable via a Hopf bifurcation, and lurching pulses bifurcation, and lurching pulses appear (Fig.1B). Under certain condition, *i.e.*, when the synaptic footprint shape is a step function, continuous and lurching pulses may coexist. Analytical calculation shows that the velocity of both continuous and lurching waves increases logarithmically with g_{syn} at large g_{syn} for an exponential footprint shape, whereas these velocities approach a finite value for a step footprint length. This work shows that lurching discharges appear in neuronal tissue if the effective delay is large enough, whereas continuous discharges propagate with small delay.

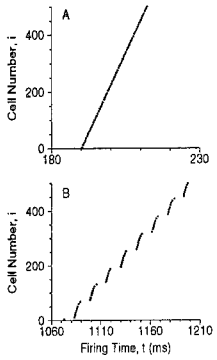

Fig. 1: Rastergram. Each solid circle represents a firing time of a neuron. A. $\tau_d = 1.7$ ms, continuous wave. B. $\tau_d = 10$ ms, lurching wave

SENSIBILITY OF MOTOR EVOKED POTENTIALS FOR DETECTING CORTICOSPINAL LESIONS IN MULTIPLE SCLEROSIS

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Motor evoked potentials(MEP) are very useful for the evaluation of multiple sclerosis(MS) patients. To know the sensibility of MEP in a group of patients with diagnosis of multiple sclerosis we carried out a transversal and descriptive study in 56 patients with definite MS. MEP using magnetic stimulation were carried out in all of them, with recordings over abductor pollicis brevis and tibialis anterioris muscles. We analyzed the sensibility of MEP and the clinical correlation of this modality of evoked potential. In 22 patients the sensibility of MEP was compared to results of other modalities such as somatosensory, auditory and visual evoked potentials(EP). Abnormalities were detected in the 87% of records, and in 18% with absence of clinical signs of corticospinal lesion. We found statistically significant differences between relapsing-remitting form of MS and the primary chronic and progressive form(Wilk's Lambda= 0.606, p=0.00), with a high lineal relation to Kurtzke scale (p<0.05). In relation to other modalities of evoked potentials, MEP were the most sensible study(68.1%), followed by somatosensory(59%), visual(45.4%) and auditory(22.5%) EP. The conclusion of this study is that MEP are a very sensible modality of EP for the detection of corticospinal lesions in MS patients, with a high degree of clinical correlation.

SEROTONIN RELEASE IN A GENETIC MODEL OF DEPRESSION

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Rats selectively bred for high and low sensitivity to the hypothermic effect of 8-OH-DPAT (DPAT), a $5\text{-}HT_{1A}$ receptor agonist, show dramatic behavioral differences. Thus, the high DPAT sensitive line (HDS) exhibits greater tendencies to depression and anxiety than the low DPAT sensitive line (LDS), as measured in the forced swimming and the social interaction tests, respectively. In order to explore possible differences in the serotonergic systems of these lines, we measured extracellular levels of 5-HT and 5-HIAA in two brain areas using *in vivo* microdialysis. A challenge dose of fenfluramine (10 mg/kg, IP), a 5-HT releaser, was given after stable basal levels were reached. Dialysates were taken every 25 min from frontal cortex and dorsal hippocampus and immediately analyzed by HPLC coupled to electrochemical detection. There was no difference between lines in the 5-HT basal dialysate concentrations (pg/20µl) from dorsal hippocampus (HDS: 3.33 ± 0.31; LDS 2.66 ± 0.12) or frontal cortex (HDS: 3.80 ± 0.32; LDS 2.98± 0.47). However, the HDS rats had a greater increase (500%) than the LDS rats (200%) in their hippocampal 5-HT levels, which were different in two consecutive samples ($p < 0.001$), following the administration of fenfluramine. Fenfluramine enhanced the extracellular concentrations of 5-HT in the frontal cortex in both lines to similar levels (about 900% and $p = 0.1$). No interline difference in the levels of 5-HIAA in either area was detected. The data suggest that the 5-HT reuptake function of the hippocampus differs between HDS and LDS lines.

LOCALIZATION OF AMINO ACID, NEUROPEPTIDES AND CHOLINERGIC NEUROTRANSMITTER MARKERS IN IDENTIFIED NEURONS OF THE BASAL FOREBRAIN PROJECTING TO THE RETROSPLENIAL GRANULAR CORTEX (RSg) IN NORMAL RATS AND FOLLOWING LOCAL ADMINISTRATION OF β-AMYLOID PROTEIN INTO THE RSg.

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Several studies have demonstrated that the RSg is involved in learning and memory. Memory impairment is an early manifestation of Alzheimer's disease (AD) in which there is reduced cholinergic innervation of cerebral cortex as a result of loss of basal forebrain neurons. The aim of the present study was (i) to examine the transmitter related characteristics of projections to the RSg from the basal forebrain by retrograde labelling with cholera toxin subunit B conjugated to horseradish peroxidase (CT-HRP) combined with immunohistochemistry and (ii) to investigate whether local administration of β-amyloid protein into RSg affects the neurochemical markers in basal forebrain neurons. In a first group of rats (Wistar, 250-300g) anaesthetized with Nembutal (45mg/Kg), injections of CT-HRP were placed into different parts of the left RSg. The animals were reanaesthetized 1-2 days later and perfused with fixative. Brains were sectioned coronally at 50 μm using a Vibratome. The sections were reacted sequentially for the histochemical detection of CT-HRP (using DAB as chromogen) and the immunocytochemical localization of choline acetyltransferase (ChAT; 1:4), GABA (1:2000), glutamate (Glu; 1:1000), enkephalin (Enk; 1:5000), neurotensin (NT) and substance P (SP) (1:2000) using the ABC method. After injections in the RSg, moderate to large numbers of ChAT+HRP containing neurons were present in the horizontal limb of the nucleus of the diagonal band of Broca (HDB) and small numbers of double-labelled (ChAT+HRP) neurons were also found in the medial septal nucleus (MS) and in the anterior subdivision of the magnocellular basal nucleus. In addition, small numbers of HRP-labelled neurons in the MS and HDB contained GABA, Glu, Enk, NT and SP-like immunoreactivity. In a second group of rats, injections of β-amyloid protein [Aβ(12-28), Research Biochemical Intern., 0.5μl/1μg-2μl/4μg] were placed into the left RSg. The animals were reanaesthetized 4 days-4 weeks later and perfused with fixative. Coronal vibratome sections, cut at 50 μm, were immunoreacted for ChAT, GABA, Glu, Enk, NT and SP. Injections of βA into RSg consistently produce changes in ChAT and Glu immunoreactivity. Degenerative changes and loss of ChAT+ neurons were seen in the basal forebrain, predominantly in the MS and HDB, together with loss of Glu+ cells and decrease of Glu-immunoreactive fibres and terminals. In addition, a slight decrease in NT-immunoreactive fibres was seen in the MS and HDB compared with the first (control) group of animals.These results suggest that the Aβ in vivo affects both the cholinergic and glutamatergic systems and that interaction between these systems may be a feature of AD (Supported by DGICYT,PM97-0091 and Junta de Castilla y León).

A TRANSPORTATION FACTOR C/EBP AND HELIX MEMORY FORMATION

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Transcription factors C/EBP are the representatives of the family CCAAT/enhancers-binding proteins. Increase of expression of these factors is connected with long-term memory formation. Phosphorilation of these factors is necessary for their dimerisation and activation. We have been studying influence of different signal systems on formation of C/EBP factors in CNS of Helix during elaboration of food-aversion conditioned reflex, using gel shift assay method. Increase of DNA-binding activity of factors C/EBP since 2-3 hours after the beginning of conditioning was found. Similar influence on activation of C/EBP was made by incubation of CNS with 5-HT or with forscolin - activator of adenylate cyclase. Increase of intracellular Ca 2+ (A 23187) did not exert significant effect on DNA-binding activity of C/EBP. Forbol aester – activator of protein kinase C (TPA) - exerted inhibitory activity. Acting in combination, 5-HT and TPA increased serotonin action on C/EBP activation at a low extent. At the same time elevation of intracellular calcium together with serotonin application increased serotonin-induced activation of C/EBP-binding activity. Hence, convergence of Ca 2+-dependent and cAMP-regulated ways can lay in the basis of long-term forms of learning. At the same time cAMP- dependent phosphorilation of transcriptional factors CRE family can induce C/EBP expression meanwhile Ca 2+ via activation of mitogen activated or Ca 2+ calmodulin-dependent protein kinase can influence on C/EBP phosphorilation.
Supported by Russian Basic Research Foundation grant.

DEVELOPMENTAL ASPECTS OF THE IBOTENIC ACID LESION ANIMAL MODEL OF SCHIZOPHRENIA

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Recently, a novel animal model of schizophrenia which addresses neurodevelopmental aspects of the disorder was introduced by Lipska and Weinberger. Using this model we could demonstrated that neonatal excitotoxic lesions of the ventral hippocampus of rats resulted in specific pattern of abnormalities only detectable after puberty. We found a hyperresponsiveness to dopaminergic stimulation in locomotion tests, disturbed latent inhibition and social interaction behaviour with a shift to more aggressive behaviour in rats tested after puberty. Biochemical investigations revealed an enhanced specific glutamate binding in the frontal cortex of adult rats and a downregulation of D1 and D2 binding sites in the striatum without changes in other brain structures.
With the intention to investigate more in detail the relevant developmental aspects of this model, we compared the effects after postnatal lesion with those of lesion of the ventral hippocampus performed in adult rats (8 weeks old). Interestingly, we found no comparable abnormalities in such lesioned rats. Latent inhibition as well as social interaction behaviour were unchanged, likewise neither dopamine binding sites in the striatum nor glutamate binding to frontal cortex were altered. In response to apomorphine we found only a slight hyperresponsiveness. The presented results comparing the consequences of postnatal and adult lesions of the ventral hippocampus support the developmental aspect of this model. Exclusively early hippocampal lesions disrupting development of the widespread cortical and subcortical circuits in which the hippocampus participates may mimic the relevant neurodevelopmental aspects of at least some forms of schizophrenia and result in the manifestation of schizophrenia-like behavioural and neurochemical alterations.

AUTOANTIBODIES TO GLIAL CYTOKINE S100 BETA AND EARLY BRAIN ALTERATIONS

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Reasons of various autoimmune diseases development in adults could take place during pregnancy and early postnatal period. It is well known that in early ontogenesis the synthesis of autoantibodies(a-Abs) is forestall the synthesis of defensive ones. When the level of fetal autoantibodies is become nonphysiological they can be consider as active pathological molecular factors during formation of specific brain alterations. But mechanisms of progressive neurodegeneration by a-Abs provocation in early ontogenesis is remain obscure. Comparative clinik-experimental analysis of autoimmune reactions to S100 beta, glial cytokine that increases intraneuronal calcium levels and promote excessive growth of neuronal processes (neurites), in offspring with various forms of prenatal hypotrophy and brain alterations was carry out. Clinical, neurosonographical, immunological and immunochemical methods during investigation were explorated. In all sera samples of infants with genetic determined forms of hypotrophy (in 27,3 % accompined by marked structural changes in brain and in 94,5 % - rough psichomotorical developmental delay, for example infants with Down-syndrom) a-ABs to S100b in titres in 2-4 time higher that control ones were revealed without any correlation with hypotrophy degree. Conformity between a-ABs S100 beta and hypotrophic degree of second form hypotrophy was determined, a-ABs level was higher in 10-20 time from control in 70 %, 75 % , 100 % cases with I, II, III hypotrophic degrees, respectively. In vitro experiments modulation of the young rat brain protein biosynthesis as influence at functional activity of neurotransmiterrs central receptors of a-Abs to S100 beta were examed.

REGULATION OF THE PHOSPHOINOSITIDE TURNOVER IN THE FRONTAL CORTEX OF THE RAT BY E-5842, A SPECIFIC SIGMA$_1$ RECEPTOR LIGAND AND POTENTIAL ATYPICAL ANTIPSYCHOTIC

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E-5842 (1-[4-[4-(4-fluorophenyl)-1-(1,2,3,6-tetrahydropyridil)]butyl]-1H-triazole citrate) is a preferential sigma$_1$ receptor ligand (K_i = 4nM) with affinity for other relevant receptors in the mid-micromolar range. E-5842 behaves as an atypical antipsychotic in preclinical behavioural and neurochemical tests. Although the sigma receptor has recently been cloned, the signal transduction pathway that mediates its response is still unknown. It has been reported that some sigma ligands can affect the phosphoinositide (PI) second messenger system, and attenuate in a dose-dependent manner the stimulation induced by muscarinic and noradrenergic agonists. Given the high affinity of E-5842 for the sigma$_1$ receptor, we have studied the effect of E-5842 (either administered acutely or chronically) on the PI pathway. The possible regulation by E-5842, either *in vitro* as *ex vivo*, of the enzyme phospholipase C (PLC) involved in the process was studied. In addition, levels of PLC and $G_{q/11}$ proteins have also been studied in different rat brain areas after chronic treatment with E-5842. Our results show that E-5842 regulates the basal activity of the enzyme PLC *ex vivo*, in frontal cortex slices. Acute administration of E-5842 (40 mg/kg, i.p., for 2 h) increases the activity of the enzyme (basal levels). Similar results can be observed after chronic (20 mg/kg, i.p. daily for 21 days) administration of E-5842. In order to confirm these results, *in vitro* experiments (with a similar protocol of administration) using synaptic membranes were performed. Chronic administration of the compound also increased the activity of the enzyme as measured by GTPγS stimulation. Immunoblotting techniques showed that there is a regulation of PLC and the $G_{q/11}$ associated protein in the frontal cortex. Our results suggest that the regulation of the PI pathway may be a significant mechanism through which E-5842 exerts its action, especially in relation to its long-term effects on brain function.

INTEGRATIVE POSSIBILITIES OF NEURONS OF PIRIFORM CORTEX.

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In a consequence of availability of the large neurons variety the classification is complicated them. In the our study we have tried to differentiate cells on dense and sparse branchy types of neurons for the account their of integrative possibilities.The research was conducted on preparations impregnated by silver on a method Goldji of 20 rats' brain. The exact figures of 205 neurons of piriform cortex (PC) were obtained by drawing-device. Primary dendrites number (d), number of the free dendrites terminations (Bd) and number of branching nodes of all dendrites (n) were counted at all neurons. We offered the following derivatives parameters for the account of branching neurons: Bd×n/d - cumulative parameter of branching one dendrite, $Bd/(d^2 \times n^2)$ - parameter of branching of all cell. Their influence on structure of a tree of dendrite was acknowledged the results of factor analysis. With allowance for these factors cluster analysis divided neurons of PC on three groups. The first group represents sparse branchy neurons with small integrative abilities (d 3-7, Bd×n/d 6,68-80, $Bd/(d^2 \times n^2)$ 0,001-0,049). Dense branchy neurons with high integrative abilities forms the second group (d 6-9, Bd×n/d 84,17-180, $Bd/(d^2 \times n^2)$ 0,0006-0.0019). The neurons of third group have intermediate branching. As the branching of one dendrite is high enough (Bd×n/d 80,04-184,8), while the general branching of a cell is insignificant at the expense of small number dendrites ($Bd/(d^2 \times n^2)$ 0,0017-0,04; d 3-5).

NEURONAL INTERACTIONS ARE MODIFIED DYNAMICALLY IN RELATION TO BEHAVIORAL EVENTS.

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To explore the hypothesis that cortical function is mediated by dynamic modulation of coherent firing in groups of neurons, we recorded neuronal activity in the frontal cortex of behaving monkeys. Dynamic cross-correlation analysis (Joint peri-stimulus time histogram (JPSTH)) was applied to examine the temporal structure of activity of the simultaneously recorded single neurons, and revealed that correlated firing between single neurons evolves within a fraction of a second, in a systematic relation to behavioral events. The averaged cross-correlation depends upon distance between neurons: while positive picks are maximal and most prevalent the closer the neurons, negatives picks are maximal and most prevalent when neurons are about a micron apart. Thus, neighboring neurons tend to exhibit positive correlations, implying that they share common inputs of the same type, whereas correlations between more distant neurons (up to few microns apart) are mixed. The pattern of correlation of each two different pairs where compared. It was found that the larger the anatomical overlap, the higher the similarity of patterns of correlation. Both average correlation and dynamic modulation of correlation contributes to this similarity. These findings suggest that the spatio-temporal organization of activity in the network allows for rapid association of neurons into a functional group, while dissociating from concurrently activated, competing groups. Functional groups are arranged in anatomical clusters. Neurons in each such cluster can form a group that is activated in a coherent manner with neurons in other clusters.

PHOSPHOLIPASE C-ß1 SIGNALLING REGULATES POSTSYNAPTIC DIFFERENTIATION AND BARREL FORMATION IN MOUSE SOMATOSENSORY CORTEX

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Phospholipase C-ß1 (PLC-ß1) is a phosphodiesterase, activated via specific neurotransmitter receptors, that is localized to an intermediate-compartment-like organelle, the *botrysome*, whose expression correlates well with the sensitive period in cat visual cortex (Kind et al., 1997, J. Neurosci. **17**:1471). In rat somatosensory cortex, PLC-ß1 is expressed in barrels during early postnatal development (Hannan et al., 1998, Neuropharmacology **37**:593). To further investigate the role of PLC-ß1 in developing cortical neurons, we examined the development of cortical barrels in PLC-ß1 knockout mice. Nissl histochemistry and bisbenzimide staining on tangential sections from homozygous knockout mice demonstrated the absence of clearly defined barrels in primary somatosensory cortex. Golgi staining of neurons in somatosensory cortex revealed a dramatic reduction in dendritic complexity in PLC-ß1 knockout mice. Immunohistochemical analysis shows that at least some of these developing neurons with abnormal morphologies are GABAergic. Labelling of thalamocortical afferents with the carbocyanine dye, DiI, demonstrated segregation of afferents into barrel-like clusters. Analysis of adjacent sections from these mice by cytochrome oxidase histochemistry revealed staining in a barrel pattern, confirming that thalamocortical axons are still able to segregate in the somatosensory cortex in the absence of PLC-ß1. These results demonstrate an active role for cortical cells in barrel development and strongly suggest that PLC-ß1 is necessary for the receptor-mediated re-arrangement of neurons and their dendrites, in response to thalamocortical activity. *Supported by the Medical Research Council, Nuffield Medical Trust and Oxford Centre for Cognitive Neuroscience.*

NEURONS IN THE MARGINAL ZONES OF THE HIPPOCAMPUS AND DENTATE GYRUS FORM AN EARLY HIPPOCAMPO-ENTORHINAL PROJECTION IN THE RAT

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The entorhino-hippocampal projection begins to develop prenatally and completes during the first postnatal weeks. The entorhinal afferents terminate in the marginal zones, the stratum lacunosum-moleculare of the hippocampus and the outer molecular layer of the dentate gyrus. Early generated neurons in these layers secrete the glycoprotein *Reelin* which plays a crucial role in the formation of the entorhino-hippocampal projection. Using *in vivo* DiI labeling, intracellular filling with biocytin and immunocytochemistry we examined whether these neurons establish an early axonal projection to the entorhinal cortex which may serve as a guiding scaffold for outgrowing entorhinal axons. Retrograde DiI tracing at different embryonic stages (E16 - E21) revealed labeled neurons in the hippocampus anlage from E17 onwards. Neurons in the marginal zones were patched and filled in acute slices of the hippocampal formation (P5 - P10) and in co-cultures taken from embryos (E19) or neonate rats and cultivated for up to 10 days. At all developmental stages examined, neurons located near the hippocampal fissure gave rise to an axonal projection to the subiculum and the entorhinal cortex. In co-cultures immunostained with an antibody against *Reelin*, a dense band of labeled neurons was found along the hippocampal fissure. These *Reelin*-immunopositive neurons showed a spatial overlap with the somatic localization of biocytin-filled neurons that project to the entorhinal cortex, and double-labeling revealed that some of the biocytin-filled neurons also contained *Reelin*. The existence of an early hippocampo-entorhinal projection of neurons in the marginal zones suggests that these neurons, among them Cajal-Retzius cells, may be involved in guiding outgrowing entorhinal fibers to their target zones. (Supported by the Deutsche Forschungsgemeinschaft: SFB 505).

REGION-SPECIFIC EXPRESSION OF THE CYP11A1 GENE IN THE FOREBRAIN OF THE PRE-HATCHING CHICK.

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ABSTRACT
Sexual differentiation is a multi-step process that is determined by the chromosomal complement of the zygote. Then, gonadal-steroid synthesis controls the evolution of sexually dimorphic structures. It has been reported that the development of sexually dimorphic nuclei in the CNS (some involved in sexual behavior) is dependent on the aromatization of gonadal androgens. There are other reports describing the synthesis and accumulation of sexual steroids, including androgens, in neural tissue (neurosteroids) independently from gonadal biosynthesis. Cytochrome P450scc (cholesterol side-chain cleavage), whose mRNA has been detected in different brain regions, is the first and rate-limiting enzyme for steroid biosynthesis. Aside from the sexual steroids produced in the gonads, secretion control of neurosteroids during development of the CNS remains unclear. The aim here, is to determine gene expression and steroidogenesis in the forebrain of the domestic fowl within the critical pre-hatching period. By RT-PCR we found evidence of region-specific alternative splicing of the CYP11A1 gene mRNA (cytochrome P450scc), in contrast to the typical expression in the gonad. Additionally, we observed the rate of conversion from 25-hydroxycholesterol to pregnenolone to be different in both regions of the anterior brain through radioimmunoassay. The role of such alternative processing may be the tissue-specific regulation of steroid secretion during sexual determination of the CNS. (Partially supported by DGAPA, U.N.A.M. and CONACYT-0694P-M9506).

CLUSTERING OF DELTA GLUTAMATE RECEPTOR IN THE DENDRITIC SPINES OF CULTURED PURKINJE CELLS -INTERACTION WITH DELTA RECEPTOR, SPECTRIN AND ACTIN CYTOSKELETON-

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Interaction of neurotransmitter receptors with underlying cytoskeleton via subsynaptic proteins is an important mechanism for the targeting of the receptors to synapses in the central nervous system. We found that agents purturbing actin caused declustering of delta subunit of glutamate receptor in cultured cerebellar Purkinje cells, suggesting that delta receptor is anchored to actin cytoskeleton through which delta receptor clusters are stabilized in the dendritic spines. As spectrin is known to link membrane proteins to actin, we next examined the interaction of C-terminal domain of delta receptor with spectrin *in vitro*. The spectrin and delta receptor were interacted *in vitro* and the interaction was inhibited 50% by 1 μM of Ca^{2+} compared with no Ca^{2+}. However, C-terminal domain of GluR1 subunit of AMPA receptor didn't interact with spectrin. Immunoprecipitation experiments using cultured Purkinje cells by anti-delta receptor antibody also supported the *in vivo* association of delta receptor with spectrin. These results suggest that delta receptor on the postsynaptic membrane of the dendritic spines of cerebellar Purkinje cell is anchored to actin cytoskeleton via spectrin, and intracellular Ca^{2+} level may modulate the receptor - spectrin - actin interaction. Further, the synaptic efficacy and plasticity may be regulated by the receptor clustering - declustering.

The comparative analysis of psychotropic drugs effects on reversible disturbance of brain functions

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In present studies of brain functions are widely used psychopathology and psychopharmacology. The existing functional disturbances have been elaborated for food conditional responses. We have been elaborating an original model of reversible functional disturbance (RFD) of avoidance response. RFD is based on unexpected changes of previously established cause-effect relations in experimental environment. Our experiments pointed out that after RFD avoidance responses in control group drastically diminished ($p<0.01$) and intertrial responses increased ($p<0.001$), which indicated emotional tension of animals. Thus RFD results in anxiety occurrence and in disturbance of previously acquired response. Piracetam (300 mg/êg) and anxiolytic phenazepam (1 mg/êg) reduced consequences of RFD. In experimental groups, neither avoidance responses diminished nor intertrial responses increased significantly ($p>0.05$). However mechanisms underlying these externally identical effects, are different. Piracetam activates mental processes and secondary, as a consequence of this activation reduces anxiety. Anxiolytic phenazepam on the contrary primarily reduces anxiety and as a consequence, eliminating the reason of avoidance disturbance, increases response reproduction. The additional information on necessity of activation of nervous processes for overcoming consequences of RFD is received from comparison of oppressing influence of neuroleptic haloperidol (0.01 mg/êg) and positive one of stimulator amphetamine (0.2 mg/êg). The obtained data show fruitfulness of the model for analysis of mechanisms of disturbance

NITRIC OXIDE-MEDIATED PLASTICITY: ROLE IN PSYCHOSTIMULANT ADDICTION AND NEUROTOXICITY

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ACTIVITY-DEPENDENT REFRACTORINESS IN CULTURED CORTICAL NEURONS

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Activity-dependence of excitability reflects the dynamic nature of membrane conductances. The availability of membrane conductances are affected by various physicochemical and biochemical processes, and are expected to modify the time course of recovery from past activity. We conduct experiments aimed at exploring the relations between past activity and the kinetics of neuronal recovery over time scales ranging from milliseconds to hours and days. We ask, how does the kinetic of neural "forgetting" (recovery from past electrical activity) relate to the activity history? Are uniquely defined time scales involved? Preliminary results from cortical neurons in cultured networks, using perforated patch configuration, demonstrate activity-dependence of refractoriness on a time scale that extends up to tens of minutes. Preliminary analysis shows that the relation between the duration of stimulation at a given frequency and the rate of recovery from the evoked firing patterns takes the form of a straight line on a log-log plot, suggesting the existence of scaling relationships. Analysis of neural activity using extracellular recordings via substrate integrated electrodes suggests that these scaling relations confer long-range temporal correlation in the activity of neurons within their networks.

ANALYSIS OF TEMPORAL PATTERNS OF AVOIDANCE RESPONDING IN RATS UNDER LOW-TO-MODERATE DOSES OF D-AMPHETAM NE AND SELECTIVE DAD1 AND DAD2 RECEPTOR BLOCKADE.

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Experiments were done on male 3-months old Möll-Wistar rats in the shuttle-avoidance task. Rats were divided into six groups which, prior to each daily experimental session, received i.p. injections of respectively, saline, three doses of d-amphetamine (0.5,1.0,and 2.0 mg/kg), haloperidol (0.05 mg/kg), and Sch-23390 (0.025 mg/kg). Cumulative response latency distributions, prepared for each group and each acquisition session independently, were compared. The major effect of chronic amphetamine administration was a dose-dependent increase in the frequency of short-latency avoidances (latencies< 2sec) in contrast to unchanged long-latency avoidance responses. The minor effect was an increase in the rate of inter-trial responses. There was no correlation between these two effects what indicates that amphetamine effect on the avoidance performance was not secondary to the amphetamine-induced increase in locomotor activity, but was rather mediated by a drug-related increase in the response eliciting properties of conditioned stimulus. D1 and D2 dopamine receptor blockers showed differential effect on the avoidance execution. Haloperidol caused significant reduction in the frequency of short latency avoidance responses. Conversely, rats receiving Sch-23390 did not differ from the control subjects in the frequency of short-latency responses, but showed lower probability of avoidances emitted toward the end of the 5 second CS-US interval. In the second part of the experiment, concomitant administration of amphetamine did not compensated for the reduction in short-latency avoidance responses in the haloperidol group, but caused increase in the frequency of short-latency avoidances well above the control level in the Sch-23390 group. These results argue for the different nature of short- and long-latency avoidance responses and suggest involvement of DAD2 receptors in the process of response initiation . Interestingly, higher dose of Sch-23390 (0.050mg/kg) when applied alone on the last training day did not produce a substantial behavioral deficit, but it resulted in a profound behavioral break-down when administered together with amphetamine. The latter observation seems to indicate that behavioral output of dopaminergic transmission may depend more on the balance of D1/D2 DA receptors than on the independent modulation of particular receptor system.

CYTOSKELETON ASSOCIATED PROTEINS IN RAT BRAIN AFTER STAB WOUNDS

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The present study concerned the distribution of the plectin, vinculin, talin and paxilin in the rat telencephalon after stab wounds. Of these proteins, the plectin is associated with the intermediate filaments, while the others are involved in the connection of the actin filaments to the cell membrane. These proteins have been demonstrated in the central nervous tissue, and the cytoskeletal elements they bounded to have important role in the glial reactions after lesions. In deep anesthesia (ketamine *plus* xylazine) the skull was drilled and a stab wound was performed with a steril disposable needle. After 3 or 7 days the animals were perfused with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. The area of the lesion was cut into serial sections with Vibratome and the sections were processed for immunochemical stainings against the aforementioned materials as well as vimentin and GFAP. The immunohistochemical stainings was developed according to the avidin-biotin method. After lesion none of these proteins exhibited so extended and intense immunopositivity like the GFAP and the vimentin. In the intact brain immunoreactive cells were found only scarcely. While a number of cells became immunopositive in the white matter, almost no immunoreactivity was found in the overlying cortex, even in the vicinity of the lesion. Along the corpus callosum the immunoreactivity extended far from the lesion site. Therefore the distribution of these proteins is corresponds to that of the vimentin rather than GFAP after lesion. Noteworthily, the distribution of the intermediate filament associated plectin was almost identical with those of are associated with the actin filaments.

NEUROMORPHOLOGICAL EVIDENCE FOR DEVELOPMENTAL DISTURBANCES OF CORTICAL CIRCUITRY IN SCHIZOPHRENIC PSYCHOSES

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A considerable number of neuromorphological studies demonstrated structural changes in several brain regions of schizophrenics. However, a comprehensive pathogenetical concept of schizophrenic psychoses has not been established until now. The aim of our investigations is the analysis of the detailed neuronal structure and circuitry of the prefrontal cortex (PFC), the anterior cingulate cortex (ACC) and the rostral entorhinal cortex (ERC) of schizophrenic patients.
Using methods from neurobiological basis research (Golgi impregnation, immunohistochemistry with antibodies against calcium-binding proteins, and fluorescence staining with the carbocyanine dye DiI), a variety of characteristic alterations of pyramidal cells and interneurons can be demonstrated in schizophrenic brains.
The PFC of schizophrenics is shown to contain different types of atypical pyramidal neurons. To a certain extent, these changes are comparable to that in other, neuropathologically characterized brain diseases. In the ACC, disturbances of neurochemically defined inhibitory interneurons can be demonstrated. These alterations are assumed to result in a decrease in neuronal output of the ACC in schizophrenic patients. The ERC of schizophrenics exhibits marked cytoarchitectural malformations probably leading to disturbances of the input of polymodal associational information into the limbic system.
In total, the detailed neuronal structure of the schizophrenic brain is found to show a characteristic pattern of alteration, which is attributable to pre- and perinatal noxious events acting on the developing brain, and leads to a severe mis-wiring in pathogenetical key regions in schizophrenia.

The Relationship between Maximal Motor Unit Firing Rate and Surface-Recorded EMG Amplitude in Young and Older Adults.

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Maximal EMG amplitude is often used to support the intensity of neural drive. This investigation was conducted to determine the relationship between maximal EMG amplitude and an independent assessment of neural drive, maximal motor unit firing rate. Experiments were conducted in seven young and eight older adults. Subjects were seated and asked to perform 3-5 maximal isometric contractions of the non-dominant limb knee extensors at 1000 of knee extension. Simultaneously, motor unit recordings were obtained from the vastus lateralis using a four-wire needle electrode. Muscle fiber signals were digitized at 51.2 kHz, filtered (1 kHz – 10 kHz), and three recorded channels were digitally stored. Off-line, individual muscle fiber action potentials were identified using customized signal recognition software. Maximal motor unit firing rate was recorded when subjects reached their maximal force level from the five shortest consecutive interpulse intervals. Surface EMG recordings were obtained using a pre-amplified surface electrode placed over the belly of the vastus lateralis and digitized at 1 kHz. Maximal EMG amplitude was obtained during a 1-2 second epoch when maximal force was reached. The results indicated a moderate relationship between maximal motor unit firing rate and EMG amplitude. Pearson correlations of $r = 0.39$ (young), $r = 0.65$ (older), and $r = 0.54$ (both groups combined) were computed. Thus, although EMG amplitude is affected by morphological characteristics of the underlying muscle fibers and technical recording factors, it also contains a component related to neural drive.
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ON DISORDERS OF PHOSPHOLIPID METABOLISM IN MOLECULAR MECHANISMS OF PATHOGENESIS OF EXPERIMENTALLY INDUCED RAT BRAIN ACUTE EDEMA

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The experimental acute brain edema of rats was induced by the intraperitoneal administration of 0.5 ml of 2.0% solution (10 mg) of tetraethyltin per 1 kg of body weight. The development of acute brain edema was accompanied by the significant increase of phospholipase A2 activity, which led to the intensification of phospholipid-glycerides deacylation, predominantly of phosphatidylcholines with the parellel formation of solid pool of lysophosphatidylcholines. These changes took place with the simultaneous increase of the quantity of mono- and especially polyenic fatty acids. Our data have shown the incorporation of fatty acids into the free radical peroxidation processes with the formation of lipid peroxides such as hydroperoxides, di- and trienic conjugates, and malonic dialdehyde (MDA) which have membranotoxic and membranolytic activities, typically performed under the conditions of acute brain edema. It was established also that the esterified arachidonic acid, the concentration of which at the pathology studied was increased greatly, seemed to be the main factor which induces alterations in fatty acids metabolism of brain tissue. Changes in the ratio of the free and esterified arachidonic acid probably play an important role in the pathogenesis of acute brain edema. Our data obtaind indicate that the disorders in phospholipid metabolism have a significant participation in iniciation, development and generalization of acute brain edema pathogenesis.

MOLECULAR MECHANISMS OF MYCOTOXIN ZEARALENON TOXIC ACTION ON CELL PHOSPHOLIPIDS METABOLIC PROCESSES

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It has been shown that mycotoxin zearalenon in concentration 2.5-15.0 mg/ml of alcohol solution activates monoamines biosynthesis, while 20.0-25.0 mg/ml of toxin studied has a contrary effect. The molecular mechanism of changes noticed is conditioned by action of zearalenon on the physico-chemical properties of proteins functioning in chromafin granules mainly in their membranes. Among these proteins the cytochrome b561, an acidic copper-containing protein and the membrane-bound form of dopamine-b-monooxigenase, which catalyses the reaction of transformation of dopamine into noradrenaline were studied. It has been established also that i/v administration of toxin in dose 1.0-15.0 mg/ml to rats leads to the development of abnormalities in mitochondrial and microsomal fractions of brain tissue in the ratio of phospholipid-phospholipid interrelations. These changes were conditioned by significant disorders in qualitative and quantitative composition of individual acidic and neutral phospholipids in cell systems studied, which were accompanied by the simultaneous depression of cell respiratory function in general. The toxic action of mycotoxin zearalenon was characterized also with the activation of free radical processes, which lead to the formation of high concentrations of lipid peroxides in the systems studied. On this background using single i/m administration of 1.0 ml of 10.0% aquaus solution of sodium thiosulfate accompanied by normalization and establishment of the initial level of phospholipid metabolism intensity and their content in the systems are studied.

THE ROLE OF ENTOPENDUNCULAR NUCLEUS IN ANIMALS LEARNING AND MEMORY

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Experiments have been done on the rats and cats using different behaviour tests with the local destruction of entopenduncular nucleus. As behaviour models were used instrumental conditional reflexes of diverse degrees of difficulties, on diverse motivation basis (learning on simultaeous and succesive differentation, tests on extinction), openfield test too.The results are compared with the effect of distraction of pallidum and of several formations of limbic system, appeared in similar conditions.It allows to discuss the data from the point of view of brain evolutionary transformations.

RELATIONSHIP OF ELECTROENCEPHALOGRAM WITH VERBAL AND SPATIAL ABILITIES OF HUMAN

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We attention to one interesting fact, concerning to a problem of the interrelation between verbal and spatial abilities in this investigation. The thirty four right handers took part in the research. The abilities were estimated with successfulness of five verbal and five spatial psychological tests. During the testing EEG was registered in five pair monopolar sites: (F3,F4; F7,F8; P3,P4; T3,T4; O1,O2). Besides that, the EEG was registered before and after the testing, when subjects have had to perform a simple sensomotoric task as a background control. The value of the EEG synchronization was calculated from EEG data under different conditions. The level of the EEG synchronization between the regions was calculated by the coefficient of linear correlation between of EEGs. The analysis showed the next. Firstly, the interhemispherical EEG synchronization correlated negatively with spatial ability scores, and correlated positively with verbal ability results; secondly, the synchronization in frontal sites revealed negative correlation with spatial abilities and positive with verbal ones. In terms of this finding we calculated the frontal-occipital gradient of the synchronization as a difference between the level of the synchronization in frontal and occipital sites respectively. These parametrs positivly correlated with the verbal ability ($r=0.49$, $p<0.01$). It has been showed, that the subjects who had a positive gradient synchronization have had higher verbal abilities, while, the subjects with negative significance of gradient have had higher spatial abilities. Thus, the subjects having a low intercorrelation between hemispheres in a background showed high activities in the parietal-occipital sites and negative gradient of synchronization characterized the higher spatial abilities and simultaneously lower verbal ones. But the subjects with inverse correlation above-named parameters demonstrated higher verbal and lower spatial abilities. We believe, that foregoing correlation with parameters of background control of EEG reflect the individual and specific mechanism of abilities, which underlies negative correlation between verbal and spatial abilities.

NEUROCHEMICAL PROFILE OF RGH-1756, A DOPAMINE D_3 RECEPTOR SELECTIVE POTENTIAL ATYPICAL ANTIPSYCHOTIC

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RGH-1756 has been discovered as a compound inhibiting apomorphine (APM) induced climbing in mice with much less action on sniffing. Its „atypical" anti-psychotic profile was confirmed by other conventional animal models described in the literature for the characterisation of antipsychotics. Here, we present the receptor profile of RGH-1756, and its actions on cerebral monoaminergic systems in mice and rats in comparison with other antipsychotics. RGH-1756 was tested on over 60 receptors in *in vitro* binding experiments. The compound was found to be the most active on cloned human D_3 receptors (K_i=0.12 nM) followed by the actions on human 5-HT_{1A} (K_i=0.96 nM) and rat adrenergic α_{1A} (K_i=2.4 nM) receptors. K_i values for human dopamine D_{2L}, D_{2S} and serotonin 5-HT_7, adrenergic α_{1B} receptors were between 10 and 50 nM whereas its effects on all remaining receptors tested were negligible. RGH-1756 enhanced DA turnover rate ((DOPAC+HVA)/DA) in mouse forebrain at doses higher than 10 mg/kg, with a maximal increase between 1 and 2 hours. At the same time, it reduced 5-HT turnover (5-HIAA/5-HT). RGH-1756 (3-40 mg/kg, p.o.) did not affect APM (2 mg/kg. sc.) induced reduction in DA turnover in the striatum and limbic areas. In the similar dose range it prevented (±)-7-OH-DPAT (DPAT, 0.1 mg/kg, sc.) evoked decrease of DA turnover. In various regions of rat brain RGH-1756 (1-30 mg/kg. p.o.) produced much less alterations in both DA (maximal increase of 30 %) and 5-HT turnover (maximal decrease of 20 %) than in mouse brain. In the rat only the highest dose examined (i.e. 40 mg/kg, p.o.) was able to partially antagonise the DPAT-induced decrease in DA turnover either in striatum or limbic brain. RGH-1756 alone (up to 40 mg/kg. p.o.) caused only slight increase of DOPA accumulation rate in striatum but not in limbic parts and a reduction of 5-HTP accumulation was seen in the limbic brain. RGH-1756 (3-40 mg/kg) slightly antagonised the reduction of striatal DOPA accumulation by DPAT in γ-butyrolactone treated rats. In microdialysis experiments RGH-1756 preferentially enhanced the extracellular DA levels in rat prefrontal cortex over that in striatum.
In summary, RGH-1756 has outstanding *in vitro* affinity to human D_3 receptors with remarkable selectivity. RGH-1756, in low doses, exerts only moderate or no actions on cerebral dopaminergic mechanisms. Increased turnover rate seen after higher doses is likely due to the D_2 antagonism. The slight or no effects of RGH-1756 (especially in lower dose rage) on dopaminergic mechanisms in rats and in mice may indicate that D_3 receptors possibly do not participate in the regulation of DA biosynthesis/turnover or release.

SUBCORTICAL GLUTAMATE/ASPARTATERGIC INNERVATION OF THE MEDIAL SEPTUM-DIAGONAL BAND-HIPPOCAMPUS COMPLEX IN THE RAT. A COMBINED STUDY BY USING [^{3}H]D-ASPARTATE AUTORADIOGRAPHY AND IMMUNOCYTOCHEMISTRY.

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The present examinations were focused on the localization and immunicytochemical character of glutamate/aspartatergic subcortical neurons projecting to the medial septum-diagonal band complex (MSDB) and to the hippocampal formation. Previously, we demonstrated (Leranth and Kiss,1996; Leranth et al., 1999) that supramammillary (SUM) area calretinin (CR)-containing neurons projecting to the septal complex contain the excitatory transmitter aspartate/glutamate. In these experiments the investigations were completed to study the exact localization, immunocytochemical character and topographical distribution of excitatory neurons which project to the MSDB-hippocampus complex from various subcortical areas.
[^{3}H]D-aspartate (^{3}HDA) as a neurotransmitter-specific retrograde tracer was injected into septal or hippocampal regions. The retrogradely labelled neurons were detected by autoradiography on brain sections previously immunostained for various neuropeptides or transmitters.
Retrogradely ^{3}HDA-labelled, supposedly aspartate/glutamatergic neurons could be observed in the SUM area after both septal or hippocampal injections, however, a significant difference was revealed in the cell density and the localization of the radioactively labelled neurons projecting to MSDB or the hippocampus. ^{3}HDA-injection into the dorsal hippocampus resulted in radiolabelled neurons in the vertical limb of the DB (vDB) and the medial septum (MS). These labelled neurons never showed CR- or parvalbumin(PA)-immunoreactivity. In sections, previously immunostained for ChAT radiolabelling was observed over ChAT-immunopositive neurons.
Our results give morphological evidence on aspartate/glutamate contain of the supramammillo-hippocampal innervation terminating in the dentate gyrus and the CA2/CA3 area of the hippocampus. We also demonstrate a further subcortical glutamate/aspartatergic projection to the hippocampus originated from the MSDB cholinergic neurons. To establish the target elements and the role of the latter projection still needs to be elucidated.
Supported by the National Fund OTKA grant T-023989 to K.J.
References: Leranth,C. and Kiss,J. (1996) J.Neurosci. 16(23):7699-7710; Leranth,C., Carpi, D., Buzsaki, G. and Kiss, J. (1999) Neuroscience, 88(3):701-718.

LONG-TERM EFFECTS OF EPILEPTIZATION ON SEIZURES THRESHOLD OF ADULT WAG/Rij RATS.

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Abstract: The development of the brain is not only determined by genetics, but also by the prenatal and postnatal environment. Seizures threshold of adult rats brain after hyperthermia convulsions during neonatal period or prenatal treatment with pentylenetetrazole (PTZ) was studied. WAG/Rij strain of rats for generalized absence epilepsy was used. Spike-wave discharges are observed in EEG beginning with the age of 3 months. Hyperthermic convulsions induced on the 3,5,7 and 9-th day in rat pups by heating them with lamp. There was no difference between albino and WAG/Rij rats to the latency periods(LP) of the beginning of the febrile convulsions. The adult rats were tested with i.p. PTZ administration. The duration of seizures (not the LP) decreased for WAG/Rij in comparison with albino rats. In the other experiments we examined the influence of prenatal PTZ (40mg/kg every other day via dams or saline as control) exposure on seizures activity of the mature brain of WAG/Rij rats. Pups were examined with PTZ once at 70, 100 or 130-th day. 70 days old rats in contrast to rats tested at 100-th day demonstrated lower seizures threshold than the control. Test on 130-th day did not show the difference between the experimental and control groups. Our data showed the possibility to modify epilepsy seizures' threshold during prenatal period, then we measured it in adult age. We are grateful to Prof. G.van Luijtelaar and Prof. T.Coenen for guidance of our researches (Nijmegen Institute for Cognition and Information, The Netherlands).

STRUCTURE-FUNCTIONAL ASPECT OF CONDITIONAL REFLEX ENERGY ENSURING

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In our previous investigations it was shown that subtle structure (microtubular disaggregation in proximal parts of dendrites) disturbance impacts the most complex forms of conditioned behaviour, in particular instrumental conditioned reflexes. In the present study we pay the special attention to distal segments of cortical apical dendrites of the I layer, as just the main target of non-specific afferents, crucial for energy ensuring of conditioned reflex. It is experimentally shown that functional «elimination» of dendrites of the I layer by means of local ischaemia (transitory photochemically induced thrombosis of the vessels of upper layers of the cortex) don't acts on highly specialised conditioned reflexes and is crucial for the elaboration and realisation of young non-specialised reflexes. Hence, the different phases of consolidation and specialisation of conditioned reflex need the different energy ensuring, which in ones turn depends upon the structure-functional peculiarities of distal parts of pyramidal neurons apical dendrites.

CHANGES IN GAD 67 mRNA EXPRESSION IN THE BARREL CORTEX DURING SENSORY CONDITIONING

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The GABA-ergic system in the cerebral cortex is regulated by neuronal activity; denervation or sensory deprivation leads to downregulation of the levels of transmitter, receptors and synthesizing enzyme(GAD). We examined the regulation of this system following sensory stimulation used in a classical conditioning paradigm. The aim of the study was to establish if stimulation affects expression of a constitutive gene coding for an isoform of GABA synthesizing enzyme, GAD67, prevalent in the cerebral cortex and responsible for 80% of GABA synthesis in the adult brain. In the conditioning paradigm stimulation of row B of vibrissae in adult mice was paired with a tail shock. Three daily sessions resulted in significant enlargement of cortical representation of the trained row of vibrissae in the barrel cortex (Siucinska and Kossut,1996). In situ hybrydization after one training session found no changes in GAD expression. Directly after 3 training sessions and 24 hours later the level of GAD67 m RNA expression in the row B of barrels was very significantly elevated. The last examined time point is 5 days after the training , when the plastic change in the cortical representation is no longer seen. The results sugggest that sensory stimulation upregulates the GABA-ergic system.

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PROTECTIVE EFFECT OF ALPHA-TOCOPHEROL ON HIPPOCAMPAL SLICE CULTURES IN THE LOW Mg^{2+} INDUCED EPILEPSY

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Patients with temporal lobe epilepsy had often febrile seizures in their childhood and present later on gliosis as well as severe loss of neurones in the hippocampus. To investigate the effects of long lasting epileptiform activity on juvenile tissue, we have used the low Mg^{2+} model of epilepsy in organotypic hippocampal slice cultures. Upon withdrawal of Mg^{2+} from the perfusion slice cultures developed epileptiform discharges. Both, the amplitude of evoked field potentials and of spontaneous events decreased with time in Mg^{2+} free cerebrospinal fluid. These cultures also showed massive cell loss as proved by the large fluorescence signal of the cell death marker propidium iodide. Free radicals produced by mitochondria may be one reason of the cell death in the seizing cultures. Therefore, we preincubated the slice cultures with alpha-tocopherol, a free radical scavenger. Alpha-tocopherol on itself did not show antiepileptic effects, but strongly protected against cell death in all regions. First experiments with the potential sensitive mitochondrial dye rhodamine123 revealed that the mitochondria depolarize during longer lasting seizures, thereby suggesting the mitochondrial origin of the free radicals in our model.

EFFECT OF RHINAL CORTEX LESIONS ON SPATIAL DELAYED RESPONSES GUIDED BY ACOUSTIC STIMULI

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Four dogs were trained in three-choice spatial delayed responses directed by auditory stimuli, with 10s delay, to a criterion of 90% correct responses in 90 consecutive trials (6 sessions). Then, they received bilateral rhinal cortex surgery (Rh). After reaching postoperative criterion, they had additional training on performance task with delays extended to 30, 60 and 120s, and with distractions, each in blocks of 90 trials. Results of the group Rh were compared to seven control dogs (group C) and six dogs submitted bilateral hippocampal surgery (group H) (see Kowalska, 1995, Hippocampus 5:363-370). Although the rhinal dogs needed some extended training to preoperative criterion (in comparison to the groups C and H), they did not show impairment on relearning the task after the surgery, whereas such impairment was observed in the group H. Opposite to the hippocampal dogs, who were significantly worse than the control dogs in the performance task with longer delays, and during training with distractions, the Rh group did not differ significantly from the group C along these stages of training. The results indicate that the rhinal cortex lesion does not impair performance on the spatial delayed responses guided by acoustic stimuli.

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Decreased GSK-3β Immunoreactivity in Postmortem Frontal Cortex of Schizophrenic Patients

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Objective- Glycogen synthase kinase-3 (GSK-3) is a protein kinase highly abundant in brain and involved in signal transduction cascades of multiple cellular processes, particularly development. In an attempt to explore possible involvement of GSK-3β in psychiatric disorders we examined its levels in postmortem brain tissue. *Methods-* Western-Blot analysis of GSK-3β in frontal cortex of 14 schizophrenic patients, 15 bipolar patients, 15 unipolar patients, and 14 normal controls was carried out. *Results-* GSK-3β levels were 41% lower in schizophrenic patients than in normal subjects ($p=0.008$). Other diagnostic groups were not different from controls. *Conclusions-* Our results are consistent with a previous report of low GSK-3 in lymphocytes of schizophrenic patients, a recent report of low β- and γ-catenin in hippocampal subregions of schizophrenic patients, and with the notion that schizophrenia and involves neurodevelopmental pathology.

PLASTICITY OF AUDITORY CORTEX IN YOUNG CONGENITALLY DEAF CATS CHRONICALLY STIMULATED WITH COCHLEAR IMPLANTS

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Deaf white cats never acquire any hearing experience. Their auditory system can, however, be stimulated electrically using cochlear implants (CIs). To investigate cortical reorganisation set up by early hearing experience, the animals were equipped with CIs at the age of 3-4 months. They were stimulated using a compressed analogue coding strategy. The signal processor was carried in a jacket. The animals could move freely. They received biologically relevant stimuli 24 h/day, 7 days/week. In addition they were classically conditioned to acoustic stimuli.

After 1 to 6 months of hearing experience the cortical activity was determined by recording field potentials and single- and multi-units (SU/MU) with microelectrodes. The results show substantial differences between naive cats and chronically stimulated cats. The area of auditory cortex responding to stimulation with biphasic pulses by the CI increased after chronic electrical stimulation. The maximal middle-latency responses increased in amplitude by a factor up to 3. Field potentials and SU/MU recordings revealed long-latency responses (>150 ms), which were absent in naive animals. Also the unit responses showed a greater variability in the shape of post-stimulus time histograms than seen in naive animals. The latter show only simple onset responses. Synaptic activity in the auditory cortex was investigated using current source density analysis. In chronically stimulated animals it revealed a nearly normal activation of cortical layers. This contrasts to the deficient pattern of layer activation seen in naive animals. The activation of infragranular layers, which is absent in naive animals, is present after the chronic stimulation of the implanted animals. Large changes were also observed in layers II/III following stimulation. The results demonstrate the capacity for plastic reorganisation of the auditory cortex in young congenitally deaf animals. The chronically stimulated congenitally deaf cats show cortical activation comparable to normal hearing, acutely deafened cats stimulated by a cochlear implant.

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PEPTIDERGIC CODING OF PROJECTIONS TO THE RAT LOCUS COERULEUS COMPLEX - IMPLICATIONS FOR CHRONIC PAIN PATHWAYS.

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The locus coeruleus complex (LCC) is activated by various sensory/noxious stimuli indicating its involvement in the integration of endogenous and exogenous pain and stress stimuli. Several neuropeptides, e.g. cholecystokinin (CCK), galanin (GAL), enkephalin (ENK), substance P (SP), appear to modulate LCC activity, however, little is known yet about the underlying anatomical pathways. To investigate this, retrograde tracing (fluorescent beads) was combined with in situ hybridization (^{35}S-radiolabelled oligonucleotide probes) for the detection of CCK, GAL, ENK, SP mRNAs. Besides known projections from the central gray (GAL, ENK mRNA), we detected for the first time retrogradely labeled cells in the cingulate and frontal cortices (expressing CCK), as well as in the central and medial amygdala (with ENK). Projection neurons were also newly found in the lateral habenula, interpeduncular nucleus (together with SP mRNA), medial preoptic (CCK/GAL), Edinger-Westphal, lateral parabrachial (both CCK) and solitary tract nuclei (GAL). Information about neurochemical coding of the multiple coeruleus afferents still has to be extended and functional implications have to be explored. In view of the present data, the LCC appears to be a brainstem analogue to the hypothalamic paraventricular nucleus (key nucleus for stress-induced activation of the hypothalamo-pituitary axis), however integrating information towards predominantly vegetative bodily reactions to stress/pain, e.g. colonic motor and bladder activity. Thus, from a clinical point of view, it is very interesting having found CCK-ergic projections from the cingulate cortex, the activity of which is altered in patients with irritable bowel syndrome - a disease that appears to be centrally generated, however the underlying pathways still have to be dissected. Supported by NIH grant DK 33061.

SELECTIVE TARGETING OF HABENULAR, THALAMIC MIDLINE AND MONOAMINERGIC BRAINSTEM NEURONS BY NEUROTROPIC INFLUENZA A VIRUS IN MICE

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Infections caused by influenza A virus have been proposed to be associated with neuropsychiatric complications, the mechanisms of which remain to be unraveled. We here report that a neurotropic strain of influenza A virus (A/WSN/33) introduced into the olfactory bulbs of C57BL/6 (B6) mice, selectively attacks habenular, paraventricular thalamic, and brainstem monoaminergic neurons. In the habenular and paraventricular thalamic areas, infection was followed by an almost total loss of neurons within 12 days. In the brainstem monoaminergic areas, viral gene products were eliminated from neurons by 12 days in B6 wildtype mice, but remained for at least 35 days in immunodefective TAP1 (Transporter associated with Antigen Presentation 1) mutant mice. In conclusion, we show that influenza A virus infection in the brain selectively targets regions, which have been implicated in neuropsychiatric disturbances, and that this virus can remain for a significant period of time in specific regions of the brains in immunodefective mice.

IMPAIRED INTERNODAL IONIC CONDUCTANCES IN MYELINATED AXONS OF PERIPHERAL NERVES FROM TRANSGENIC MICE WITH DIFFERENT NEUROFILAMENT EXPRESSION.

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Neurofilaments (NFs) are the major cyoskeletal component in large myelinated axons of the peripheral nervous system, and are composed of three protein subunits, NF-L, NF-M and NF-H, with molecular weight of approximately 70 kD, 145 kD and 200 kD, forming the rope like structures. NFs play a critical role in determining axonal caliber and consequently conduction velocity of large myelinated fibers. The aim of this study was to examine electrophysiological properties of myelinated axons with altered morphology in transgenic mice with different NF-H expression (NF-H knockout mice and mice overexpressing human NF-H, line 200 mice). Single axons membrane properties were measured on isolated sciatic nerves in vitro using intracellular standard current-clamp technique. Transgenic animals showed several functional deficits in physiological properties. In NF-H knockouts voltage responses revealed increased steady state input resistance (dRss) during depolarization (12.1±1.0 MΩ, control vs. 15.5 ±1.0 MΩ) and increased peak resistance during evoked hyperpolarization (hRp) (33.5±2.4 MΩ , control vs 42.6±3.9 MΩ) (control n=28, NF-H-/- n=14). Axons from line 200 transgenic mice showed significant increase in peak (dRp) (14.2±0.95 MΩ control vs 23.11±1.7 MΩ) and dRss (12.07±0.82 MΩ vs 20.44±1.12 MΩ) (control n=28, line 200 n=15) during depolarization associated with impaired inward rectification . Our studies demonstrate that targeted NF-H disruption or human NF-H hyperexpression in transgenic mice affect functional characteristics of large myelinated fibers (ion channel defects), suggesting a complex structure/function relationship distinct from previously established structural role of NF.
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ROLE OF THE CYTOSKELETON IN CALCIUM SIGNALING IN RAT PERITONEAL MACROPGAGES

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According to conformational coupling model the coupling between depletion of intracellular stores and activation of plasma membrane Ca^{2+} channels is suggested to occur through a physical interaction between the IP_3 receptor and the Ca^{2+} channel. Such an interaction could be either a direct protein-protein interaction, or could perhaps involve interaction with the cytoskeleton. Thus we investigated the effects of disruption of actin and tubulin cytoskeletons on Ca^{2+} signaling in rat peritoneal macrophages. Using Fura-2 microfluorimetry it was shown that in cells pretreated with cytochalasin B, cytochalasin D or dihydrocytochalasin B, agents that disrupt actin microfilaments, the ability of thapsigargin or cyclopiazonic acid to empty Ca^{2+} stores and activate store-dependent Ca^{2+} influx was significantly attenuated. These compounds were also able to reduce capacitative Ca^{2+} entry after it had been fully activated. Similar experiments with microtubule disrupters vinblastine, colchicine or colcemid to depolymerize the tubulin network yielded identical results. On the contrary, microfilament and microtubule disrupters did not effect ATP- or UTP-induced increases of $[Ca^{2+}]_i$ indicating that release of Ca^{2+} from intracellular stores through the inositol phosphate pathway was intact. The results suggest that an intact cytoskeleton is required for capacitative Ca^{2+} entry but not for agonist-induced Ca^{2+} signaling. The necessity for an intact cytoskeleton favors a conformational coupling model for store-dependent Ca^{2+} entry.

SIMULATION OF tTHE DINAMICAL BEHAVIOR OF NEURON ACTIVITY ON THE SUBCORTICAL LEVEL OF THE VISUAL SYSTEM.

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The responses of single neurons or neuronal pools of the cat lateral geniculate body to the presentation of the visual stimuli with various spatio-brightness parameters were investigated. The power spectrum analysis methods was used for the evaluation of the influence of the stimulus properties upon appearance and development of gamma-oscillations (20-80Hz) in the neurons responses. The pronounced gamma-oscillations have been shown to appear in responses of neurons when their activity was in excess of the threshold, certain for each neuron. When the activity level decreases in consequence of the change of the stimulus parameters, several additional peaks arise in the response spectrum, i.e. the spectrum form becomes typical for chaotic dynamics of systems. Similar threshold of the activity level is also exhibited by uneven alteration of the correlation interval, which determines average length of the regular phase. Quantitativive evaluation of the stimulus-evoked synchronization have been obtained by comparison of the correlational dimension calculated for the pool responses to the stimuli with various brightness parameters. The concepts given above were used for mathematical discription of the proceses of appearance and development of gamma-oscillations based on the nonlinear transformation, characteristic of the intermittent transition to deterministic chaos. The results of the model investigations are in a good agreement with the experimental data.

VARIETY OF ASSOCIATION OF MYASTHENIA GRAVIS AND OTHER AUTOIMMUNE DISEASES

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Myasthenia Gravis (MG) is widely recognized as an autoimmune disease with various disease associations, but there is no common point of view about the frequency of immunological disorders among the patients with MG. In our research in 81 Myastenics and their 802 relatives was found an extremely higher rate of collagenoses, allergic diseases, diabetes mellitus, thyroid diseases and malignant tumors (50,6% patients, 15,2% siblings, 37,7% parents, 13,8% grandparents, 7,9% children), than in the general population of Georgia. In 11 patients MG was combined with demyelination disorders. Diagnosis was based on the typical clinical picture, neurophysiological, immunological and x-ray findings. The following hypothesis about the mechanism of combination of MG and demyelination diseases was expressed: the primary activation of myelin reactive T-cells by microbial antigen or superantigen takes place in periphery. Activated T-cells preferably penetrate into the thymic medulla. Myelinreactive activated T-cells mediate inflammation process directed against myelin basic protein (MBP) epitope, not only in brain, but also in thymus. Thus single BMP epitope would give rise to the first attack of the target organ, that would result in T-cells mediated inflammation and development of multiple autoimmune reactions due the widespread sensitization. This mechanism would enhance autoantigen presentation or release of immunogenic cell components that would give rise to secondary autoimmune response to acetylcholine receptor.

Thus, the high frequency association of MG and other autoimmune diseases is based on generalized disturbance of immunoregulation and the associated disease needs special treatment.

ROLE OF CYTOSCELETON IN DIFFERENT SYNAPTIC COMMUNICATIONS OF CEREBELLUM GRANULAR AND PURKINJE NEURONS.

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In 1987 we revealed electrophysiologically long-term increase of glutamatergic parallel fibre-Purkinje cell synaptic efficacy of frog Rana Temporaria cerebellum in vitro /1/ after joint stimulation of climbing and parallel fibres (PFs). Then in 1991 we have found electronmicroscopically morphological correlates of this effect (connected with the problem of long-term memory), looking as electrondense globulas (about 100 nm width) penetrating a synaptic cleft /2/. Stimulation of PFs (1 h) led to bouton encapsulation of spines without globulas. In the norm we observed mainly spine encapsulation of boutons. Incubation (2h) of cerebellum in pure Ringer solution increased the number of nonencapsulated PF-PC-synapses and enhanced the process of desynaptization /3/. So we have observed four types of PF-PC-synapse profiles: globulated, nonencapsulated («face-to-face»), bouton- and spine-encapsulated (apparently as a result of cytosceleton change in encapsulating synaptic terminal). In the consequence of our analysis of different influences on structure and configuration of PF-PC-synapses we supposed the possibility of existence of continued connection between cytosceleton elements of Purkinje and granular cells. In the case of joint stimulation of climbing and parallel fibres the globulas, penetrating a synaptic cleft, were such bouton-spine connecting elements, and in the case of encapsulation this connecting role was realized by the interneuronal matrix of synaptic contact. Configuration of encapsulated synaptic profile reflected the direction of information gradient (for example bouton encapsulation was observed under PF stimulation). In the case of pathological pressure increase (high concentration of reagents in vitro, long time of their action, PF elecrostimulation parameters) it was observed in 1994 (in glutamate, 1mM in vitro) the pathological fusion of neurons with the appearance of cytoplasmic continuity /4/. Thus we supposed in 1999 that between the absence of cellular contact (desynaptization) and pathological contact with cytoplasmic continuity (as a result of neuronal fusion) there is the diapason of neuronal communication (besides mediator) in which it is possible the appearance of INFORMATIONAL CYTOSCELETON CONTACT between neurons through the synaptical matrix.

REFERENCES: 1) Dunin-Barkovski V.L., et. al. Neirofiziologija, v.19, №2, pp. 156-164, 1987. 2) Larionova N.P., et. al. In: Intercellular Communication. Ed. F. Bukauskas, Manchester and New-York, pp.77-90, 1991. 3) Larionova N.P., et. al. Dokladi Akademii Nauk, v.333, №2, pp. 260-263, 1993. 4) Samosudova N.V., et. al. Dokladi Akademii Nauk, v.336, №3, pp. 406-409, 1994.

MODULATION OF THE NOCICEPTIVE NEURON RESPONSES IN THE CAT CEREBRAL CORTEX

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Intracellular recordings of the nociceptive neurons of the somato-sensory cortex I (*Gyrus coronarius*) have shown responses to the supraliminal single stimulation of the tooth pulp, which consisted of EPSP-peak-IPSP. Ipsilateral stimulation of the central gray matter (CGM) elicited an inhibition of the background activity for 150-250 ms, and hyperpolarization of the membrane potential (MP) by 5-10 mV. Conditioning stimulation of the CGM suppressed the nociceptive response altogether. Intravenous injection of morphine (0.3 mg/kg) decreased a frequency of spike activity without affecting neuron's MP, and than partly (1 min) and completely (2 min) suppressed a pulp stimulation-induced postsynaptic potentials. Meanwhile the neuron activity elicited by the non-noxious stimuli did not change at all. Suppression of the postsynaptic potentials of the nociceptive neurons elicited by the tooth pulp stimulation during both conditioning stimulation of the CGM or morphine administration is realized probably through the afferentation input, on the segmentary level, as well as presynaptically - on the cortical neurons.

BACK-PROPAGATING ACTION POTENTIALS DECREASE THE THRESHOLD FOR CALCIUM ACTION POTENTIALS IN LAYER 5 NEOCORTICAL PYRAMIDAL NEURONS

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Layer 5 (L5) neocortical pyramidal neurons have at least 2 zones for generating action potentials (APs). One zone, in the axon, has a low threshold and generates sodium APs (Na+-APs) which propagate along the axon and back into the dendrites. The other zone has a higher threshold and generates so-called calcium action potentials (Ca2+-APs) in the distal dendrites of L5 pyramidal neurons. These Ca2+-APs are broad and usually cause bursts of Na+-APs in older animals (>P28). The question arises as to what physiological conditions may lead to the initiation of Ca2+-APs. Here we show that the back-propagating Na+-AP can also facilitate the generation of a Ca2+-APs when timed so that it arrives in the dendritic initiation zone at the same time as the distal EPSP input (within a 20 ms time window). Furthermore, we show that inhibitory input onto the dendrite has a far larger blocking effect on the generation of Ca2+-APs than Na+-APs. This therefore constitutes a novel mechanism whereby inputs leading to the generation of Na+-APs can be associated with inputs arriving in the distal dendrites leading to a burst of axonic Na+-APs. Dendritically located inhibition, on the other hand, can selectively abolish the Ca2+-AP and the subsequent burst without affecting the ability of the neuron to fire normal Na+-APs.

METHYLATED-DNA BINDING TRANSCRIPTION FACTOR MECP2 IN THE POST-NATAL AND ADULT RAT BRAIN

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MeCP2 is a transcription factor protein that binds to methylated cytosine bases in the promoters of genes and represses their transcription[1]. It is essential for embryonic development, and for NGF-induced neuronal differentiation. We examined the expression of MeCP2 in paraformaldehyde-fixed post-natal and adult rat brain, both in vivo and in cell culture, using two specific and high-affinity antisera.

Immunoreactivity for MeCP2 was restricted to the cell nucleus, and it occurred in neurons but never glia. At post-natal day 1 it was not present in the spinal cord, but various brain areas showed weak nuclear staining. Definitive immunostaining first appeared in the spinal cord and brain at post-natal day 4, and this subsequently increased in intensity to reach adult levels by day 26.

In the adult brain, MeCP2 usually appeared in all neurons within any one area, but the intensity of staining varied markedly between different areas throughout the brain. MeCP2 was absent in many areas. These included the caudate putamen, the medial and lateral geniculate, the mediodorsal thalamus, and the mammilary, pontine and accumbens ncl. Weak staining was present in areas such as the septum, the cochlear and gigantocellularis ncl, and the raphe magnus. Moderate staining occurred in the olivary ncl, piriform cortex, tegmentum, and the superior colliculus. Intense immunoreactivity was seen in the central grey, the parabrachial ncl, the habenula, the hypothalamus, and the zona incerta. In many areas, such as the hypothalamus, the immunoreactivity was distributed evenly throughout the nucleus of each neuron; whereas in other areas, such as the hippocampus, it instead had a punctate distribution within each nucleus.

The levels and distribution of MeCP2 were unaffected by nerve stimulation or a variety of neuroactive compounds, or by axotomy or ischemia.

Thus transcription factor MeCP2 varies in its distribution throughout the brain, being absent in some areas. Other methylated-DNA binding transcription factors must exist in these areas.

1 Nan X, Campoy FJ and Bird AP, *Cell* **88** (1997) 471-481.

QUANTITATIVE ANALYSIS OF THALAMOCORTICAL SYNAPSES IN DEVELOPING MOUSE BARRELS.

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This study focuses on the development of synapses made by thalamocortical afferents to mouse barrel cortex. Goals are to determine the timing of halamocortical (TC) synaptogenesis, whether TC synapses are subject to overproduction and elimination, the numbers of boutons per axon length and the number of synapses per bouton at various developmental stages, the identities of postsynaptic elements and whether changes occur during development in specific synaptic p a t t e r ns. TC axon terminals are labeled by the anterograde transport of (1) DiI injected and photoconverted postmortem, or (2) BDA injected in vivo. The tissue is then processed for electron microscopy and serial thin sectioned. Results to date indicate that some TC synapses are formed as early as P7. Up to P11, labeled boutons form only single, asymmetrical synapses, whereas in the adult, boutons of thalamocortical afferents typically form 2 to 4 synapses; en passant synapses with no varicosity at all are observed also. As in adult barrels, 80% of the TC synapses are axospinous, whereas 20% are axodendritic. The similarity in the distribution of thalamocortical synapses onto spines vs. dendrites in developing and mature barrels indicates that adult synaptic patterns already are specified at the onset of thalamocortical synaptogenesis.

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NOCICEPTIVE ACTIVATION OF LAMINA I NEURONS PROJECTING TO THE CAUDAL VENTROLATERAL MEDULLA IS MODULATED BY THE TARGET

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Neurons in lamina I of the spinal cord belong in four structural groups and play a major role in pain transmission. Nociceptive activation of lamina I neurons varies according to the morphological cell class, the supraspinal target and the nature of the noxious stimulus. Several supraspinal lamina I targets originate spinopetal pain control pathways and are reciprocally connected with lamina I neurons. In this study, we evaluate whether the descending actions triggered from a particular supraspinal pain inhibitory area, the caudal ventrolateral medulla (VLM), account for the control of nociceptive activation of lamina I neurons projecting to it. Male Wistar rats were injected in the left VLM with 0.3µl of the retrograde tracer cholera toxin subunit B (CTb) under halothane anaesthesia. Three days later, the animals were reanaesthetised and the right VLM injected with 0.3 µl of either saline (control group) or quinolinic acid (lesioned group). After another three days, animals were subjected to noxious pinching or to formalin stimulation of the right thigh, under chloral hydrate anaesthesia, and perfusion-fixed after 2 hours. Coronal sections of segments T_{13}-L_3 were processed immunocytochemically for CTb and the Fos-protein. Numbers of CTb-labeled neurons of each lamina I cell class (fusiform, pyramidal and flattened) exhibiting Fos-immunoreactive nuclei were compared by multiway ANOVA. Numbers of CTb-labeled neurons immunoreactive for Fos increased in VLM-lesioned animals after both kinds of stimulation, although statistical significance was only reached for fusiform cells activated by mechanical stimuli and flattened cells activated by chemical stimuli. The present results support the possibility that the nociceptive responses of lamina I cells is under the control of supraspinal lamina I targets. Supported by Biotech project no. BIO4-CT98-0076.

EMERGENCE OF SYNCHRONY IN A SIMPLE FEED-FORWARD NETWORK WITH BALANCED EXCITATION-INHIBITION: IMPLICATIONS FOR RATE CODING

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Balanced excitation-inhibition (BEI) has been used in a number of models addressing both the problem of neural gain control (i.e. how to make the neuron fire with a rate identical to the average rate of its inputs) and of temporal variability in firing patterns. Recently, Shadlen and Newsome (S&N, J. Neurosci. 18:3870:3896, 1998) presented a model of information transmission by average firing rate of groups of neurons in a strictly feed-forward network with BEI. On the basis of simulations of the transmission of spikes in a single layer they concluded that firing rate can propagate in a stable way in a multilayered network. In addition, for percentage of shared connections up to 40%, the spike correlations between neurons in each layer remain low, a feature which is essential for the ability to extract rate information by population averaging. To test S&N hypothesis we have simulated a multilayered feed-forward networks with S&N parameters. In contrast to previous claims we found a rapid decay of firing rate between subsequent layers. The reason is that, because of shared connections, neurons tend to synchronize their firing times. Due to the BEI, inhibitory neurons become synchronized with the excitatory neurons and cancel the excitatory input to the next layer. We conclude that a simple feed-forward model consisting of identical layers with excitation-inhibition balance is incapable of stable transmission of an unsynchronized rate.

EFFECT OF ACTIVE MEMBRANE CONDUCTANCES ON DENDRITIC INTEGRATION

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Recent experiments have clearly shown that the dendritic membrane contains a myriad of voltage-dependent ion channels. Some of these channels participate in the initiation and prorogation of dendritic spikes but most of them are also active in the sub-threshold regime. Interestingly, some of these channels are distributed non-uniformly over the dendritic membrane. Numerous suggestions were provided regarding the possible role of these channels, from boosting of distal synaptic signals to linearization of synaptic potentials to binding between the synaptic input and the back-propagating dendritic spike. However, a systematic study that explores the interaction between these ion channels and their effect on sub-threshold synaptic integration is still missing. The present study utilizes detailed compartmental modeling in order to integrate the available experimental data. This includes the spatial distribution of different channel types over the dendritic surface, the morphology of the dendritic tree and the properties of its synaptic inputs. Specifically, this study highlight the effect of fast inactivating A-type K^+ channels, persistent Na^+ and the slow hyperpolarization-activated I_h current, all known to be present in dendrites of cortical pyramidal neurons. Rules which determine the input-output properties of dendrites containing these channels are highlighted. We show that, while insights that were gained from passive cable models of dendrites are still relevant, dendritic nonlinearity enriches the repertoire of synaptic integration that could be implemented by the dendritic tree.

SYNAPTICALLY COUPLED EXCITATORY SPINY NEURONS IN LAYER 4 OF RAT BARREL CORTEX WITH A COLUMNAR ORGANIZATION OF THEIR AXONS

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The so-called 'barrel field' in layer 4 of rodents is the cortical representation of the whisker pad on the muzzle. Excitatory neurons in layer 4 are the main targets of thalamic afferents and are therefore in a key position to process and distribute information coming from extracortical areas. Here we describe morphological features of two classes of synaptically coupled excitatory neurons, spiny stellate and star pyramidal cells. The dendritic domain is largely confined to a single barrel with the exception of the apical dendrite of star pyramidal neurons that terminates in layer 2/3. In contrast, the axonal projection spans the entire cortical laminae with the most prominent projections in layers 4 and 2/3, but was largely restricted to a cortical column. Horizontal collaterals in layers 5 and 6 were found to project across columns. The total number and density of boutons/100 µm axonal length was highest in layers 4 and 2/3. Electron microscopy revealed that the majority (90%) of synaptic contacts were established on basal dendritic shafts and spines of excitatory neurons in these laminae. The largely columnar organization of the axons with a strong and predominant projection within cortical layers 4 and 2/3 suggests that spiny layer 4 neurons may serve as key elements in amplifying thalamic inputs and distributing excitation within a cortical column. Therefore, these neurons are fundamental for the control of information flow in the barrel field of the somatosensory cortex.

THE ROLE OF ARTERIAL BLOOD SUPPLY AND VENOUS CEREBRAL OUTFLOW WITH MULTIPLY SCLEROSIS (MS)

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The subject of the work is multiple sclerosis. The purpose is to study the hemodynamical changes of cerebral blood sypply in MS patients. 17 such patients aged 21-42 years old have been observed at Ukrainian Scientific Medical Centre. The present observation included neurologic status, EEG and brain blood condition inspection-ultrasound dopplerography(USDG) using the methods of our own for a clinical interpretation of the USDG data of external cerebral arteries and veins. We have noted decreased linear circulation rate in cerebral arteries with systole below 50% from normal rate in 82% of patients. In all patients, we diagnosed the pronounced cerebral arterial hypertension. 43% of these patients had the increased intracranial pressure rate mainly at the level of the diastolic arterial blood pressure. Concerning venous cerebral outflow, we diagnosed the pronounced venous cerebral discirculation in one or both internal jugular veins because of various types of venous distony and the absence of venous collateral as a compensatory mechanism with venous congestion. Due to our methodology of venous cerebral disorders diagnostics and the individual approach to medicinal vasoactive treatment we received some positive results in treatment of neurologic deficiency, speach improvements, frontal psychics correction, improvement of extremities' functions. Our first experience has shown that the pathogenetic treatment with above stated diseases has to be long term taking into account the circulation condition.

AXONAL PROJECTIONS FROM THE HYPOTHALAMUS TO THE MEDIAN EMINENCE AND POSTERIOR LOBE IN RATS DURING ONTOGENESIS: DII TRACING STUDY

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This study has determined the schedule of the arriving of the axons of the hypothalamic neurosecretory neurons in the median eminence (ME) and posterior lobe (PL) in rats during ontogenesis by using the fluorescent lipophilic dye, 1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate (DiI) as a retrograde tracer. In the 1st series of experiments, DiI was implanted postmortem in the ME on the 13th embryonic day (E13), E14, E15, E16, E17, E18, E20 and on the 2nd postnatal day (P2). In the 2nd series of experiments, DiI was implanted in the PL on E16, E17, E18, E20, P3 and P10. According to our data, the axons of the hypothalamic neurons first reached the ME at E14. >From E14 to E16, the number of the neurons projecting their axons to the ME increased abruptly, that was accompanied by their redistribution throughout the hypothalamus from the region around the third ventricle to the places of their final settling. Some neurons from the preoptic and more rostral region also send their axons to the ME. From E17 till P2 DiI-labelled neurons were mainly located in the diagonal band, preoptic region, supraoptic (SO) and retrochiasmatic (RC) nuclei, as well as in the magnocellular and parvicellular divisions of the paraventricular nucleus (PV), and the arcuate nucleus (ARC). Few neurons in the anterior periventricular nucleus became visible from E20. It should be mentioned, that the visualisation of the magnocellular neurons after the DiI application on the ME may be a result of the labelling of the hypothalamo-pituitary tract fibers in the internal zone of the ME. As follows from the 2nd series of experiments, the SO contained fluorescent neurons as early as on E16, suggesting the arrival of their axons in the PL at the very beginning of the neuron differentiation. The initial axons of the neurons of the PV appeared to reach the PL later, at E18, and the number of these cells was not large even postnatally. The magnocellular neurons of the RC sent their axons to the PL first on E17-E18, whereas the axons, originated from other accessory nuclei, reached the PL only after the birth. Thus, the axons of parvocellular and magnocellular neurons reach the ME and PL unexpectedly early in embryogenesis, attracting the attention to the possible functional significance of neurohormones in fetuses, before the establishment of the neuroendocrine regulations.

THE CONTENT OF SEROTONIN IN THE CNS DURING LEARNING IN HELIX

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It is known, that the serotonin mediates sensitization,as well as, constitutes an important part of a noxious reinforcement in learning (Balaban P.M., 1987).We are concerned with studing the effect of learning on the level of the serotonin in the CNS in Helix. The experiment was carried out on snails (Helix lucorum). Animals to be conditioned received 4 paired stimuli (conditioned stimulus - a piece of carrot, and unconditioned stimulus electric stimulation of the lips) at 15 min intervals. The level of serotonin was determined spectofluorimetrically (Curson G., Green A.R., 1970). The Fluorescence of samples was measured using a Hitachi spectrofluorimeter at 475 nm. The light used to excite fluorescence had a wavelength of 360 nm. The level of serotonin was calculated in micrograms per gram of tissue wet weight. The data obtained were treated statistically according to Student-Fisher. According to results obtained the content of serotonin in the CNS during avoidance conditioning in Helix decreased. In 1 hour after training the level in serotonin in the CNS is fairly stable. It appears reasonable to assume the avoidance conditioning in Helix may be controlled by the seroninergic neurones.

PEPTIDE-INDUCED CHANGES OF ADRENOREAKTIVE PROPERTIES CORTICAL NEURONS

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The characteristic outline of up-to-date stage of the problem of neurochemical mechanisms of learning and memory is the study of the particular role of peptides in these processes.

The experimental results showed that cortical neurons reactions on electrical stimulation of ascenting noradrenergic brain system (Locus coeruleus) on the background of peptides actions are of different character. The analysis of neurons excitations showed the redistribution of relative number of exciting and inhibiting neurons as well as the rearrangement of their impulsation pattern on the vasopressin background. The level of observed changes in neurons active part gives the grounds to consider the existence in the cortex the specialized cell-targets, possessing the high sensitivity to noradrenaline and vasopressin.

In overall estimation of the data, it is important to emphasize that the efficiency of interactions of adrenergic and peptidergic impacts on the cortical neurons is determined not only by modulation of noradrenaline synthesis and secretion under the vasopressin influences, but as well by changing of cortical postsynaptic structures mechanisms of changing of adrenoreactive features of cortical neurons under the noradrenaline impact.

ANALYSIS OF DRAWINGS BY A MONKEY

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Are complex drawing-motions composed of primitives? This question may be studied by analyzing drawing movements made by a monkey, as well as the neuronal activity during this behavior. We report here on the methods of analysis of the movements.

A Fascicularis monkey was trained to "draw" while holding a manipulandum. Although no explicit guidance on the form, speed, etc... was provided, it seemed that the monkey developed a few prototypical elements of drawings, which it tended to concatenate into more complex figures. Thus we wish to whether the monkey generates complex figures out of simpler drawings which in turn may be composed of yet simpler strokes.

We first separated periods of fast motions from periods of relative rest. Then, parameters of motion were extracted by computing the tangential velocity, and the radius of curvature at each point. Using the tangential velocity, the fast motions were broken into strokes from one extremum of the tangential velocity to the next. Each stroke was fitted by finding the parameters beta and K that allowed for the best fit to the power-law:

The goodness of fit was estimated from the correlation coefficients within each stroke. In most stokes the fit was very good ($r>0.95$). Marked deviations were found at the beginning, end, and sometimes in the middle of a stroke.

Motion parameters were not constant throughout the training period. The number of strokes per movement and the length of each stroke increased during training. Beta started well above 1/3 and approached 1/3 during training (1/3 fits harmonic motion, and is reported to be the value for human drawings).

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CONTRASTING EFFECTS OF SELECTIVE HIPPOCAMPAL LESION ON DIFFERENT MEMORY EXPRESSIONS OF SPATIAL DISCRIMINATIONS IN MICE

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There is controversy as to whether the hippocampus of rodents is specialized for spatial memory or whether it mediates a more general function in all forms of relational/declarative memory. In the present study, the discriminative performance of mice with ibotenic hippocampal lesion were assessed in a three-stage spatial task. The information acquired in the initial learning stage was the reward contingency associated with six (3 positive and 3 negative) arms of a radial maze . The only parameter which was varied between the successive phases was the way of presenting the arms to the mouse. Training began using a go-nogo discrimination procedure during which the arms were presented one by one. This acquisition phase was followed by two test phases in which the arms were first presented by pairs (2-choice discriminations) and then 6 at a time (6-choice discriminations).

1°) As compared to controls, lesioned mice displayed lower performance in the initial acquisition. 2°) However, even those lesioned mice which succeeded in acquisition, were impaired in subsequent 2-choice discriminations. 3°) Finally, whatever their previous performance, hippocampal mice tended to be more accurate than controls in 6-choice discriminations.

The presently observed dissociation between different expressions of memory for the same piece of acquired experience suggests that the role of the hippocampus is critical to a declarative form of memory and not limited to spatial memory.

MODULATION OF HIGH VOLTAGE SPINDLES BY THE LATERAL PARAFASCICULAR NUCLEUS IN FREELY MOVING RATS

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Rats with spontaneous rhythmic synchronized waves at 6.5-9.5 Hz (high voltage spindles, HVS) are considered as model of human generalized absence epilepsy. HVSs occur during awake immobility in some strains of rodent, and arise suddenly out of a normal desynchronized EEG background. In the present experiments, we used young Sprague-Dawley rats which are not genetically prone to HVS. Animals were previously implanted with standard electrodes to monitor EEG and EMG to assess the behavioral state of vigilance. The power spectra in EEG activity were computed on line. The rats received stereotaxic unilateral microinjection (under ketamine anesthesia) of the excitotoxin ibotenic acid into the lateral parafascicular nucleus (PF). In this report, we concentrate on the immediate-early postoperative effects. Immediately after the end of the injection, the EEG activity of the hemisheres was asymmetric as regards the power spectra in the 4-7 and 7-14Hz bands. These bands increased contralaterally to the injection, while the distribution of slow waves remained symmetrical. Twenty five min after the injection, sporadic high amplitude HVS, in which the spike component predominated, arose bilaterally. These peculiar EEG rhythms simulated quite closely in form and sequence the EEG patterns of the recruting responses in cats (Morison and Dempsey, 1942). Gradually, the EEG activity was ipsilaterally depressed, while bursts of spindling occurred contralaterally. During the first postop day, HVSs were of higher amplitude with the wave component also evident, and occurred mainly contralaterally. Their density (incidence reached 6/min) and frequency (from 5 to 9 Hz) increased. They were interspersed with long runs of theta waves (6-9Hz), and were present not only in the quiet waking, but also during REM. During slow wave sleep, spindling activity was also increased contralaterally. Behaviorally, HVSs were accompanied by immobility. The asymmetry as regard immobility-related HVS and sleep spindles was a short-term deficit recovering rapidly and disappeared after 48h. This period corresponds to the early excitation induced by the drug. The lesions were histologically analyzed and only results obtained from rats with circumscribed lesion restricted to the lateral PF were included in this study. These results stress the contribution of lateral PF neurons to the generation of 2 brain rhythms: HVS and spindles. The multiplicity of connections of the lateral PF neurons, recently described, may define a network capable of generating dynamic patterns of ensemble activity and mediate the contralateral effect.

A NEURON-SPECIFIC GENE, nel, ENCODING A CYSTEINE RICH PROTEIN INCLUDING SIX EGFL-REPEATS.

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The nel gene encodes a 91Kd cysteine rich secretory protein including 6 EGFL-repeats. The amino acid sequences are well conserved between species, and all cysteine residues are conserved. Although the gene is ubiquitously expressed in early embryos, expression is restricted to neural tissues in adults. *In situ hybridization* analysis and immunohistological studies revealed that gene expression occurred in neurons, and that the protein was localized in the cytoplasm including that of axons and dendrites of the neurons, despite the fact that the protein contains a signal peptide domain. In order to clarify the localization of the protein in the cells, a tag (FLAG) conjugated nel plasmid was introduced into COS cells and neuroblastoma (CL8c4.7) cells expressing nel, and the gene product was monitored with an anti-FLAG antibody. The Nel protein was detected in the culture supernatants of COS cells. In the CL8c4.7 neuroblastoma, secretion of FLAG-Nel protein was observed in only one of three stable transformant cell lines tested. The cytoplasm, including the processes, was stained with the antibody in a stable transformant from which the antigen was not secreted, similar to that of the brain neurons. The antigens were accumulated in ER lumens of the cells as revealed by electronmicroscopic analysis. These results suggested that Nel is possibly a ligand type factor of which secretion is controlled by physiological conditions of the cells, although the target cells are not yet known.

Studies on the effects of chronic cyanide administration on behaviour and brain neurotransmitters in albino rats

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Much of the toxicological interest in cyanide has been focused on its rapid lethal action; however, its most widely distributed toxicological problems are due to its chronic toxicity from dietary, industrial and environmental factors which have not been studied in detail. Hence the effects of chronic sublethal dose of cyanide on albino rats was studied with behavior and brain neurotransmitters as the evaluation parameters. Chronic sublethal dose of cyanide significantly alters the emotional status, reduces the memory and impairs motor co-ordination. It also alters the neurotransmitters dopamine, norepinephrine, epinephrine and serotonin in the corpus striatum, cerebellum, hippocampus and hypothalamus. This study revealed that even very low dose of cyanide when given over a period of time is capable of producing significant alteration in behavior and brain neurotransmitters.

IPSILATERAL RETINOCOLLICULAR PATHWAY REFINEMENT IS DISRUPTED IN NITRIC OXIDE SYNTHASE DEFICIENT MICE

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The free radical nitric oxide (NO) has been implicated as a retrograde messenger in synaptic plasticity, including pathway refinement during brain development. In normal mice, the pathways from the retina to the superior colliculus (SC) are initially exuberant but gradually refine into an appropriate topographic pattern by eye opening. We have been studying whether NO plays a role in this process by examining the refinement of the ipsilateral retinocollicular pathway in NOS inhibited mice and in gene knockout mice in which either the neuronal or both neuronal and endothelial NOS genes have been disrupted. NOS inhibited mice were injected intraperitoneally daily with n;-nitro-R-arginine from P1 until P14 and perfused at P15. Gene knockout mice were raised until various ages (P3 to P42) before perfusion. The anterograde tracers DiI or WGA-HRP were injected into the ipsilateral eye 48 hours before sacrifice. The labeled area in SC was measured from digital micrographs using a digitizing tablet and measurement software. The ipsilateral pathway in both control and single nNOS knockouts was initially spread across much of the mediolateral extent of the rostral SC, but retracted to the rostral and medial SC by P15. By contrast, the pathway in double e, nNOS knockouts remained spread throughout much of the rostral and mediolateral SC at ages P21-P28 and did not fully retract until adulthood. Quantitative comparisons of the distribution of label in double knockouts vs control animals showed that this delay in retraction was statistically significant. NOS inhibited animals also had an aberrant ipsilateral retinocollicular pathway when compared to vehicle controls. We conclude from these results that NO contributes to pathway refinement in the rodent SC, but only when both isoforms of NOS are disrupted and only during the initial phase of refinement.
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PERSISTENCE, COMPRESSION AND BEHAVIORAL DEPENDENCE OF SPIKE SEQUENCES IN THE HIPPOCAMPUS

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According to the two-stage memory model, hippocampal pyramidal cells selectively replay the sequence of spikes during slow-wave sleep that was earlier generated during exploratory behavior. We tested the "replay" hypothesis by considering multi-cellular triplets and quadruplets of spikes where the sequential order of spikes and the relative timing were both constant. The temporal structure of the 4 to 28 simultaneously recorded CA1 and CA3 hippocampal pyramidal cell spike train revealed stable repetition of spike sequences that was persistent across different behavioral states. (1) The joint probability of spikes within 200 msec interval was significantly higher than by chance using Fisher's exact probability test and Monte Carlo statistics. (2) The temporal distribution of spikes relative to each other was discrete and time locked to the ongoing field activity (theta, gamma, sharp wave-related ripple activity). (3) Long (50-200 msec) sequences were predominant during theta activity. In contrast, short (<50 msec) sequences were coincident with sharp wave-related ripple activity. (4) Sequences recurred during slow wave sleep were 4-5 times compressed in time relative to the same sequence (4) Behaviorally induced sequences were persistent during subsequent sleep episodes but they were absent during the preceding sleep phase. The behavioral induction and persistence of spike sequences may implicate their role in information representation and memory consolidation. Supported by NINDS, NIH, HFSP.

THE VISUAL MEMORY SYSTEM IN THE CAT : NEUROBEHAVIORAL APPROACH

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As is well known ,visual memory in mammals involves two different forms: one - the nonspatial visual memory , and the other - the spatial visual memory. The specific object of study is elucidation of the cortical structures in the cat involved in the performance of the tasks, handling seperately nonspatial and spatial varieties of the visual memory . The delayed matching to sample (DMTS) was used to assess the abilities of normal and cortically lesioned cats to solve the nonspatial visual memory task , while the classical delayed response (DR) and Parkinson' s test (PT) were used in assessment of the spatial visual memory . The tasks used were presented to animals in two varietes (a) using manipulatory responses, and (b) using locomotor responses. 20 trials per day were presented during experimental sessions until animals have learned the tasks to criterion (no more than 2 errors in the 20 consecutive trials) on the all tasks used . Comparable in size lesions were performed by subpial suction in dorsolateral prefrontal cortex (mainly g . proreus) and in posterior parietal cortex (mainly areas 20 and 21) , which we view as a functional analog of the IT cortex in primates . Results obtained have shown , that normal cats were superior in the learning of the DR and PT tasks if learning proceeded in the locomotor version instead of the manipulatory one , while the contrary was observed for the DMTS task . It follows , that in the normal cats the visuospatial memory dominates the nonspatial visual memory if the locomotor responses are used in the process of solution, which probably is the case in natural behaviors in this species . Nonspatial visual memory was severely disturbed in the cats after the bilateral lesions in the posterior cortical association areas (areas 20 and 21), while the spatial visual memory was severely handicaped after bilateral lesions in the prefrontal association cortex. Unfortunately the so called double dissociation of symptoms cannot be inferred from data obtained because the less severe deficit was found on the learning of the DMTS task in the cats after prefrontal lesions too, if they were tested on this task under the manipulatory version .This conclusion is supported by the neuronal data , obtained with the extracellular recordings of the single cells from rhe above mentioned cortical association areas in the cats trained to perform the relevant behavioral tasks - measuring spatial (DR), and nonspatial (DMTS) visual memories. Discriminant analysis of the neuronal data has rewvealed that activity of the units in the parietal cortex was related to the visual characteristics of the to be remembered stimuli irrespective to their spatial locations while unit activity in the prefrontal cortex was related either to the spatial location or to both the spatial location and visual characteristics of the to be remembered stimuli . This work was partially supported by the grant MX 200 from the Soros foundation to the first author .

PROTEIN S100 beta AND NEURONAL PLASTICITY

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Participation of S100 beta, calcium-binding protein from S100 protein family with neurotrophic and neurogrowth activities, in mechanisms of neuronal plasticity in mature brain was investigated. Experiments were carried out on snail Helix lucorum command neurons of defensive behaviour. By S100 beta specific ELISA S100 beta quantitative level in nervous ganglia and muscle of snail Helix lucorum was analyzed. It was detected that in nervous ganglia of snail Helix lucorum the S100 beta level is 11,4±1,9 ng/mg tissue and in 3-4 times higher than in snail's muscle. Administration of rabbit polyclonal antibodies to S100 beta in doze 0,1 mg/ml to Helix lucorum command neurons caused the increasing of excitability of plasmatic membrane and decreasing of nervous cell's expression to responses at sensory stimuli. Increasing of excitability of neuron's membrane and it's decreasing during antibodies to S100 beta application was registrated in snails during elaboration of noneceptive sensitization. No influence of antibodies against S100 beta at command neuron's synaptic facilitation of sensitizated animals was marked. Nonimmune rabbit IgG in dozes 0,1 mg/ml and 0,01 mg/ml also as antibodies to S100 beta in doze 0,1 mg/ml didn't evoked any changes of registrated parameters of activity in investigated nervous cells. Experimental data testified the involvement of neurotrophic factor of S100 beta in providing of plasticity in snail's neurons.

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EFFECT OF OLANZAPINE ON COGNITIVE DYSFUNCTION INDUCED BY FG 7142 AND DIZOCILPINE

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The present study examined the effect of atypical antipsychotic olanzapine on FG 7142- and dizocilpine-induced cognitive impairment in passive avoidance paradigm and elevated plus maze in mice. Both FG 7142 (5 mg/kg) and dizocilpine (0.1 mg/kg) increased the latency to reach shock free zone both during training and retention session in passive avoidance paradigm. This effect was reversed by olanzapine (0.063, 0.125, 0.25 and 0.5 mg/kg). Similarly, FG 7142 (5 mg/kg) increased transfer latency on both first and second day and dizocilpine on second day in elevated plus maze. The lower doses of olanzapine (0.063 and 0.125 mg/kg) reversed the effect of both FG 7142 and dizocilpine on second day in elevated plus maze but higher doses (0.25 and 0.5 mg/kg) failed to modify their effect. Eventhough olanzapine (0.063, 0.125 and 0.25 mg/kg) failed show any effect per se in passive avoidance task, in higher dose (0.5 mg/kg) it increased the latency to reach shock free zone on second day. Olanzapine (0.063, 0.125, 0.25 and 0.5 mg/kg) did not show any per se effect on transfer latency in elevated plus maze on first day while on second day, it (0.125, 0.25 and 0.5 mg/kg) increased transfer latency as compared to control group. The reversal by olanzapine of dizocilpine-and FG 7142-induced impairment of cognitive process might be due to blocking of excessive DAergic activity in the prefrontal cortex.

Effect of noxious chemical stimulation on afferns of cardiovascular and respiratory systems

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Aim of Investigation: This study tested the hypothesis that mechanoceptors responding to activation of cardiovascular and respiratory systems are involved in the perception of visceral pain.

Method: Wistar rats were anesthetized with hexenal (0.25 g/kg i.p.). Actions potentials were recorded from single afferents of vagi nerve fibers. Bradykinin (BK) was using as the noxious chemical stimul. BK (5, 10 and 20 μg) was administered i.v.

Results: Units were selected on the basis of their sensitivity to activation of cardiovascular and respiratory organs. The duration of impulses activity spikes was 2.4± 0.1 msec and 2.7± 0.7 msec respectively. The inhibitory effect of this viscероceptores was observed in 10 sec after BK (10 μg) injection. The maximum of block was up to 20 sec. The irregular spontaneous activity (60-80 imp/sec) has elicited in the part of units (10 %) only at time BK influence.

Conclusion: The peripheral mechanism of visceral pain exciting by noxious chemical factors are associated with activation of BK- sensitive units, block of mechanoreceptors function and accordingly with breach of cardiovascular and respiratory organs control the work.

THETA WAVES RECORDED FROM THE CEREBELLAR CORTEX DURING DESYNCHRONIZED SLEEP IN RATS

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Introduction. The cerebellum has been shown in the past decade to be involved in several neural processes in addition to control of motor functions. Memory, learning and visual processing are among such functions (see Bloedel et al. Progr. Brain Res. 1997, 114:499-509, and Strick, P.L. Mol. Psychiatr. 1996, 1:429-433). In the present work a presumable involvement of the cerebellum in sleep was investigated. Method. In twelve adult Sprague-Dawley rats we recorded: 1) the electro-oscillograms of neocortical Krieg's areas 10 and 3 (rostral portion of F2 and caudal portion of F1, respectively, according to Zilles), of the hippocampal field CA1 and of the anterior lobe cerebellar vermis; 2) head (H), rostrum+vibrissae (R) and eye (E) movements. Results. Theta waves (6-8 Hz, nearly 100 m V) occurred in the cerebellar cortex, in A3 and in CA1 during preparadoxical and paradoxical (desynchronized, DS, REM) sleep, increasing in voltage and frequency when H, R and E appeared, thus unveiling involvement of the cerebellum in the DS mechanisms. During attentive wakefulness, when theta waves are always present in area 3 and in the hippocampus, theta activity was scanty in the cerebellum. Comments. Theta waves are clearly involved in DS, keeping a close relationship to the hippocampal electro-oscillograms. No direct connections are known to exist between the cerebellum and the hippocampus that might explain such a functional pattern. However, many projections connect with the cerebellum several neocortical regions in which theta predominates during wakefulness and DS. In addition, the pontine nuclei, some of which seem to participate in the generation of theta waves (Sim es, Valle & Timo-Iaria, Braz.J.med.Biol.Res. 1996, 29:1645-1650), project directly to the cerebellar cortex, via a pathway that may be in parallel with the branches that send theta waves to the hippocampus.

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SEARCH FOR THE CHOLINE TRANSPORTER BY FUNCTIONAL COMPLEMENTATION IN A MUTANT YEAST WITH AN ELECTRIC LOBE LIBRARY

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Despite many attempts and diverse approaches, neither a 'general' choline transporter nor that which is thought to be specific for cholinergic nerve terminals has been clearly identified for any multicellular organism; even though choline is important for membrane synthesis and involved in intracellular signalling as well as being the precursor for acetylcholine. We decided to apply the strategy used to clone the choline transporter from yeast (Nikawa et al., JbiolChem 265, 15996) to this problem by trying to complement the yeast choline transport mutation using a yeast expression library made with electric lobe cDNA. The electric lobe of torpedo has already proven to be an excellent source of cholinergic clones. Among the clones selected for choline dependent growth of mutant yeast under selective conditions, the cDNA coding for torpedo inorganic phosphatase showed the strongest rescue phenotype, perhaps due to its proposed role in regulating phosphatidylcholine synthesis. Several other clones were associated with weak rescue, some of which seemed to code for membrane proteins. One of these was able to promote choline uptake by transformed yeast, and was identified as being a truncated homologue of a 9-10 TMD protein of unknown function indicated by the genomic sequence of *C. elegans*. The full length torpedo cDNA was isolated and expressed in Xenopus oocytes: no clear increase in total choline uptake was observed, but the hemicholinium-3 sensitive component of choline uptake was found to have doubled. Northern analysis shows a single 4 kb band of mRNA which is predominantly expressed in the electric lobe but also found in non-cholinergic tissues such as the electric organ. One explanation of these results is that the complementation paradigm selected a choline transporter with characteristics reminiscent of the erythrocyte choline transporter, also endogenous for cultured cells and oocytes; indeed, screening by PCR has not yet uncovered a negative cell line. Defining the nature and metabolic role of this new protein will take some effort, but should help establish more propitious conditions in the search for the neuronal choline transporter.Financial support for this work was partly provided by a grant from the Association Francaise contre les Myopathies.

RATIO BETWEEN DIAMETERS OF THE CEREBELLAR PURKINJE CELLS AND THEIR NUCLEI IN COURSE OF RAT'S ONTOGENY: MORPHOMETRIC DATA

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The aim of the study was to compare vertical and horizontal diameters (VD and HD respectively) of rat's cerebellar Purkinje cells (PC) and their nuclei in the course of ontogenesis. The results were obtained on four groups of Wistar rats: newborn, one week, three weeks old and adult animals. The animals were sacrificed under deep urethane anaesthesia, the cerebellum was removed, fixed in mixture of 10% formaline with ethanol, after treatment by different percentage alkaline solution, then it was embeded in paraffin and serially sectioned in parasagittal plane in 15 μm section. The sections were stained with cresyl-violet by the Nissl method. We study the ratio between the vertical and horizontal diameters of cerebellar PC and their nuclei in different age groups. It was shown that in the course of ontogenesis the ratio between VD of PC decreases from 1.49 μm in newborn rats to 1.12 μm in adult one, while ratio between the HD of PC decreases on more value - from 1.96 to 1.28 μm. The same tendency in ratio between diameters of rat's PC nuclei was observed. These observations permit to conclude that tendency to decreasing of horizontal diameters ratio during ontogeny is more pronounced in comparison with the same index of vertical diameters. Such type of cerebellar PC neurogenesis is determinant factor of pear-like shape of the cerebellar PC at the adult stage.

A PROJECTION SYSTEM FROM THE MIDLINE DORSAL TEGMENTUM WITH CROSSED REACTIVITY WITH THE GENOSYS FOS ANTISERA

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We have observed that Genosys antibody anti Fos protein gives rise to two kinds of immunoreactivity: one nuclear and stimulus-dependent and one cytoplasmic and stimulus-independent. We have called the cytoplasmic labeling as Genosys extranuclear fos-like immunoreactivity (GENFLI). GENFLI labeling localized in neuronal somata of three places: the ventral tegmental area, the ventromedial periaqueductal grey and a group of nuclei in the midline dorsal tegmentum composed of the nucleus O, the central grey pars alpha and the nucleus raphe pontis. From these places tracts of fiber labeling courses rostrally through the medial forebrain bundle. In the cerebral cortex GENFLI terminal-like labeling was observed in the frontal, retrosplenial and occipital cortex and in the dorsal endopyriform nucleus. Dense plexus of fibers were present in the entorrinal cortex, medial and basolateral amygdala and hippocampus. The densest GENFLI plexus was observed in the medial septum-diagonal complex but intense labeling was also present in the rest of septal nuclei. The hypothalamus also contained dense GENFLI labeling except of the medial and the lateral mammilary nuclei. Some GENFLI plexuses were seen in the midline thalamic nuclei (paraventricular, reuniens and rhomboid). In the brainstem dense labeling was observed in the ventral tegmental area, periaqueguctal grey, rafe nuclei and interpeduncular nuclei. Tracer injections in areas with dense GENFLI plexuses resulted in retrograde labeling in the VTA, periaqueductal grey and midline dorsal tegmentum.

IDENTIFICATION AND MOLECULAR CLONONG OF DIFFERENTIALLY REGULATED GENES ASSOCIATED WITH THE NEURONAL ACTIVITY-DEPENDENT SURVIVAL OF CEREBELLAR GRANULE NEURONS.

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Depolarizing stimuli promote the survival of many neurons. The cerebellar granule neuron is one of the typical examples whose survival is neuronal-activity dependent. In the presence of sub-depolarizing concentration of KCl and/or NMDA in the culture medium their survival is greatly enhanced. We used the mRNA differential display to identify genes which are regulated by an activity-dependent manner and found multiple genes were regulated. One of the genes was up-regulated by stimulating the cells with 25 mM KCl or the combination of 150 microM NMDA and 15 mM KCl. The expression of this gene was confined to these survival-promoting conditions and virtually no expression was observed in the absence of these reagents, the condition of which induces apoptosis. Addition of AP-5 to the NMDA-supported culture of the granule neurons induced apoptosis within 24 hours. The expression of this gene was rapidly down-regulated within 5 hours under this condition. The another gene was up-regulated under the non-depolarizing condition which induced apoptosis of the granule neurons. No expression of this gene was observed under the survival-promoting conditions. Addition of AP-5 to the NMDA-supported culture induced the expression of this gene within 5 hours. These results indicate that those two genes are regulated by the depolarizing stimuli and highly correlated to the activity-dependent regulation of survival of the granule neurons.

CYTOSKELETAL CONTROL OF THE C-JUN AND THE GLUCOCORTICOID SIGNALING PATHWAY

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Glutamine synthetase (GS) is a glial specific enzyme that constitutes in neural tissues an endogenous 'neuroprotective mechanism' which amidates the neurotoxic amino acid glutamate to the non-toxic amino acid glutamine. GS fails to prevent glutamate neurotoxicity under pathological conditions which are often accompanied by massive proliferation of glial cells (gliosis). While it is not clear whether glial cell proliferation is stimulated by the release of soluble factors or by the disruption of glial-neuron cell interactions at the site of injury, we have demonstrated that both growth factors and disruption of cell contacts are sufficient to cause a dramatic decline in GS expression. In this study we investigated the possible role of the cytoskeletal network in cell contact control of GS expression. We showed that depolymerization of the cytoskeletal network in cells of intact retina can mimic the effects of cell separation and inhibit the expression of GS. The molecular basis for inhibition of GS expression involves, in both cases, the transcription factor c-Jun and the glucocorticoid receptor protein, which is a key factor in the transcription machinery of GS. Depolymerization of the actin or the microtubule network rendered the glucocorticoid receptor transcriptionally inactive, although its cellular level remained unchanged. Depolymerization of the cytoskeleton also activated the JNK and p38 pathways and induced a massive increase in the level of c-Jun. The increase in c-Jun, which can form a protein-protein complex with GR and can block its transcription activity, is causally related to the decline in GR activity. Induction of c-Jun expression by depolymerization of the cytoskeleton is dependent on tyrosine kinase activity and is restricted to Müller glial cells, the only cells in the tissue that express glutamine synthetase and maintain the ability to proliferate upon disruption of cell contacts. Our findings suggest that the c-Jun signaling pathway can sense changes in the state of the cytoskeletal network and convert them into changes in GR activity and glutamine synthetase expression. Changes in the cytoskeletal structure might have a role in the transduction of cell contact signals to the nucleus.

GENETIC DETERMINATION OF MORPHOLOGICAL (CT, MRI) PECULIARITIES OF THE VENTRICLES IN THE FAMILIES OF SCHIZOPHRENICS.

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On order to study genetic determination of morphological peculiarities of the ventricles in schizophrenia we examined 68 families of schizophrenics (223 subjects at all) and 162 controls. 135 subjects (42 probands, 70 parents and 23 sibs) underwent CT, 88 subjects (26 probands, 47 parents and 15 sibs) - MRI examination.

The methods included determination of linear dimensions of different regions of lateral ventricles, third ventricle, their indices and individual deviations from controls (22 CT and 14 MRI parameters at all). The method of component expansion of phenotypical dispersion signs was used.

The enlargement of anterior horns in the region of caudate nucleus and central parts of lateral ventricles in schizophrenics, their sibs and parents as compared with controls was established. The majority of analyzed parameters (19 CT and 9 MRI) had high (>50%) and medium (30 - 50%) heritability coefficients.

The results show significant genetic dependence of ventriclemegalia in the families of schizophrenics. From practical point of view, CT parameters of ventricles with high level of heritability are very promising for development of criterion for identification of subjects with high predisposition to schizophrenia

NITROXIDES INHIBIT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental autoimmune encephalomyelitis (EAE) is an experimental model for multiple sclerosis. The neurological disease is caused by activated T cells that cause tissue inflammation. Infiltration of momonuclear cells acting in concert with adhesion molecules, chemokines and proinflammatory cytokines cause demyelination. It is believed that the detrimental effects are partly mediated by free radicals. Attempts to treat EAE with scavengers of free radicals such as catalase and superoxide dismutase were not effective in inhibition of clinical signs mainly because they cannot readily penetrate the blood brain barrier. Nitroxides are stable free radical scavengers and are used to mimic superoxide dismutase activity. Moreover, nitroxides have the advantage to penetrate any viable cell membrane. Therefore, we have administered a nitroxide compound (TEMPOL) to treat EAE in rats. TEMPOL (200 mg) was administered intraperitoneally starting at day 7 post inoculation of MBP+CFA. Clinical signs of EAE were followed in this treated group and compared to vehicle treated rats. We found that TEMPOL treatment resulted in marked inhibition of clinical signs of EAE and of disease incidence (3/16 vs 16/18 in control group). Inhibition of EAE was observed also in cell transfer experiments, where recipients were treated with TEMPOL. We have shown also that TEMPOL inhibits lymphocyte transformation in vitro. We conclude that depletion of excess oxygen free radicals from the target tissue may result in inhibition of the detrimental effector mechanisms in autoimmune diseases. Furthermore, indirect evidence indicate that depletion of oxygen free radicals by TEMPOL prevent the production of peroxynitrite and thus contribute to the beneficial effect to brain tissue. Modulation of free radicals together with other immunotherapies may result in greater effect on patients suffering from multiple sclerosis.

CONVERGENCE OF SENSORY PULSES OF DIFFERENT MODALITIES ON FOREBRAIN NEURONS OF RUSSIAN STURGEON (Acipender guldenstaedi)

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The evoked reaction (summarized and neuronal) in sturgeon forebrain in response to stimulation of visual and olfactory nerves were studied by electrophysiological methods. It was found that in the dorsolateral area of the forebrain there is the zone of combined representation of visual and olfactory analysators. In particular, as the evoked neuronal activity analysis have shown, in this zone there are bimodal neurons, reacting both on visual and olfactory stimulation. The previous stimulation of olfactory input of such neurons caused response inhibition on following visual nerve stimulation. The such convergent interrelations underlying the brain integrative activity, show that on the level oflowest vertebrates the forebrain is already not only the projectional center but as well the integrative center, participating in realization of behavior complex forms.

EVALUATION OF ANTI-PSYCHOTIC ACTIVITY OF CALCIUM CHANNEL BLOCKERS

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Recent evidence suggests that calcium channel blockers (CCB) may have a beneficial effects in the treatment of psychiatric disorders. Therefore, the present study was undertaken in rodents and primates to confirm their behavioural and antipsychotic effects. In rodents, Verapamil (10-80 mg/kg) and nimodipine (25-100 mg/kg), i.p. produced a dose-dependant decrease in spontaneous motor activity (SMA) and sterotypy as obtained in Digiscan Animal Activity Monitor, without producing any catalepsy. Verapamil (40 mg/kg,i.p.) antagonised amphetamine (AMP) induced hyperactivity and amp and L-dopa induced jumping behaviour. In primates studies, graded dose of Verapamil (5-20 mg/kg, im) and nimodipine (7.5-30 mg/kg, im) produced a mild decrease in social and solitary behaviour in rhesus monkey living together in a social colony without producing any cataleptic posture. For neuroleptic effect, CCB's were studied on AMP induced psychosis model. Pretreatment with Verapamil (20 mg/kg, i.m.) significantly suppressed Amp induced hypervigilance, sterotypy, oral hyperkinesia and tachypnoea but was unable to reverse other AMP induced behavioural effect. On the other hand, nimodipine have insignificant antipsychotic effects in both the animals. These results suggest that Verapamil have a definite antipsychotic effect without any extrapyramidal side effects and thus may have a clinical significance in the treatment of psychosis.

THE RETICULAR REGULATION OF RETINA'S FUNCTION AND MECHANISMS OF ITS REALIZATION

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The problem of existence and neurophysiological mechanisms of centrifugal control of retina distal section's functions, participating in Electroretinogram formation is very controversial. In our investigations performed on awake rabbits was shown that as a response on single pulse stimulation of Mesencephalic Reticular Formation the evoked potential of short latency was registered in «Reticular-Retinal-Response» (RRR) that interacted specifically with Retina's response to light. That intravitreal injections of glycine and Strophantin-K were used as inhibitors. The experiments demonstrated that in the case of these inhibitors effect the clear blockade of Retina response on light stimuli was observed. Glycine inhibits all ERG components except «a»-wave, which significantly increases that possibly reflects the elimination of return inhibitory influence from amacrine units on Retina receptor cells. The main RRR components are also inhibited in this case. Under the influence of Strophantin-K the total inhibition of all Retina response components both to light and to reticular stimuli is observed. Is shown the existence of «return» light stimulus influences on RRR formation. In the case, when light flash precedes the reticular stimulus, in certain time intervals the significant elevation of RRR formation is observed. The interrelations of ERG and RRR reflect the role of local processes interrelations mechanisms and of regulatory efferent influences in Retina function realization. The whole picture of obtained data is considered as argument of existence of centrifugal influences from mesencephalic reticular formation to Retina's distal sections, participating in ERG formation.

A PURINERGIC COMPONENT OF THE EXCITATORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS.

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The excitatory synaptic transmission in the CA1 area of hippocampal slices of 17-19 days old rats has been investigated in situ by using patch clamp and intracellular calcium concentration ($[Ca^{2+}]_i$) measurements. Excitatory post-synaptic currents (EPSCs) were elicited by stimulating Schaffer collaterals at frequencies below 0.2 Hz. A small component of the EPSC remains uninhibited after full inhibition of glutamatergic transmission by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and 2-amino-5-phosphovaleric acid (D-APV, 100 μM). The amplitude of this residual EPSC (rEPSC) comprised 25±11% of the total EPSC when measured at a holding potential of -50 mV. The rEPSC was blocked by bath application of the selective P2 purinoreceptor blocker pyridoxal phosphate-6-azophenyl-2'-4'-disulphonic acid (PPADS, 10 μM) or the non-hydrolizable ATP analogues, ATP-γ-S and α,β-methylene-ATP at 50 and 20 micromolar concentrations respectively. The rEPSC was considerably potentiated (to 250±170% of control) by external Zn^{2+}(10 M) in 70% of the cells tested and was inhibited for 25 ±11% in the rest of the cells. Dithiothreitol (DTT) reversed both effects of Zn^{2+} in all cells investigated. In another series of experiments, exogenous ATP was applied to the CA1 neurons *in situ* and elicited the inward current as well as the changes in $[Ca^{2+}]_i$. Inward currents as well as $[Ca^{2+}]_i$ -transients were inhibited by PPADS to the same extent as the rEPSCs. It is concluded that about one fifth of the postsynaptic current generated at the excitatory input to CA1 neurons of rats is due to purinergic transmission which uses various subtypes of P2X receptors.

STRUCTURAL BASIS OF CORTICAL EPILEPTIFORM DISCHARGE SYNCHRONIZATION IN SOMATOSENSORY CORTEX OF RATS.

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The changes in V layer pyramidal neurons number and synchronization of interictal epileptiform discharge in somatosensory undercut cortical slab of 30 and 90 days survival were investigated in 33 white mature rats. In control animals the epileptiform potentials evoked by penicillin were completely synchronous in neocortex sites disposed on distance of 4 mm from each other. The number of cells have decreased in 90 days isolated cortical slabs on 49 %, and the synchronization of potentials was absent in spatially separated parts of the slab. The number of the large and medal size pyramidal neurons in layer V of 30 days isolated cortical slab has decreased on 25 % and the degree of synchronization of the interictal epileptiform discharge was less than in control animals. Our data show, that the losses of large pyramidal neurons of layer V, accompanied a notable loss of the spatial synchronization of electrical potentials. It mast be noted, that the main axonal collaterals of the large pyramidal neurons of layer V of somatosensory cortex could be followed horizontally for a distance of up to 2 mm. Based on this evidence, we have suggested, that the neuronal network formed by the large pyramidal neurons of layer V, provides spatial synchronization in neocortex.

DIFFERENT PROJECTION PATTERN OF THE BASILAR PONTINE NUCLEI (BPN) AND THE NUCLEUS RETICULARIS TEGMENTI PONTIS (NRTP) TO THE PALEOCEREBELLUM

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This study firstly showed that the projection pattern of the BPN and the NRTP to the paleocerebellum is different, in variance to previous studies that often considered the BPN and the NRTP as a whole in reason of their apparent projections to comparable areas of the cerebellum. Two lots of albino rats were used in this study. The rats of the first lot were injected with biotinylated dextran amine in the BPN or the NRTP and the projections to the vermal cortex and the fastigial nucleus were studied. Injections in the BPN labeled projections to the lobules VI-IX of the vermis whereas no fibers were traced to the fastigial nucleus. Injections in the NRTP labeled projections to the lobules II-IX of the vermis as well as to the fastigial nucleus of both sides. The rats of the second lot were injected with rhodamine dextran amine in the fastigial nucleus of one side. The labeling of the BPN and the NRTP always concerned symmetrical areas of the two sides. The labeling concerned both cell bodies and fiber terminals in the NRTP and only fiber terminals in the BPN. Thus, the projections of the NRTP differed by those of the BPN because the former reached both the vermal cortex and the fastigial nucleus whereas the latter only to the vermal cortex (and not to the fastigial nucleus). Moreover, the cortical areas of the vermis projected upon from NRTP and BPN were only partially overlapped. Finally, the different anatomical features of the projections of the BPN and the NRTP obviously support different functional activities. Thus, the NRTP and the BPN are likely concerned in different control of the paleocerebellum.

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PHYSICAL TRAINING DURING EARLY ABSTINENCE PERIOD IN OPIOID DEPENDENTS DECREASES WITHDRAWAL SYMPTOMS BUT ENHANCES SYMPATHETIC ACTIVITY.

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Studies have shown that regular engagement in physical training leads to changes in cardiovascular autonomic regulation. Objective: Our aim was to investigate the effect of physical training on autonomic function and opiate withdrawal score in opioid dependence syndrome patients during post- detoxified period. Method: Before recording the parameters, the patients (age 31±8.4 years) were detoxified with buprenorphine or dextropropoxyphene for 9 days. Then they were randomized into physical training (PT, n=22) and control (CT, n=21) groups. The PT group underwent supervised 30-min bicycling a day for two weeks. The load of exercise was based on heart rate that was kept between 110 - 130 bpm. The other group did no exercise. The autonomic function and opiate withdrawal scores were recorded before and after the training period. The autonomic function was assessed using standard battery of five reflex tests (deep breathing, Valsalva maneuver, lying to standing, cold pressor & handgrip) and activity measurement (heart rate variability & QT/QS2 ratio). The data presented are either mean ± SD or median (range). All patients consented for the study and ethics committee of the institute approved the study protocol. Results: The short-term physical training in PT resulted in reduction in withdrawal symptoms when compared within the group [7(0-19) vs. 1(0-7) p<. 01] and with CT [4(0-11) vs. 1(0-7) p<. 01]. The PT group showed significantly high resting heart rate (78.2±8.3 vs. 72.1±10 bpm p<. 05), and mean arterial pressure (MAP 113.04±9.49 vs. 105.6±7.86 mm Hg, p<. 05) in comparison to CT. In intragroup comparison, they also had significantly decreased heart rate variability [normalized unit of high frequency band [19.58 (2.96-49.23) vs. 9.55(3.19-36.22) p<.05],SDNN (msec) [47.28(23.26-96.89) vs. 40.1(18.17-83.94, p<. 05), CV [5.74(3.31-10.21) vs. 4.43(2.42-9.95) p<. 05], SDSD (msec) [163.41(0-977.2) vs. 147.17(0-2.1.7), p<. 05]. In handgrip test the MAP difference (delta) was decreased (27.89±10.2 vs. 22.21±7.58 mm Hg p<. 05) in comparison to CT. The absolute MAP response to lying-to-standing was increased (116.95±12.34 vs. 109±10.99 mm Hg, p<. 05). There were no differences in parasympathetic reactivity ratios (Valsalva, 30:15 and E: I) and in QT/QS2 ratio. Conclusion: It is concluded that short-term physical training in opioid dependents during post-detoxified period causes increased sympathetic activity with reciprocal decrease in vagal activity. It also decreases sympathetic reactivity and opiate withdrawal symptoms possibly by eliminating the residual exogenous opioid activity.

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SUBTHALAMIC RESPONSES TO INTRASTRIATAL ADMINISTRATION OF APORMORPHINE.

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The subthalamic nucleus is a key structure in the physiology of the basal ganglia. The enhanced activity of this nucleus in Parkinson's desease resulting from decreased activity of the external segment of the globus pallidus, would be the cause of the over-inhibition of thalamocortical pathaway and the motor cortex. In accordance with this hypothesis the inactivation of the subthalamic nucleus improves motor perforance in Parkinson's patients. Our interest was to analyze the response of subthalamic neurons to activation of striatal dopamine receptors in normal rats. In anesthetized animals with urethane, single unit activity was recorded in the subthalamic nucleus by mean of glass microelctrodes, filled with 2M NaCl with pontamine sky blue dye. The dopamine receptors were stimulated by intrastriatal microinjection of apomorphine (10 μg/0.5 μl). The spontaneous activity of the neurons in the intact rats had a mean of 4.67 ± 0.9 Hz (n=20). All the subthalamic neurons respond to apomorphine. The 75% were inhibited and the 25% excitated. The neurons that were inhibited had an irregular pattern of discharge (CV 1.15 ± 0.14, n=15), while the excitated had a more regular (CV 0.1 ± 0.39). The inhibited subthalamic neurons decrease their basal firing rate by 73% (4.55 ± 1.2 Hz vs. 1.23 ± 0.51 Hz, n=15, P< 0.03, Newman-Keuls, after significant ANOVA). The duration of this effect was 17.5 ± 1.3 min., with a latency of 7.2 ±1.5 min. The excitatory responses, produce an increase of the basal firing rate of 97% (5.05 ± 0.7 Hz vs 9.94 ± 1.8 Hz, n=5, P< 0.05, N-K test). This effect had a duration of 14.5 ± 0.7 min. and a latency of 9.5 ± 3.8 min. Solvent of apomorphine microinjected in the striatum was ineffective. The above results indicate that activation of D_1 and D_2 striatal dopamine receptors modify the activity of subthalamic neurons, according to the model suggested for the function of the basal ganglia. The most of the subthalamic cell recorded were inhibited, however a relatively high percentaje were excited.

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SLOW $GABA_A$ INHIBITORY POSTSYNAPTIC CURRENTS SUPPRESS THE FIRING OF INHIBITORY INTERNEURONS IN AREA CA1 OF THE RAT HIPPOCAMPUS

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Two kinetically distinct classes of $GABA_A$ inhibitory postsynaptic currents (IPSCs) have been found in hippocampal CA1 pyramidal neurons[1]. $GABA_{A,fast}$ synapses decay rapidly ($\tau_{decay} \approx 8$ ms), are concentrated near the soma, are sensitive to blockade by furosemide, and are resistant to presynaptic $GABA_B$ modulation. $GABA_{A,slow}$ synapses are dendritic, decay slowly ($\tau_{decay} \approx 50$ ms), are resistant to furosemide, and exhibit strong presynaptic $GABA_B$ modulation. These synapses arise from different populations of presynaptic interneurons with different firing properties, as spontaneous $GABA_{A,slow}$ IPSCs are extremely infrequent, whereas spontaneous $GABA_{A,fast}$ IPSCs are seen in pyramidal neurons at approximately 15 Hz[2]. We found previously that stimuli that elicit $GABA_{A,slow}$ also produce a prolonged (400 ms) pause in the ongoing spontaneous inhibitory activity[3], a phenomenon we refer to as Suppression of Fast Inhibition (SFI). We have now tested whether SFI is produced by $GABA_{A,slow}$ synapses onto $GABA_{A,fast}$ inhibitory interneurons.

Whole cell recordings were made from hippocampal slices prepared from young Sprague-Dawley rats (14-40 days). SFI in pyramidal neurons was elicited by stimulation of stratum lacunosum-moleculare (SL-M). Both $GABA_{A,slow}$ currents and SFI were found to be reduced by the $GABA_B$ agonist baclofen, and prolonged the GABA uptake inhibitor NO-711. Recordings from interneurons in stratum radiatum that project to somatic and perisomatic sites revealed that SL-M stimuli elicited a long-lasting IPSC with kinetics similar to $GABA_{A,slow}$. Computer simulations showed that the kinetics of decay of $GABA_{A,slow}$, but not $GABA_{A,fast}$ or $GABA_B$, produced the appropriate duration of SFI.

We conclude that both $GABA_{A,fast}$ interneurons and pyramidal neurons are inhibited by $GABA_{A,slow}$ synapses. This interaction produces SFI, and may allow coordination of complex circuit phenomena, such as network oscillations, that depend on inhibitory interactions at different time scales.

1. RA Pearce, *Neuron* **10**: 189-200 (1993).
2. MI Banks, TB Li, and RA Pearce, *J. Neurosci.* **18**:1305-1317 (1998).
3. MI Banks and RA Pearce, *Soc. Neurosci. Abstracts* **24**:362.4 (1998).

TRANSCRIPTION FACTORS IN THE HIPPOCAMPUS AFTER C-FIBER STIMULATION

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Sensory input induces transient expressions of numerous inducible transcription factors (ITFs) such as the Fos, Jun and Krox families, in areas activated by the input 1. Many studies have focused on ITF expressions following a single, brief input; but neurons in the brain are activated by multiple inputs and ITF expression arising from interacting inputs remains to be studied. Moreover, no studies have reported ITF expression in the hippocampus in relation to nociception, which is surprising considering its role in learning and the potent saliency of such stimuli.We examined the expression of Fos, Jun, Krox, as well as phosphorylation of CREB, in the hippocampus after single, repeated or simultaneous stimulation of hindlimb sensory nerves in halothane-anaesthetized rats. Two stimuli were used: electrical stimulation of the sciatic nerve at C-fiber strength for 10 mins, and/or clamping the ventral hindpaw skin contralaterally for 10 min. After fixation in paraformaldehyde, cryostat-cut sections were immunocytochemically processed. With single stimuli, both c-Jun and Krox-24 fell dramatically (5- and 20-fold) for up to 24 hours. c-Fos and Krox-20 were not induced; whereas FosB increased 2- to 10-fold for longer than 24 hours. With repeated stimulation, i.e. when the clamp stimulus was given to the opposite paw 6 hours after electrical stimulation of the sciatic nerve, it now caused a lesser reduction in c-Jun and Krox-24 (to 2.5- and 4-fold, respectively). Also, it was able to induce both c-Fos and Krox-20; and produced an even greater expression of FosB. With simultaneous stimulation, i.e. when clamps were applied to opposite paws at the same time, there was no reduction in c-Jun and up to a 10-fold increase in Krox-24 compared to what would be expected from summing the expressions caused by two single stimuli. As with repeated stimul-ation, simultaneous stimulation induced Krox-20, (but not c-Fos), and increased FosB expression. There was no consistient CREB phosphorylation with any of the stimulation paradigms, probably resulting from innate differences between animals.Thus: (1) a noxious stimulus causes both inductions and repressions in ITFs in the hippocampus, (2) two noxious stimuli can interact to produce markedly *different patterns* of ITF expression than either single stimulus, (3) an initial noxious stimulus can cause a *potentiation* of either the induction or repressions in ITFs caused by a second stimulus, (4) some ITFs were induced *only* in response to repeated or simultaneous stimuli, pointing to conditional factors connecting the activation of a neuron's genetic and electrical responses.

KINEMATIC MODIFICATIONS DURING MOTOR LEARNING IN MAN

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The aim of this study was to describe the modifications of arm kinematic parameters in man during a motor task that require an optimization process. This approach can serve for investigating the control strategy used by the nervous system during motor learning. Six healthy, right-handed and untrained subjects were studied using an ultrasound system that acquires the 3D coordinate from 4 markers placed on the shoulder, elbow, wrist and hand. Subjects launched a tennis ball into a basket by a pendulum movement of the arm; they learned the task by trial-and-error in a series of successive trials. For each trial we measured: performance improvement, by means of distance from the center of basket and the point were the ball fell; arm acceleration and arm geometry (i.e. combination of shoulder-elbow-wrist joint angles) at launch time; total movement time; single values of joint angles and acceleration acquired at shoulder, elbow and wrist at launch time. Linear regression analysis revealed that changes of arm acceleration, arm geometry and movement time were correlated with the performance improvement (R^2>0.8). Changes of joint angle were correlated with the movement improvement only for the wrist angle. Shoulder and elbow acceleration variations were correlated with the performance improvement but not the wrist acceleration changes. The results indicate that task improvement depends on changes of global arm parameters that reflect a strategy at single joints level. In fact, the arm acceleration variations depend on changes of acceleration at shoulder and elbow while the geometry adjustments are related to wrist angle changes. This modality permit the nervous system to reduce the degree of freedom and improve the movement by considering the three joints as two functional biomechanical systems to control acceleration and geometry of the arm

THE THOUGHT TRANSLATION DEVICE: 24-HOURS UNASSISTED COMMUNICATION IN LOCKED-IN PATIENTS

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Self-regulation of Slow Cortical Potentials (SCP) is used in patients with locked-in syndrome, and allows them to communicate verbally and to manage their environment by means of a Thought Translation Device (TTD). However, in order to introduce the technique into the everyday communication needs an unassisted TTD spelling i.e. a reliable and prolonged contact of such patients with TTD: this was realized by a voluntary switch from a "stand-by" mode to a "writing" mode and back; this switch is turned on by the SCP of the patient. In order to supply TTD with such an option a special additional TTD-software was created. A shaping procedure was developed by which the patient learned to produce a special sequence of SCP signals in order to operate the switch for 24 hours. The results of two completely paralyzed patients confirm that this is possible to communicate with the TTD and with the 24-hours stand-by-system without any muscular activity and without any assistance.

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5,7 - DIHYDROXYTRYPTAMINE LESION OF THE DORSAL RAPHE AFFECTS ETHANOL-INDUCED CONDITIONED TASTE AVERSION IN RATS

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Accumulating evidence suggest an inverse relationship between ethanol (EtOH) drinking and central serotoninergic function. Interestingly, some lines of rats genetically selected for high EtOH intake and preference show hypofunction of the brain 5-HT system. Furthermore, these animals (e. g. P line) show an innate reduced sensitivity to the aversive effects of EtOH as compared with the EtOH-nonprefering animals (NP line). Thus, it may be hypothesised that 5-HT deficit could be responsible for higher threshold for EtOH aversivity, thus leading to increased rewarding properties and voluntary EtOH consumption. Therefore, it was the aim of the present study to investigate whether selective lesion of the main serotoninergic nucleus in the brain, the dorsal raphe nucleus (DRN) influences aversive effect of EtOH in the conditioned taste aversion (CTA) procedure. The rats were anaesthetised with ketamine and stereotaxically injected with 5,7- dihydroxytryptamine (5,7-DHT, the 5-HT selective neurotoxin) into the DRN. The test was started 7-8 days after the 5,7-DHT treatment. EtOH-induced CTA to saccharin solution was unaffected by 5,7-DHT lesion when evaluated in one-bottle choice test. However, in two bottle choice test, the aversive effect of lowest EtOH dose (1 g/kg) was significantly reduced. This result suggests that lesion of the DRN may attenuate the aversive properties of ethanol, but the effect is limited to the low/moderate dose range.

MEASURING BEHAVIOR VARIABLES ON A PSYCHIATRIC WARD

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We developed some mathematical measures in order to evaluate the dynamics of some behavior variables trying to describe the interactions among patients from a psychiatric ward. The protocol used to evaluate the state of a patient consists on measuring sociability and restlessness daily on scales according to standard procedures ([1],[2]) .We collected data along 62 days from 68 individuals consecutively admitted into a female psychiatric unit for acute patients([3]) attributing a grade for each parameter, per patient. The grades were checked by different specialists and tables of incidence were constructed for the whole set and grouping through diagnostic([4]) and we studied several methods to analyze the data ([5]) and we noticed that power laws seem to provide good fitness, suggesting that either the whole ward or the diagnostic groups show self-organizing criticality ([6]).

References

[1] Sanson-Fisher, R.W. & Martin, C.J. (1981). Standardized interviews in psychiatry: issues of reliability. Br. J. Psychiatry **139**, 138-143.
[2] Wittenborn, J.R. (1972). Reliability, validity, and objectivity of symptom rating scales. J. Nerv. Ment. Dis. **154**, 70-87.
[3] Piqueira JRC, Monteiro LHA, Magalhães TMC, Ramos RT, Sassi RB & Cruz EG (1998). Zipf's law organizes a psychiatric ward – submit. to Journal of Theoretical Biology.
[4] World Health Organization} (1991). Mental and behavioral disorders: clinical description and diagnostic guidelines. In: *The ICD-10 Classification of Mental and Behavioral Disorders*. Geneve: World Health Organization.
[5] Ramos, RT, Piqueira, JRC & Sassi, RB(1998). Modeling pathological behavior in psychiatric patients. In: *31-st Meeting of Society for Mathematical Psychology*. Nashville: Vanderbilt University.
[6] Per Bak (1997). *How Nature Works*. Oxford: Oxford University Press.

ESTIMATION OF MULTIPLE SCLEROSIS ACTIVITY BY H^+ NMR SPECTROSCOPY.

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75 patients with multiple sclerosis (MS) were examined, severity of the disease was equal to 1-6 balls by J. Kurtzke's scale (1983). The H^+ NMR spectroscopy of MS foci was implemented simultaneously with NMR by mean of the unit Magnetom Vision (1,5 T, Siemens).

Identical foci of demyelination in MRI showed different spectra in the H^+ NMR spectroscopy which conformed to their heterogeneity. In active foci there were noted the NAA reduction, Ins increase and Lipid appearance. Ratio: NAA/Cr-0,53-0,85; NAA/Cho-1,20-1,26; Cr/Cho-1,42-1,47; NAA/Ins-1,50-1,54. In MS remission: NAA/Cr-1,21; NAA/Cho-1,83; Cr/Cho-1,54; NAA/Ins-2,50.

In severe cases of the disease a sharp reduction of the NAA contents was observed.

Application of the MRI and H^+ NMR spectroscopy can help in the determination of the MS activity degrees..

HISTAMINE EFFECTS ON EXCITABILITY MEDIATED BY I_H IN MEDIAL VESTIBULAR NEURONS.

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Antihistaminergic H_1-receptor antagonists are therapeutically effective in vertigo and motion sickness. Neurons of the medial vestibular nucleus (MVN) are likely sites of excessive release of histamine, modulating the sensory conflicts in such syndromes. Immunohistochemical studies have demonstrated a presence of H_1-receptors in the MVN. The modulation of vestibular information processing also may involve histaminergic interactions with H_2- and H_3-receptors. We have bath applied histamine, its receptor-agonists and -antagonists to brainstem slice preparations (gerbils) and examined their effects on the membrane properties of MVN neurons, recorded with whole-cell patch-clamp techniques. Application of histamine (5-50 μM) or dimaprit (H_2-agonist; 0.3-50 μM) usually increased the input conductance and excitability. Some neurons exhibited a burst response, enhanced by histamine, at the offset of imposed hyperpolarizing pulses. Pyrilamine and thioperamide, H_1- and H_3-antagonists, respectively, did not alter the effects of histamine. ZD-7288 (10 μM) or Cs^+ (1-3 mM), selective blockers of the I_H current, eliminated the voltage-sag and rebound spike-burst responses and histamine effects. Histamine caused a depolarizing shift of the activation curve for I_H along the voltage axis. Cimetidine (10 μM) blocked the effects of histamine on I_H-activation and rebound spike-burst. These results are consistent with a faster turn-on of I_H due to interactions of histamine with H_2-receptors.

ELECTRICAL BRAIN ACTIVITY AT READING OF NORMALLY AND CONTINUALLY WRITTEN TEXT

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With the wish to approach the nature of stuttering process, our aim was to follow electrical brain activity at reading of the normally written text and at reading of the text without space between the words, i.e. continually. Method: EEG of a stuttering male subject, 15 years old. Recording was performed at states: 0- at rest, with open eyes; 1- reading of the text in capital Latin letters without space between the words; 2- reading of the text in capital Latin letters with normal spaces between the words. Results have shown statistically significant differences between the observed states, in regard with the fields as well as frequency ranges, either in the direction of decrease or increase of electrical activity. Besides the important differencies between the observed states and fields wich appeared and will be shown, the special significance in our work is represented by the difference between the state 2- reading of the normal text and state 1-text without spaces, in the sphere of delta frequences, in the field T5, which also appears between fields T6 and T5, for the benefit of greater electrical activity T5 (left). Special significance of this result is that the development of the sentence in speech happenks in the period of development when delta electrical brain activity is dominant.

SYNAPTIC PLASTICITY IN THE BASOLATERAL NUCLEUS OF THE AMYGDALA

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It is well known that the basolateral nucleus of the amygdala (BLA) is involved in the storage of implicit memories of fears. The cellular mechanisms underlying synaptic plasticity in this nucleus are largely unknown. Electrophysiological experiments were performed to study synaptic plasticity in the BLA using an in vitro slice preparation. Population spikes (PS) were evoked in the BLA by stimulating either the external capsule or the lateral amygdala. Tetanic stimulation with a single train (1x 100Hz/1s) produced only short-term potentiation. Five trains of tetanus (5x 100Hz/1s; 10s ISI) induced long-term potentiation (LTP; 115.2% ± 6.2, after 60min). This form of LTP was completely blocked by AP5 (50μM). We also examined LTP in slices which have been exposed to picrotoxin (10μM), in order to block GABAergic inhibition. In picrotoxin treated slices, LTP induced by five trains was more pronounced (140.4% ± 7.1; after 60min). Low frequency stimulation (1Hz/900pulses) induced either long-term depression (LTD; 71.5% ± 4.4, after 60min) or depotentiation. LTD could not be blocked by infusion of either AP5 (50μM), nor by the L- and T-type Ca^{2+}-channel blockers nifedipine (20μM) or Ni^{2+} (50μM), respectively. In contrast, higher doses of AP5 (100μM), attenuates the extent of LTD. The PS amplitude did not reach control levels (93.6% ± 1.1, after 60min). However, the depression of LTD was totally blocked by picrotoxin (10μM), indicating that the GABAergic system plays an important role in the expression of LTD in the BLA.

IN VIVO TEST OF DIFFERENT GENETICALLY ENGINEERED PSEUDORABIES VIRUS STRAINS: USABILITY FOR TRACT TRACING STUDIES

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In order to enhance the transneuronal tract-tracing properties of the commonly used Bartha strain of pseudorabies virus (PrV-Ba), in this study three mutant pseudorabies virus strains have been constructed. By introducing mutations to specific locations of the viral genome of PrV-Ba, virulence of the virus has been reduced, resulting in more specific spreading characteristic. We inserted the bacterial lacZ gene to the PrV genome. This reporter gene was driven by the human cytomegalovirus immediate early 1 promoter (P_{hCMV}) which conferred several advantageous features of the virus. The immediate early kinetics of the reporter gene expression allowed the identification of infected neurons in an early stage of the infection, when neural proteins and mRNA are still expressed at near-normal levels, an important prerequisite for the neurochemical identification of these cells. In addition, the time-delay between the expression of the reporter genes and the viral proteins detected by immunohistochemistry can be used to reveal the hierarchical order of synaptic linkage of neurons.
For the *in vivo* test of these new strains small amounts of live viruses were injected into the kidney of Sprague-Dawley rats. After survival times (2 to 5 days) the viral infection was examined by double label immunohistochemistry at different regions of the central nervous system (spinal cord, medulla oblongata, hypothalamus) and the dynamics of the propagation were established for each virus strain tested. In order to follow the route of the infection in restricted areas of central nervous system neurons, the most adventageous strain was used. To visualize the marker gene products and viral proteins in same neuronal cells, the A1/C1 catecholaminergic cell group was investigated by double label immunohistochemistry. Results indicate that the utility of viral labeling has been improved significantly, making neurotropic viruses an even more interesting tool for transneuronal tract-tracing.

FINE STRUCTURE OF THE AUDITORY M100 IN SCHIZOPHRENIA AND SCHIZOAFFECTIVE DISORDER

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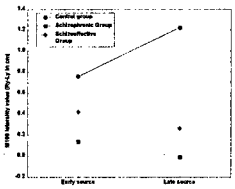
The 100 msec latency auditory evoked field component (M100, thought to index echoic memory) , demonstrates anomalous lateralization (less lateralized, or more variable) in schizophrenia (1,2). This component may actually by generated by two active regions separated in time by 25 msec and in space (later source about 0.1 cm anterior in the L hemisphere, and 0.8 cm anterior in the right hemisphere (3). This study examined both early and late M100 components in both hemispheres of 23 controls, 16 patients with paranoid schizophrenia, and 12 patients with schizoaffective disorder, all males. Stimuli were 30 msec duration 1kHz 85dB SPL tone pips. MEG was recorded with a BTI model 607 7 channel 2nd order gradiometer, and source localizations performed with custom software using a single equivalent current dipole model in a conductive spherical volume. Superior temporal gyral volumes, for most subjects, were computed from MRI scans (coronal series, 124 1.7 mm thick slices). In controls, the second source was more lateralized than the first. In both patient groups, neither source was lateralized (see Figure 1). Lateralization was independent of STG volume, which also differed by diagnosis (see Figure. 2). Both schizophrenic and schizoaffective subjects appear to exhibit anomalous lateralization, especially affecting the second source. The two sources may index different aspects of echoic memory (4).

Figure 1. Mean M100 laterality values



1. Reite, M. et al. Arch Gen Psychiat. 54:433, 1997
2. Hajek, M. et al. Schiz. Res. 24:329, 1997
3. Teale, P. et al. NeuroReport 9:2647, 1998
4. Sams, M. et al. J. Cog. Neurosci. 5:363, 1993

RELATIONSHIP BETWEEN DIRECTION PREFERENCE MAPS AND INTRACORTICAL SYNAPTIC CONNECTIONS IN FERRET VISUAL CORTEX

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In the visual cortex neurons showing similar stimulus selectivity form columnar clusters which are organized in systematic representations ('maps') of stimulus features such as position, orientation, direction or ocular dominance.
Here we have used a combination of optical imaging *in vivo* and scanning photostimulation of synaptic inputs to individual neurons *in vitro* to characterize the relationship between iso-direction domains, iso-orientation domains and intracortical synaptic connections. Following optical imaging of functional maps and injection of fluorescent beads as alignment markers the imaged visual cortex was removed and tangential slices (400 μm thickness) were prepared. The slices were transferred to a recording chamber mounted to the stage of an upright microscope equipped with epifluorescence illumination. Whole cell patch clamp recordings from individual neurons were performed and presynaptic inputs stimulated by focal photolysis of caged glutamate using a fiber optic attached to a 100 W mercury arc lamp source.
We recorded orientation and direction maps from three animals (P37 – P41). Iso-orientation domains tended to be subdivided into regions preferring opposite directions of stimulus motion, as reported previously by Weliky et al (1996). Our studies indicate that the local circuitry within an orientation domain is organized as follows: Local excitatory connections are restricted to iso-direction subregions within an orientation domain. Interestingly, the direction tuning histogram of inhibitory inputs shows two peaks – inhibitory connections originate from regions preferring the same as well as from regions preferring the opposite direction of stimulus movement (n = 11 neurons). Long range horizontal excitatory connections were either orientation- or direction specific. This indicates that the emergence of a second feature map forces intrinsic excitatory connections into a higher degree of specificity. The horizontal, orientation specific projection system appears subdivided into direction and orientation specific subsystems. A comparison of locations of origin of synaptic inputs with the axonal branch pattern of postsynaptic cells indicates reciprocity of intracortical connections.

References:

Weliky, M., Bosking W. H. and Fitzpatrick, D. (1996) A systematic map of direction preference in primary visual cortex. Nature 379: 725-728.

THE EVALUATION OF THE DEVELOPMENT OF PAIN IN EXPERIMENTAL MODELS.

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To study the aetiology, pathogenesis and possible pharmacological influencing of the pain, many animals´ models were developed. In our laboratory we are using three models of pain in rats:
1.Our own model of neurophatic pain.
2. The model of the arthritic pain in the strain Long Evans.
3. The model of deafferentation pain in the strain Wistar.

Results: In the first model the different changes of the brain metabolism were studied. It was found out that HDL cholesterol was increased during the short lasting nociceptive stimulation, while LDL cholesterol is increased during the chronic long lasting nociceptive stimulation The free oxygen radicals MDA and both enzymes (SOD and GSHPx) as well as antioxidative capacity were increased after the painfull stimulation. This effect is compensate by the vitamine coctail (vitamins A,C, E, and selen), and especially in the combination with NSAIDs (aspirin) and morphin. The effect of melatonine is effective in the higher doses (60 to 80 mg / kg). In the model of adjuvant arthitis the different parametres like the protein spectrum, the prolactin, corticosterone etc. were studied. It was found out that characteristics of adjuvant arthritis are possible to compensate by the starvation (up to 60 %). In deafferentated rats the activity of the medial thalamic nuclei (CM, pF and CL) was studied. After the deafferentation in Long Evans rats it was found out that the electrical activity differs completely from the normal activity especially the type of frequence and arrangement of spikes. After the deafferentation the spikes are organize in groups and this organization remind during the whole life span. We are trying now to find the compensation of this effect by the electrical stimulation of the cortex. Using our models it is possible to describe different biochemical and electrophysiological changes during the pain and used this models for study the different effects or physical or chemical influence of the pain, which could be used also for testing the pharmacological agents for possible treatment.

CONFOCAL FLUORESCENCE IMAGING OF INTRACELLULAR CALCIUM AND SODIUM CHANGES IN CULTURED CELLS, EVOKED BY α OR β SCORPION TOXINS.

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Mechanisms that modify Ca^{2+} influx have significant modulatory effect on neurotransmitter release (e.g. by altering the activation/inactivation of Na^+-channels). Using confocal microscopy, we have examined the increase of $[Na^+]_i$ and $[Ca^{2+}]i$ evoked by scorpion toxins in N-18, SN-56 and GH3 cells. Tityustoxin (TsTX) is an α-type toxin (delays inactivation of Na^+ channels), and toxin-γ (TiTX-γ) is a β-type toxin (facilitates activation of the channel at more negative potentials). Cells were loaded with the fluorescent probe SBFI-AM or Indo-1-AM (5μM) at 37□C and perfused in presence with TsTX or TiTX-γ (2μM). Images were collected using a Zeiss Axiovert 100 microscope coupled to a Bio-Rad MRC 1024UV laser scanning system. TsTX was able to promote $[Na^+]_i$ and $[Ca^{2+}]i$ increases in all three types of cells studied and its effect was blocked by tetrodotoxin (5μM). However, TiTX-γ had an effect only in GH3 cells, where its effect was also blocked by tetrodotoxin. These results suggest that the tested cells have different types of voltage sensitive sodium channels or that GH3, due to its spontaneous potential oscillations, have sodium channels on a kinetic state that favors the binding of TiTX-γ. In addition, we report here the successful use of a sodium probe (SBFI) in confocal imaging.
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DYNAMICS OF ELECTRICAL ACTIVITY OF HUMAN BRAIN IN THE COURSE OF BI-MODAL (VISUAL AND AUDITORY) ALTERNATING STIMULATION. SWITCHING PHENOMENA.

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The Purpose of Investigation was to study the spontaneous regulation of electrical activity in the human brain when it is involved in distribution of recourses ("attention") among two targets -visual and auditory ones - presented in alternating order during the course of a long term EEG recording session. The beginning of this Project belongs to the late 1960's [1,2]. The framework of the theory and the results achieved in animals during the previous stage have been published in [3]. Current results ("switching paradigm") with human subjects are presented.

Methods. Subjects: 7 men. Stimuli: auditory and visual, alternating with 1 Hz frequency. Time of stimulation/recording:10 min. Auditory stimuli: Binaural Clicks 30 dB SL. Visual stimuli: Alternating checker Board. EEG Recording: Neuroscan System, 16 scalp electrodes. Bandpass Filter 1- 30 Hz. Averaging: N= 5 and 25 trials.

Results. A rich phenomenology of cooperative behavior in successions of AEPs and VEPs has been revealed in the course of the test. In particular; **a**. if the whole time interval of the test is taken into consideration, the preference for *weak negative correlation's* between AEP and VEP amplitudes appears at all electrode sites; **b.** through definite time intervals during the test alterations of AEPs and VEPs found to be of two types - either of the same direction ("coherency") or mostly of opposite direction ("switching"); **c.** the long term EEG epoch of switching on many channels simultaneously is registered with a high reliability – *"switching phenomena"*; **d.** both modes of regulation - *coherency* and *switching* - were found to be predominant in the *left* and *frontal* areas of human cortex **e.** new type of evoked potential is introduced – Two-dimensional Long term EP (TLEP).

Conclusions. It is suggested that the Switching paradigm is an effective method in the analyses of regulation of electrical activity in the human brain. In particular it may help to understand the methods of spontaneous prevention of epileptic type seizures in man. This approach may also be applied to Schizophrenia oriented studies as well.

References 1. Rosenstein, G. Sh. (1969) *Biofizika, 14(4), 765-767 2.* Meschersky, R.M.& Rosenstein. G. Sh.(1969), *BrainResearch,13, 367-375* 3. Rosenstein, G-Z. Income and Choice in Biological Control Systems, Lawrence Erlbaum, Ass., Publishers, Hillsdale, Hove and London. 1991.

EFFECTS OF SENSORY DEPRIVATION ON THE DEVELOPMENT OF SYNAPTIC PATTERNS IN MOUSE BARRELS.

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The effects of deprivation of whisker activity on synaptic development in barrel D4 of PMBSF cortex was examined by pulling the large mystacial vibrissae on the contralateral snout from P6, or by daily triming of the vibrissae from P0. P0 was chosen as the starting point for whisker trimming to maximize the sensory deprivation effects of this relatively benign approach. Whisker pulling is performed at P6 because gross barrel formation appears complete by this time and presumably would be unaffected by subsequent damage to the whisker follicle. At P20 the mice are perfuse-fixed. P20 was chosen because the period of rapid synaptic growth during barrel development terminates several days earlier (White et al., '97). Hemispheres contralateral to the deprived side are osmicated, sectioned at 40 µm and embedded in plastic for thin sectioning. Light microscopic examination of sensory deprived tissue indicates that gross barrel formation including the tangential extent and height of barrel D4 is normal. In contrast, electron microscopic examination evidenced a significant decrease in the density of asymmetrical synapses in both the hollow and wall neuropil of deprived animals; the density of symmetrical synapses was not altered. The diameter of asymmetrical synaptic junctions is decreased, but by an amount (~10%) determined to be insufficient to explain the decrease in density.

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DIFFERENT EFFECTS OF NMDA ANTAGONISTS, NOOTROPIC SUBSTANCES, DIAZEPAM AND NALOXONE ON DEVELOPMENT OF PTZ-KINDLING, HIPPOCAMPAL POTENTIATION PHENOMENA AND COGNITIVE DEFICIT IN RATS

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Pentylenetetrazol (PTZ) kindling which is an accepted model for a primary generalized epilepsy induces a novel form of potentiation, i.e. the long-lasting enhancement of hippocampal evoked potentials following application of a subconvulsive test dose of PTZ. Similarly, it causes also a diminished learning performance in an active avoidance task (shuttle box). With the intention to investigate the relations between development of convulsive behaviour, kindling-induced potentiation (KIP) and learning deficits rats were kindled under pretreatment with the competitive (CGP 43487) and the non-competitive (MK 801) NMDA receptor antagonist, the nootropic substances piracetam and methylglucamine-orotate, the GABA-benzodiacepine agonist diazepam and the µ-opioid receptor antagonist naloxone. The substances differently influenced the three parameters of kindling. Taken together the results show no absolute correlation between seizure development, learning deficit and KIP. NMDA receptor antagonists depress seizure development, learning deficit and KIP. Diazepam only suppresses seizure development, but has no effects on learning deficit and KIP. In contrast, piracetam and naloxone do not influence seizure development but improve learning performance of kindled animals and depress KIP. The influences of the substances on KIP and learning deficit seem to fit well. Summarizing it could be suggested that development of KIP may be involved in the kindling-induced learning deficit.

SIMULATION OF LONG-LASTING CHANGES OF SYNAPTIC TRANSMISSION

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A model of synaptic transmission, possessing properties of long-lasting changes of efficacy of synaptic transmission depending on presynaptic stimulation, is suggested. The model is developed from the previously designed model of chemical synaptic transmission, adequately describing short-term changes of synaptic efficacy. The new model is based on the hypothesis of regulatory influence of postsynaptic neuron on the presynaptic terminal through a feedback mechanism. The proposed feedback mechanism is turned on and off depending on the level of depolarization of postsynaptic membrane, and produces long lasting redistribution of neurotransmitter between pools of presynaptic terminal, as well as changes in total amount of neurotransmitter. Computational experiments with the model showed that it is able to qualitatively reproduce the effects of both long-term potentiation (LTP) and depression (LTD), depending on the frequency and pattern of presynaptic stimulation. The character of model parameters' changes during different forms of LTP and LTD was analyzed and the values of parameters were estimated. It was shown, that the regulatory influence of the feedback may affect different model parameters (such as the amount of immediately available neurotransmitter, release probability, time constants of neurotransmitter redistribution in presynaptic pools), causing various forms of changes of synaptic efficacy.

ELECTROPHYSIOLOGICAL STUDY OF NEURONES IN THE PONTINE MICTURITION CENTRE WHICH SEND AXON TO THE SPINAL CORD IN CATS.

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Two types of neurones have been reported in the pontine micturition centre: one increases activity when the bladder contracts and the other decreases. The present study aims to examine whether both types of the neurones send axon to the spinal cord and which level the axon reaches. Neuronal activities were recorded with glass microelectrodes in α-chloralose-anaesthetised or decerebrated cats. (1) All neurones that were antidromically activated by electrical stimulation of the dorsolateral funiculus of the lumbar or sacral cord showed a former type of the firing pattern. Majority of neurones had spontaneous activities and gradually increased firing rates soon after relaxation of the bladder, and further increased firing rates during micturition contraction. These neurones, however, were silent when the intravesical pressure was near 0 mmH2O. Remaining neurones fired only prior to and during micturition contraction. (2) All neurones antidromically activated from the upper lumbar cord sent axon to the sacral cord. The caudal end of the axon was between S1-S3. The latency of the antidromic spike from the most caudal level of the sacral cord was 16-36 ms, suggesting that the descending neurones activate the sacral preganglionic neurones mainly polysynaptically, since electrical stimulation of the pontine micturition centre has been reported to activate the sacral preganglionic neurones with much longer latency (45-60 ms). The conduction velocity of the axon from the cell body to the upper lumbar level was estimated to be 10-25 m/s and it remarkably decreased from the lower lumbar cord, suggesting that axons branch at lumbar as well as sacral level.

INTERFERENCE BETWEEN THE ACQUISITION OF TWO MOTOR SKILLS.

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Previous work has shown that acquisition of a new motor skill is degraded when another, similar skill has just recently been acquired. This interference is strong when the interval between the two training sessions is a few minutes, and subsides for intervals beyond 5 hours. Our study expands this finding to another type of skill (tracking rather than pointing), and scrutinizes its time course.

A visual target moved smoothly along an upredictable path, and subjects tracked it with a joystick-controlled cursor. Target, cursor, and joystick movement occurred in the same (horizontal) plane. Performance was quantified as RMS tracking error over episodes of 60 s. In a first training session, ten minutes of "warm-up" practice were followed by 30 minutes in which the relationship between joystick and cursor movement was left-right reversed. In a second session, 30 minutes with up-down rather than left-right reversal were administered.

Left-right reversal in the first session led to a dramatic initial increase of RMS error, followed by gradual normalization. Up-down reversal in the second session yielded an *even larger* initial increase. This increment confirms the existence of interference; it was strongest when both sessions were only minutes apart, but persisted even when the interval was 24 hours. Furthermore, RMS errors at the end of the second session were *even lower* than those at the end of the first, suggesting the existence of facilitation ("learning to learn").

THE GOLGI ARCHITECTURE – BASIS FOR THE ANALYSIS OF THE NEURONAL ORGANIZATION OF THE FUNCTIONAL STRUCTURE IN SCHIZOPHRENIC PSYCHOSES

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The pre-requisite for the detection of histomorphological changes elucidating the pathogenesis of schizophrenic psychoses are special methods for the demonstration of the fine structure of the brain. With the Nissl staining technique, only the neuronal somata can be demonstrated. A more detailed view on alterations of the functionally disturbed schizophrenic brain can be achieved with the demonstration of the Golgi architecture. The Golgi architecture is based on staining methods, which label the neurons with all their dendrites and axons. There are only a few methods, which demonstrate the neurons of the human brain with their functionally relevant detailed structures exactly and correctly. The characterization and functional classification of neurons is a basic requirement for the analysis of cortical circuits. The pyramidal neurons (PN) are demonstrated with the Golgi silver impregnation method. With this technique, PN can be classified and the number and quality of visible dendritic spines can be analysed. The complete axonal ramification field can be shown with the DiI fluorescence method. The differentiation of interneurons (IN) becomes possible with immunohistochemical techniques using antibodies against the calcium-binding proteins, which allow an exact morphological classification of IN.
The histomorphological analysis of all neurons is conducted with confocal microscopy. With this technique, also axonal connexions can be demonstrated. The dendritic structure of the single neurons is analysed with the Neurolucida computer system. With these tools, also pathological alterations of the functionally relevant neuronal parts can be detected.
The Golgi architecture represents the basis for a better understanding of brain function. Such specific methods and the quantitative analysis of the single neuron allow more comprehensive conclusions concerning the pathogenesis of schizophrenic psychoses.

Characterization of mouse inositol monophosphatase genes

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The enzyme inositol monophosphatase (IMPA) is a key enzyme in the phosphoinositide signaling system. IMPA is uncompetitive inhibited by therapeutically relevant concentrations of lithium, a widely used mood-stabilizing medication, yet, the molecular mechanism of its therapeutic effects is unknown. Berridge first proposed that the physiological effect of Li's inhibition of IMPA is depletion of brain free inositol and the consequent attenuation of neurotransmitter-driven phosphoinositide second messenger signal generation. Recently we have found that the activity of the IMPA in transformed lymphoblastoid cell lines of bipolar patients is significantly lower than those from a normal comparison group. When the bipolar patients were grouped according to clinical response to Li therapy, Li responders exhibited significantly lower IMPA activity compared to poor Li responders.
Two human IMPA genes have recently been cloned. The IMPA1 gene was found on chromosome 8q21.13-21.3 and IMPA2 was located on chromosome 18p11.2. Several studies have indicated the presence of a susceptibility locus for bipolar disorder on chromosome 18p11.2. Therefore IMPA genes are candidate genes for genetic studies in bipolar disorder.
We presently report the structure of mouse IMPA-2 cDNA including the 5' and 3' untranslated regions and the gnomic structure of both mouse IMPA genes.
This is the first step toward the production of knockout mice lacking the IMPA genes.

NEW VIEW AT CYTOARCHITECTURE OF THE CENTRAL AMYGDALOID NUCLEUS

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Mechanisms of the central amygdaloid nucleus participation in securing such important and complicated processes as adapting behavior, emotions, memory, stress, visceral and endocrine functions regulations are not exposed as yet. It indicates at the necessity of a further detailed analysis of the peculiarities of its cyto-architecture in order to work out a rational scheme of investigation of its structural-functional organization. Cyto-architecture of the central amygdaloid nucleus was studied at 22 rats (Wistar) in series of sections made in three planes of space and died with crezile violet. The authors developed a new classification of the central nucleus subnuclei on the basis of the peculiarities of the topography, neuron density, their sizes and the nuclei-cytoplasmatic correlation. This classification correlates with the McDonald's data (1982), but is more detailed.

BEHAVIOR OF HINDBRAIN NEURONS DURING LATENCY OF EVOKED LOCOMOTION IN SALAMANDER

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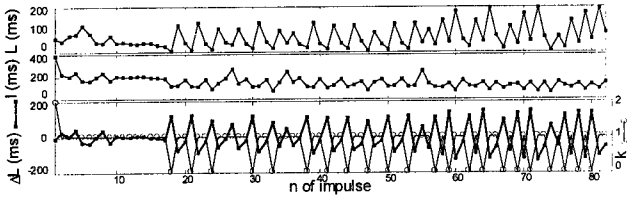
Transition from resting state to locomotion was elicited by repetitive electric stimulation of the low threshold "locomotor site" in the midbrain of salamander *Ambystoma tigrinum*. Delivering near threshold train of stimuli, the latency can be distended up to 15 s. Current (of 1 ms duration, usually 6 to 10 μA) and interstimulus interval (ISI, ordinarily 80 to 200 ms) of the applied train are interchangeable for the macro-effect in certain span. Dozens of impulses of a single neuron were recorded during the transitory period. There were neurons, which responded to stimulation even if the train did not elicit locomotion. Impulses can be time-locked to stimuli, distributed in ISI or form composite response. Both latency and probability of firing could change gradually or shift abruptly during the trial. Irregular alternation of $k \geq 1$ and $k+1$ stimuli between adjacent impulses was common. Accompanying fluctuations of latency (L), shown as difference between successive latencies ($\Delta L_i = L_{i+1} - L_i$) could be either related to these alternations or independent. Portion of interimpulse intervals (I) containing k stimuli remained constant or diminished during subthreshold train. This portion could increase during near threshold train, either gradually or abruptly. Sometimes new unstable state $k-1$ arose instead of $k+1$ and began to alternate with k-state (see Inset). Modes of latency of time-locked impulses were mostly 18 to 40 ms, and the distribution of latencies could be bimodal. Later impulses could gather too, either around the middle of ISI or in its second half. The early time-locked impulses and the late pre-stimulus ones could arise in the same ISI ($k=0$).



A SEARCH FOR THE GENE RESPONSIBLE FOR VAN DER KNAAP MYELINOPATHY

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Van der Knaap syndrome is a rare myelin degenerative disease recognized from infancy by pronounced macrocencephaly with retarded motor development. Motor deficits progress slowly within two decades to severe paralysis. In Israel, twelve affected children from 6 Libyan-Jewish families and from one extended Turkish-Jewish family have been identified. Seven are offspring of consanguineous marriages, implying an autosomal recessive mode of inheritance. To search for a putative responsible gene, we performed a genome wide linkage screen on these families using a panel of dinuclotide and tetranucleotide microsatellite repeat markers that show a high degree of polymorphism between individuals. Allelic homozygosity found only in affected children in each family would indicate close proximity of the DNA marker to the disease locus. In a study of 170 polymorphic markers the genome was screened at 20 cM intervals. The highest lod score achieved so far was 2. The panel could yield a lod score of 7 if all families exhibited linkage. Further screening is now continued between gaps. *Supported by grant No 6279397 from the Israel Ministry of Science.*

INFLUENCE OF SHORT-TERM PREGNANT FEMALE EMOTION-PAIN STRESS ON CYTOGENETIC TRAITS OF DEVELOPING BRAIN OF THE RAT LINES WITH DIFFERENT EXCITABILITY OF THE NERVOUS SYSTEM.

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It is known that stress of pregnant female can influence on morphogenesis of embryonal brain,results the change of its structure/function peculiarities and later evoke as normal as pathological behavior alterations in adult animals.The genetical mechanisms of such influence and its dependence on basic characteristics of the nervous system are not known. The aim of the present study is to analyse cytogenetical traits of neuroblasts of 16-17 day old embryos of rat lines selected according to nervous system excitability in the norm and after stress (modified methods of K.Hecht).It was shown: 1) Proliferation activity,level of chromosome aberrations and degree of chromatin condensation (general pool and C-region) correlate with nervous system excitability in the norm. 2)The pain-emotion stress result in increase of mitotic index and chromosome aberrations in neuroblasts and decrease of the interphase condence chromatin and C-heterohromatin area in interphase nuclei of neuroblasts.These changes depend on linear characteristics of nervous system excitability. Our data demonstrate that the changing of gene expression also as perturbances of processes of origin (proliferation activity) and destruction (chromosome aberrations) of neuroblasts can underlie of female stress influence on morphogenesis of embryonal brain and on the pathaloglcal alterations of adult rats behavior in connection with functional state of nervous system. Supported by RFFI.

DISTRIBUTION OF A POTASSIUM-CHLORIDE COTRANSPORTER (KCC2) IN THE RAT HIPPOCAMPUS

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GABA, the main inhibitory neurotransmitter in the adult brain, causes hyperpolarizing responses in hippocampal pyramidal neurons via activation of $GABA_A$ receptor/channel complexes. The transmembrane distribution of Cl^- determines the direction of the Cl^- flux gated by $GABA_A$-receptors: In the early postnatal period (approximately until P5) $GABA_A$ receptors mediate depolarization that is sufficient to activate voltage-dependent Ca^{2+} channels, but in the adult brain it results in hyperpolarization. We investigated the cellular and subcellular localization of the outward directed, neuron specific, K^+-Cl^- cotransporter, KCC2, during development and in the adult rat hippocampus. In animals older than 5 days the pattern was similar to adults at the light microscopical level. KCC2 immunostaining produced a strong diffuse labeling in CA1-CA3 str.oriens and str.lacunosum-moleculare as well as in str.moleculare of DG, while faint KCC2 staining was observed in str.radiatum, in the principal cell layers and hilus. The strength of the diffuse staining matches with the density of GABAergic terminals. Besides the diffuse staining, somata and dendrites of subpopulations of interneurons were outlined by strong KCC2 staining in all areas. Electron microscopical immunogold labeling confirmed that KCC2 is located in dendritic and somatic membranes, but due to the lower sensitivity of immunogold techniques, KCC2 could only be demonstrated in the dendrites of interneurons. In the immature rat hippocampus (from P1-P5) only weakly stained interneurons appeared in the dentate hilus and CA1-CA3 str.oriens, but the vast majority of neurons were only labelled after the fifth day of postnatal life. The present study shows a high degree of heterogeneity in the temporal and spatial profile of KCC2 expression. In future work, it will be of interest to examine the electrophysiological as well as developmental consequences of the expression pattern of KCC2.

ROLE OF DIFFERENT STRUCTURES OF THE HYPOTHALAMUS IN DEPRESSIVE-LIKE BEHAVIOR OF IMMUNIZATIONED MONKEYS

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Our previous works have demonstrated that presence at an organism of antibodies to β-endorphin (β-En) have been associated with depressive-like behavior in monkeys *(Soc. Neurosci. Abstr. (1997); 723.4)*. In this study, we were active immunized 7 adult monkeys (*Macaca mulatta, Macaca fascicularis*) against β-En: bovine serum albumin conjugated to β-En (BSA-βEn: 250-300 μg/kg) was dissolved with immunoamplifier (complete Freund's adjuvant) to give in 2.0 ml of emulsion injected. Booster immunization was conducted for 10 days. The effects of bipolar electrical stimulation (ES; 100 Hz, 0.5 msec, 20-50 μA) of the hypothalamus (Hp) on the classical motor-defensive conditioned reflexes (CR) was studied on monkeys with different types of nervous systems (strong, middle, weak), using the Protopopov method. The CR was represented by positive (defensive) and negative (differentiation and delayed inhibition) reflexes. EEG, motor and vegetative components (respiration (Rp) and heart rate (Hrt) of the CR were recorded. Free behavior was tested inside of soundproof room. It was shown that at immunized monkeys: 1) ES of dorsomedial Hp (DMH) increased the percent of correct defensive motor responses (CR), extended of latency period (LP) and increased number of defensive reactions (DR). The background of Hrt rate and frequency of Rp, as well as the motor components of the CR were increased. Motor activity, food intake and intragroup contacts of monkeys was increased; 2) ES of posterior Hp (mamillary body) slightly increased CR and quantity of defensive reactions. Differentiation and other negative reflexes did not changed during ES of the DMH; 3) ES of anterior Hp (lateral preoptic area) did not influenced essentially on depressive-like behavior of monkeys. The received dates suggest, that chronic (during 5-6 days) eletrostimulation of the DMH of the immunized monkeys was decreased of depressive-like behavior symptoms.

LOSS OF DIFFUSION ANISOTROPY AND PARALLEL GLIAL PROCESS ORGANIZATION IN HIPPOCAMPUS OF AGED RATS WITH IMPAIRED LEARNING ABILITIES.

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Diffusion in the hippocampus in young adult rats is anisotropic (Mazel et al., Neuroreport 9: 1299-1304, 1998), i.e. diffusion is facilitated in the mediolateral direction, whereas it is restricted in the rostrocaudal direction and even more in the dorsoventral direction. This could be due to the transversely-oriented fiber system in the hippocampus (mediolateral direction) and the sheet-like structure of cellular agregates in the hippocampus (perpendicular to the dorsoventral axis). Three extracellular space (ECS) diffusion parameters, volume fraction a (ECS volume/total tissue volume), tortuosity l (l^2 = free diffusion coefficient/apparent diffusion coefficient) and nonspecific uptake k', were studied *in vivo* in the hippocampus of aged rats (26-32 months old), which were classified according to their performance during place learning. Two groups, bad and good learners, were selected. Diffusion measurements were performed using the real-time iontophoretic tetramethylammonium method along three orthogonal axes (mediolateral, rostrocaudal, and dorsoventral). After the measurements, the animals were perfused transcardially and the brain sections were immunohistochemically stained for glial fibrillary acidic protein (GFAP), chondroitin sulphate proteoglycans and the cell attachment fragment of fibronectin. Significant differences in ECS diffusion parameters between good and bad learners were observed in the deeper layers of the hippocampus, dentate gyrus (DG) and CA3: a was significantly lower in bad learners than in good learners; moreover, there was a loss of anisotropy in bad learners. GFAP staining revealed astrogliosis in the brains of all aged rats. The typical radial organization of astrocytic processes was decreased in good learners and even further diminished in bad ones. A decrease in the staining for chondroitin sulphate proteoglycans as well as for fibronectin was observed during aging. While there was a clear decrease in the staining around neurons and their processes (forming so-called perineuronal nets) in CA1, CA3 and DG in good learners, there was a complete loss of staining in bad learners. In this study we provide evidence for a decrease in ECS volume and a loss of diffusion anisotropy in the hippocampus of aged rats. The loss of anisotropy could be explained by the loss of the radial organization of glial processes and a decrease in the amount of extracellular matrix molecules. These changes could affect extrasynaptic transmission, "cross talk" between synapses and long term potentiation (LTP) and could explain memory deficits (Nicholson and Syková, TINS 21: 207-215, 1998).
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TRANSIENT RE-EXPRESSION OF A JUVENILE PHOSPHORYLATED MAP 1B ISOFORM IN THE MOUSE BARREL CORTEX DURING POSTNATAL DEVELOPMENT

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Microtubule associated proteins (MAPs) are expressed in numerous isoforms which are known to be developmentally regulated. Juvenile MAPs are implicated in neuritic plasticity. A model system to investigate neuronal plasticity is the barrel cortex - a part of primary somatosensory cortex of rodents. In order to test whether the plastic potency of nearly mature barrel cortex is reflected by the presence of early MAPs we have examined developmental expression of the two phosphorylation modes of MAP-1B in the mouse barrel cortex. The MAP-1B phosphorylation mode I has been reported to disappear during maturation, whereas the MAP-1B phosphorylation mode II increases along with neuronal maturation. To visualize the phosphorylation mode I and II of MAP-1B we have used antibodies 150, and 125, respectively. The 125 immunoractive fibres are not yet detectable at postnatal day 5 (P5), they are already present at P12 and become more evident at P21. In the barrel cortex of P90 mice they are much less pronounced. In contrast, a very faint 150 immunostaining detected at P5 is not seen at P12. At P21 , however, apparent immunopositive fibers reappear. They are no longer present at P90 in the barrel cortex. The re-expression of the MAP-1B phosphorylation mode I which is a juvenile isoform characteristic for growing axons may imply induction of processes providing mouse barrel cortex neurons with potency for plastic changes at the terminal stage of synaptogenesis.
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DIRECT RECORDINGS OF PATTERNED ACTIVITY FROM SPINAL NETWORKS IN RAT SLICE CULTURES

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Patterned spontaneous activity appears in motoneurons under conditions of increased excitation in neonatal rat spinal cord as well as in fetal slice cultures. This finding suggests that local spinal networks are able to generate a patterned output to motoneurons. We directly tested this hypothesis by multiple recordings from spinal networks in slice cultures. Extracellular multisite recording was performed using planar microelectrode arrays fabricated by lithographic microtechnology. Patterned extracellular signals of biological origin were recorded by the microelectrode arrays mainly but not exclusively under conditions of increased excitation induced either by lowering extracellular magnesium or by disinhibition. Patterned activity consisted of irregular low rate bursting (1 - 10 / min) and occasionally of regular high rate oscillations within the bursts (2 – 8 Hz). Activity was highly synchronised over both hemispheres of the slice on the level of the bursting as well as on the level of the oscillations. To study the development of the networks in vitro we compared the pharmacological sensitivity of the activity patterns at different ages in culture. We found that the sensitivity to NMDA and AMPA antagonists changed within the second week in culture, suggesting alterations in the major receptor systems underlying pattern generation. These findings demonstrate that multielectrode arrays are an appropriate tool for studying the formation of pattern generating networks in spinal slice cultures.

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CLOZAPINE IN PATIENTS WITH SCHIZOPHRENIA AND SUBSTANCE ABUSE

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Introduction Substance use disorders are particularly common in patients with schizophrenia and contribute notably to the overall morbidity of the illness. It has been hypothesized that schizophrenic patients abuse these various substances in order to self-medicate underlying negative symptoms or alternatively to ameliorate effects of dysfunctional dopamine-mediated brain reward mechanisms. Unfortunately typical antipsychotic medication appear to have no effect and may even exacerbate substance abuse. It remains unclear what the role of clozapine, an atypical antipsychotic medication, has on substance abuse in such patients. **Method** In a pilot study, 58 patients comorbid for schizophrenia (or schizoaffective disorder) and substance abuse, and treated with clozapine, were investigated retrospectively by chart review and clinician interview. The survey involved an assessment of change in substance use and global clinical symptoms during the course of clozapine treatment. **Results** During the course of clozapine treatment over 85% of patients who were active substance abusers at the time of clozapine administration decreased substance use, with approximately 72% achieving abstinence. There was a significant correlation between decrease in substance use and decrease in global clinical symptoms. **Conclusion** Our pilot findings support previous observations that clozapine may reduce substance use in schizophrenia. We have hypothesized that clozapine may have this effect by virtue of its mechanism of action which includes release of dopamine in the prefrontal cortex. This may in turn enhance the brain reward circuit and improve negative symptoms, thus lessening the self-medication requirement in such patients. Our data suggest the need for prospective studies of clozapine in this "dual diagnosis" population.

SPINAL PATTERN GENERATOR FOR LOCOMOTION IN PRIMATES

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Eventhough functional recovery after spinal cord injury was extensively studied in the past, there exists considerable disagreement about the nature of recovery, the mechanism responsible for it and methods of measuring it. The aim of the present study is to investigate the extent of recovery of function after spinal cord injury in bonnet monkeys using a battery of quantitative tests to measure reflex responses and different components of locomotion. Experimental monkeys were subjected pre-operatively to different motor/reflex testing viz., Grasping reflex, Righting reflex, Extension withdrawal reflex, Placing reflex, Hopping reflex and were also trained in bipedal walking in Wide runway, Narrow beam runways, Grid runways, Inclined plane and Treadmill and were assessed by "Combined behavioural score" (CBS). Surgical hemisection was performed in the right side of spinal cord at T12 / L1 level of pre-trained monkeys and the effect of lesion and recovery of function was assessed using the CBS. Dragging movement was noticed in the right hind limb while walking during the early post-operative period. The right hind limb was not used for supporting the body weight. At the end of first month of post operative period, animals had a mild deficits in hopping and extension withdrawal response in the right hind limb, but greater deficits to other reflex responses were noted. However, the pattern of locomotion in the wide runway, inclined plane and treadmill in the experimental monkeys were near normal at the end of first month post-operative period. These preliminary observations point to the fact that the near normal pattern of locomotion seen after spinal cord hemisection may be attributed to the "spinal pattern generator for locomotion" (SPGL) in primates, similar to that has been reported for rodents.

MEMORY AND LEARNING ACTIONS OF RGH-1756, A NEW D_3 RECEPTOR SELECTIVE POTENTIAL ATYPICAL ANTIPSYCHOTIC

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Memory impairment is a characteristic symptom of schizophrenia and other psychotic states. Since the conventional neuroleptics have no effect on it or even themselves induce memory deficits, the need of treating the cognitive decline poses a considerable challenge to new, atypical antipsychotics. RGH-1756 (1-(2-methoxy-phenyl)-4-{4-[4-[(6-imidazo[2,1-b]thiazolyl)-phenoxy]-butyl}-piperazine) is under development as a potential antipsychotic compound with remarkable memory improving activity. The compound possesses a unique receptor profile (100 fold selectivity for D_3 over D_2 receptors), a certain degree of limbic selectivity and reduced extrapyramidal side-effect.

The drug proved to be active in several traditional antipsychotic tests. However, in contrast to haloperidol and clozapine, RGH-1756 (60 mg/kg po.) had no effect on conditioned avoidance response. Moreover, in much lower doses it exhibited beneficial effects in various learning tasks. One mg/kg po. daily dose of RGH-1756 restored the impairment of acquisition induced by the benzodiazepine agonist diazepam in a water-labyrinth system. The same dose of the drug reversed the benzodiazepine inverse agonist FG-7142-induced learning deficit in this task. Daily treatment with 20 mg/kg ip. FG-7142 caused an impairment in learning performance in the 8-arm radial maze, as well, reflected by the elevated number of total errors and reduced number of initial correct responses during acquisition. RGH-1756 (1 mg/kg po. daily) counteracted the disruptive effect of FG-7142. The action of RGH-1756 developed parallel with time. In the radial maze the drug also had a dose-and time-dependent improving action against scopolamine-induced memory impairment. 0.3 mg/kg po. daily dose caused a significant decrease in the number of total errors only on day 5 of acquisition, whereas the 1 mg/kg dose significantly improved the performance of scopolamine treated rats alrea dy from day 3.

Therefore, RGH-1756 may be a promising antipsychotic compound with effective memory improving potential.

UNITARY FIELD POTENTIALS GENERATED BY INDIVIDUAL VB THALAMOCORTICAL AXONS ENTERING AN S1 BARREL

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Action potentials were recorded from VB thalamocortical neurons of awake rabbits while spike-triggered averages (STAs) of field potentials were generated at various depths within the topographically aligned S1 barrel column. VB action potentials elicited a cortical response in layer 4 with three components: (1) a biphasic, initially positive response (latency < 1 ms) reflecting activation of VB axon terminals (the AxTP). (2) a brief (~ 4 ms) negative potential (focal synaptic negativity, the FSN), interpreted to reflect monosynaptic excitation. (3) a long-lasting positive potential (the FSP), interpreted to reflect di/polysynaptic inhibition. The amplitudes of the AxTP, the FSN and the FSP peaked near layer 4 and were highly reduced in both superficial and deep cortical layers and in neighboring (miss-aligned) cortical barrels. We examined the contribution of the autocorrelogram of the VB triggering neuron and of presynaptic synchrony among VB neurons. Although the AxTP and the FSN were little affected, the amplitude of the FSP was strongly influenced by weak synchrony among neurons of the same VB barreloid. We conclude that single thalamic impulses entering an S1 barrel engage monosynaptic excitatory and di/polysynaptic inhibitory mechanisms. We suggest that the single-fiber access to intracortical inhibition is facilitated by sharp synchrony among feed-forward GABAergic interneurons of S1 (Swadlow et. al., J. Neurophysiol, 1998), and that the FSP reflects a consequent synchronous wave of feed-forward inhibition within the barrel. Supported by grants to H.A.S. from the U. S. National Science Foundation and to A. G. from the Russian Foundation of Basic Research .

EFFECTS OF ANTIDEPRESSANTS ON IMMUNOLOGICAL FACTORS IN CHRONIC MILD STRESS MODEL OF DEPRESSION.

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It has been hypothesized that major depression may be accompanied by an acute phase response (apr) as indicated by increased plasma levels of positive acute phase proteins (apps). Of the various apps measured in depression, the most prominent alterations were found in levels of haptoglobin (Hp) and alpha-1-acid glycoprotein (AGP). We have investigated the effect of two SSRI: fluoxetine (Flx) and fluvoxamine (Flv) on immune function in the chronic mild stress model of depression in rats. Plasma concentration of two acute phase proteins: Hp and AGP as well as one of „early cytokins"- tumor necrosis factor α (TNFα) were investigated before and after treatment with Flx and Flv. Male Wistar rats (about 350g) were used. In CSM model, rats were subjected to a variety of extremely mild stressors, showing a gradual decrease in their response to rewarding stimuli which was monitored by substantial decrease in their consumption of a 1% sucrose solution. The difference in sucrose intake between stressed and controled animals was significant after 3 weeks of treatment. For the subsequent 4 weeks they received antidepressant treatment: Flx (5 mg/kg) and Flv (15 mg/kg) p.o. Flx and Flv had no significant effect on control animals, in stressed rats they caused a gradual increase in sucrose intake. The plasma concentration of Hp and AGP were measured by rocked immunoelectrophoresis with human anti-Hp cross reactive with rat Hp and anti-rat AGP. TNFα concentration was measured with ELISA. Plasma concentrations of AGP, Hp and TNFα were elevated after three weeks of stress in CMS. Antidepressant effect of Flx and Flv was accompanied by decrease of Hp, AGP and TNFα concentrations. These results demonstrate that in „anhedonic" rats in CMS model of depression there is an immune activation with elevated plasma levels of Hp, AGP and TNFα. This finding may have important relevance to human major depression. Antidepressants could regulate immune changes which might be a part of their antidepressive properties.

NOCICEPTIVE - SPINAL MEDULLARY CATECHOLAMINERGIC INTERCONNECTION

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Neuronal inputs to spinal cord nociceptive neurons were investigated by transneuronal tract-tracing using Pseudorabies virus (Bartha strain - Ba-PRV) in intact rats and in rats with surgical transections at spinal cord and the medulla oblongata (midsagittal section through the medulla, spinal cord hemisections, ipsi- or contralateral to the site of virus injections). Three to five days after Ba-PRV inoculations into the right hind limb subcutaneously, rats were perfused with a fixative solution intracardially, the brains were removed, cut in serial coronal sections, and the infected cells were visualized by the avidin-biotin immunohistochemical technique. Labeled neurons were seen in dorsal root ganglionic cells in lumbar segments (L2-L5) exclusively one side, ipsilateral to the viral inoculation. In the spinal cord (also in L2-L5), most of the labeled cells located in the ipsilateral marginal zone but infected cells also occurred in lamina 2 (outer layer), 3,4 and 5 ipsilaterally, and in lamina 10 bilaterally. In supraspinal levels, labelled cells appeared at first (3.5 days after inoculation) in the ventrolateral pons (A5 catecholaminergic cell group), which was followed one day later by labeling of neurons in the ventromedial medulla (in the giganto- and para-gigantocellular, the raphe magnus, obscurus, pallidus nuclei) and in some cells in the rostral portion of the nucleus of the solitary tract. By the day 5, labelled cells were seen in the locus coeruleus, the A7 catecholaminergic cell group, the midbrain central gray, the hypothalamic paraventricular nucleus and in the lateral hypothalamus. All of above labeling were bilateral . Observations in operated animals clearly show that the major descending catecholaminergic (from the A5, A7 and locus coeruleus neurons), as well as serotonergic and peptidergic neurons of the medial ventromedial medulla cross over in the spinal cord. Fibers from the midbrain and the hypothalamus may cross-over at supraspinal levels. The nociceptive neurons may not receive direct neuronal inputs from cells in the lower medulla oblongata or from extrahypothalamic forebrain areas including the central nucleus of the amygdala.

SENSITIVITY OF CEREBELLAR PURKINJE CELLS TO MODERATE DOSES OF HARMALINE IN OLD RATS.

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It is a matter of common knowledge that the alkaloid harmaline induces a rhythmic tremor in mammalians and corresponding synchronous rhythmic climbing fiber activation of cerebellar Purkinje cells (PC). The aim of the study was to investigate sensitivity of rat's cerebellar PC to harmaline. The experiments have been performed on old Wistar rats 25-36 month of age. The harmaline in 15 mg/kg dose was injected intraperitoneally (i.p.). The activity of PC had being recorded by glass microelectrodes for 10 min. before injection and 30-60 min. after it. Two types of alterations in PS activity were detected. 1) The frequency of complex spikes (CS - result of synaptic PC activation by climbing fibers) remained almost without change, whereas that of simple spikes (SS - result of synaptic PC activation by mossy fibers) was decreased. The inhibitory pause, which follows CS was elongated. 2) The frequency of CS was increased, whereas no SS were registered. As it was shown previously the latency of PC responses on harmaline injection (time between i.p. injection and beginning of simple spikes vanishing) was increased with age of animals. In fact, the latency (30 - 40 min.) of cerebellar PC responses to moderate harmaline dose injection in old rats in several times overshoots the reaction time of PC in young rats. It has also been shown that in old animals after i.p. harmaline injection the PC responses by complex spikes were more expressed. So, the frequency of CS following i.p. harmaline injection in the second type reaction was 5.58±0.46 imp/s in old rats and in young rats it was 3.45±0.62 imp/s. In this way the obtained dates allow to conclude, that cerebellar PC activity of old rats is more sensitive to harmaline, than young rats one.

CHRONIC MILD PRENATAL STRESS EFFECTS IN AN ANIMAL MODEL OF SCHIZOPHRENIA.

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Adverse intrauterine events apart from genetic vulnerability seem to contribute to the pathogenesis of schizophrenia. We investigated the influence of prenatal stress in the rat offspring using the latent inhibition (L I) procedure. The chronic prenatal stress (CMS) consisted of transitory and variable changes in the rat's living conditions, using the approach of Willner *et al*, 1987 (Psychopharmacology 93, 358-364). Pregnant Wistar rats were divided in stressed and non stressed groups (N =10, each), during the last two weeks of pregnancy. Male offspring at adult age were subdivided into 4 groups: No Preexposed Controls (NPC), No Preexposed Stressed (NPE), Preexposed Controls (PC) and Preexposed Stressed (PS). Preexposure consisted of tone presentation before the classical tone- shock conditioning. During the test, the tone was presented to the animals and the time to complete 10 licks immediately before and during the tone was recorded. LI results were evaluated by means of suppression ratios. Both stressed and control animals when preexposed to the tone were able to demonstrate LI . There was no difference between suppression ratios from NPC and NPS groups or retardation of learning produced by prior exposure to the tone at the PC and PS groups. The results suggest that the alterations produced by prenatal CMS may not affect LI in animals.
Financial support: CNPq and CAPES.

LITHIUM TREATEMENT PREVENTS THE INHIBITION OF LEUKOCYTE MIGRATION INDUCED IN HELPLESS RATS.

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Depression is known to affect various physiological functions, including the immune system. We have examined the influence of the learned helplessness model of depression and the effect of an antidepressant on an inflammatory response. Female Wistar rats(N=97) drank either tap water or a 20 mM LiCl solution (serum level of 0.5 mEq/l) *ad libitum* for 28 days. Each group was then divided into: 1) stressed rats which received 60 escapable 1mA footshocks, 2) helpless rats which received inescapable shocks yoked to group 1, and 3) control rats which received no shocks. In all cases, neutrophils migration was evaluated in peritoneal cavity, 4 h after the injection of carragenan (150 ug /cavity) or LPS (100 ng/cavity) while the migration of monocytes was assessed 96h after an i.p. injection of 10ml of a 3% solution of thioglycollate. Sterile saline served as control. ANOVA followed by Duncan's test revealed that monocyte migration was inhibited in both shocked groups while neutrophil migration was only inhibited in helpless rats. Lithium treatment did not provoke peritoneal inflammation and prevented the inhibition of monocyte and neutrophil migration in helpless rats only. We concluded that untreated depression affects the release by peritoneal resident cells of factors that are chemotactic for both neutrophil and monocyte.
Financial support: CNPq and FAPESP.

EFFECTS OF CHRONIC LITHIUM ADMINISTRATION ON MU-OPIOID RECEPTOR IMMUNOSTAINING IN THE RAT FOREBRAIN

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Lithium alters opioid receptor transduction mechanism by interfering with the metabolism of phosphoinositides, and partially blockade the increase in dopamine receptor sensitivity induced by opioids, generating an antimanic effect. Male Sprague-Dawley rats (n = 6) were administered with lithium carbonate (10 mg/kg/day, i.p.) for two weeks and brain coronal sections were immunostained for mu-opioid receptor by the avidin-peroxidase technique and examined with an optic microscope and a image analysis system. Lithium treatment increased the density of neural cells expressing mu-opioid receptors in the piriform, parietal and frontal cortices, the caudatus-putamen, the lateral septum and the dentate gyrus, with respect to controls. These data suggest that high dose chronic lithium administration increases mu-opioid receptor expression in the rat forebrain. This could be a compensatory mechanism induced by the effects of lithium on mu-opioid receptor transduction mechanism, and could partially explain the controversial interactions of lithium salts with antidepressant drugs and its prophylactic effect in bipolar disorders.

DECODING OF VIBRISSAL TEMPORAL INFORMATION BY THALAMO-CORTICAL LOOPS: THEORY AND EXPERIMENTAL VERIFICATION

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Spatial features are encoded by the moving whiskers of the rat by using both spatial and temporal cues. An object's location is encoded spatially by the identity of the activated rows of whiskers, and temporally by the time interval between receptor firing at the onset of a whisking cycle and receptor firing due to perturbation of whisker motion by the external object. Spatially- and temporally-encoded information are probably decoded in parallel by the 'lemniscal' and 'paralemniscal' systems (see Sosnik et. al., this meeting). We examined how temporal decoding can be achieved, and suggest how the slowness of the 'paralemniscal' pathway is advantageous for temporal decoding at the frequency range of whisking. The principle of the suggested decoding scheme is that the cortex, by using oscillatory neurons, produces an expectation for the timing of the next input from the whisker. The timing of these oscillatory cortical outputs is compared at the thalamus (POm) with the input timing . The comparison is implemented by simple gating, in which the cortical signal gates the vibrissal signal. The result of the comparison, the "error signal," is fed back to the cortex for further processing. This signal is now rate-coded, by populations of thalamocortical neurons, and is thus ready for integration with other neuronal signals, mainly those that arrive via the 'lemniscal' pathway, which probably carries spatially-encoded information, coded by rate. The slowness of the 'paralemniscal' pathway is important, since the working range of the thalamo-cortical loops is limited by the range of temporal delays (between the whiskers and the cortex) that the thalamocortical neurons can detect, i.e., translate to a rate-code. This range is limited by the width of the afferent signal. The optimal width for decoding is probably around half of the whisking period (i.e., 40 - 100 ms), which is the range of POm response widths. Brief responses, like those that occur in the 'lemniscal' pathway, are optimal for temporal decoding with higher frequencies, like those that occur during texture identification. One prediction of this model is that whisker frequency is represented by cortical spike counts (an inverse relation) and by cortical latency (a direct relation). This indeed was found when applying frequency modulated (FM) whisker stimuli. A sudden perturbation of the whisker frequency, which simulated an external object, produced a sudden change in the cortical spike counts and latencies, which approved another prediction of the model. Overall, our data, together with previous vibrissal data, are consistent with an active temporal decoding process performed by POm-cortical loops. *Supported by Grant no. 97-222 from the US-Israel Binational Science Foundation, Israel. S.H. supported by The Center for Absorption in Science, Ministry of Absorption, Israel.*

OPTICAL RECORDING OF ENHANCEMENT OF THE NEURAL ACTIVITY DURING PROPAGATION FROM THE SUBICULAR COMPLEX TO THE POSTERIOR CINGULATE CORTEX OR TO THE ENTRHINAL CORTEX.

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To visualize functional connection from the subiculum to the entrhinal cortex (EC) or to the posterior cingulate cortex (PCgC), we applied a voltage-sensitive dye to the brain slice, and optically recorded enhancement of the neural activity during the propagation from the subicular complex (SC) to the EC, or to the PCgC. We used a high speed, high resolution optical recording system (Deltaron 1700, FujiFilm; with an area sensor of 128 x128 pixels, 0.6 msec/frame). We also recorded electrically the field potentials, units and synaptic potentials at the same time, and confirmed the optical signals really reflect the neural events. Single weak stimulation of the subiculum evoked spread of the neural activity to the EC; one via the SC, and the other via the perforant path. In the nominally Mg ion free solution both of these enhanced but more strongly in the former path, and spread further over the rhinal fissure. The enhancement is blocked by CPP or APV, NMDA receptor antagonists, suggesting the NMDA receptors are involved. In a coronal section including the SC and the PCgC, single weak stimulation of the subiculum evoked a neural propagation to the boundary of the SC. After a pause, a weakened response enhanced in the deep layer of the SC, which spread to the superficial layer, and further to the PCg. The enhancement of the neural activity seems to happen in the bursting type of neurons in the deep layer, and the GABAergic inhibitory mechanisms are involved. In this experiment functional connection from the SC to the EC, or to the PCgC is visualized, and enhancement of the neural activity is identified in the SC and the EC.
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AMYGDALA: NEW DATA ABOUT SEX DIMORPHISM ZONES

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The main sex dimorphism zones of Amygdala are dorsomedial, basolateral and anterior cortical nuclei. We have first discovered neurons with morphological characteristics of secretote activity in these nuclei. We identify these neurons: they have the characteristics of karyochromic neurons in light microscopy: large basophylic cell nucleus the surface of which is uneven and extremely narrow perikarion. The method Goldgi show that these neurons are long-axonal sparcely dendritic neurons mostly short dendritic neurons. The concentration of these neurons in the sex dimorphism zones show that they have straight attitude to regulation of neuroendocrine processes.

ATTENTIONAL MODULATION OF ACTIVATION IN OBJECT-RELATED AREAS OF HUMAN VISUAL CORTEX.

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It is still unclear to what extent activity in object-related areas of the human visual cortex can be modulated by shifts of visual attention. To address this question we conducted fMRI experiments in which subjects viewed pictures of complex, natural objects. A small central arrow was superimposed on each picture, with its direction changing randomly from picture to picture. Subjects were instructed to attend and covertly name either the objects or the direction of the arrow. Three regions of preferential activation for objects were identified: area V4, the lateral occipital complex (LO), and dorsal foci that consisted of area V3a and a region slightly anterior to it. In all three regions attending the objects yielded stronger activation than attending the central arrow, while an opposite effect was observed in the motion sensitive area MT/V5 (attention index: V4=16.0%±3.6 (SEM); LO=7.5%±2.5; V3A+=24.0%±4.5; MT=-34.5%±6.5; N=9). For each of these areas, we calculated a stimulus selectivity index, which measures the differential activation by the objects vs. the arrows alone. A striking correlation was observed between stimulus selectivity and the level of attentional modulation across all areas, (r=0.81). Control experiments excluded either eye movements or attention to specific retinotopic locations as the source of the attentional effect. These results suggest that feature-based attention plays a role in modulating the activity in the LO complex.

SEASONAL VARIATIONS IN MORPHINE WITHDRAWAL SIGNS. PREVENTION WITH BACLOFEN.

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Baclofen (BAC), a selective GABA B agonist, as well as morphine (MOR), a selective opioid agonist, were found to elicit a dose-dependent antinociceptive effect in mice. In previous studies we have demonstrated a possible interaction between the GABAergic and opioid systems involved in the antinociceptive effect of BAC. On the other hand we have studied the development of MOR antinociceptive tolerance and the cross-tolerance to the BAC effect. Being well known the opioid drugs addiction, the aim of the present study was to examine whether BAC prevents the MOR abstinence signs in mice. The experiments were performed in two seasons (summer and winter) on male Swiss-Webster albino mice weighing 25 to 35 g. Mice received MOR (2 mg/kg, i.p.), twice daily for 10 days. After the last dose of the opioid agonist, naloxone (NAL) (6 mg/kg, i.p.), an opioid antagonist, was administered and the following signs were determined: locomotor activity, normal feces, liquid feces, rearing, wet-dog shakes and sniffing. In a group of MOR dependent mice, the last dose of MOR was followed by the administration of BAC (2 mg/kg, i.p.). This treatment was performed before the NAL-precipitated abstinence syndrome. In winter the results showed a significant increase in certain morphine abstinence signs compared with the summer group: liquid feces (65.25 %, $p<0.001$); wet-dog shakes (46.1 %, $p<0.01$); sniffing (35.34 %, $p<0.001$). BAC administration before NAL-precipitated abstinence syndrome showed a significant decrease in all the parameters compared with the abstinence group, both in summer and winter.
These results suggest that the MOR withdrawal signs in winter are stronger than in summer. The finding that BAC diminished the MOR withdrawal signs suggest that this drug could be useful for the treatment of opioid drugs addiction.

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Here is my abstract for the 5 th IBRO World Cogress of Neuroscience, submitted for poster presentation. Registration form and check follow by mail.

THE EFFECT OF ETHANOL ON FREE N-ACETYL-HISTIDINE LEVELS AND ON UPTAKE OF 14C-LABELED L-VALINE BY THE LENS OF THE GOLDFISH IN VITRO

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N-acetyl-L-histidine (NAH) and N-acetyl-L-aspartate (NAA) are major natural amino acid constituents of vertebrate lens and brain and appear to be metabolic analogs. In the eye, these amino acids are synthesized in the lens and hydrolyzed in ocular fluids. The cycling of these acylamino acids between the lens and ocular fluids suggests a pump mechanism, and there is some evidence that their metabolic role in the lens may be to function as molecular water pumps (MWP's) [Baslow, M.H., J. Mol. Neurosci. 10(3):193-208, 1998]. Ethanol, which appears to affect water homeostasis in the brain may, at very low blood-alcohol concentrations, also produce subtle effects on metabolic pathways at lower levels than those that produce discernible CNS impairment. In this study, the interaction of ethanol on the level of free NAH in the lens, and the transport of L-valine (Val) into the lens were investigated. Isolated lenses of the goldfish, Carassius auratus, weighing between 2-4 mg with a water content of 53.5%, placed in a lens support medium (NaCl, 0.9%; Ca 2+, 4 meq/L; D-glucose, 5 mM) at pH 7.4 and 240C, were utilized. Control lenses contained 2.5 ± 0.9 mmol/g of free NAH. After 3 h of incubation in the presence of a metabolic indicator (0.1 mM Val tagged with 0.2 mCi of 14C UL Val), there was an efflux of NAH and residual NAH was 1.2 ± 0.7 mmol/g. During the incubation period there was also an active uptake of Val, and the Val concentration factor (CF), measured as CPM per ml of lens water/ CPM per ml of medium was 6.5. The addition of ethanol at 0.01, 0.05, 0.10 and 0.25% to the medium in this assay resulted in the almost complete absence of lens NAH at all ethanol concentrations tested, whereas the CF for Val was graded and decreased to 4.7, 3.2, 5.0 and 2.2 respectively. In addition, the absence of NAH could be correlated with the appearance of a histidine-like substance in the lens. It is speculated that the striking and specific effect of ethanol on residual lens NAH, while active transport is only moderately affected, may involve the possible role of NAH as a MWP. This effect may also occur in poikilotherm retina and brain, and NAA, a metabolic analog of NAH in poikilotherms and homeotherms, may have a similar interaction with ethanol.

5-HYDROXYTRYPTAMINE 1B (5-HT1B) RECEPTORS MODULATE THE REINFORCING EFFECTS OF COCAINE : CONVERGING EVIDENCE USING 5-HT1B KNOCKOUT MICE AND THE 5-HT1B/1D LIGAND MODULINE.

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Serotonergic transmission has been suggested to modulate some effects of cocaine. However, the specific receptor underlying this phenomenon has not been identified. The 5-HT1B receptor could be a good candidate because mice lacking this receptor show increased vulnerability to cocaine in a self-administration procedure. In order to study the involvement of 5-hydroxytryptamine 1B (5-HT1B) receptors in the subjective rewarding effects of cocaine, we compared cocaine-induced place conditioning in wild type mice (129/Sv-ter) and in mice of the same strain lacking the 5-HT1B receptor. Cocaine (5 and 20 mg/kg) induced place preference in wild type mice while it did not elicit any effect in 5-HT1B knockout mice at doses ranging from 2.5 to 40 mg/kg. Furthermore, intracerebroventricular administration of the 5-HT endogenous peptide moduline (100 µg) completely blocked the place preference exhibited by C57BL/6 mice after cocaine (5 mg/kg) while the scrambled peptide (100 µg) had no effect. It is concluded that the 5-HT1B receptor is implicated in the ability of cocaine to induce rewarding effects.

EXPERIMENTAL ETHANOL CRAVING: EFFECTS OF NALTREXONE AND NMDA RECEPTOR ANTAGONISTS IN THE EXTINCTION/REINSTATEMENT PARADIGM.

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Ethanol has been reported to attenuate various NMDA receptor-mediated responses *in vitro*. In line with the above, NMDA receptor antagonists mimic some central effects of ethanol both in animal subjects and human alcoholics. However, their utility in the treatment of alcohol addiction remains to be established. Recently, a non-selective opioid receptor antagonist, naltrexone has been shown to reduce relapse rates in detoxified alcoholics. Thus, the aim of the present study was to compare the effects of naltrexone and selected NMDA receptor antagonists on: (i) ethanol self-administration; (ii) parameters of extinction and reinstatement of ethanol-seeking (*i.e.* experimental craving). Male Wistar rats were trained (30 min/day) in an oral self-administration procedure where a single lever press resulted in presentation of 0.1 ml of 8% ethanol from a liquid dipper. The selectivity of drug's actions was estimated by studying its effects on water-reinforced behaviour in separate control experiments. In the 30-min reinstatement session, lever pressing was first extinguished for 20 min. Then, various kinds of stimuli were non-contingently delivered and reinstatement of lever pressing was assessed. Preliminary experiments revealed that fifteen random (RT15 s) presentations of different sets of ethanol-associated cues, *i.e.* the dipper containing either ethanol (4-8%) or water, potently reinstated ethanol-seeking. Only repeated treatment with naltrexone (3 mg/kg) selectively reduced ethanol intake. On the other hand, acute administration of naltrexone (1-3 mg/kg) enhanced extinction of ethanol-reinforced responding. Moreover, naltrexone dose-dependently inhibited reinstatement of ethanol-seeking. An uncompetitive NMDA receptor antagonist, MRZ 2/579 (5-7.5 mg/kg) selectively reduced ethanol self-administration and increased extinction of lever pressing for ethanol. However, this compound did not alter reinstatement of ethanol-seeking. A glycine$_B$ site antagonist, MRZ 2/576 did not produce any selective effects on ethanol-reinforced behavior. These results indicate that (i) repeated treatment with naltrexone leads to progressive reduction of ethanol self-administration; (ii) naltrexone potently decreases experimental ethanol craving; (iii) the uncompetitive NMDA receptor antagonist, MRZ 2/579 suppresses ethanol self-administration and eliminates at least some conditioned aspects of ethanol reinforcement.

INTERACTION BETWEEN OPIATERGIC SYSTEM AND ANXIOLYTICS

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Interaction between benzodiazepines and opioids was previously described. Naloxone blocks the antianxiety effect of benzodiazepines in several animal models of anxiety, and it has similar effects in humans. Opiate antagonists does not bind significantly to receptors thought to mediate anxiolytic activity, therefore it is likely that opiate antagonists block anxiolytic activity through an action on opiate receptors. We hypothetized, that this anti-anxiolytic action of opiate antagonists is not limited to the activity of benzodiazepines but to any other anxiolytics regardless their mode of action or chemical structure.
The aim of present study was to establish the activity of naltrexone on the effect of anxiolytics with different mechanism of action. The anxiolytic activity of the compounds was assessed using three different models. Anxiolytics but not naltrexone significantly elevated the number of shocks accepted in Vogel lick-conflict, increased the number of open arm entries in the elevated plus-maze test, and increased the movement time almost back to control values in mCPP treated rats in the mCPP-induced anxiety model. Naltrexone pretreatment succesfully antagonized the anxiolytic effect of the drugs acting on benzodiazepine-binding site, the activity of serotonine receptor antagonist and the anxiolytic effect of the 2,3-benzodiazepine in each model. In conclusion, naltrexone was able to antagonize the anxiolytic activity of the compounds studied regardless their mode of action or model used. The present data indicate, that the anti-anxiolytic effect of opiate antagonists is not limited to benzodiazepines, but to other anxiolytics as well.

THE ROLE OF PRETECTAL COMMISSURAL FIBRES IN TRANSFER OF THE OPTOKINETIC INFORMATION IN RATS.

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Optokinetic nystagmus (OKN) is an ocular reflex allowing gaze stabilisation despite animal self-motion. In mammals, the nucleus of the optic tract (NOT) is considered essential for horizontal OKN. In lateral-eyed animals, monocular stimulation evoked an asymmetry in the response of the contralateral NOT cells related to direction of motion: activation or inhibition were elicited by nasalward or temporalward moving stimuli, respectively. Moreover, in rats not all NOT cells are binocularly driven as only about a 50% respond to ipsilateral eye stimulation, in any case unrelated to the paucity and inefficacy of ipsilateral retinal afferents. Recently, it has been shown in the opossum that inactivation of one NOT leads to disappearance of binocularity in neurons of the opposite nucleus suggesting an interplay between NOTs throughout the commissural fibres. Based on this result we investigated whether neurons in each NOT were recruited by monocular or binocular horizontal optokinetic stimulation (hOKS) and, for the monocular hOKS, in relation to the direction of stimulus motion. We stimulated hooded rats with a prolonged hOKS and studied the appearance of c-Fos protein in the NOT. Surprisingly, c-Fos protein was found in both NOTs, independent of vision condition and stimulus motion. The highest number of c-Fos positive cells was counted in the NOT contralateral to the nasalward stimulated eye after either binocular or monocular hOKS. The lowest labelling was detected following monocular temporalward hOKS. However, c-Fos labelled cells found in the NOT contralateral either to the closed or temporalward stimulated eye suggest a transfer of optokinetic signal between the NOTs throughout the pretectal commissure, finally assuring an appropriate optokinetic performance.

STRUCTURE AND FUNCTION OF THE NEURAL CELL ADHESION MOLECULE, NCAM

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The external part of NCAM is composed of five immunoglobulin (Ig)-like domains and three fibronectin type III (F3) domains. An NCAM molecule on one cell is capable of binding to an NCAM molecule on another cell through so-called homophilic binding. This type of binding is presumably residing in the first three Ig domains. The first and second Ig domains are supposed to bind to each other in a double reciprocal interaction and the third domain is supposed to bind to itself. NCAM is also able to undertake heterophilic binding to a series of carbohydrate ligands including heparin, heparan sulfate (agrin), chondroitin sulfate, and an oligomannosidic glycan. Binding to the oligomannosidic glycan is thought to mediate binding to another neural cell adhesion molecule, L1. NCAM is also believed to interact with the fibroblast growth factor receptor, supposedly in a cis-interaction. Finally, NCAM is capable of binding and hydrolysing ATP. We have studied the role of these binding interactions in cell-cell adhesion, neurite extension, and cell motility. Both synthetic ligands identified by combinatorial chemistry and natural ligands have been used for these investigations and new compounds have been developed, which may be of clinical interest in the treatment of neuro-degenerative diseases.

EXCITATORY GLUTAMATERGIC NEUROTRANSMISSION IN THE TOLERANCE TO AND AFTER A SINGLE DOSE OF LORAZEPAM (LZ).

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Benzodiazepines (BZ) constitute a group of drugs that, due to its safety and efficacy in the treatment of insomnia and anxiety disorders, have become of frequently use all over the world. Persistent administration of these drugs causes pharmacodynamic tolerance to their various pharmacological effects. Several changes in the inhibitory gabaergic neurotransmission have been demonstrated during the tolerance period. The aim of our work was to evaluate the existence of compensatory changes in the excitatory glutamatergic neurotransmission, in animals tolerant to and treated with a single dose of BZ. Male Wistar rats (180-200 g) were treated with 3 mg/kg/day (i.p.) of LZ, a dose proved to be sedative. Control animals were treated with vehicle. Tolerance to LZ after 21 days of treatment was evidenced by the absence of sedative effect of the last dose, tested on an open field experiment 30 minutes after the injection; control animals were injected with a dose of LZ. Separated groups of animals were treated with a single dose of either 3 mg/kg of LZ or vehicle and sacrificed 30 minutes later. The endogenous content of glutamate and aspartate in cerebral cortex of tolerant and acute treated rats was quantified by Reverse Phase HPLC (C18), pre column derivatization with o-Phthaldialdehyde in alkaline solution, in the presence of β-Mercaptoethanol. An electrochemical detector, at an oxidation voltage of +0.6 V was used. No differences were found in the cortical content of glutamate neither in the tolerance (2086±178 *vs.* 2186±329 μg/g tissue, tolerant *vs.* controls) nor in the acute treatment (1183±85 *vs.* 1265±75 μg/g tissue, acute treated *vs.* controls). The content of aspartate was not different either (432±40 *vs.* 424±51 μg/g tissue, tolerant *vs.* controls; 523±67 *vs.* 509±66 μg/g tissue, acute treated *vs.* controls). We also studied the binding of ^{3}H-glutamate to cortical NMDA receptors from tolerant and acute treated rats. There was a statistically significant difference in the K_D value of the tolerant animals (132±15 *vs.* 74±14 nM; tolerant *vs.* controls, $p<0.05$), while no differences were found in the Bmax value (6809±479 *vs.* 5253±1367 fmoles/mg protein; tolerant *vs.* controls). There were no differences in the parameters obtained from acute treated rats (K_D: 80±17 *vs.* 48±6 nM; Bmax: 4201±316 *vs.* 3461±328 fmoles/mg protein; acute treated *vs.* controls). We conclude that, although no significant changes neither in the content of glutamate and aspartate nor in the NMDA receptor density in cerebral cortex were found, there is a diminution in the affinity of these excitatory amino acid receptors for glutamate in the tolerant rats. There was no evident change neither in the content of the quantified amino acids nor in the NMDA receptor parameters studied after an acute administration of a sedative dose of LZ.

MODULATION OF NOCICEPTIVE TRANSMISSION BY NMDA/GLYCINE SITE RECEPTOR IN THE VENTROPOSTEROLATERAL NUCLEUS OF THE THALAMUS

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NMDA-type glutamate receptors are involved in the generation and maintenance of altered pain states. In the present study, we examined the effect of an NMDA-glycine site antagonist, GV196771A [E-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-ylidenemethyl)-1H-indole-2-carboxylic acid sodium salt], on responses to noxious stimuli both in normal rats and during peripheral mononeuropathy induced by chronic constriction injury (CCI) of the sciatic nerve. In one series of experiments, activity of nociceptive neurons in the ventroposterolateral (VPL) nucleus of the thalamus was recorded in response to pressure stimuli to the contralateral hindpaw. Intravenous injection (iv) of the glycine antagonist had no effect on these cells in normal rats. When tested in rats with CCI induced 2-3 weeks previously, however, GV196771A (0.125, 0.5 and 2.0 mg/kg) blocked noxious stimulation in a dose-dependent and reversible manner. Morphine (0.5 mg/kg, iv) and the NMDA channel blocker MK801 (0.1 mg/kg, iv) suppressed noxious stimulus-evoked activity of VPL neurons in both normal and CCI-treated rats. MK801 also decreased the responses of non-nociceptive neurons to brush stimulation in both sets of animals, in contrast to the glycine antagonist which did not alter the responses of these cells. Similar results were obtained from a series of behavior experiments in which the latency for paw withdrawal from heat stimulation was measured in normal and CCI-treated rats. GV196771A (3 and 10 mg/kg) injected orally, reduced the hyperalgesic response in the treated rats but did not change the withdrawal latency in normal rats. Taken together, these findings suggest that the NMDA receptor-channel complex decreases nociceptive neurotransmission at the level of the thalamus and can modulate hyperalgesic states. GV196771A and glycine antagonists in general may represent innovative and safe agents for the treatment of neuropathic pain.

SCOPOLAMINE AND DIAZEPAM AMNESIA AS A RESULT OF THESE DRUGS STIMULUS PROPERTIES

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Scopolamine (Sc) and diazepam (Dz) are ordinar amnestic agents. The corresponding memory impairments are explained by cholinergic and GABA/benzodiazepine alters. From the other hand this drugs have stimulus properties and lead to dissociation in learning. The aim of this study was to consider the role of state dependent learning in postscopolamine or postdiazepam amnesia in passive and active avoidance paradigms. Male white rats were trained in such very popular models as step-through passive avoidance and two-way active avoidance tasks. Passive avoidance retention was tested 24 h after training, retired recalling of active avoidance was tested in 44 days after the acquisition. Passive avoidance retention was disturbed if Sc (0,5 mg/kg, i.p.) or Dz (6 mg/kg, i.p.) had been administered 30 min before training. Sc treatment 30 min before testing broke the skill too. However double administration of these drugs before training and testing did not affect the retention. Sc administration 15 min before every trial during active avoidance training did not prevent acquisition but the retired retention of this conditioned response was defected if Sc had not been administered before it. From the obtained data it is concluded that Sc- and Dz-indused amnesia is a result of state dependent learning but not of consolidation or memory trace storage deficit. The state dependent learning can be a general mechanism in amnestic processes. The same brain state in training and in retention is necessary for memory trace expression according to concept of B.E. Kotliar.

PROCESSING-LEVEL HIERARCHY WITHIN FRONTAL AND TEMPORAL LOBES DURING VISUAL DISCRIMINATION TASK - DEPTH ERP STUDY

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Visual event-related potentials were simultaneously recorded from different anatomical structures within frontal and temporal lobes in twelve epileptic patients. A simple discrimination task was performed to complement previous studies on the localization of P3 generators in the human brain. The role of multiple cortical structures in the generation of both P3a and P3b components was confirmed. Activities contemporary to a visual P3b were recorded in the hippocampus, amygdala and temporal pole. Anterior cingulate and orbito-frontal cortices generated activities that were closely related in time to the surface P3a. Earlier events related to visual discrimination took place in more lateral sites of the frontal lobe, but their contribution to the scalp P3 remains uncertain. Subsequently, mutual temporal relations among single generators were analyzed. The results suggested a processing-level hierarchy within the neural network for directed attention with the key role played by the dorsolateral prefrontal cortex.

IDENTIFICATION OF COMPLEX TWO-DIMENSIONAL IMAGES IN SINGLE VISUAL CORTEX CELLS OF THE CAT.

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A previous evidence is given that area 18 in cats (De Weerd et al., *Eur J Neurosci.*, 5: 1695 [1993]) and V2 in monkeys (von der Heydt and Peterhans, *J. Neurosci.*, 9: 1731 [1989]) represent the first level of the contour abstraction process. Thus, we have studied the ability of single cells in area 18 of cats to "identify" animate objects in their receptive field. The responses of 27 cells were examined to visual patterns: 1) cat's body contours (the whole contour and 3 levels of degradation), 2) scrambled cat's contours, 3) inverted cat's body contours (closed contours, same quantity of lines) and 4) ellipses. The various patterns were presented monocularly and repeatedly to the same eye within the cells' receptive fields. Then, the same patterns were presented binocularly: 1) the same patterns to the two eyes, 2) rivalrous patterns, and 3) fusible patterns. The results showed: 1) the whole cat's contour monocularly presented elicited more intense response than scrambled contours (W-paired test; $p<0.01$), but no difference was found when presented binocularly ($p=0.61$). Contrary, monocular responses to the whole cat's contour and ellipse did not differ ($p=0.72$), but the response to ellipses was higher in binocular presentation ($p<0.01$). 2) The responses to the whole cat's contours were much more intense than to the degraded contours ($p<0.01$). 3) Responses to different inverted cat's contours were similar ($p=0.20$). 4) The responses to binocular presentation of the whole cat's contours (identical patterns) were similar to responses to the scrambled contours ($p=0.61$) and fusible patterns ($p=0.18$), but were much stronger than to rivalrous patterns (the whole cat's contour to one eye and scrambled contours to the other) ($p<0.05$). We thus conclude that coding of complex contours occurs already in visual cortex area 18 of the cat. Apparently, different mechanisms are involved in processing of the monocularly and binocularly presented stimuli. During monocular presentation cells react as merely "edge detectors". During binocular presentation (whether same, fusible or rivalrous stimuli) a different mechanism of whole contour analysis is engaged, which may involve cognitive brain processes.

DEOXYGLUCOSE-UPTAKE IN THE CORTICAL WHISKER REPRESENTATION OF BARREL-LACKING MICE (*brl*, Tg8).

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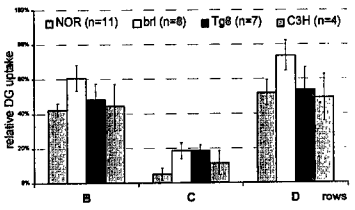

Sensory information from the mystacial whisker follicles in mice is processed in a part of the somatosensory cortex that contains, at the level of layer IV, cytoarchitectonic units named «barrels». In several mutant mouse strains barrels fail to form. We investigated levels of ^{14}C-deoxyglucose (DG) uptake within the cortical whisker representation in two such strains, i.e. *brl* - a mouse lacking functional adenylyl-cyclase I (*Welker et al., 1996, Science 271:1864; Abdel-Majid et al., 1998, Nat.Gen. 19:289*) and Tg8 – which lacks monoamine oxidase A (*Cases et al., 1996, Neuron 16:297*). DG-uptake was also determined in the strains that form the genetic background of these mutants, i.e. NOR (for *brl*) and C3H (for Tg8). All whiskers in adult animals were clipped unilaterally, except for the three posterior-most whiskers of rows B and D. After DG injection (0.165µCi/gr b.w.) mice explored a large, object-filled cage for a period of 45 minutes. Then, they were deeply anaesthetised, perfused and their hemispheres oriented for tangential sectioning of the somatosensory cortex. Autoradiography revealed that the size, orientation and spacing of the cortical representations of the spared whiskers of rows B and D is identical in the four strains. The level of stimulus-dependent DG-uptake expressed relative to a non-activated part of the somatosensory cortex is also identical, except for *brl* mice in which it is about 20% higher (see values for B and D in the graph). The level of DG-uptake in the representation of the clipped whiskers of row C is higher in the barrel-lacking strains than in their controls. In *brl* mice, the ratio between the metabolic activity of the cortical representations of spared and clipped whiskers remains, in this barrel-lacking strain, as high as in barrel-rich controls. In contrast, the ratio is smaller in Tg8 mice. In conclusion, the topological organisation of the whisker representation in barrel-rich and barrel-lacking strains is similar; however, sensory discrimination based on the difference in metabolic activity between the cortical representation of stimulated and non-stimulated whiskers seems to be specifically impaired in Tg8 mice. Supported by the European Community grant BMH4 CT97 2412.

NOREPINEPHRINE CHANGES THE TIME COURSE OF THE EVOKED QUANTAL RELEASE AT FROG NEUROMUSCULAR JUNCTIONS VIA ADENYLYL CYCLASE ACTIVATION.

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Recently we showed that norepinephrine (NE) shortened the period over which evoked quanta release at frog neuromuscular junction. NE changed the histogram of the real synaptic delays distribution: the number of evoked uni-quantal endplate currents (EPCs) with longer synaptic latencies was decreased as was the histogram modal value. It was concluded that NE synchronizes the evoked quantal secretion from nerve terminal. Pharmacological analysis showed that presynaptic beta1-adrenoceptors mediated NE action on the time course of the evoked quantal secretion. In the present investigation the hypothesis that NE acts on the secretion time course via the change in adenylyl cyclase activity was tested. The fluctuations in latencies of nerve evoked uni-quantal EPCs were recorded with extracellular pipettes together with nerve spikes from superficial fibres of the cutaneus pectoris muscle of the frog Rana ridibunda. In 0.2 mM Ca2+ and 4.0 mM Mg2+ Ringer solution NE (1*10-5 M) shortened the secretion time course. The effect of NE was mimicked by the dibutyryl- cAMP. The adenylyl cyclase activator forskoline and the phosphodiesterase inhibitors 3-isobutyl-l-methylxantine and teophilline decreased the synaptic latencies fluctuations as well. Addition of NE to the superfusion solution containing forskoline, dibutyryl-cAMP or teophilline failed to synchronize the secretion by NE. It was suggested that the effect of NE on the secretion time course may be due to adenylyl cyclase activation. Abstract for poster

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TYROSINE HYDROXYLASE IS REDUCED IN THE NUCLEUS ACCUBENS THREE DAYS FOLLOWING PROPOFOL INFUSION

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Propofol is an intravenous anaesthetic used for the induction and maintenance of general anesthesia. It's non-anesthetic actions include cardiovascular depression, antiemetic and neuroexcitatory effects, as well as behavioral changes, which may be due to effects on specific neurotransmitter systems. Immunohistochemical studies indicate a significant decrease in tyrosine hydroxylase (TH) in the nucleus accumbens (Acb) three days following a six hour, sub-anaesthetic, propofol infusion. Rats receiving propofol exhibit a decrease in amphetamine stimulated rearing, a behavior mediated by dopamine release in the Acb. We used Western Blot to quantify TH levels in male Wistar rats infused with propofol or Intralipid® (control). Three days post-infusion the animals were decapitated, the brains removed and frozen. Micropunches of neural tissue were obtained from the Acb, caudate putamen, ventral tegmental area, the lateral frontal, medial prefrontal and insular cortices. Proteins were separated using SDS-PAGE, electrophoretically transfered onto nitrocellulose paper and TH localized using immunochemistry with a chemiluminescent substrate. Densitometry was used to quantify TH. The results indicate a 46% ($p < 0.05$) reduction in TH levels in the Acb of animals receiving propofol anesthesia compared to the Intralipid® group. No change was observed in the other regions. A reduction in TH in the Acb suggests a decrease in the activation of the dopaminergic system consistant with the behavioral results. Overactivity of the dopaminergic system in the mesolimbic system is believed to be involved in the manifestation of schizophrenic symptoms. A reduction in TH provides evidence that propofol may be an effective anti-psychotic agent.

IDENTIFICATION AND LOCALIZATION OF A SECOND ISOFORM OF THE NEUROPEPTIDE GONADOTROPIN-RELEASING HORMONE IN THE BRAIN OF HUMAN AND RODENTS

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Gonadotropin releasing hormone-I (GnRH-I), (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), originally isolated from the mammalian hypothalamus, is the neurohormone that regulates the reproductive functions. Today, a dozen isoforms of GnRH are known in vertebrates, all are decapeptides that are conserved by 90-50% as compared to GnRH-I. In general, at least two different isoforms of GnRH are expressed in the brain of each of the lower vertebrate species. One isoform, [His^5, Trp^7, Tyr^8] GnRH-I, named GnRH-II, is expressed in almost all vertebrate classes . The form of GnRH which varies across vertebrate classes, is localized mainly in the hypothalamus and is responsible for the regulation of reproduction, whereas GnRH-II is localized mainly in extrahypothalamic brain areas, mostly in the midbrain. Thus GnRH-II is an ancient isoform of GnRH and is structurally conserved for over 500 million years of evolution, suggesting that its neuronal functions are of utmost importance. In this study we demonstrate, for the first time, that GnRH-II is present in the brain of the mouse, rat and human. Human and rat brain extracts contain two isoforms of GnRH, GnRH-I and GnRH-II, which exhibited identical chromatographic properties to the respective synthetic peptides, in high performance liquid chromatography. Using immunohistochemical techniques we have found that GnRH-II is present in neuronal cells that are localized mainly in the periaqueductal area as well as in the oculomotor and red nuclei of the midbrain. In addition, substantial concentrations of GnRH-II are also present in the human hypothalamus and stored in the pituitary stalk. It is of interest to note that in the hypogonadal mouse although the GnRH-I gene is deleted, GnRH-II is present. By using RT-PCR we have also found that while GnRH-II is not expressed in the human cerebellum, it is expressed in all three structures of the brain stem: midbrain, pons and medulla oblongata. Confocal microscopy and double fluorescence labeling have indicated that GnRH-II and GnRH-I are co-localized in the same neuronal fibers in the median eminence of the mouse. These results therefore imply that, in the hypothalamus, GnRH-II is produced by the same neurons that produce GnRH-I. We suggest that GnRH-II may have dual functions: as a neurohormone at the hypothalamic-pituitary gland axis and as neurotransmitter or as a neurotrophic factor in the brainsteam.

QUINOLINIC ACID LESION INDUCES CHANGES IN RAT STRIATAL GLUTATHIONE-RELATED ENZYMES.

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Although the involvement of oxidative mechanisms in the cytotoxicity of excitatory amino acids has been well documented it is not known whether the intrastriatal injection of quinolinic acid (QA) induces changes in glutathione-related enzymes. In this work, the activities of the enzymes glutathione reductase (GRD), glutathione peroxidase (GPX), and glutathione S-transferase (GST) were studied in the striatum, hippocampus, and frontal cortex of rats 1 and 6 weeks following the intrastriatal injection of QA (225 nmol). One group of animals remained untreated. This lesion resulted in a 20 % decreased striatal GRD activity at both the 1- and 6-week postlesion times, while GST suffered a 30 % activity increment in the lesioned striatum observable only 6 weeks after the lesion. GPX activity remained unchanged. Enzyme activities from other areas outside the lesioned striatum were not affected. GST activation could represent a beneficial compensatory response to neutralize some of the oxidant agents generated by the lesion. However, this effect together with the reduction in GRD activity might enhance the reported QA-induced deficit in glutahione availability and, consequently, further disrupt the oxidant homeostasis of the injured striatal tissue. Therefore, these results provide evidences that the in vivo excitotoxic injury to the brain might affect oxidant/antioxidant equilibrium by eliciting changes in glutahione-related enzymes.

EFFECTS OF NERVE GROWTH FACTOR ON GLUTATHIONE-RELATED ENZYMES IN RAT MODELS OF BASAL FOREBRAIN DEGENERATION.

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Modulating effects on antioxidant defenses have been postulated to underlie neurotrophic influence on neuron survival and maintenance. However, the in vivo influences of nerve growth factor (NGF) on glutathione system in rat models of basal forebrain degeneration are not well documented. To test whether NGF is capable of inducing changes in glutathione-related enzymes, glutathione reductase (GRD), glutathione S-transferase (GST) and glutathione peroxidase (GPX) activities were measured in brain areas from rats suffering age-related cognitive impairment or septohippocampal pathway transectio n following NGF or placebo (cytochrome c) administration. The aged cognitively impaired rats showed increases in the activities of septal and hippocampal GST, as well as, in the hippocampal, striatal and cortical GPX. On the other hand, the lesion resulted in a decreased hippocampal GRD and septal GST activities, as well as, in an increase in GPX activity from frontal cortex, striatum, and septum. The enzyme activity increases could be interpreted as compensatory responses to cope with the oxidative damage that has been accumulated by the aged or lesioned brain. The changes in GPX and GRD activities were attenuated or abolished by NGF treatment, a fact possibly linked to the known neuroprotective action of this neurotrophin. These results point out GRD and GPX as possible targets of the neurotrophic effects.

„CONIOTOMY OF THE BRAIN" (STEROID THERAPY END „VASCULAR TUNNEL"CREATION IN ACUTE TREATMENT AT SEVERE BRAIN INJURY)

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Abstract: In case of spinal chord injury the steroid-therapy is obligatory, but it's effect in cerebral injuries has not been verificated as yet, morcover this way of treatment is not recommended. The anatomical borderline between the brain and the spinal chord is at the level of C 0. Structural changes begin in the diencephalic region, and from here on becomes the CNS more and more abounding in cells. Effect of steroid therapy may be partially based on this anatomical situation. Severe traumatic cerebral edema may result brainstem herniation, and damages of the cerebral trunk. For the reduction of ICP I propose a large-sized, bilateral craniectomy and durotomy as well . By the special incision of dura a hexagonal shaped aperture is created and the brain protude into it. The polygon (hexagon) should be shaped on the way, that the main arteries and veins entering the herniated area, be situated at the bisecting point of the sides of the hexagon, where the dura is stretched as a roof of a tent on the surface of the brain. In this way a vascular tunnel is created at the middle of the sides of the hexagon, where the tension caused by shear and pressure to the tissue of the brain is lower then at the corners. This is a reason, why there are less chance for vessels occlusion .The mortality of patients can be considerably reduced with this two new methods. I would like to demonstrate our early experiences based on 20 cases.

FUNCTIONAL ANALYSIS OF PATHOLOGICAL MUTATIONS IN THE NEURAL CELL ADHESION MOLECULE L1.

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L1 is one of a host of neural cell adhesion molecules which are important components of the network of guidance cues and receptors that influence axon growth and guidance during development. Mutations in the L1 gene are responsible for a clinically variable X-linked neurological disorder. These mutations provide the foundation for studies aimed at relating the nature of the mutation to the patient phenotype. Over one third of the mutations described are missense, highlighting important residues for L1 homophilic and heterophilic interactions. Mutations distributed throughout the extracellular surface (ECD) of the protein, can alter residues which either affect the structural integrity of the domain or the surface properties. We have engineered L1-ECD disease-causing missense mutations into different vectors for expression in mammalian cells. Expression of mutated L1 as a soluble fusion protein allowed us to conduct ligand binding assays. The results show that several, but not all, of the mutations located throughout the ECD reduce homophilic binding. This suggests the patient pathology is due to several types of L1 dysfunction. The patient pathology indicates a more severe phenotype is evident when mutations affect key structural residues. In contrast, surface mutations are more likely to result in a less severe phenotype.

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GANGLIOSIDE a/b RATIO IN THE DIFFERENT RAT BRAIN REGIONS AFTER CHRONIC DIAZEPAM TREATMENT

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The main purpose of this study was to determine the a/b ratio of gangliosides in the different rat brain regions after prolonged diazepam treatment. Male Wistar rats were maintained on a nutritionally adequate diet and diazepam was administered in a dose of 10 mg/kg/day. Control animals were pair-fed an adequate diet. Feeding was continued for 180 days. Total gangliosides were extracted according to Harth et al. (1978) and total ganglioside-NeuAc (N-acetylneuraminic acid) was determined by Svennerholm's resorcinol method (1957), modified by Miettinen and Takki-Luukkainen (1959). The effects on a/b ganglioside ratio in rat cerebral cortex, hypothalamus, nucleus caudatus, hippocampus, thalamus and cerebellum were studied. It was found that the ratio remained unchanged in rat thalamus (0.75 in control vs. 0.72 in DZP group), hypothalamus (0.47 in both experimental and control group) and cerebellum (0.74 in control vs. 0.79 in diazepam-treated group). It was slightly decreased in nucleus caudatus (1.23 in control vs. 1.02 in experimental group) and hippocampus (1.11 vs. 0.90), but it was not statistically significant. The drastic decrease ($p<0.01$) was found in rat cerebral cortex (1.28 in control vs. 0.42 in DZP group). The alterations in ganglioside pattern differ in distinct brain regions (strong dominance of the b-pathway gangliosides in cerebral cortex and slight increase in b-series in nucleus caudatus and hippocampus). Our results suggest that altered a/b ratio of brain gangliosides may reflect the adaptive changes that occur upon prolonged exposure to diazepam.

A PET INVESTIGATION OF THE NEURAL SUBSTRATE INVOLVED IN THE ATTRIBUTION OF INTENTION TO OTHERS

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Several authors have argued that theory of mind, i.e. the capacity to infer intention, desire or belief to others, is associated with a cortical pattern of activity involving the left medial prefrontal cortex. Our study was designed to identify the neural regions activated during the attribution of intentions to others using non-verbal material. PET measurements of rCBF with the $H_2{}^{15}O$ method were performed in eight volunteers during three activation conditions and one base-line condition :
- Attribution of intention (AI).
- Physical causality with characters (PC).
- Physical causality with objects (PO).

In all conditions, comic strips were visually presented and subjects had to select the logical end of each story.

AI versus PC was associated with rCBF increases located in the middle and medial prefrontal cortex (BA 9), in the inferior prefrontal cortex, in the inferior temporal gyrus in the right hemisphere, in the left superior temporal gyrus, left cerebellum, bilateral ly in the anterior cingulate and middle temporal gyri. **PC** versus PO showed the activation of lingual and fusiform gyri, middle and superior temporal gyri on in both sides.
Attribution of intentions to others is thus associated with a complex neural network involving right medial prefrontal cortex when a non-verbal task is used. The lateralization of this function may be modulated by the nature of the sensorial input.

POSTNATAL MATURATION OF THE ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF RAT HYPOTHALAMO-NEUROHYPOPHYSIAL NEURONES.

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Magnocellular neurones projecting to the neurohypophysis differentiate into oxytocin (OT) and vasopressin (AVP) secreting neurones during prenatal life. However, this differentiation is not complete since each class of neurones continues to express small amounts of the mRNA coding for the other hormone in adulthood. In addition, a subset of neurones expresses both hormones and their proportion varies according to physiological conditions. Thus, the real differentiation appears to be functional and probably occurs at the time when the neurones get connected to specific networks. In order to elucidate the extrinsic or intrinsic factors participating in this differentiation, we analyse the postnatal development of electrophysiological and pharmacological properties of magnocellular neurones from the supraoptic nucleus (SON).

Patch-clamp recordings performed in horizontal slices from the ventral hypothalamus indicate that rat SON neurones can fire action potentials from the day of birth. The resting potential is initially very unstable and we often observe the occurrence of a spontaneous and irregular activity. During the two subsequent weeks, a stabilisation of the resting potential and a decrease of the incidence of an inwardly rectifying current (Ih) tend to decrease the occurrence of this erratic activity, which is thereafter replaced by the typical activity of adult SON neurones: phasic for AVP neurones and tonic for OT neurones.

Both OT and AVP are known to be released at somato-dendritic sites and exert an autocontrol on their respective secreting cells in adults. The question then arises whether they can also control the functional differentiation of SON neurones. During the three postnatal weeks studied, we observed that most SON neurones respond to either OT or AVP (rarely to both) by a depolarisation and an increased activity. This raises the possibility that each peptide participates in the differentiation of the neurones that secrete it, by favouring synchronous activity in neurones with the same phenotype.

ANTIPROLIFERATIVE EFFECT OF GnRH AGONIST ON HUMAN BREAST CANCER CELLS

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GnRH analogs are commonly used as adjunctive therapy in the treatment of breast cancer patients by means of pituitary-gonadal down regulation and desensitization that leads to reduced steroidogenesis. Studies have suggested that GnRH agonists may have a direct inhibitory effect on human breast cancer cells. Our study is designed to confirm the direct effect of the GnRH agonists on MCF-7 cells using Lupron (GnRH analog). The data obtained clearly demonstrated the antiproliferative effect of Lupron on ER positive human breast cancer cells (MCF-7) but not on ER-negative human breast cancer cells . Moreover, the effect of Lupron on MCF-7 cells are reversible after Lupron was removed. The antiproliferative effect of Lupron is dose dependent. Flow cytometry analysis showed that the MCF-7r cells without Lupron grew approximately 43% in G1/G0 phase, 33% in S phase and 24% in G2 and M phase. However, the cells treated with Lupron grew about 73% in G1/G0 phase, 7% in S phase, and 20% in G2 and M phase. This result demonstrates a great decrease of cell numbers in S phase, suggesting that Lupron inhibits tumor proliferation by preventing the cell from entering S phase and thus the cell is unable to divide. A significant decrease of cyclin D1 in the Lupron treated MCF-7 cell was observed. Moreover the inhibition of cyclin D1 gene expression was increased following increased treatment time. In contrast, there was no great change for cyclin B within these groups. This result indicates that Lupron specifically inhibits cyclin D1 gene expression in Lupron treated human breast cancer cells.

ACETYLCHOLINE-DEPENDENT EXPRESSION OF FUNCTIONAL PLASTICITY IN THE BARREL CORTEX OF THE RAT

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Peripheral manipulations of vibrissal activity induce a reorganization of the barrel cortex that depends on the integrity of the cholinergic innervation. While the role of acetylcholine (ACh) in the induction of cortical plasticity has been studied extensively, little is know about the role of ACh in the expression of these functional changes. We found a novel type of plasticity, in which both the induction and expression depend on ACh, in the barrel cortex of the anesthetized adult rat. We recorded units extracellularly using combined electrodes, which are composed of a tungsten core surrounded by six glass pipettes for iontophoretic applications. Temporal-frequency tuning curves were obtained by mechanically stimulating the principal whisker at different frequencies (2 to 14 Hz), first without and then with ACh. During conditioning, stimulation at a single frequency was paired with iontophoresis of ACh. Following conditioning, the tuning curves were re-tested, with and without ACh application. Pairing resulted in a significant frequency-specific modification of the response in 29% of the cells when tested without ACh (11/38, of which 73% were depressions). On a different population (42%) of the same neurons, the tuning curve modification was revealed only when tested in the presence of ACh (16/38, of which 63% were potentiations). These functional changes are associative, long-lasting (up to 45 min), input-specific, and reversed by a second pairing at a different frequency. The ACh-dependent expression of this plasticity may represent a cellular analog of state-dependent learning, a phenomenon in which the retrieval of newly-acquired information is possible only if the animal is in the same behavioral context and physiological state as during the encoding phase.

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EFFECTS OF HEMISPHERECTOMY ON IMMOBILITY TIME IN THE ROTATIONAL SWIMMING BEHAVIOR OF SWISS MICE

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Previously we reported that sex difference in immobility time on the rotatory swimming test (RSWT) depends on laterality. Here, we investigated sex-differences in immobility time during RSWT of unilateral hemispherectomized adult mice. The hemispherectomized group consisted of 25 adult mice. Fourteen animals received a sham operation. After 15 days of recovery from the surgical procedures, each mouse was placed in a container (diameter = 21cm) filled with water for 5 min on 3 different days (test-retest time interval = 48h). Vigorous movements from 30° to 30° were counted and consistency of laterality was defined considering the persistence of the same preferred turning side in three sessions. The time that the animals remained immobile was measured for each session. After the behavioral tests, the animals were anesthetized and perfused. The brains were coronally cut and stained with cresyl-violet. In all animals, the forebrains were entirely eliminated. In the hemispherectomized group 90% of the animals were classified as side-consistent rotators whereas in the control group only 50% were side-consistent rotators. Hemispherectomized males exhibited a significant higher immobility time than hemispherectomized females. Moreover, this sex-difference is greater than that exhibited for side-consistent normal animals. Therefore, hemispherectomy seems to exacerbate sex-differences.

WHISKING FREQUENCIES AND TEMPORAL TUNING IN THE CORTEX OF NORMAL AND Atm DEFICIENT MICE

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Unlike with the rat, the whisking properties of mice have not been characterized as yet. By using a fast camera (Fujix HR Deltaron 1700), we determined that the whisking frequencies in mice are in the range of 14 - 20 Hz (mean of modal frequencies 15.7 ± 1.5 Hz, n = 9), roughly twice as fast as those of rats. We recently found that Atm mice have a massive reduction in the number of mesencephalic dopamine (DA) containing neurons, reduced projections to the basal ganglia, and altered levels of DA receptors in their barrel cortex. We compared the frequency of whisking in normal mice with those of Atm deficient mice, which are known to have motor deficits. The Atm deficient mice exhibited a significantly lower whisking frequency (range of 10 - 16 Hz ; mean of modal frequencies 11.9 ± 2.5 Hz, n = 5), than wild-type mice. Treatment with L-dopa increased the whisking frequency in the Atm mice to 16 – 20 Hz (mean of modal frequencies 17.3 ± 2.5 Hz; n = 5). In rats, the 'paralemniscal' pathway is tuned to process temporal whisker information within the rat's whisking frequency range (5 – 11 Hz; see Sosnik et. al. and Ahissar et. Al., this meeting). Whether neurons in the barrel cortex of mice are similarly tuned to their whisking frequencies was examined by applying frequency modulated (FM) whisker stimulations (3 to 24 Hz) to rats and mice and examining the resulting cortical representations. FM vibrissal signals are represented by both latency and spike counts of cortical responses. The frequency ranges in which those representations form inverted monotonic functions of the whisker frequency are potential working ranges for thalamocortical temporal decoding of vibrissal information (see Ahissar et. al., this meeting). Preliminary results (21 multi-unit recordings from the mouse and 30 multi-unit recordings from the rat) suggest that mouse cortical neurons are indeed tuned for frequencies that are significantly higher than those for which rat cortical neurons are tuned (latency of 7 - 16 vs. 5 – 10 Hz; spike count of 12 – 20 vs. 4 – 15 spikes/cycle, respectively). These preliminary results suggest that in both the rat and mouse sensory neurons are tuned for optimal decoding in the frequencies generated by whisking.

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PRESYNAPTICALLY LOCATED CB1 CANNABINOID RECEPTORS REGULATE GABA RELEASE FROM AXON TERMINALS OF SPECIFIC HIPPOCAMPAL INTERNEURONS

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To understand the functional significance and mechanisms of action in the CNS of endogenous and exogenous cannabinoids, it is crucial to identify the neural elements, which serve as the structural substrate of these actions. We used a recently developed antibody against the CB1 cannabinoid receptor to study this question in hippocampal networks. Interneurons with features typical of basket cells showed a selective, intense staining for CB1 in all hippocampal subfields and layers. Most of them (85.6%) contained cholecystokinin (CCK), which corresponded to 96.9% of all CCK-positive interneurons, whereas only 4.6% of the parvalbumin (PV)-containing basket cells expressed CB1. Accordingly, electron microscopy revealed that CB1-immunoreactive axon terminals of CCK-containing basket cells surrounded the somata and proximal dendrites of pyramidal neurons, whereas PV-positive basket cell terminals in similar locations were negative for CB1. The synthetic cannabinoid agonist WIN 55,212-2 (0.1-1 M) reduced dose-dependently the electrical field stimulation-induced [^{3}H]GABA release from superfused hippocampal slices. The CB1 cannabinoid receptor antagonist SR 141716A (1 M) prevented this effect, whereas by itself it did not change the outflow of [^{3}H]GABA. These results suggest that cannabinoid-mediated modulation of hippocampal interneuron networks operate largely via presynaptic receptors on CCK-immunoreactive basket cell terminals. Reduction of GABA release from these terminals is the likely mechanism by which both endo- and exogenous CB1 ligands interfere with hippocampal network oscillations and associated cognitive functions.

MARIJUANA, ANANDAMIDE AND OLEAMIDE: HIGH OR SLEEP?

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Since the discovery of oleamide as an endogenous sleep-inducing agent, controversy has arisen on oleamide's mode of action. We have suggested previously that anandamide may play a role in sleep since oleamide elevated concentrations of the endogenous cannabinoid receptor (CB1) ligand, anandamide (Mechoulam et al., Nature 389,15,1997), while others have suggested that oleamide promotes sleep by interacting with serotonin (5-HT2) receptors (Huidobro-Tobro and Harris, PNAS 93, 8078,1996; Boger et al.,PNAS 95, 4102,1998). In the typical assay for cannabinoid-like activity, ketanserin (a 5-HT2 receptor antagonist) exerted similar effects compared to THC (the major psychactive compound in marijuana) or anandamide, but did not bind to CB1 receptors (Fride etal., ICRS symposium, 1998). In the present study, we attemped to resolve (1) whether anandamide and oleamide display similar effects when sleep behavior is studied and (2) whether anandamide and oleamide exert effects typical of CB1 receptor activation and /or of 5-HT2 receptor interaction. Female Sabra mice were subjected to detailed behavioral observations after administration of oleamide, anandamide, THC, ketanserin or αMethyl-5-HT (a 5-HT2 agonist). Results indicated that ketanserin, but not αMethyl-5-HT -injected mice, displayed sedation which was indistinguishable from sleep in uninjected mice. THC produced quiescence, which was very different from normal sleep. Oleamide and anandamide produced sedation which included aspects of both ketanserin- and THC-like effects. We conclude from these results that from the compounds studied, only ketanserin produces 'true' sleep, similar to normal sleep. Further, oleamide's effects are similar to those of anandamide, but different from sleep, with properties of both ketanserin and THC. We propose that oleamide and anandamide have similar modes of action which involve interaction with both 5-HT2 and CB1 receptors.

PICTURE PERCEPTION AND THE EMOTIONAL STATE ON PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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The present experiment assesses the hypothesis that the emotional experience, especially those which are tightly coupled on motor functions e.g. flight-fight-responses, are reduced in patients with ALS (Amyotrophic Lateral Sclerosis).
Our patients (eight men) view 64 pictures from the International Affective Picture System (IAPS) (Center for the Study of Emotions and Attention, 1994) depicting in 8 scenes from 8 affective categories: erotic couples (1), babies, happy children and families (2), neutral people (3), household objects (4), attack (5), mutilations (6), food (7) and sports(8). The experiment is divided into three parts: During the first two shows, EEG, EMG, heart rate and skin conductance is recorded. In a third show the patients rate each picture on the dimensions of valence, arousal, dominance and movement association.
Results from subjective exhibited a general higher sensibility in comparison to healthy subjects, i.e. neutral pictures were rated more pleasant and negative pictures lead to higher levels of arousal.
A strong correlation between arousal and heart rate acceleration as measured for healthy people was found, but no correlation found between skin conductance and arousal and between facial muscle responses and valence.
The most pronounced ERP components P100, P400 show a more positive ERP for pleasant pictures than for negative or neutral pictures, whereas the positive slow wave at 800ms reveals an arousal effect e.g. pleasant and unpleasant pictures were associated with a more positive ERP than neutral ones.
Therefore parts of the results contradict our hypotheses, but we found also some correspondence between the motor disturbance and the emotional response on affective pictures.

DISTINCT LAMINA I CELL TYPES OF THE RAT SPINAL CORD HAVE PARTICULAR MEMBRANE PROPERTIES

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Lamina I neurons of the spinal cord dorsal horn belong in four morphological classes. Electrophysiological recordings failed to disclose the functional meaning of such heterogeneity, since several kinds of peripheral stimuli activated cells of the same morphological type. This study attempts to elucidate whether this diversity correlates with specific firing patterns.

Whole-cell recordings were performed in 150 µm thick spinal cord slices of young rats (10-19 days). The response of lamina I cells to 20 mV step current injections in the soma was used to characterize their firing pattern. Recorded cells were stained with Lucifer Yellow (0.3%) or Biocytin (2%) added to the recording pipette solution.

Thirty-two lamina I cells were fully characterized as to their morphology and responses to current injection. Fusiform cells showed no adaptation, and their trains of spikes presented fast after-hyperpolarizations (AHP). Pyramidal cells also responded with no adaptation, but their spike trains presented very slow AHP's. Both flattened and multipolar cells showed great adaptation, with most of the multipolar cells responding with only one spike, and the flattened cells firing with spike trains to weak current injections but adapting very quickly to higher step depolarizations. The calcium-dependent AHP effects were only seen in older animals (aged over 15 days).

These results show that different ionic channels or different channel densities are characteristic of each morphological type of lamina I cell, therefore suggesting that their physiological distinctiveness resides on the way they process sensorial information rather than on the kind of input they receive. Detailed knowledge of the local networks that these cells establish will be crucial for the understanding of pain modulation at the spinal cord.

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UTILIZATION OF TEMPORAL AND SPATIAL CUES BY THE HUMAN TACTILE SYSTEM

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When textured surfaces are scanned by human fingers, the nervous system is provided with information encoded in both time and space. The temporal coding is based on changes in receptor activation over time, whereas the spatial coding is based on differential activation of adjacent receptors. A two-alternative forced-choice tactile discrimination task was designed to test whether the human somatosensory system prefers one coding over the other. Ten subjects (Ss) were asked to scan two metal gratings, each with a different hand, and to report which grating was more dense. Utilization of spatial and temporal cues was tested by elimination. In order to eliminate temporal cues, finger motion was limited to vertical (parallel to the bars' orientation) motion. To eliminate spatial cues, the Ss scanned the gratings with gloves to which a single pin was attached. The spatial frequencies of the gratings were between 200 to 800 bars/m. Analysis of the success rates and finger velocities revealed a clear separation of the Ss into two groups of 5 Ss each. Group 1 exhibited better performance with the glove than during normal scanning, whereas Group 2 exhibited the opposite. For both groups, vertical scanning impaired performance. The improved performance with gloves of Group 1 was associated with changing motion profiles and scanning velocities, which is indicative of an active temporal mechanism that was probably masked during normal scanning. Group 1 changed from simultaneous motion by both hands during normal scanning to alternating (each hand separately) scanning while using the gloves, and they reduced scanning velocities to produce receptor activation frequencies around 30 Hz, which are optimal for decoding by comparison to somatosensory cortical oscillations. Group 2 produced frequencies close to 30 Hz with both normal and glove scanning. With Group 1, both hands produced the same temporal frequency while scanning with the glove. For Group 2, similar frequencies were produced by both hands in the normal scanning. For both groups, utilization of the same frequency by both hands was correlated with better performance. We conclude that (i) temporal cues are essential for tactile perception, (ii) human subjects execute different strategies of texture exploration that can activate spatio-temporal (Group 2) and hidden-temporal (Group 1) mechanisms, and (iii) both hemispheres should optimally be provided with similar temporal information for this task.

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IMIDACLOPRIDE-INDUCED MODIFICATIONS OF NERVOUS FUNCTIONS AND OF BRAIN CYTOCCHROME OXIDASE ACTIVITY IN THE HONEYBEE

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We have previously shown that nicotinic antagonists injected in the honeybee brain induced an impairment of olfactory conditioning. The insecticide imidaclopride could act as a nicotinic agonist and hence, modify learning abilities to subletal doses.First, we cheked the ability of imidaclopride to alter sensorimotor functions and its effects on a non-associative learning. Then we specified the cellular brain targets of imidaclopride using the histochemistry of cytochrome oxidase (CO).
Response threshold to antennal sucrose stilmulation was enhanced as soon as 15 mn after a topic application of imidaclopride to the doses of 5,10, and 20 ng/ bee. Motor activity evaluated in an open-field-like apparatus decresead significantly from 30mn to 2h after imidaclopride application in the same conditions. To the dose of 2.5 ng/bee imidaclopride induced a faster habituation of the proboscis extension reflex.
CO histochemistry was evaluated 30 mn after an intracranial injection of imidaclopride to different doses (0.5 µl, 10-4 M,10-6 M,10-8 M). A staining increase was observed to the highest dose in antennal lobes and in the alpha lobes of mushroom bodies.
Imidaclopride-induced sensory deficits are associated to the drug-induced modifications of CO activity in the first relay of chemosensory informations, the antennal lobes.
Modifications of staining in alpha-lobes which were previuosly shown to be involved in memory processes coul be linked to the facilitation of habituation.

SUBCELLULAR DISTRIBUTION OF PYROGLUTAMYL-PEPTIDASE I ACTIVITY IN HUMAN BRAIN CORTEX

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Pyroglutamyl-peptidase I is an enzyme capable of degrading certain neuropeptides such as TRH, LHRH, neurotensine, and bombazine. However, its role in the neuropeptidic synaptic transmission is very controversial, because the vast majority of the written works define it as a cytosolic enzyme. In order to throw some light on the role of pyroglutamyl peptidase I activity in the peptide degradation, in this work we have measured fluorimetrically pyroglutamyl peptidase I activity, using pyroglutamyl–β–naphtilamide as substrate, in several subcellular fractions of the human rat cortex. The studied fractions were soluble and membrane-bound synaptosomal, microsomal, nuclear and mitochondrial fractions and cytosolic fraction. Pyroglutamyl peptidase I activity is present in all the fractions studied in both soluble and membrane-bound forms. Soluble activity is slightly higher than membrane-bound activity. Membrane-bound activity does not correspond with pyroglutamyl-peptide II because the measured activity is EDTA activated. The highest levels of both soluble and membrane-bound activities were found in the synaptosomal fraction (in soluble synaptosomal fraction 2 fold higher than in the cytosolic fraction) and the lowest in the microsomal fraction. These results seem to demonstrate the existence of a particulate pyroglutamyl-peptidase I. They also suggest to us that soluble pyroglutamyl peptidase I could play a role in degrading peptide neurotransmissors or neuromodulators in or near the synapsis.

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THE LATERAL SPREAD OF ORIENTATION-TUNED AND UNTUNED ACTIVITIES REVEALED BY OPTICAL IMAGING USING VOLTAGE SENSITIVE DYES IN CAT VISUAL CORTEX

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Evidence from anatomical and physiological studies indicates the existence of long range horizontal connections in cortex, and activation also spreads through recurrent connections within and between cortical areas. Such connections have been implicated in a number of psychophysical effects. We set out to examine the speed at which retinotopic activation travels horizontally in cortex when a limited region of the visual world is stimulated, and the extent to which the remote activity depends on stimulus orientation. We used optical imaging of voltage dye RH1692 in cat area 18 using half-field visual stimuli consisting of orthogonal pairs of high contrast moving gratings. We ascertained that this stimulus retinotopically activated only a small region of the imaged cortex. We visualized the appearance of orientation maps with millisecond precision, following stimulus onset. We observe a wave of activation travelling from a directly activated region outwards. The velocity of this wave is 0.1-0.2 m/s. We found that the later activated regions have a patchy appearance being strongest in an orientation specific manner. These delayed patchy orientation columns coincided with the orientation columns obtained by direct activation with full field gratings. It remains to be explored which fraction of this activity is contributed by the long range horizontal connections.

SHORT NOVEL PEPTIDES PROVIDE BROAD NEUROPROTECTION

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The 28 amino acid peptide, vasoactive intestinal peptide (VIP) has been shown to protect neurons against electrical blockade. The specific object of the study was to identify active neuroprotective sites for VIP and related proteins. A superactive analogue of VIP containing a lipophilic moiety attached to the N-terminal of the VIP backbone; stearyl-Nle[17]-VIP (SNV) was developed as a potential neuroprotective drug. Recent studies have shortened the 28 amino acid SNV to stearyl-Lys-Lys-Tyr-Leu-NH_2 (Stearyl-KKYL-NH_2, Proc. Natl. Acad. Sci. USA, 96, 4143-4148, 1999), mapping the active site of VIP to a core of four amino acids. VIP induces increases in the mRNA of Activity-Dependent Neuroprotective Protein (ADNP, 828 amino acids, pI 5.99, J. Neurochem. 72, 1283, 1999) in glia and in cells of neuronal origin. Furthermore, VIP induces the secretion of glial neuroprotective proteins, including the hsp60-like, activity-dependent neurotrophic factor (ADNF). ADNP and ADNF contain homologous femtomolar-acting peptides ADNF-9 (9aa) and NAP (8aa), respectively. Preconditioning neurons (pheochromocytoma, PC12) with VIP and related peptides resulted in partial neuroprotection against toxicity associated with H_2O_2 (oxidative stress). VIP/SNV/NAP conditioned neurons treated with iodoacetate (ischemia-like conditions - glycolysis inhibition at the step catalyzed by glyceraldehyde phosphate dehydrogenase) were also partially spared. Mitochondrial activity (MTS) and the amount of extracellular lactic dehydrogenase (LDH) were used to assess the degree of neuronal survival. Taken together, the novel short peptides may be important lead compounds in search for neuroprotective agents. Original studies identified protective properties for SNV/ NAP/ stearyl-KKYL-NH_2 against Alzheimer's related neurodegeneration *in vitro* and *in vivo* (see above references) these studies are now extended to defense against ischemia and oxidative stress.

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THE EFFECT OF SMA LESION ON BIMANUAL COORDINATION, NEURONAL ACTIVITY AND INTER- HEMISPHERIC INTERACTIONS IN PRE-CENTRAL MOTOR FIELDS

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Previous studies suggest that SMA is essential for bimanual coordination (BC). However recent studies contradicted this notion. To test the hypothesis that additional frontal cortical fields play a role in BC, we recorded neural activity in precentral motor cortex during performance of unimanual and bimanual reaching movements. Local field potentials (LFPs) and single unit activity were simultaneously recorded from the two hemispheres by 8 microelectrodes before and after ibotenic acid (axon sparing) uni- and bilateral lesions of the SMA arm areas.

Immediately following the lesion the monkey did not perform some or all bimanual movements (for a day) and movements of the contralateral hand were less frequently performed (for several days). Since the effects were transient and BC was rapidly restored, we conclude that bimanual movements do not depend solely on SMA. Rather, they seem to be mediated by several motor areas, which can engage compensatory mechanisms. Indeed, we found significant alterations of neuronal activity in the spared motor areas after the lesion. The average single-units firing rate (in premotor cortex, and less so in MI) was increased and the bilateral tuning of cells (see Donchin et al, this volume) was modified. Furthermore, LFP synchronization between the hemispheres was strongly increased after the lesion suggesting a need for increased level of interactions between the spared cortical areas in order to recover bimanual the coordination between the arms.

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FUNCTIONAL MAGNETIC RESONANCE ADAPTATION REVEALS INVARIANCES OF OBJECT REPRESENTATION IN THE HUMAN VISUAL CORTEX.

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Retinal images of objects are altered by changes in viewing conditions such as illumination and viewpoint, but recognition is largely unaffected by these factors. We investigated to what extent human object-related areas exhibit such invariance. To that end, we used a rapid shape-adaptation effect in which repeated presentation of identical images reduces the functional magnetic resonance (fMR) signal. We then tested how sensitive this adaptation was to changes in size, position, illumination, or viewpoint. Our results (obtained in 17 subjects) show that voxels in the object-related Lateral Occipital complex (LO), were strongly activated by different images of the same semantic category. Presentation of identical images reduced the signal to 54±10% of maximum. The profile of the fMR-adaptation revealed two subdivisions within the LO complex: the occipital subdivision (LO) showed sensitivity to all image manipulations; activity largely recovered even upon changes in object position (90±10% of max). In contrast, the anterior-ventral part of LO (LOa) was adapted by changes in object position (73±11%) and size (72±12%) but not by illumination (93±13%) or views (92±11%). Our results indicate putative subdivisions within the object-related LO complex, with the more anterior subdivision showing a higher degree of invariance. Our results also indicate enhanced sensitivity to viewpoint and illumination changes compared to position and size in high order object representations.

SIMULTANEOUS MULTI-SITE RECORDINGS, COUPLED WITH MICROIONTOPHORETIC DRUG AND DYE APPLICATION, AT DIFFERENT LEVELS OF THE VIBRISSAL SENSORY PATHWAY

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Vibrissal sensory information about the location and texture of external objects is encoded both spatially and temporally, and is conveyed along ascending sensory pathways, through the brainstem and thalamus, to the somatosensory cortex. Our previous results indicate that decoding of vibrissal information is based on interactions between cortical and thalamic neurons. In order to study the processing of vibrissal information along the vibrissal afferent pathway, we developed a setup that permits simultaneous extracellular recordings (coupled with iontophoretic drug applications) from the barrel cortex, thalamus, and brainstem. This setup includes terminals (Haidarliu et al., *J. Neurosci. Methods*, 1995, **56**:125) that are stereotaxically oriented and each contain up to four tungsten-in-glass and/or combined electrodes. For recordings from brainstem trigeminal nuclei, four electrodes were passed through the visual cortex and cerebellum, thus avoiding large blood vessels. By simultaneous recordings from multiple neurons in the barrel cortex and thalamic nuclei (POm and VPM) in anesthetized rats, we found that cortical and thalamic spikes were strongly coupled. Periodic whisker stimulation (2 to 14 Hz) provoked fluctuations of thalamocortical activity between stabilized (phase-locked to stimulus) and non-stabilized states. During stabilized states, these neurons exhibited typical closed-loop behavior. In contrast, brain stem neurons, in both the Pr5 and Sp5I, exhibited a straight forward relay operation, which replicated the vibrissal information. This method of multi-site recording can be used to reveal the transformations of input data along sensory afferent systems.

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EFFECT OF EXTERNAL NOISE ON SPATIAL VISION: COMPARATIVE ELECTROPHYSIOLOGICAL AND PSYCHOPHYSICAL INVESTIGATION

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We studied the influence of additive wide-band visual noise on spatial frequency characteristics of the visual system. Visual evoked potentials (VEP) in response to sinusoidal gratings were registered in the occipital area both in presence and absence of additive noise. Amplitude of the main VEP components was plotted vs. spatial frequency (SF). When stationary noise was added to the test grating, the early VEP components strongly reduced and the late negative wave N180 increased for all SF's; the amplitude of the late positive component P240 slightly declined for the mid-range and high SF's. In psychophysical experiments, observers were to estimate the subjective contrast of the gratings based on six-grade scale - from «0» (the worst visibility) to «5» (the best visibility). The average subjective contrast was plotted vs. SF. For both conditions (with and without noise), the resulting curves were similar to those of the dependence of the P240 amplitude on SF. The results are discussed from the viewpoint, which is based on the correlation between the late positive component P240 and pattern detection (Kulikowski & Leisman, 1973). Reducing the early VEP components and increasing the late N180 in the presence of visual noise suggests that extracting of the images from the noise presumably takes place at the high levels of the visual system.

PROLACTIN REGULATING FACTOR (PRF) FROM A MOUSE PITUITARY CELL LINE.

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Substantial evidence indicates that the pituitary intermediate lobe (IL) contains a prolactin regulating factor (PRF). A cell line (mIL5) was established from an IL tumor obtained from a transgenic mouse carrying a POMC-Tag construct. These cells produce and secrete a potent PRF, as determined by an *in vitro* bioassay (GH_3/luc) that measures both prolactin (PRL) gene expression and release. Our objectives were to: 1) characterize the biochemical properties of this PRF, 2) determine if it differs from FGF-2 or HB-EGF, 3) isolate PRF and resolve its primary structure. PRF activity from cell extract or conditioned media (CM) binds to heparin at pH 7.3 and elutes between 0.6-1.0M NaCl (PRF-1) and 1.7-1.9M NaCl (PRF-2) from a heparin affinity column using a linear salt gradient. PRF-1, but not PRF-2, binds a strong cation exchanger (SP) at pH 7.3 and elutes at 0.9M NaCl. The molecular weight of these proteins is estimated as less than 30,000 daltons. RT-PCR of total RNA from mIL5 cells showed the expression of two heparin-binding growth factors, FGF-2 and HB-EGF. Since both compounds induce the PRL gene in a dose-dependent manner (ED_{50} 200pg/ml), they could have accounted for the PRF activity in mIL5 cells However, their affinity for heparin differ from that of PRF-1 or PRF-2. Immunoneutralization of either growth factor abolished their PRF bioactivity, but did not affect that of the heparin-purified mIL5 cell extract or CM. Western blots did not detect either growth factor in purified fractions of mIL5 cells. These data suggest that the PRFs produced by mIL5 cells are not FGF-2 or HB-EGF and likely represent novel, possibly related, proteins. Purification of these PRFs from 5-6 liters of CM, using heparin-affinity, cation exchange and reversed phase HPLC is currently being used to resolve the primary structure of these PRFs. (supported by NIH NS13243 to NBJ and NRSA DA05737 to RH)

IN SITU EXPRESSION OF NEUROPEPTIDE Y IN THE SPLEEN OF THE SPONTANEOUSLY HYPERTENSIVE RATS BY IN SITU RT-PCR

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Neuropepitide Y (NPY) is one of the most abundant and widespread peptides in the mammalian nervous system. It is a 36 amino acid peptide. This peptide serves as a neurotransmitter or neuromodulator to participate in vasoregulation. Although the NPY mRNA is quantitatively analysed in central and peripheral organs,there are rare reports in molecular morphology. We investigate the NPY mRNA expression in the spleen of the spontaneously hypertensive rats, compared to normotensive Wistar-Kyoto rats, by in situRT-PCR.

THE ACTIVITY-DEPENDENT GLYCAN MODULATION IN THE HIPPOCAMPAL LTP: THE INFLUNCES ON NCAM AND NITRIC OXIDE PRODUCTION*

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Recent work has shown that agents which disrupt the extracellular interactions of neural cell adhesion molecules (NCAMs) block the expression of LTP. Adhesion receptors may involve in neural signal transduction and modulation of other membrane receptors ,the glycan of NCAMs also may play an important role in morphological or physiological plasticity.NO has proved to be require for LTP. To address whether the changes of glycan modulate NCAM and NO production or not , we examined the effects of tunicamycin (an inhibitor of glycan synthesis) in LTP in the CA1 region of rat hippocampal slices, using immunohistochemical and biochemical techniques , slices whole-cell recordings (in blind) methods . When slices were exposed to tunicamycin for 30 min before tetanic stimulation, baseline synaptic responses were unaltered, but LTP could not be induced. Similarly, expression of NCAM and activation of NOS were also prevented . however, postsynaptic synaptic currents via NMDA receptor was not affected at the early stage of LTP. These results suggest that activity-dependent carbohydrate structure modulations in LTP exert a evident influence on NCAM function ,further directly or indirectly control releases of retrograde neuromessengers .

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FUNCTIONAL PROPERTIES OF NEURONS IN THE BLOB AND INTERBLOB REGIONS OF CAT VISUAL CORTEX

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We have previously shown that the cytochrome oxidase blobs in cat area 17 coincide with domains responding best to low spatial frequency stimuli, while the interblob regions have a preference for higher spatial frequencies (Shoham et al., Nature 385, 529, 1997). Here we address the question whether neurons in both compartments also differ in other functional properties. We first localized the blob and interblob regions *in vivo*, taking advantage of the fact that the cytochrome oxidase staining pattern corresponds to the spatial frequency map, which was visualized with optical imaging of intrinsic signals. Targeted electrode recordings were then employed to study the receptive field properties of single blob and interblob neurons in detail. Cells in both compartments did not exhibit any obvious differences in their orientation selectivity, their ocular dominance, and their direction selectivity. Overall, neurons in the blobs had somewhat broader orientation tuning curves, but this might simply reflect the known fact that a given cell's orientation tuning curve broadens when lower spatial frequencies are used to stimulate it. We did, however, find a difference in the contrast response functions: blob cells tended to have higher contrast sensitivity compared to interblob cells, whose contrast response curves were shifted towards higher contrast values. Thus, similar to macaque striate cortex, neurons located in the blobs of cat area 17 are characterized by a combination of low spatial frequency preference and high contrast sensitivity. These observations are compatible with our and other anatomical findings, which indicate that geniculate Y-cells terminate preferentially in the blobs.
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SPATIAL ATTENTION IMPROVES VISUAL ACCUITY IN HUMAN AND NON-HUMAN PRIMATES

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One of the open questions in the study of visual attention has been if spatial attention is able to improve performance on visual discrimination tasks. In order to contribute to this debate, we measured visual acuity based on a paradigm, requiring the discrimination of the two possible orientations of a conventional Landolt "C" optotype. To be able to address later the neuronal mechanisms underlying the attentional modification of discrimination performance, using a monkey model, we performed a comparative psychophysical study of humans and monkeys. Subjects had to indicate the orientation of a Landolt "C", whose size was varied according to a PEST staircase procedure. The "C" was presented in locations along the horizontal and vertical axes with eccentricities of 3, 9 or 15 degrees of visual angle. We compared acuity thresholds, defined as the minimum size of the "C"-gap, yielding 75% correct responses. Three types of trials, differing with respect to whether a spatial cue was absent, present and valid or present, but invalid, were presented randomly interleaved. The spatial cue appeared 500 ms after the onset of maintained fixation of a little spot in the center of the display, stayed on for 150 ms and after a gap of 100 ms, the "C" was presented for 150 ms in one out of two mirror symmetric positions on one of the cardinal axes (choice of axes and eccentricities blocked), while the subject maintained fixation. Monkeys indicated their perceptual decision by eye movements, well after the presentation of the „C" and human subjects used response buttons. As expected, we found that acuity thresholds increased with increasing eccentricity. We also observed a strong influence of perceptual learning, reflected by the fact that acuity thresholds kept declining asymptotically over about 5 weeks of daily measurements. However, even after having settled to a more or less constant threshold, spatial cueing remained able to modify acuity thresholds. Shifting the monkey's attention to the correct location by spatial cueing improved acuity thresholds on average by 20% , independent of eccentricity. Conversely, shifting spatial attention to the false location raised thresholds by a comparable amount, largely independent of eccentricity. While absolute acuity thresholds for the rhesus monkey studied most heavily so far were significantly higher than those of human subjects, tested on the same task, the modulatory influence of spatial cueing on the performance seems to be the same in the two primate species explored. We conclude that spatial attention is able to enhance spatial visual resolution in monkeys and man, based on a mechanism which might be independent of the mechanism(s) underlying perceptual learning.

TYROTROPIN RELEASING HORMONE (TRH) DEGRADING ENZYMES IN DISCRETE AREAS OF RAT AND HUMAN BRAIN

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The tripeptide TRH (pGlu-His-Pro-NH2) is primarily degraded in the brain by three enzymes. Pyroglutamyl peptidase I and II that hydrolyces the N-terminal pyroglutamyl residue and prolyl endopeptidase that catalizes the deamidation of the tripeptide. Although the regional distribution of these enzymes in human brain regions. In this report, we describe the regional distribution of TRH degrading enzymes in several regions of the human and rat brain. Pyroglutamyl-peptidase I and prolyl-endopeptidase activities have been measured in soluble and particulate fractions. Enzyme activities were measured using these substrates: pyroglutamyl-β-naphtilamide, for pyroglutamil peptidase I, TRH-β-naphtilamide and inhibitors for the pyroglutamyl-peptidase II and using Z-Gly-Pro-β-naphtilamide for the prolyl endopeptidase. Soluble prolyl-endopeptidase specific activity is x-fold higher in the human brain areas than in the rat brain areas. Moreover, we have obtained higher activities in frontal, parietal and occipital cortices than in the striatum, the hippocampus, the amygdala, the hypothalamus and the cerebellum both in human and in the rat brain. Although membrane bound prolyl-endopeptidase activity is lower than the soluble one, the profile of regional distribution is similar. On the other hand, pyroglutamyl peptidase activities are more homogeneously distributed in the brain. However, it can be noted than the activity is higher in the hypothalamus and brain cortices and lower in the striatum and the cerebellum. In pyroglutamyl-peptidases, we have not found statistical differences between rat and human brain areas. These results suggest to us that prolyl endopeptidase activity could be related with the filogenetic development of the rat brain.
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A FUNCTIONAL ANTAGONISM BETWEEN NITRIC OXIDE AND ATP IN NANC RESPONSES OF GUINEA PIG SMALL INTESTINE

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The role of nitric oxide (NO) and ATP in non-adrenergic non-cholinergic (NANC) responses elicited by electrical stimulation (0.8 ms duration, 40 V, 1-20 Hz, 20 s trains) was investigated in non-precontracted longitudinal muscle layer of isolated guinea pig ileum. The NANC, tetrodotoxin (0.1 μM)-sensitive responses during the electrical stimulation consisted of relaxation phase followed by phase of contractions, twitch-like and tonic contractions. N-G-nitro-L-arginine (L-NNA, 0.1-0.5 mM) depressed the relaxation phase. L-arginine (0.5 mM) restored the relaxation phase in L-NNA-pretreated (0.5 mM) preparations. D-arginine (0.5 mM) was ineffective. The relaxation phase was enhanced after ATP-induced purinoceptor desensitization and in the presence of suramin (1 μM) but was reduced by L-NNA during purinoceptor desensitization. This phase was abolished by apamin (5 μM). The phase of contractions was increased by L-NNA (0.1-0.5 mM) and restored to the initial level by L-arginine. This phase was decreased after ATP-desensitization and in the presence of suramin or apamin. L-NNA enhanced the phase of contractions during ATP-desensitization. Sodium nitroprussid (1-100 uM) induced concentration-dependent relaxations. ATP (5-50 μM) evoked contractions which resembled the phase of contraction of the responses to electrical stimulation. The contractile effects of ATP were tetrodotoxin- and L-NNA-resistant and suramin- and apamin-sensitive. It is concluded that NO and ATP are involved in these two-phasic ileal NANC responses. NO, acting as an inhibitory transmitter, mediates the relaxation phase while ATP seems to contribute via smooth muscle P_2 purinoceptors to the phase of contractions as an excitatory transmitter, which suggests a functional antagonism between NO and ATP at postjunctional level. The inhibition of the nitrergic relaxation by apamin assumes that interactions between NO and apamin may occur at the level of calcium activated potassium channels.

DIRECTION AND DEGREE OF HAND PREFERENCE IN TRANSSEXUALS.

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Several theories have proposed that sex hormones act on the fetal brain, influencing functional cerebral asymmetry, including handedness. It was demonstrated that prenatal exposure to a high level of testosterone (T) resulted in higher percentage of non-right-handers (nRHs) and, paradoxically, in stronger hand preference, at least among right-handers (RHs). Prenatal exposure to atypical level of T, as some data suggest, also plays a role in etiology of transsexuality.

The aim of the present study was to investigate direction and degree of hand preference in transsexuals. Edinburgh Handedness Inventory was administered to 199 transsexuals (163 anatomical females, 36 anatomical males) and 483 controls (333 females, 150 males).
The findings showed higher percent of nRHs in transsexuals than in controls, and among RHs, stronger right hand preference in transsexual groups. This data support the view that transsexuality and handedness are interrelated. This relationship might result from the influence of the prenatal testosterone on the developing brain, however other factors cannot be ruled out.

RAPID PHOSPHORYLATION OF ELK-1 TRANSCRIPTION FACTOR AND ACTIVATION OF MAP KINASE SIGNAL TRANSDUCTION PATHWAYS IN RESPONSE TO VISUAL STIMULATION

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The AP-1 transcription factor (composed of various combinations of Fos and Jun proteins) is believed to be a key participant in molecular processes that guide activity-dependent changes in gene expression. In this study, we investigated the function of different MAP kinases, possibly involved in transducing signals from cell membrane to AP-1 activation—ERK, JNK/SAPK, and p38 MAPK—and their nuclear targets—Elk-1 and c-Jun—in rat visual cortex after light stimulation. The transcription factor Elk-1 (a possible regulator of *c-fos* expression) was found to be transiently modified by phosphorylation (as shown by specific antibody) when visual stimulation was applied after a period of dark rearing. In vitro kinase assay with Elk-1 as substrate showed that light stimulation activated MAPK/ERK in visual cortex but not frontal cortex. Furthermore, ERK activation was temporally matched to onset of Elk-1 phosphorylation. The activities of JNK1 (c-Jun N-terminal kinase 1) were elevated at 2-6 hours after visual exposure and was also temporally correlated to increase of endogenous P-c-Jun levels and its appearance within the AP-1 DNA-binding complex. The activities of p38 MAP kinases did not change significantly. These results demonstrate the differential engagement of MAPK signaling pathways following sensory stimulation and their relative effects upon AP-1 expression in the intact brain.

Low doses of naloxone suppress morphine consuming on opiate addiction model.

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This work was performed on two models of narcotic dependance in Wistar rats and mices C57BL/6 by means of chronic injections of increasing doses of morphine-hydrochloride (5-30 mg/kg twice per day with 8 hours rest). Animals were kept after in free choice to morphine access. Low doses of naloxone (0,2 and 0,3 mg/kg) injected i.p. Single naloxone injection decreased morphine consuming in 1,5-2 time in rats and 2 time in mices. 4-days chronic naloxone application highly suppressed morphine consuming. Also, low doses of naloxone strongly decreased nociception level. Testing blood serum in chronic morphine rats (using ELISA method) after 10-days morphine deprivation showed decreasing autoantibodies level to 5-HT-BSA, whereas application of low doses of naloxone precludes from this decreasing. Thus, obtained results allow to recommend using of low doses of naloxone for suppressing drugs addiction.

COMPARATIVE STUDY OF OSPHRADIAL CHEMORECEPTION IN MOLLUSCS.

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The using of comparative morpho-functional method in the study of chemoreception function allows revealing of most common, generically fixed structurally functional principles in the chemosensory systems. The chemosensory osphradial system of molluscs represents the convenient plant for such researches as it is homologous for principal classes of *Mollusca* type and contains the series of primitive characteristics of osphraduim organization.

Most simply organized osphradia are in *Polyplacophora*, representing the pair zones of poorly specialized epithelium containing primary sensing cell which axons enter the pleuro-visceral nervous trunk. The extracellar recording from it has revealed a diminution of frequency of impulsation on hyperosmotic and decrease-on hypoosmotic stimulations of osphradial surface. The osphradia of *Archaeogastropoda* and *Bivalvia* are organized similarly. The least the migration of receptor cells into subepithelial layer forms the primitive osphradial ganglion. The formation of the special peripheral ganglion containing the receptor, gangliar and effector nervous cells and being a center of primary processing of sensory information represents a major stage in the development chemoreceptory function. Just in such a way the osphradial organs of *Caenogastropoda* and *Pulmonata* are arranged. Electron-microscopically the synaptic organization of ganglions at principal representatives of classes was investigated. The existence of synaptic glomerules in osphradial neuropile were revealed.

By extracellular recording from an osphradial nerve and patch-clamp whole cell configuration method the receptor and neurosecretory cells of osphradial ganglion Lymnaea stagnalis has been studied. The registration of nerve activity revealed the sensivity of osphradium to hyperosmotic pressure, sodium chloride and aminoacids. Besides, osphradium detects the quality of water, where the animals were kept. The membrane currents caused by NaCl(10^{-3}M) and aminoacids(10^{-5}M)applications on ganglion surface were found out. The neurosecretory and receptor cells demonstrate various sensitivity to neurotransmitters. 5-ÍÒ and GABA (10^{-5}M) exits the large gangliar cells, while Ach (10^{-6}M) - inhibited. The sensory cells were excited by ACh and 5-HT and inhibited by GABA. Ca^{2+} acting in a cell on voltage-activated membrane L- type channels takes part in action potential generation of neurons.

SPATIO-TEMPORAL PATTERNS OF CORTICAL POPULATION ACTIVITY PREDICT SINGLE NEURON FIRING RATES

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The visual cortex is engaged in a highly complex pattern of ongoing population activity which to a large degree determines the subsequent response to stimulation, as demonstrated by real-time optical imaging. The precise nature of the population activity and its relation to spontaneous and evoked firing of single neocortical neurons is not known. To study this relation, we analyzed data obtained both in the absence and presence of visual stimulation. We used optical imaging of voltage sensitive dyes (RH795 or RH-1692), combined with spike discharges of two isolated neurons recorded from a micro-electrode, inserted into the exposed area (12x12 or 128x128 sites covering 2x2 or 7x7 mm^2 of areas 17 & 18 of anesthetized cats). We find that the probability of a neuron's firing at any instant can be predicted by the corresponding spatio-temporal pattern of the population activity in the area surrounding the neuron, extending up to 3 mm from it. Moreover, the population state at which the probability of firing is maximal has a similar shape in the spontaneous and the evoked regimes, which indicates that a neuron is driven by the same constellation of inputs in both these regimes. In the evoked regime, we are able to predict spike activity even if the stimulus used is a non-optimal one for the neuron. Preliminary results using the dye RH-1692, suggest that the state optimal for the neuron, corresponds to the functional map in which the optimal stimulus is used. We conclude that there exists a set of population states, presumably determined by a pattern of intra-cortical connections. External optimal stimulation temporarily pushes the system into a particular state where the firing rate of a neuron tuned for this stimulus is maximal.

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THE SPEED OF SIGHT AND THE PIPELINE ARCHITECTURE OF VISION

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Some models of visual processing in the brain emphasise stimulus competition and feed-back connections yet behavioral and electrophysiological data indicates that stimulus discrimination can be too fast to rely on feedback. To study the speed of visual processing, we presented macaques with sequences of unrelated natural images, instead of single or pairs of images. We recorded cells in the temporal cortex of the macaque monkey (area STSa) that responded selectively to complex patterns (e.g. faces) at a latency of 108ms. We found that 57% of cells retained their stimulus selectivity at rates faster than previously studied (13.9ms/image). Thus, at each moment during such sequences, the visual system processed more than 7 different images (108/13.9). Cell responses discriminated between stimuli within 10ms of response onset and outlasted stimulus duration by ~40ms, creating temporal overlap between neural representations of successive stimuli. These findings suggest that under time pressure, the visual system can use feed-forward processing with different stages processing different stimuli independently ('pipeline' architecture) and neural coding that allows multiple stimuli to be represented simultaneously within one area without winner-take-all competition.

SYNAPTOPHYSIN (p38) ALTERATIONS IN THE HUMAN AND RAT BRAIN INDUCED BY OPIATE ADMINISTRATION AND WITHDRAWAL

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Synaptophysin (p38) is an integral protein of synaptic vesicle membrane, which may be involved in the exocytotic process of neurotransmission (Greengard et al., 1993). The presence of p38 is assumed to correlate to synaptic density (Masliah et al., 1990). Quantitative alterations of p38- immunoreactivity (IR) were observed following experimental brain lesions (Walaas et al., 1988) and in the brain of cases of some CNS diseases (Masliah et al., 1991). We hypothesized that drug addiction is associated with changes in synapses. To investigate this human and rat brains exposed to drugs of abuse were examined using p38 as a presynaptic marker. The brains of control rats (n=4), 7 days morphine-treated rats (60 mg/kg, i.p., n=4) and rats which were 7 days abstinent after withdrawal of morphine (n=4) were studied. The p38-IR was studied in the serial frontal brain sections using indirect immunofluorescence and quantitative confocal microscopy (Belichenko et al., 1996). The areas of p38-IR were measured in the frontal cortex, nucleus accumbens, hippocampus, striatum and thalamus. There were no significant changes of p38-IR areas in any of the brain regions after morphine administration, as compared with control. However, in the 7 days withdrawal group significant decreases in p38-IR were observed in layer III of the frontal cortex, in nucleus accumbens and in hippocampus compared to control. Thus, the significant changes of p38- IR in the rat brain were detected after withdrawal, but not after one week of morphine treatment. The study of changes in presynapses was also carried on postmortem brains of drug users (n=4) and of control cases (n=4). In drug users an increase in p38-IR areas was observed in the frontal, parietal and temporal neocortical areas. Alterations in p38-IR areas are considered to reflect plasticity of presynaptic structures in the rat and human brains induced by opiate addiction.
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PULSATILE SECRETION OF GONADOTROPIN-RELEASING HORMONE (GNRH) IS AN INHERENT FUNCTION OF GNRH NEURONS, AS REVEALED BY THE CULTURE OF MEDIAL OLFACTORY PLACODE OBTAINED FROM THE EMBRYONIC RAT

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To know whether GnRH neurons deriving from the nasal placode can drive GnRH secretion in a pulsatile fashion, we developed an assay system capable to measure GnRH released into the culture medium. The medial nasal placode containing the primordium of the vomeronasal organ (VNO), which was obtained from day-13.5 rat embryo was cultured. In some cases, the VNO was co-cultured with the forebrain or the hypothalamic tissue. After 2-4 weeks-culture, whole amount of the culture medium was collected at 8-min intervals over a period of 2 hours for the determination of GnRH concentration by ELISA. Also, all the cultures were fixed for the immunohistochemistry, irrespective of whether sampled or not. We found that GnRH concentrations in the medium fluctuated significantly, showing several GnRH pulses in the 2-hour period. The interval between such GnRH pulses in the VNO culture was 29.3 ± 2.3 min (mean ± SE, n=9). The VNO co-cultured with brain tissues showed GnRH pulses with similar intervals. We then found that GnRH neurons in cultures which showed GnRH pulses intermittently always exhibited a very weak immunoreactivity to GnRH or GnRH associated peptide, and cell bodies and fibers were hardly detectable. In contrast, cultures that had not been sampled at all had clear immunoreactive GnRH neurons and fibers. We conclude that pulsatile GnRH secretion is an inherent function of GnRH neurons. Further, GnRH immunoreactivity is affected by the frequent sampling.

EFFECT OF THE TRANSCRANIAL ELECTROSTIMULATION (TES) ON THE FUNCTION OF SKIN MECHANORECEPTORS BOTH INTACT AND RESTORATING AFTER NERVE DAMAGE

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TES is well known to cause analgesia, which is in turn conditioned by an activation of the opioidergic system. An opioidergic system is involved in the variety of organism's functions including immune and stress reactivity, blood circulation and reparative processes. To reveal participation of the peripheral nervous system in the TES mechanisms, the activity of foot skin mechanoreceptors in the rat has been examined via registration of discharge of single fiber of the sciatic nerve. An inhibitory effect of TES on the activity of both noxious and low threshold (touch skin) mechanoreceptors has been demonstrated. The technique as applied, allow, thereby, blocking not only central nociceptive structures, but also peripheral afferents, which take part in the reception of pain.
The TES effect on recovery of the function of the sciatic nerve afferents after transection and microsurgical suture has been examined. Electroanalgesia was found to promote the regenerative outgrowth of injured nerve fibers by an average 25%. Moreover, the TES was favorable with the respect to recovery of the conductivity in damaged afferents. Accordingly, TES have been shown to affect the peripheral receptors, inhibiting their activity under normal conditions while facilitating the post traumatic restoration of their functions.

FUNCTIONAL PROPERTIES OF SKIN MECHANORECEPTORS IN THE RAT: EFFECT OF RHIZOTOMY

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The receptor structures are known to be controlled by the central nervous system (CNS) via the efferents ways, where an inhibitory effect from the side of the sympathetic nervous system most frequently occurs. Nevertheless the ability of CNS to affect skin receptors via the afferent ways by the retrograde axonal transport is still unclear. Sensory neurons of spinal ganglia, branching both to the central and peripheral nervous systems, comprise the most convenient model to the study preferred. Activity of mechanoreceptors and appropriate afferent nerve fibers was examined in the hind foot of normal rats in comparison with the animals after right-side extensive rhizotomy (L4-L6). Conduction velocity of the afferent fibers has been shown to increase considerably at the operated side in comparison with the contralateral one. Evoked nerve impulses were recorded from the afferent fibers of the n. tibialis. Persisted functioning of the decentralized receptive structures has been revealed. Examination of a single nerve fiber activity has demonstrated the elevated mechanosensitive thresholds. Moreover, the adaptive properties of the receptors has been deteriorated. Rapidly and very rapidly adapting receptors dominated in intact animals, whereas slowly adapting receptors prevailed in the rhizotomized ones. Accordingly, alterations in functional properties of the afferent structures suggest a trophic influence from the side of CNS on the skin receptors activity, that is implemented via central roots of sensory ganglia.

OSTROGEN REPLACEMENT REVERSES THE EFFECT OF OVARIECTOMY ON HYPOTHALAMIC BLOOD FLOW AUTOREGULATION DURING HEMORRHAGIC HYPOTENSION IN RATS.

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The influence of ovariectomy and oestrogen replacement on hypothalamic blood flow autoregulation was investigated in anesthetized, artificially ventilated, temperature controlled rats. In order to study the changes of the hypothalamic blood flow (H_2-gas clearance technique), at the lower limit of autoregulation systemic arterial pressure was reduced in a stepwise manner to 80, 60, and 40 mmHg, by hemorrhage. Autoregulatory mechanisms of the hypothalamic vessels remained effective and hypothalamic blood flow showed no significant reduction until the arterial pressure was reduced to 60 mmHg in the sham-operated control rats. Ovariectomy however, induced a significant downward shift of the lower limit of the autoregulation: othalamic blood flow started to decrease onlyat the 40 mmHg arterial press- -vel. Oestrogen replacement (450 mg/kg/week, i.m.) resulted in a restora- of the original pattern of hypothalamic blood flow autoregulation observed ... the control group. Alteration of the lower limit of regional cerebral blood flow autoregulation following ovariectomy and it's restoration by selective hormone replacement by oestrogen suggests an important role of gonadal steroids in hypothalamic autoregulatory mechanisms during hemorrhage-induced hypotension.

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ETHANOL DISCRIMINATION IN RATS: THE ROLE OF 5-HT NEURONS IN THE DORSAL RAPHE NUCLEUS

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The drug discrimination paradigm is a useful animal model of the subjective effects of drugs in humans. This procedure is also very helpful for identifying receptors that might mediate the stimulus effects of drugs. Ethanol, as other drugs of abuse, may produce interoceptive (discriminative) cues that the animal can identify and associate with a particular behavioral response (*e.g.* responding on a given lever). It has been reported that ethanol may induce a mixed ('compound') discriminative stimulus mediated, at least partially, through serotonergic 5-$HT_{1/2}$ receptors, the $GABA_A$/benzodiazepine receptor complex and the glutamatergic NMDA receptor subtype. The purpose of this study was to assess the role of brain 5-HT in the discriminative stimulus effects of ethanol in Wistar rats. We focused on the dorsal raphe nucleus (DRN) because of its extensive ascending innervation sent to different forebrain structures thought to be involved in the ethanol drinking behavior. Selective lesions of 5-HT neurons located in the DRN were induced by a 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). Control rats received injections of ascorbate vehicle. After a two-week recovery period, food deprivation was started and the subjects were trained to discriminate between 1 g/kg ethanol and saline in the standard two-lever operant procedure. On a given day the animals were injected with ethanol or saline and required to press one of the levers to obtain sweetened milk reinforcement. The acquisition of the ethanol discrimination and the ED_{50} derived from the ethanol generalization curve did not differ between the 5,7-DHT- and vehicle-treated animals. A non-selective benzodiazepine agonist, diazepam almost completely generalized from the ethanol cue in both groups. A 5-$HT_{1/2}$ receptor agonist, m-chlorophenylpiperazine (mCPP; 0.1-0.9 mg/kg; i.p.) did not substitute for ethanol in either group. However, mCPP substantially decreased the rate of operant responding the effect being significantly stronger in the sham animals. A selective 5-HT_{1A} receptor agonist, 8-OHDPAT (0.05-0.4 mg/kg; i.p.) partially substituted for ethanol in the 5,7-DHT- but not in the vehicle-treated subjects. Concluding, these data suggest that (i) the 5-HT neurons of the DRN do not play an important role in the ethanol discrimination; (ii) the depressant effects of mCPP on responding for palatable food are mediated, at least in part, by the 5-HT neurons located in the DRN.

LONG-TERM ETHANOL CONSUMPTION IN RATS: THE ROLE OF 5-HT NEURONS IN THE DORSAL RAPHE NUCLEUS.

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Alcoholism is a chronic, relapsing disorder with phases of drinking, remission (abstinence) and relapse appearing in succession. Hence, prevention of relapses is the most important goal of treatment of detoxified abstinent alcoholics. Both animal and human studies indicate an important role of brain serotonergic (5-HT) pathways in ethanol consumption. In general, decrease in 5-HT neurotransmission increases alcohol intake. The majority of 5-HT neurons is localized in the brainstem and midbrain raphe nuclei. One of them is the dorsal raphe nucleus (DRN) which sends extensive ascending innervation to forebrain structures (*e.g.* prefrontal cortex, hippocampus, nucleus accumbens). Given the above, the purpose of the present study was twofold. First, the pattern of long-term, voluntary ethanol consumption with repeated deprivation periods was assessed in Wistar rats. In addition, relationship between the alcohol intake and behavioral parameters from the open field and saccharin drinking tests was studied. Second, the role of the 5-HT neurons in the DRN in the long-term ethanol drinking was estimated in rats treated with a 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). Saccharin drinking parameters positively correlated only with acceptance of low ethanol concentrations in a two-bottle choice test (2-6% ethanol *vs.* water). However, this relationship disappeared during further weeks of drinking when ethanol was available in a three-bottle choice situation (8% ethanol *vs.* 16% ethanol *vs.* water). In addition, none of the behavioral parameters measured in the open field test (*e.g.* forward locomotion or rearings) correlated with subsequent ethanol drinking. The total ethanol intake in six consecutive 28-day drinking periods was relatively stable (range: 3.5-4.5 g/kg). In contrast to previous studies, the alcohol deprivation effect (*i.e.* increase in alcohol intake after forced abstinence) was weak or even absent in our model. The DRN lesion affected none of preliminary behavioral parameters except of water intake that was significantly greater in 5,7-DHT-treated group. In general, 5,7-DHT lesion did not exert any major effect on the long-term ethanol drinking behavior. In a separate experiment, the three-bottle ethanol drinking procedure was started seven months after the lesion. Interestingly, this manipulation led to a significantly higher intake of 8% ethanol in the 5,7-DHT-injected subjects. This latter finding would underline the importance of neuroadaptive changes for subsequent alcohol acceptance in the lesioned animals.

TYROSINE PHOSPHORYLATION OF PKCδ PLAYS A ROLE IN THE PROLIFERATION AND DIFFERENTIATION OF GLIAL CELLS

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PKC plays an important role in the proliferation and differentiation of glial cells. In a recent study we found that overexpression of PKC δ reduced glial cell proliferation and the expression of the astrocytic marker glutamine synthetase (GS). The roles of the regulatory and catalytic domains of PKCδ on GS expression and cell proliferation were examined using PKC chimeras. We found that only chimeras between the regulatory domain of PKC δ and the catalytic domains of PKC α or ε inhibited the expression of GS and cell proliferation, similar to the inhibition exerted by overexpression of PKC δ. Similarly, tyrosine phosphorylation of PKC δ in response to PMA and PDGF occurred only in chimeras that contained the PKC δ regulatory domain. Cells transfected with a PKC δ mutant (PKC δ5), in which five putative tyrosine phosphorylation sites were mutated to phenylalanine, showed markedly diminished tyrosine phosphorylation in response to PMA and PDGF and increased levels of GS and cell proliferation. Transfection of the cells with a PKCδ mutant in which only tyrosine 155 was mutated to phenylalanine blocked the inhibition of cell proliferation induced by PMA but not that of GS expression Our results indicate that tyrosine phosphorylation in the regulatory domain of PKC δ mediates the inhibitory effect of this isoform on the expression of GS and cell proliferation. Tyrosine phosphorylation of PKCδ on tyrosine 155 appears to play a selective role in the inhibitory effect of PKCδ on cell proliferation but not on glutamine synthetase expression.

A STUDY OF UAING INHIBITOR OF ENZYMIC BARRIER TO TREAT VASOGENIC BRAIN EDEMA

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Vasogenic brain edema (VBE) has frequent occurrence in the clinic with increased permeability of blood-brain barrier, accumulated plasma filtrate fluid in extracellular space,especially in the white matter. It's difficult to treat. Here we used a new drug LBQ which inhibit the activity of enzymic barrier in cerebral capillariy endothelial cells to treat VBE and found it could alleviate brain edema. There were 93 Wistar rats came into our experiment and were randomly divided into 4 groups::normal group(n=19), VBE group(n=21), treating group(n=17),control group (n=22).VCE model was made by injecting Phenylephrine into peritoneal cavity ,mannitol was uesd as control. Water content of cerebral gray and white matter were measured respectively with Moisture Analyzer(Denver Ins.).We found that the water content in gray and white matter of the 4 groups mentioned above are 66.646.34% 61.717.47%; 78.287.53% 76.358.14%; 65.098046% 58.247.48%;65.389.01% 67.155.85% respectively. Compared with the control group, treating group had no marked statistic significance in water content of gray matter($P>0.05$), while that of whiter matter much less than control group($P<0.01$). Our results indicated that LBQ has special selective effect on white matter and is a new effective drug to treat VBE.It also suggest enzymic barrier has close relation to formation of VBE.

MATERNAL MORPHINE INCREASES DENDRITIC SPINE DENSITY IN NTORHINAL CORTEX OF NEONATAL MOUSE.

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Prenatal drug-exposure leads to significant deficits in sensory function, motor activity, exploratory behaviour, learning and memory performances (Maharajan et al. 1996). These behavioural alterations are often correlated to structural modifications at cellular and subcellular levels in specific brain regions in the neonates maternally exposed to opioids. Earlier studies have revealed neuroanatomical alterations in opioid exposed neonatal brains along with changes in neurotransmitters, neurofilaments, neuropeptides, growth factors and opioid receptors and in macromolecular synthesis (Zagon and McLaughlin 1984). In the present work we have studied dendritic structures in the entorhinal cortex (EC) of neonatal mice maternally-exposed to morphine. Female Swiss mice were daily administered saline or morphine (30 or 60 mg/kg body wt) for a period comprising 7 days before mating, gestation and 21 days post-partum. Their pups were sacrificed on postnatal day 18 and the brains were examined for histology and Golgi-staining. The number of cresyl violet-stained cells in the EC, does not change significantly in morphine-treated groups as compared to control. But we find a remarkable increase in the density of dendritic spines of the pyramidal and stellate neurons of the EC in morphine-treated neonates. The increased number of dendritic spines might represent increased synaptic activity. The EC forms part of the limbic structures and projects to the hippocampal complex via perforant pathway as well as it receives powerful projections from the hippocampus (Lopes Da Silva et al. 1990). The roles of the hippocampus and the EC in the processes of learning, memory, cognition and emotion are well documented. Thus modifications in the synaptic patterns of the EC in morphine-exposed neonates could be related to the behavioural alterations recorded in neonates, maternally-exposed to addictive drugs.

References
1) Lopes da Silva F.H. et al. (1990) Physiol. Rev., 70:453-511.
2) Maharajan V. et al.(1996) Acta Medica Veterinaria, 42:91-97.
3) Zagon I.S. and McLaughlin P.J. (1984) In: Neurobehavioural Teratology, Ed: Yanai J., Elsevier, Amsterdam. pp197-233.

IMMUNOLOGICAL DISTURBANCES IN DIABETIC FETOPATHY/ENCEPHALOPATHY.

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Diabetic fetopathy (DF) with disturbances in the nervous system (NS) development is one of the most serious complications in fetus/newborn from insulin-dependent diabetes mellitus (IDDM) suffered mother. The pathogenic mechanisms of DF are still obscure.

Sera from women with IDDM and their newborns were analyzed for specific antibodies (AB) to insulin and insulin receptors by ELISA and immunoaffine chromatography. Alongside with 2-3 fold increase of AB of IgG class to insulin and its receptors in sera of diabetic mothers and their newborns the prominent crossreactivity of anti-insulin AB with nerve growth factor (NGF) was revealed. Increase of AB to brain-specific proteins S100b and GFAP was also found in such newborns.

Since IgG pass freely through the placental barrier, AB to insulin receptors (were identified as anti-idiotypic AB to anti-insulin AB) could extensively block corresponding receptors and lead to generalized metabolic disturbances in NS and other organs in fetus. AB to insulin can bind and block NGF and other (immunologically related) neurotrophins crucial for NS development in fetus. Hence, increased level of anti-insulin/anti-NGF AB in pregnant woman may play a fundamental role in fetus NS damage. Immunological correction of elevated specific AB levels before and/or during the pregnancy thus may be a perspective strategy to avoid NS damage in fetuses from IDDM suffered women.

THE EFFECTS OF CALLOSAL AGENESIS ON THE SUSCEPTIBILITY TO SEIZURES ELICITED BY PENTYLENETETRAZOL IN ADULT MALE BALB/cCF MICE

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In humans, callosal agenesis is frequently associated with epilepsy. Here we tested adult male mice from a strain in which 20% to 30% of the animals have partial or total callosal agenesis. We injected 40 mg/kg (n = 42) or 50 mg/kg (n = 43) of pentylenetetrazol (PTZ; i.p.) and videotaped all tests. We used the following classification: 1) no abnormal behavior; 2) myoclonic jerks; 3) running bouncing (RB) clonus; 4) tonic hind limb extension. For the 40 mg/Kg dose we obtained the following distribution of classification in acallosal mice (n = 11): 18.2% had no abnormal behavior; 18.2% presented myoclonic jerks and 63.6% presented RB clonus. For the normal mice (n = 31) the observed distribution was: 54.8% had no abnormal behavior; 16.1% presented myoclonic jerks and 29% presented RB clonus. For the 50 mg/Kg dose, all acallosal mice (n = 12) had RB clonus, whereas in normal mice (n = 31) 9.7% had no abnormal behavior; 9.7% presented myoclonic jerks and 80.6% presented RB clonus. These results suggest that susceptibility to epileptic seizures induced by PTZ is higher in acallosal mice.

P2 RECEPTORS IN THE RAT PINEAL GLAND.

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The rat pineal gland possesses P2 receptors which potentiate the effect of noradrenaline-induced N'-acetyl-5-hydroxytriptamine (NAS) production. (Br J Pharmacol. 112:107-10,1994). **AIM**: Characterize the P2 receptor present in rat pineal glands according to agonist selectivity and signal transduction mechanisms. **METHODS** Two days cultivated pineal glands were incubated with one concentration of agonist (30μM-1mM, 5h) in the presence of noradrenaline (10nM). NAS and cAMP content was determined by hplc and RIA, respectively. **RESULTS:** 2ClATP, 2MeSATP, ADPβS, ADP and ATP, but not UTP, increased noradrenaline-induced NAS production, in a concentration-dependent manner. NAS basal noradrenaline production was 15.6±1.8 ng/well and 2.5±0.4 ng/gland (n=23). 2MeSATP was the most efficient agonist, potentiating NAS production in 4.4 times. The P2 receptor antagonist (PPADS, 10–60μM) attenuated, in a concentration-dependent manner, 2MeSATP effect. The PLC inhibitor, U73122 (10μM) blocked 2MeSATP effect, while this agonist neither induced the production of cAMP, nor inhibited its formation when the glands were stimulated by forskolin (0.1mM). **CONCLUSION**: Considering that: **1**- the agonists tested, except UTP, potentiated noradrenaline-induced NAS production; **2**-PPADS blocks the effect of 2MeSATP, **3**-the receptor is coupled to a G protein that stimulates PLC, but does not modify the activity of adenylyl cyclase; it is concluded that the effect herein studied is mediated by a $P2Y_1$-like receptor.

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REGENERATION OF THE SPINAL CORD AXONS IS PROMOTED BY THE CHOROID PLEXUS TRANSPLANTS IN THE ADULT RAT

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We examined the effectiveness of choroid plexus ependymal cells on the axonal regeneration in the adult rat spinal cord. The choroid plexus was excised from the fourth ventricle of donor rats, and stained with fluorescent dye (DiI or CFSE). The spinal cords of recipient rats were exposed from the dorsal, and cut transversely with a scissors, making a small lesion in the dorsal funiculus, to which the fluorescence-labeled choriod plexus was implanted. The rats were fixed by perfusion 1 and 3~4 weeks after grafting, and the spinal cord including the lesion was sectioned 10 um thick using cryostat. Axons were visualized by immunohistochemistry for neurofilament protein. Confocal laser scanning microscopy (CLSM) showed that a large number of regenerating axons extended into the graft, in which axons were closely surrounded by the fluorescene-labeled cells. Immunoelectron microscopy, in combination with CLSM, revealed that fluorescent cells were in close contact with axons, and that some of them were situated next to basal laminae so that they were in the normal choroid plexus. This feature showed that the fluorescent cells in association with axons were ependymal cells of grafted choroid plexus. These results indicate that choroid plexus ependymal cells can support and fascilitate axonal regeneration in the injured spinal cord of the adult rat.

AFTER HYPERPOLARIZATIONS IN MAMMALIAN SYMPATHETIC NEURONES INVOLVE MULTIPLE TYPES OF Ca2+ AND K+ CHANNEL

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The action potential in neurones of mammalian sympathetic ganglia is followed by a long afterhyperpolarization (AHP) which depends on Ca2+ influx during the action potential. This AHP is responsible for limiting the firing rate of postganglionic neurones during high levels of preganglionic activity. One K+ conductance is common to neurones in ganglia of several species: it has a rapid onset and decays exponentially with a time constant of 100-150 ms (termed gKCa1). In a subclass of guinea-pig neurone with an AHP lasting several seconds, a second slow onset conductance decays with a time constant of ~1.4 s (termed gKCa2). We have used selective pharmacological blockade to identify the subtypes of Ca2+ and Ca2+-activated K+ channels involved in gKCa1 and gKCa2. Recordings were made in guinea pig ganglia using single electrode voltage clamp at RMP. gKCa1 was activated mainly by Ca2+ entering through N-type channels and was largely apamin-sensitive but partly iberiotoxin-sensitive. In contrast, gKCa2, which was substantially inhibited by blocking CICR with ryanodine, was blocked partly by iberiotoxin and partly by apamin and was partly insensitive to these and other K+ channel antagonists, but had invariant kinetics. Ca2+ for activation of CICR to produce gKCa2 originated from P-type, L-type and N-type channels. Thus Ca2+ entering through different subtypes of channel is destined to activate particular K+ conductances. Further gKCa2, although kinetically similar to a similar conductance underlying the AHP in hippocampal neurones, differs in that it involves multiple subtypes of Ca2+ and K+ channel.

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SEX DIFFERENCES IN SENSITIVITY TO PENTYLENETETRAZOL IN ADULT SWISS MICE.

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In the present study we tested 71 male and 75 females adult Swiss mice for sex-differences in epileptic susceptibility to PTZ. The doses used (i.p. injected) were 40 mg/Kg (21 males, 20 females); 50 mg/kg (35 males, 39 females); 60 mg/Kg (15 males, 16 females). The tests were recorded with a video-camera and two independent observers analyzed the recordings. The behavior of each animal was classified in: 1) no abnormal behavior; 2) myoclonic jerks; 3) running bouncing (RB) clonus; 4) tonic hind limb extension. There was a high interobserver reliability (Pearson correlation coefficient; r = 0.96). A sex difference was observed in the 50 mg/Kg dose: males exhibited a behavior distribution with 48.6% normal, 11.4% myoclonic, 40% RB clonus and 0% hind limb extension, whereas females exhibited a different behavior distribution (2.6% normal, 12.8% myoclonic, 82.1% RB clonus and 2.6% hind limb extension). These results suggest that females are more susceptible to PTZ induced seizures than males.

CLINICAL STUDIES OF BINOCULAR RIVALRY

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We have recently reported that the rate of perceptual alternation in binocular rivalry (BR) is slow in euthymic, medicated and unmedicated individuals with bipolar disorder compared with control subjects[1]. A test-retest correlation of greater than 0.8 suggests that slow BR rate may represent a reliable trait-dependent biological marker for bipolar disorder. Here we report data from ongoing clinical studies. Bipolar subjects (n=38, median=0.27Hz) remain highly distinguishable from controls (n=101, median=0.59Hz, $p<0.0005$) by BR rate. We have observed slower than normal switch rate in unipolar depressives (n=10, median=0.38Hz) and slow BR rate in some unaffected relatives of bipolar probands. In monozygotic twins, preliminary data further supports a genetic contribution to alternation rate (n=16 pairs, r=0.72). We are currently assessing BR rate in schizophrenia and state-dependency of switch rate in bipolar inpatients. We have postulated that the molecular defect in bipolar disorder is reduced cationic channels in neurones responsible for interhemispheric switching[1]. Based on the perceptual interference effects of unilateral caloric vestibular and transcranial magnetic stimulation, we have suggested that interhemispheric switching mediates the alternations of both BR and reversible figures such as the Necker cube[2]. Our finding of slow BR rate in bipolar disorder is therefore consistent with the same finding for Necker cube switch rate reported some 66 years ago[3].

1. Pettigrew, J.D. & Miller, S.M. (1998) A 'sticky' interhemispheric switch in bipolar disorder? *Proc. Roy. Soc. Lond.* B., 265, 2141-2148.
2. Miller, S.M., Liu, G.B., Ngo, T.T., Hooper, G., Riek, S., Carson, R.G., Pettigrew, J.D. Interhemispheric switching mediates perceptual rivalry. *(submitted).*
3. Hunt, J.M. & Guilford, J.P. (1933) Fluctuation of an ambiguous figure in dementia praecox and in manic depressive patients. *J. Abnorm. Social Psychol.*, 27, 443-452.

A CASE WITH NEURO-BEHCET'S SYNDROME PRESENTING WITH DENSE AMNESIA

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The neuropsychiatric symptoms of neuro-Behcet's syndrome include personality change, mood change, intellectual decline and memory impairment. We present a patient with neuro-Behcet's syndrome whose clinical symptom was limited to dense amnesia.

Case report. A 48-years-old male public official had suffered from abdominal pain and recurrent aphtous and skin eruptions for 6 years when he was diagnosed as having gastro-intestinal Behcet's syndrome in 1996. In April 1998, he was noticed to be forgetful and unable to complete his work sufficiently. Physical, neurological and neuropsychological examination did not show abnormalities except for dense amnesia and frontal-executive dysfunction. General intelligence was well preserved. He was indifferent to his cognitive deficit. CT scan and MRI were essentially normal, but SPECT demonstrated hypoperfusion in the frontal regions and the brain stem. PET showed significant hypoperfusion/hypometabolism in the cerebellar corteces. Mild elevation of protein level was noted in the cerebrospinal fluid.

Conclusion. The present case suggests that neuro-Behcet's syndrome may present with chronic and stable amnesic state similar to Korsakoff syndrome without other focal neurological signs.

ORIENTATION DETECTION AND RELATED VISUOMOTOR PERFORMANCE IN A V6/V6A-LESIONED MONKEY

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It is not clear which cortical areas are responsible for orientation detection and wrist rotation. In order to study the role of areas V6/V6a in these processes, we trained an adult, female *Cercopithecus aetiops* to detect the orientation of a central grating and to signal it by touching corresponding points on a touch screen. A second test required picking up pieces of food from a narrow slit presented at different orientations. After the training period, the monkey underwent to a restricted cortical lesion, first in the anterior bank of the parieto-occipital sulcus of the left hemisphere, and then, after two months, in the corresponding region of the right hemisphere. The tests, repeated after each lesion, demonstrated that damage to the V6 complex compromised the correct orientation of the contra-lesional wrist only, while orientation detection deteriorated more seriously only after the right-hemisphere lesion. It is to be supposed, therefore, that wrist orientation and orientation detection are governed by at least partly separated modules and use different lateralization logic. Experiments are in progress to validate these observations in more animals.

DEVELOPMENT AND DISTRIBUTION OF ZINC TRANSPORTER IN THE MOUSE CNS.

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Zinc exhibits a diverse array of functions in the mammalian brain, including acting as an enzyme co-factor and as a key component of zinc finger transcription factors. We have examined the distribution of a newly cloned zinc transporter, ZnT-1, during pre- and postnatal development, using immunocytochemistry and western blots. ZnT-1 positive cells were observed as early as E-13. In the postnatal brain, neuronal perikarya and processes immunoreactive for ZnT-1 were most heavily concentrated in the cortex, hippocampus, globus pallidus and cerebellum. In these areas, patterns of ZnT-1 immunoreactivity were revealed with respect to stage of development, region and cell layers. Neurons in layers II and IV of the cortex, for example, exhibited robust staining relative to other layers. Labeling was largely eliminated by prior incubation of the antiserum with the ZnT-1 immunizing peptide. ZnT-1 also co-localized with the synaptic vesicle protein, synaptophysin in axon terminals. Co-localization of ZnT-1 with specific calcium-binding proteins was also observed in neuronal processes and somata in several areas and with the neuroglial marker GFAP throughout the CNS, with heavy concentrations in the corpus callosum and external capsule. Differences in the distribution of labeled elements were confirmed in western analysis of tissue samples taken from different brain regions. Interestingly, an apparent nuclear to cytoplasmic shift of ZnT-1 intracellular distribution was observed between P6 and P9 in several regions. The early appearance and the wide and asymmetric distribution of ZnT-1 in the mouse brain suggest involvement in numerous activities influencing development and function in the mammalian CNS.
Supported by a Ben-Gurion Research & Development Grant.

LACK OF ZOLPIDEM ANXIOLYTIC ACTIVITY IN COMPARISON TO DIAZEPAM EFFECTS IN CHRONICALLY ETHANOL TREATED RATS

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In our previous study the positive ZPD influence on memory performance tasks using passive avoidance test in chronically ethanol treated rats [ECTR] was found (Mikolajczak *et al.*, in press). Due to the impossibility of ruling out the sedative or anxiolytic component from the used test (Griebel *et al.*, 1996) it prompt us to evaluate ZPD anxiolytic and sedative properties in ECTR groups. *Materials&Methods:* ETOH preferring [PRF], nonpreferring [NPF] and control [CR] - male Wistar rats were treated with ZPD (2.0 mg/kg, *po*) or DIAZ - (2.0 mg/kg, *po*) for 21 consecutive days (21x). For the evaluation of anxiolytic properties the two-compartment exploratory test was used (Crawley, 1981). Assessment of sedative activity was performed by measuring the locomotor activity paradigm and a sleep time period produced by ETOH (3.0 g/kg, *ip*). *Results:* The decrease of locomotor activity of 21xZPD treated rats in all investigated groups was observed, whereas no effect of 21xDIAZ administration was found. DIAZ treatment led to a prolongation of ETOH-induced sedation in PRF and NPF groups while ZPD-induced prolongation of ETOH sedative effect in PRF rats was only noticed. In the experiment with ZPD and DIAZ effect on performance of anxiolytic tasks it was found that DIAZ showed the anxiolytic activity in CR animals and partially in PRF rats. ZPD treatment led to worse fulfillment of these tasks in all groups. *Conclusions:* These results suggest that ZPD has rather no anxiolytic properties in all investigated groups and probably sedative component of its action affects anxiolytic responses.

SEXUAL BEHAVIORS AND NEURAL STEROID RECEPTORS IN FEMALE ZUCKER RATS

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This study was designed to test the hypothesis that deficiencies in neural steroid hormone receptors contribute to obese Zucker rats' behavioral hyporesponsiveness to ovarian steroid hormones. Ovariectomized (OVX) lean and obese Zucker females were tested for sexual behaviors following injections of physiological (15 μg/kg) or supraphysiological (100 μg/kg) doses of estradiol benzoate (EB) followed by progesterone (P, 2 mg/kg). Subsequently, females received vehicle, 15 μg/kg or 100 μg/kg EB prior to perfusion for progestin receptor (PR) immunocytochemistry (ICC). Other OVX fat and lean Zuckers were perfused for estrogen receptor$_\alpha$ (ERα) ICC. Following treatment with 15 μg/kg EB+P, lordosis quotients (LQs) were significantly lower in obese (39 ± 10) vs. lean females (76 ± 10). 100 μg/kg EB+P induced LQs in obese females (49 ± 11) not different from those in lean animals receiving 15 μg/kg EB+P. No genotypic differences in neural ERα-immunostaining were found. EB injection (vs. vehicle) induced PR-immunoreactivity (PR-IR) in both genotypes. After injection with 15 μg/kg EB, lean rats had significantly more PR-IR cells in the medial preoptic area (MPOA) than obese females (904 ± 102 vs. 544 ± 112). However, obese females receiving 100 μg/kg EB had similar numbers of PR-IR cells (898 ± 95) in the MPOA as lean females injected with 15 μg/kg EB. These parallel differences in MPOA PR-IR and sexual receptivity in obese vs. lean females are consistent with the hypothesis that MPOA PR deficiencies contribute to behavioral hyporesponsiveness to physiological doses of ovarian hormones in obese Zucker females. (Supported by NSF IBN-9720633.)

THE INFLUENCE OF GENDER AND ETHNICITY ON THE EXPRESSION OF CANNABINOID RECEPTORS AND THEIR GENE TRANSCRIPTS

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There is overwhelming experimental evidence for an endogenous marijuana (cannabinoid) system in the brain and peripheral organ systems in humans. This previously unrecognized elaborate network provides a new means for systematically studying the biology of marijuana use/abuse/dependence and the role of the cannabinoid system(s) in normal physiology. Previous studies in women (Kendler and Prescott: Am. J. Psy., 155: 1016-1022, 1998) and in men (Tsuang et al., Am. J. Med. Genet. 67: 473-477, 1996) report that heavy cannabis use and dependency are highly heritable (with genetic basis) and that women have similar rates as men in the use of cannabis. Blood samples (6 ml) were obtained from 23 adult male and female subjects who did not meet DSM-IV criteria for alcohol or marijuana dependence. The samples were analyzed for CB1 and CB2 cannabinoid receptor (Cnr) gene expression using PCR and blot hybridization. Western blotting analysis was performed on the samples using Cnr CB1 polyclonal antibody. The results obtained show that the human Cnrs and their gene transcripts can be analyzed in blood samples when combined with PCR. Primer pairs from CB1 and CB2 cDNA coding region sequences showed identical amplified DNA band sizes in both DNA-PCR and reverse PCR, with human templates, suggesting that these genes are intronless at least in their coding regions. The advantage of being intronless in the coding region may have implications related to the biological functions of these proteins. The expression of Cnrs appears to vary according to ethnic background with whites>blacks>Asians. While the implication of these findings is subject to a number of speculations, the data suggest the existence of an elaborate human cannabinoid system that can be exploited therapeutically.

IN-VITRO AND IN-VIVO ACTION OF CANNABINOIDS

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In our continuing effort to understand the molecular basis of the neurobehavioral effects of cannabinoids we are using multidisciplinary approaches to study cannabinoid receptor (Cnrs) gene expression and function. We are testing the general hypothesis that the Cnr, CB1 alone may not be responsible for all the myriad cannabinoid induced neurobehavioral alterations in mice. In this study we have used mouse in-vivo models of motor function and anxiety tests and in-vitro Xenopus laevis oocytes expression system to examine the influence Cnr agonists and the antagonist SR 141716 for CB1. For the in vivo study the effect of SR 141716A (0.03-3.0 mg/kg) in 2 mouse models of anxiety was evaluated. The ability of the antagonist to block the effect of the Cnr agonist, methanadamide was also determined. SR141716A induced an anxiolytic profile that was dependent on strain and the test utilized. The acute anxiogenic and cataleptogenic effects of methanadamide were antagonized by pre-treatment with SR141716A. In the voltage clamp studies we investigated the effects of anandamide on recombinant AMPA GluR3 subunit currents generated by kainic acid in oocytes expressing the AMPA glutamate receptor. We present evidence that anandamide inhibited the kainate activated currents in oocytes expressing the AMPA glutamate receptor via a cannabinoid independent mechanism. WIN 55,212-2 and arachidonic acid had no significant effects on the kainate activated currents. SR141716 had no effect on the anandamide inhibition. However, the effect of anandamide was potentiated by forskolin whereas MDL-HCl reversed the anandamide effects. These findings indicate that SR141716A is a putative anxiolytic in vivo. The in- vitro study indicate that anandamide's action which may involve cAMP transduction is independent of a Cnr mechanism. ESO is supported by NHLBI-HL03319.

IONOCHROMIC PROPERTIES OF LONG-WAVE-SENSITIVE CONES IN THE GOLDFISH RETINA

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Vertebrate visual pigments with λ_{max} longer than about 520 nm are sensitive to the concentration of Cl^- ions in their ionic environment. Reduction of Cl^- concentration causes a spectral displacement to shorter wavelengths, e.g. a rhodopsin λ_{max} normally at 565 nm is hypsochromically shifted to about 540 nm in a Cl^--free environment. The question we have addressed is whether the spectrally altered pigment is still able to initiate transduction. Microspectrophotometry was used to demonstrate that the ionochromic effect occurred in the LWS cone porphyropsin of the goldfish, *Carassius auratus*.

Measurements of the aspartate-isolated late receptor potential were used to determine the functional properties of the pigments. Responses to alternate red (675 nm) and green (530 nm) flashes were recorded from isolated retinas either incubated or perfused with normal cyprinid Ringer's solution or salines in which the Cl^- concentration were varied by replacement of chloride with gluconate. Under photopic conditions the red flash will stimulate primarily the LWS cones (λ_{max} 620 nm), but the green flash will stimulate both the LWS and MWS cones (λ_{max} 535 nm). If reduction in Cl^- concentration leads to a functional spectrally-displaced LWS pigment, then we would expect to see a reduction in response to the red flash, but an increase in response to the green flash. In contrast, if the spectrally displaced LWS is nonfunctional, both the green and red responses should be reduced. In Cl^- free solution, responses to the red flash were reduced to almost zero, whereas responses to the green flash were also reduced, but only to about 60% of normal. The effect was reversible and dependent on Cl^- concentration. Under scotopic conditions, when only rods were stimulated, no ionic effect was observed. Our data support the hypothesis that the spectrally shifted LWS pigment is nonfunctional. We presume that removal of Cl^- from the binding pocket in the in he extracellular loops of LWS opsin leads to a conformational change that not only causes a spectral shift, but also inhibits the activation of transducin.

Key words: visual pigment, spectral shift, transduction.

RESTRAINT STRESS UPREGULATES GLUCOCORTICOID BINDING IN MOUSE SPLEEN CELLS

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Reciprocal pathways of interaction between the nervous and immune systems during stress may be regulated by stress-induced secreted glucocorticoids (GC) that act via type II GC-receptors (GR). The aim of the present study was to investigate whether restraint stress is capable of upregulating GR in lymphocytes as a mechanism of negative feedback. We used male Balb/C mice which were adrelalectomized seven days before exposure to restraint stress. The results showed that exposure to 4 hrs of restraint stress caused a significant increase in the binding of [^{3}H]-Dexamethasone to GR in the cytosol of splenocytes (Bmax= 43 fmol vs 72 fmol) but not in the binding affinity (Kd= 21 nM vs 23 nM). In correlation with this increase in binding, restraint stress caused an increase in the amount of GR protein as determined by western blot using specific anti GR antibodies. We wanted to establish the relation of the nervous system to this stress induced effect. To this aim we blocked the autonomic innervation to the spleen with the ganglionic blocker chlorisonedamine. The treatment abrogated the stress induced increase in the binding of [^{3}H]-Dexamethasone to GR and in the GR protein levels. These results suggest that the stress induced increase in the level of GR is mediated by the sympathetic nervous system. It is possible that stress modulation of lymphocyte GR levels may be implicated in the bidirectional communication between the nervous and the immune systems.

NITRIC OXIDE (NO) AND HYPEROSMOTIC BLOOD BRAIN BARRIER DISRUPTION (HBBBD).

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The mechanism by which the blood-brain-barrier BBB is modulated is not clear although control of blood pressure is crucial to disruption. In the present study we wanted to investigate the contribution of central nitric oxide to the osmotic disruption of the blood brain barrier. We administered L-NAME, or aminoguanidine (NOS inhibitors) and either NOR-3 or NOR-4 (NO donors) into the left cerebral ventricle (ICV) of Fischer rats. BBB disruption was achieved by intracarotid injection of mannitol (25%). The extent of disruption was evaluated quantitatively by Evans blue (EB 2%) dye extravasation. ICV injection of saline followed by intraarteial mannitol resulted in EB leakage into the ipsilateral hemisphere of 111.6 ± 10 (μg EB/mg tissue) vs 11.9 ± 2 in the contralateral side. ICV injection of L-NAME (without BBBD) abrogated the cerebellar NOS activity and caused an increase in mean arterial blood pressure. BBBD following ICV injection of L-NAME induced increase in EB leakage into both hemispheres: ipsilateral, 178.3 ± 20 (60% increase), contralateral 45.5 ± 5 EB μg/mg tissue. ICV injection of aminoguanidine (inhibitor of inducible NOS) did not change the blood pressure and did not influence the leakage of EB into the disrupted hemisphere. Administration of either NOR-3 or NOR-4 (150 μg) caused a moderate fall in blood pressure (95 ± 4 mmHg) and a marked inhibition of the EB leakage into the disrupted hemisphere (20 μg EB/mg tissue). Inhibition of BBBD by the NO donors showed a clear cut dose response relationship. We conclude that BBBD is associated with significant inhibition of constitutive brain NOS activity but not the inducible NOS. Exogenous extracellular NO causes a dose dependent inhibition of BBBD. Inhibition of brain NOS activity facilitates the BBBD. Manipulation of the NO system using NO donors or NO inhibitors may be relevant for relative delivery of drugs into the normal parenchyma or into brain tumors.

FEATURE SEARCH: A DRAMATIC DIFFERENCE BETWEEN CENTRAL AND PERIPHERAL CAPABILITIES

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Feature search for a light bar with one orientation (or color) embedded in an array of bars with a very different orientations (or color) is quick, easy and independent of the number of array elements. Search for a conjunction task has a linear response time dependence on the number of distractors. We report that these properties are not valid for eccentric stimulus presentation. Subjects were tested on orientation and color pop-out tasks, and a conjunction search with arrays of 3x3, 5x5 or 7x7 elements. Orientation pop-out stimuli were arrays of vertical white bars on a black background with a diagonal target present on half of the trials. Color pop-out stimuli consisted of 60°-oriented bars with blue distractor and yellow target elements on a gray background. For conjunction task we used as a target a yellow line element with an orientation of 60° embedded in a group of blue elements with the same orientation and yellow elements with an orientation of 30°. The arrays were presented centrally or peripherally in the right or left hemifield at eccentricities of 0°, 1.5°, 3° and 4°. Most subjects have better and worse hemifields. During a second testing session improvement was so much greater for the non-preferred hemifield that sometimes the preference was switched. Surprisingly, preferred hemifield performance actually declined for some subjects. Thus, the effect seems related to competition, and perhaps an automatic attention directing mechanism. We reconfirmed the central presentation set-size independence but found a great difference between large and small arrays when presentation was lateral. There are two sources of this array size effect: 1- Target eccentricity, demonstrated by comparing performance for different target locations with the same array size. 2- Target location uncertainty, seen by comparing performance for different size arrays when the pop-out elements appeared at the same locations.

PRESERVED PERCEPTUAL GROUPING IN BRAIN-DAMAGED PATIENTS WITH VISUAL EXTINCTION

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Extinction is manifested in conditions of bilateral simultaneous stimulation, as a failure to detect the stimulus contra-lateral to the side of a cerebral lesion, while the same stimulus is correctly detected there when presented in isolation. We have recently shown, using pairs of Gabor patches as stimuli, that pair detection is possible when the two stimuli are proximal, co-oriented and co-axial (Pavlovskaya, Sagi, Soroker, Ring, 1997 *Cognitive Brain Research* **6** 159-162), indicating preserved pre-attentive grouping interactions. Here we show that these interactions are contrast dependent. Eight patients were tested with briefly presented stimuli containing one or two targets, each being a horizontal or vertical Gabor signal, having to report the number of detected targets (0, 1, 2). Contrast (high and low) was manipulated using iso- or aniso-contrast pairs. We found that severity of extinction was affected by the relative saliency of both stimuli (being more severe when contrast of ipsi-lesional signal exceeded that of the contra-lesional one). The further advantage of co-oriented co-linear stimuli was shown exclusively with iso-contrast stimulus pairs, and was significantly enhanced when the contrast level of the stimulus pair was low. The findings suggest that extinction (attentional rivalry) ameliorate when stimulus properties tend to enhance perceptual grouping. This correlates with the response characteristics of long-range interactions observed in the primary visual cortex. The present demonstration of contrast dependency in such processing strengthens our previous conjecture that even in the presence of significant, extinction producing, parietal damage, the primary visual cortex preserves the capacity to encode, using long-range lateral interactions, an image description in which visual objects are already segregated from background.

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ACTIVITY OF SPINOCEREBELLAR PURKINJE CELLS DURING PASSIVE FORELIMB MOVEMENTS IN ANESTHETIZED RATS.

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The present study was carried out to investigate the sensory representation of arm movements in the activity of spinocerebellar Purkinje cells of anesthetized rats. The left hand of the rat was attached to a computer controlled robot arm programmed to move the rat limb passively through circular hand paths at constant speed. The activity of Purkinje cells located in lobules 3-4-5 and in the paramedian lobe was extracellularly recorded and histograms of simple-spike activity from responses to 4 consecutive movement cycles were computed. Principal components (PC) analysis was used to quantify the variety of Purkinje cell responses.It was observed that the activity of 55/180 neurons was significantly modulated by forelimb movements. PCs analysis showed that Purkinje cell responses exhibited a strong first PC capable to explain the 65.6% of the total variance; higher order PCs explained each a fraction of the variance that never exceeded 5%. All responses showed a strong positive correlation with the first PC, suggesting that Purkinje cells throughout the spinocerebellum responded with the same pattern. As a matter of fact, all cycle histograms showed a firing rate increase while the arm was moved forward and downward, and almost constant level of firing during the rest of the cycle. The possibility that the activity of spinocerebellar neurons could reflect the movement speed was also examined. Average movement speeds of 0.6 cm/sec and 0.4 cm/sec were used, in separate blocks of trials, to quantify the speed sensitivity of Purkinje cells. For each neuron, we computed the percent firing rate variation between responses to two independent blocks of movement cycles at different speed or at the same speed. We observed that only one subgroup of Purkinje cells shows movement speed sensitivity and that the overall sensitivity across neurons is rather weak.The present results suggest that a transformation of sensory information occurs in the cortex of spinocerebellum. This transformation may reduce the multiplicity of spatial tuning observed across precerebellar spinal neurons into a unique and stereotyped representation of arm movements.

MEMBRANE DEPOLARIZATION INDUCED PRESYNAPTIC [CA2+] ELEVATION AND NEUROPEPTIDE RELEASE FROM NERVE TERMINALS VIA COUPLING BETWEEN L-TYPE Ca2+ CHANNELS AND RYANODINE RECEPTORS.

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The presynaptic C nerve terminals in bullfrog sympathetic ganglia release a neuropeptide, luteinizing hormone-releasing hormone (LHRH). In half of these terminals, Ca2+ released through the ryanodine receptor channels is necessary to raise the intraterminal [Ca2+] beyond the threshold level required for LHRH release (Peng, 1996, J. Neurosci. 16:6703-6712). An increase of this Ca2+ release by caffeine (10 mM) enhances the amount of LHRH release (Cao and Peng, 1999, Neurosci.). I tested whether membrane-depolarization alone could induce Ca2+ release through the ryanodine receptor channels to evoked Ca2+ elevation and LHRH release from these nerve terminals. Intraterminal [Ca2+] was monitored by fluorimetry of fura-2-filled nerve terminals. When Ca2+ influx through the voltage-gated Ca2+ channels was prevented by bathing the ganglia with solutions that have no added Ca2+ and Mg2+ (4 mM) or Co2+ (4 mM) or in Ca2+-containing Ringer's solution (1.8 mM) with added Cd2+ (400 mM), electrical stimulation to the presynaptic nerves evoked 84±8% (mean±s.e., n = 9), 45±11% (n = 13), and 19±2% (n = 19) of the net peak Ca2+ elevation in the Mg2+, Co2+, and Cd2+ solutions, respectively. Ca2+ transients in these solutions were blocked by either nifedipine (10 mM) or ryanodine (10 mM). WhenLHRH release was monitored as LHRH-ergic epsps in the postsynaptic neurons, 82±13% (n = 15), 35±8% (n = 3) and 24±3% (n = 7) of the peak amplitudes remained in the Mg2+, Co2+, and Cd2+ (200 mM) solutions, respectively. These LHRH-ergic epsps were completely blocked by10 mM ryanodine whereas the postsynaptic cells still responded to exogenous LHRH. Thus, direct mechanical coupling between L-type Ca2+ channels on the plasma membrane and the ryanodine receptors induced Ca2+ release through the latter which was sufficient for LHRH release. This work was supported by the Alfred P. Sloan Foundation and by NIH (NS32429).

STUDY OF PHYSIOLOGICALLY ACTIVE POLYPEPTIDES SECRETED BY NERVE CELLS IN DIVERSE FUNCTIONAL STATES.

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During electrophysiological experiments on surviving rat brain olfactory cortex slices, the perfusates corresponding to four functional states: quiet, low-frequency stimulation, posttetanic potentiation and posttetanic depression, were collected. It was shown that the potentiated perfusates evoke depression in intact slices, and vice versa, the "depressive" perfusates evoke potentiation in intact slices. This physiological activity is attached to polypeptides, because the perfusates treated with immobilized trypsine showed no physiological activity. Electrophoretic analysis of the perfusates showed differences in polypeptide spectra secreted in diverse functional states (see Mokrushin, Plekhanov in: Neurochemistry, ed. Teelken and Korf, Plenum Press, N.Y., 1997, p. 519-521).

Immunochemical study of the perfusates showed that BASP1 (NAP-22, CAP-23) synaptic protein and HMG1/2 proteins are secreted by nerve cells in all the functional states studied, and GAP-43 presynaptic protein (B-50, neuromodulin) is not secreted. The secretion of HMG1 and/or HMG2 is minimal in quiet, but is significantly increased even by minimal electrophysiological activity.

EFFECTS OF DEXAMETHASONE AND MIFEPRISTONE IN RAT NEONATAL MODEL OF AXOTOMY-INDUCED MOTONEURONAL CELL DEATH

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Axotomy is a widely used model for studying the mechanisms of the neuronal cell injury and for testing the effects on the motoneuronal survival of various agents. In this study have been determined the effects of dexamethasone and the glucocorticoid receptor antagonist mifepristone (RU486) on the motoneuronal cell death and the nuclear and somatic morphology changes occurring after peripheral nerve transection in the neonatal rats. The study was performed on 3 days old Wistar rats. Animals were divided into 4 groups - control, axotomised, axotomised and dexamethasone-treated and axotomised and mifepristone-treated. The nereve transection was performed bilaterally. On day 7 after the operation the animals were sacrificed and the motoneurons in segments L4 and L5 in the spinal cord were counted and their morphology was analysed. 25.88% cell loss was found in the axotomised group ($p<0.001$ vs. control). Dexamethasone significantly decreased the number of the surviving motoneurons yielding 43.33% cell loss in the dexamethasone-treated and axotomised animals ($p<0.05$ vs. axtomised), while mifepristone had no such effect ($p>0.05$ vs. axotomised). Dexamethasone induced shrinkage of the lesioned motoneurons while mifepristone abloished this effect. Our results propose a possible hazard towards the application of dexamethasone in the treatment of the new-borns with concomitant nerve injuries.

DIFFUSION COEFFICIENTS OF POLYMERIC DRUG CARRIERS IN THE EXTRACELLULAR SPACE OF THE BRAIN MEASURED BY INTEGRATIVE OPTICAL IMAGING

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Diffusion properties of two types of copolymers of N-(2-hydroxypropyl)methacrylamide (HPMA), developed as water-soluble anti-cancer drug carriers, were studied in rat cortical slices: a) HPMA polymeric chains with M_r = 220 kDa and b) star-like systems, containing either albumin (179 kDa) or immunoglobulin (IgG) (319 kDa) in the center with HPMA side branches [1]. Using the integrative optical imaging method [2] and pressure microinjection of fluorescein-tagged polymers, we determined the apparent diffusion coefficients (*ADC*) in rat cortical slices and compared them with the free diffusion coefficients (*D*) in 0.3% agarose gel.

For HPMA-polymer chains, $D = 2.03 \pm 0.02$ and $ADC = 0.75 \pm 0.02$. For star-like copolymer HPMA-albumin, $D = 2.34 \pm 0.05$, $ADC = 0.45 \pm 0.01$, and for HPMA-IgG, $D = 1.19 \pm 0.02$, $ADC = 0.26 \pm 0.01$ ($\times 10^{-7}$ cm^{2}s^{-1}, mean ± S.E.M.). These data imply that the extracellular space tortuosity of the cortex, defined as $\lambda^2 = D/ADC$, is 1.58 ± 0.07 for HPMA-polymer chains, while for star-like copolymer HPMA-albumin, $\lambda = 2.27 \pm 0.06$, and for HPMA-IgG, $\lambda = 2.12 \pm 0.06$. The tortuosity for HPMA chains is therefore smaller than the tortuosity for star-like copolymers and is also smaller than those previously obtained for dextran (M_r = 70 kDa) [2] or albumin (M_r = 66 kDa) [3].

Our data suggest that rather than M_r, the shape of the anti-cancer drug carriers is the limiting factor in their movement through the extracellular space.

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[1] Ulbrich, K., J. Bioac. Comp. Polymers 6, 348-357, 1991.
[2] Nicholson, C. & Tao, L., Biophys. J. 65, 2277-2290,1993.
[3] Tao, L. & Nicholson, C., Neurosci. 75: 839-847, 1996.

SEROTONIN STIMULATORY CONTROL OF THE GnRH NEURON MIGRATION IN RAT FETUSES

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This study has evaluated the possible influence of serotonin (5-HT) on the GnRH neuron migration in rat fetuses. For this aim, the GnRH was measured by radioimmunoassay in the rostral forebrain and in the rostral hypothalamus of fetuses on the 16th embryonic day (E16) and E21 following daily systemic administration of p-chlorophenylalanine (pCPA), an inhibitor of serotonin synthesis, to pregnant rats from E11 up to E15 or E20. Moreover, the quantitative immunocytochemistry was used to estimate the fractions of GnRH neurons along the trajectory of their migration at E18 following the inhibition of 5-HT synthesis from E11 till E17. The control animals received saline. On E16 the GnRH concentration in the rostral forebrain highly exceeded that in the hypothalamus both in the control and pCPA-treated fetuses suggesting an accumulation of GnRH neurons in the former in the course of their migration towards the latter. On E21 in pCPA-treated fetuses, the GnRH concentration remained significantly higher in the rostral forebrain than in the hypothalamus whereas there was a reverse in control fetuses. These data suggest the deceleration of the GnRH neuron migration under 5-HT deficiency or, in other words, the stimulatory influence of 5-HT on this process. This hypothesis was proved by our quantitative immunocytochemical observations. Namely, the fraction of GnRH neurons in the rostral forebrain occurred to be significantly higher in pCPA-treated fetuses compared to the control fetuses. However, there was a reverse for the hypothalamic fractions of GnRH neurons in pCPA-treated and control fetuses. Thus, 5-HT appears to stimulate the GnRH neuron migration to the places of their final settling in the septo-preoptic area.

EXPRESSION OF CLINICALLY-DERIVED MUTATIONS IN THE L1 NEURAL CELL ADHESION MOLECULE IN CELLS OF THE CNS

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The L1 cell adhesion molecule is an important player in the formation of CNS connections and structures during development. Mutations in the human L1 gene result in CNS malformations that are linked to a variety of neurologic syndromes, including mental retardation. Distinct L1 mutations can generate a spectrum of clinical signs and symptoms for unknown reasons. L1 knockout mice exhibit some of the same CNS defects. To begin to understand how pathogenic L1 missense mutations affect L1 protein function, we are expressing full-length mutant L1 in cells of the CNS using defective herpes simplex virus (HSV) vectors. Defective HSV vectors are an efficient means to introduce genes into post-mitotic cells in vitro and in vivo. We have generated human L1-expressing vectors encoding the single amino acid mutations R184Q, D598N and S1194L. Infection of astrocyte cultures results in the expression of full-length L1 protein. However, the L1 proteins with mutations in the extracellular Ig-like domains (R184Q and D598N) are not expressed on the cell surface and have altered mobility on SDS-PAGE. Expression of wild-type L1 in astrocytes provides a permissive substrate for neurite outgrowth and neuronal migration of cerebellar granule cell neurons in vitro. Among the mutants, only the cell surface expressed L1 mutant, S1194L, is able to support enhanced neurite outgrowth. This suggests that the inability of some L1 mutations to reach the cell surface might be responsible for the observed clinical symptoms, rather than altered function of the mutated extracellular domain.

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LEVELS OF AMINO ACID NEUROTRANSMITTERS DURING MOUSE OLFACTORY BULB NEUROGENESIS AND IN HISTOTYPIC OLFACTORY BULB CULTURES

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The developmental changes in the levels of amino acid neurotransmitters were analyzed by HPLC during mouse olfactory bulb neurogenesis, from embryonic day (E) 13 until young adult age, between postnatal day (P) 30-40. During the embryonic period, between E13-21, high levels of excitatory Glu and Asp as well as the inhibitory GABA transmitters were obtained, with the values of GABA about two-fold higher than those of Glu and Asp. At birth (P0), the levels of these transmitters decreased significantly by about two-fold indicating the influence of birth stress on these neurotransmitters. During the first two postnatal weeks, the Glu content increased gradually showing maximal values at P5 and P11, and then diminished thereafter to adult levels. The Asp contents were about half-fold the values of Glu and significant increments were seen at P3 and in young adult age. The postnatal GABA concentrations had almost equivalent values as those of Glu, varying slightly during the first two postnatal weeks with peak levels at P3 and P11. The relation between the glutamatergic and GABAergic synaptic transmission during olfactory bulb development, however, can be more clearly seen from the calculated Glu/GABA ratio showing a constant relation of 0.5 from E15-P3 and ±1.0 from P5 until young adult stage. These data suggest that inhibitory GABAergic predominates over excitatory glutamatergic synaptic transmission from E15 until P3, and thereafter, the unique dendrodendritic reciprocal synaptic interactions in the olfactory bulb start to have influence on sensory processing, coinciding with the period of maximum synaptogenesis in the mouse olfactory bulb. On the other hand, relatively low and constant levels of Gly were obtained throughout the studied developmental period, whereas the Tau contents increased gradually from very low concentrations at E13 to about the same levels as GABA and Glu in the young adult animal. The intrinsic neurotransmitter production was also studied by analyzing their contents in primary histotypic olfactory bulb cultures prepared at P10 and comparing the in vitro levels with the results obtained in young adult animals. The comparative analysis demonstrated that there is a high endogenous Gly production and that most of the Tau are extrinsic to the olfactory bulb. Moreover, the Glu/GABA ratio showed homologous results in vitro and in situ, indicating that an equal proportion of Glu and GABA operates intrinsically in the olfactory bulb internal circuitry and that this region of the brain receives afferent excitatory as well as inhibitory innervations also in equal proportion.

PURSUIT ROTOR PERFORMANCE: EFFECTS OF AGE ARE MEDIATED BY SHRINKAGE OF THE CEREBELLUM AND THE PUTAMEN.

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Objective: To examined age differences in motor performance in conjunction with differential aging of CNS structures. Method: Healthy adults (age 22-80) performed a pursuit rotor task (four blocks of 20 15-second trials each). Volumes of the cerebellar hemispheres, the caudate nucleus, the putamen , the globus pallidus, the dorsolateral prefrontal cortex and the hippocampus were obtained from MRI (SPGR sequence, 124 axial slices, reformatted in the coronal plane). The performance index was time on target (TOT); greater TOT indicated better performance. Results: Linear improvement trend was evident across trials, and across blocks. The slope of improvement was age-invariant. However, younger participants showed significantly longer TOT. In a hierarchical linear regression analysis, only the volumes of the cerebellum and the putamen showed significant association with the mean TOT: reductions in the volume of the cerebellar hemispheres and the putamen predicted shorter TOT. Neither age, nor the volume of the other brain regions showed association with motor performance. Conclusion: In healthy adults who show no clinical signs of motor system disease, procedural learning is preserved, and motor performance is reduced only in individuals with significant shrinkage of the cerebellum and the putamen. Longitudinal study is necessary, however, to rule out possible effects of pre-clinical degenerative disease of the motor system.

AIT-082, A UNIQUE PURINE DERIVATIVE WITH NEUROREGENERATIVE AND NEUROPROTECTIVE PROPERTIES.

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AIT-082 (leteprinim potassium, 4-[[3-(1,6-dihydro-6-oxo-9-purin-9-yl)-1-oxopropyl] amino]benzoic acid, potassium salt), is a hypoxanthine derivative containing a p-aminobenzoic acid moiety. AIT-082 has been shown to improve memory in young adult mice and to reverse age-induced memory deficits in aged mice. It has also been shown, when given in the drinking water, to delay the onset of age-induced memory deficits in mice. These effects were observed across a wide range of doses. AIT-082 has been shown both in vitro and in vivo to increase expression of mRNA for a variety of neurotrophic factors in the brain and spinal cord after aging or injury. In addition, increased expression of neurotrophic factor proteins have been seen in brain tissue. The implications for this expression pattern include use of AIT-082 for nerve regeneration. In addition, AIT-082 has neuroprotective effects when administered after excitotoxic injury. Human clinical trials have been initiated for this compound in patients with mild to moderate Alzheimer's disease. The results of these studies have demonstrated safety at doses up to 4,000 mg and a plasma half-life for AIT-082 in excess of 12 hours. AIT-082 was well tolerated and produced cognitive improvements after single doses in healthy elderly volunteers and after 28 days of treatment in patients with Alzheimer's disease.

STRUCTURAL DETERMINANTS OF α_1-SELECTIVITY IN THE BENZODIAZEPINE BINDING SITE OF THE $GABA_A$ RECEPTOR

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The imidazopyridine zolpidem is a selective hypnotic drug which acts through the same modulatory site of the $GABA_A$ receptor as the benzodiazepines. Studies with recombinant receptors (namely $\alpha_{1\text{-}5}$, $\beta_{2\text{-}3}$, γ_2) have illustrated that α_1-containing receptors exhibit high affinity and α_5-containing receptors exhibit low affinity (Kd>10 µM) for zolpidem. Chimeras between α_1 and α_5 were designed, chosen amino-acids of the α_5 subunit were replaced by their α_1 counterparts by site directed mutagenesis and zolpidem affinity used to map the α_1-selective binding site on these chimeric subunits. One mutant α_5 subunit, bearing P162T, E200G and T204S substitutions, exhibited high affinity zolpidem binding similar to that of the α_1 subunit. To elucidate the interaction between these amino acids and zolpidem, the affinity of this mutant subunit was measured for analogues of zolpidem that differed in their side chain composition. We propose a pharmacophore model where α_1S204 establishes a hydrogen bond with a carbonyl in zolpidem and α_1T162 and α_1G200 have a conformational influence on the binding pocket.

MATERNAL STRESS IMPAIRS NORADRENERGIC CONTROL OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS DIFFERENTLY IN MALE AND FEMALE ADULT RATS

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Perinatal hormone and neurotransmitter influences on the mammalian developing brain are capable of modifying neuroendocrine control of physiological functions in adulthood. Formerly we have found a decrease of pituitary-adrenal cortex response to an acute stress (1 h immobilization) in adult rat male offsprings, not in females, resulted from maternal stress (1 h immobilization daily during a last week of gestation). In particular, the retention of corticotropin-containing granules in the pituitary gonadotropocytes indentified by electron microscopy and a decrease in blood plasma corticosterone level under an acute stress were observed in male offsprings. Following that study, hypothalamic noradrenaline (NA) response to an acute stress was investigated in prenatally stressed (PS) adult males and females (experiment 1). Blood plasma corticosterone levels were measured with 30 min intervals during 90 min in conscious, freely moving offsprings after injection of 10 μg NA bitartrate into the 3rd brain ventricle (experiment 2) or during 120 min after β 1-24 corticotropin challenge (Synacthen-depot, 60 μg/kg b.w. i.m.; experiment 3). 8-9 days before the experiment steel cannula was installed in the 3rd brain ventricle using stereotaxic coordinates. Blood samples were taken from silastic catheter that was placed into external jugular vein 24 hrs prior to the experiment. In contrast to normal males and PS females, PS males did not respond to an acute stress with a depletion of hypothalamic NA content. Surprisingly, PS caused an enhancement of adrenocortical secretion elevation after NA intraventricular injection in males. This reaction was suppressed in female offsprings despite moderate augmentation of corticosterone secretion response under an acute stress. Both males and females have demonstrated normal plasma corticosterone reaction to Synacthen. Conclusions: 1) maternal stress modifies noragrenergic reactivity of HPA axis of male and female offsprings in opposite directions; 2) inhibitory effect of maternal stress on HPA stress-reactivity in male offsprings is not mediated by alteration of noradrenergic reactivity at the hypothalamic level; 3) a failure of the adrenal cortex stress-reactivity in male offsprings of the dams exposed to strict immobilization during pregnancy is caused by the absence of adequate NA release in the hypothalamus.

SELECTIVE NEUROTENSIN ANTAGONISTS, SR-48692 AND SR-142948a POTENTIATE AMPHETAMINE SENSITIZATION PRODUCED BY D-Tyr[11]NEUROTENSIN.

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Previous studies have shown that neurotensin (NT) plays a role in the development of amphetamine (AMPH) sensitization. Blockade of central NT receptors with SR-48692, for instance, prevents AMPH sensitization while repeated central NT receptor activation sensitizes to AMPH. In this study, we tested the effect of two selective NT receptor antagonists, SR-48692 and SR-142948a, on the development of AMPH sensitization produced by D-Tyr[11]NT. Experiments were performed on male rats implanted with a guide cannula above the lateral ventricle. During the initial training phase, locomotor activity was measured on four occasions, every second day (day 1,3,5 and 7) and for two hours, after an ICV injection of 18 nmol/10 μl of D-Tyr[11]NT, or an equal volume of saline; on each training day, rats were pretreated with SR-48692 (160 or 640 μg/kg, ip), SR-142948a (160 μg/kg, ip), or the vehicle. Ambulatory and non-ambulatory movements were measured in plexiglass boxes equipped with 30 infrared photocells. One week after the training phase, locomotor responses to a single injection of AMPH (0.75 mg/kg, ip) were measured in all rats (sensitization test). Results show SR-48692 and SR-142948a potentiated, D-Tyr[11]NT-induced inhibition of locomotion on day 1, and attenuated this effect on day 7. The NT antagonists also potentiated D-Tyr[11]NT-induced AMPH sensitization, and increased sensitivity to locomotor activating effect of AMPH when given alone during the training phase. These results suggest that the development of AMPH sensitization produced by repeated treatments with D-Tyr[11]NT is likely mediated by a sub-type of NT receptor insensitive to SR-48942 and SR-142948a. Supported by MRC Canada.

STIMULUS DISTRIBUTION AFFECTS UNSUPERVISED FORMATION OF VISION-BASED CLASSES

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We are studying mechanisms underlying the formation of perception-based subordinate classes. In order to focus on perceptual processes, we analyzed unsupervised classification learning. We find that in the absence of other cues, subjects base classification on stimulus distribution. Subjects performed a free sorting task during sequential exemplar presentation over many trials and using a large stimulus set. The stimuli were simple and differed only in the value of one continuous property. Different groups of subjects were shown the same range of stimuli, but with different stimulus distributions. They were not informed of the stimulus distribution, the sorting principle or the expected number of classes. No feedback or labeling was given. We found that subjects were not aware of the stimulus distribution, and that nevertheless their pattern of classification reflected stimulus distribution. Subjects tended to use classes centered on stimuli at distribution peaks and with boundaries near distribution minima. There was an edge effect – a tendency to devote one or more classes to stimuli near the edges of the stimulus range. Reaction times were longest near class boundaries, shorter near class centers and at the edges of the stimulus range. Learning rate and consistency level were higher for subjects sorting more informative, multi-peaked distributions than for subjects sorting a uniform stimulus distribution. We conclude that supervision is not necessary for learning classification based on implicit perception of stimulus distribution.

ENHANCED SYNAPTIC TRANSMISSION ASSOCIATED WITH INCREASED ODOR- LEARNING IN THE RAT PIRIFORM CORTEX

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Cellular modifications were studied in rat piriform cortex brain slices following olfactory operand-conditioning. Rats were trained to discriminate positive cues in pairs of odors, until they demonstrated dramatic increase in performing the same task with unfamiliar odors ('rule learning'). Paired-pulse facilitation (PPF) of EPSPs evoked by stimulating the intra cortical axons was significantly smaller in the 'trained' rats, (e.g. at inter-stimulus interval of 50 ms: 1.14±0.11, mean ± SD, n =19 in 'trained' group, vs. 1.33 ± 0.15, n=38 in 'pseudo trained', and 1.27 ± 0.22, n=23 in 'naive' group, $p<0.02$, one way ANOVA test). This PPF reduction, which appeared 3 days after training, returned to control value within 8 days. PPF of EPSPs evoked by stimulating the afferent fibers, originating from the olfactory bulb, increased transiently for 1-2 days after training (1.37±0.13, n =7 in 'trained', vs. 1.21 ± 0.13, n=23 in 'pseudo trained', and 1.22 ± 0.1, n=11 in 'naive' , $p<0.03$). The ratio between stimulus intensity and intra cortical axonal volley, as measured by extracellular recordings (fPSP), did not differ between 'trained' and the control group, indicating that training did not alter the intrinsic axonal excitability in the cortex. However, the ratio between fPSP amplitude and axon volley increased significantly after training. Our data suggest that synaptic transmission is transiently enhanced after learning. This pathway-specific enhancement is related to increased learning capability, which is reduced within few days after training, rather than to the memory for specific odors, which lasts for weeks.
Supported by a grant from the Israeli Science Foundation.

OPTICAL SIGNALS RECORDED FROM BULLFROG SYMPATHETIC GANGLIA USING A VOLTAGE-SENSITIVE DYE: PRESYNAPTIC AND POSTSYNAPTIC COMPONENTS

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Multiple-site optical recording of neural activity, using a fast voltage-sensitive merocyanine-rhodanine dye (NK2761) and a 12 x 12-element photodiode array, was employed to monitor the synaptic activity from many sites in the bullfrog lumbar sympathetic ganglion. When the presynaptic nerve fibers were stimulated, the transmembrane voltage-related optical (absorbance) signals were more compound in shape: the signal showed a duration of 20 - 30 ms with two or three peaks which could be distinguished by their shapes and timing, and their later phase was reduced in a low calcium bathing solution or in a solution containing d-tubocurarine(d-Tc, 0.25 mM). We conclude that the first phase of the optical signals evoked by the presynaptic stimulation corresponds to the electrical activity of the presynaptic neurons. In the presence of d-Tc, the amplitude of this signal was decreased by reducing the concentration of Ca2+ or by the application of Cd2+(1 mM). On the other hand, the signals evoked by the postsynaptic stimulation showed relatively simple shape reflecting the action potential in the ganglion cells: a fast spike-like signal with the duration of about 10 ms was often followed by an undershoot which lasted for about 100 ms. Supported in part by Grants from the Monbu-sho of Japan.

CORRELATION BETWEEN THE SEVERITY OF THE TRAUMATIC SPINAL CORD INJURY AND THE METHILPREDNISOLONE DOSE NECESARY FOR INHIBIT THE LIPID PEROXIDATION PROCESS.

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Methilprednisolone (MP) megadose (30mg/kg of body weight) in the acute phase after a traumatic spinal cord injury (TSCI) is at date the unique treatment accepted for human beens. However, the results are disputables even in experimental models. The reason may be that exists a correlation between the severity of the TSCI and the MP required doses for inhibit the lipid peroxidation process (LPP). The LPP was assessed in the spinal cord of 84 adult rats divided in Group 1: uninjured (n=12), Group 2: rats with light TSCI (n=24), Group 3: rats with moderate TSCI (n=24), Group 4: rats with severe TSCI (n=24). Animals were subjected to a TSCI using the weight-drop contusion model. Five minutes after the TSCI rats received MP as a single dose of 15mg/kg, 30mg/kg or 45 mg/kg of body weight (8 rats of each injured groups for each dose). Twenty-four hours after, the LPP was studied by espectrophotometric and flurometric techniques at the epicenter of the injury or at the correspnding area in the uninjured group. Results showed that in the group 2, 15mg/kg of MP prevented the LPP ($p>0.05$), while 30 mg/kg increased it ($p>0.04$). In group 3, 15 and 30 mg/kg increased the LPP and 45 mg/kg decreased it ($p>0.07$). In group 4, all doses decrease the LPP, but 30 mg/kg was the best one ($p<0.03$). This showed a correlation between the TSCI severity and the MP dose required for decrease the LPP that should be taken in consideration before to design a therapeutic schem.

PATCH CLAMP STUDY ON NEURONS AND GLIAL CELLS IN FRESHLY ISOLATED MYENTERIC GANGLIA

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The myenteric plexus consists of neurons and glial cells located in the wall of the gastrointestinal system and plays essential role in motility control. The physiology of this plexus was often studied in myenteric ganglia attached to the longitudinal muscle. This preparation has several disadvantages, such as the occurrence of muscle movements and the presence of basal lamina over the ganglia, which prevents patch clamp recordings. To overcome these limitations we used isolated myenteric ganglia from the guinea-pig ileum. In this preparation movement was eliminated as muscle was absent. Due to the apparent lack of basal lamina, gigaseals were obtained and whole cell recordings were made from neurons and glial cells. During the recordings the fluorescent dye Lucifer yellow was injected into the recorded cells to allow their morphological identification. Neurons showed a high input resistance (392 MOhm). Hyperpolarizing voltage steps evoked only small inward currents. At depolarizing potentials fast inactivating inward currents and slower activating, sustained outward currents were evoked. Pharmacological experiments revealed the fast inward currents to be Na^+ and Ca^{2-}-currents. The proportions between both currents varied among the cells. However, all neurons with identified Ca^{2+}-currents also displayed Na^- currents. Immunostaining for Na^- channels confirmed that all myenteric neurons display these channels to a varying degree. Eight of ten neurons responded to the application of GABA, demonstrating that neurotransmitter receptors were retained. Glial cells had lower input resistances (90 MOhm) and expressed mostly "passive" currents, which were reduced by octanol -- a blocker of gap junctions. Octanol caused the appearance of voltage-dependent currents. Additional application of Ba^{2-} blocked the inward currents, indicating the existence of inwardly rectifying K^--channels. Glial cells were found to be dye coupled. These results demonstrate the advantages of isolated ganglia for studying myenteric neurons and glial cells. The patch recordings from glia are noteworthy because the small size of these cells has so far precluded their physiological investigation. Supported by BSF and ISF.

INFLUENCES OF HANDEDNESS AND GENDER ON THE GROOVED PEGBOARD TEST

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We studied performance on the Grooved Pegboard Test upon repeated trials and transfer of training between the hands in the first trial. We employed three trials for each hand and two different protocols for the order in which the hands started the test. For the three trials combined, women were faster than men. From the first to the second trial, there was an improvement in performance for both sexes. Within the first trial, sex differences reached significance and the protocol interacted with handedness. In this trial, only left-handed men were found to benefit from previous opposite-hand performance. It is speculated that a larger corpus callosum in left-handed men allows for the greater transfer of training between the hands.

EFFECT OF LATERALITY ON SEX-DIFFERENCES IN IMMOBILITY TIME DURING THE ROTATORY SWIMMING BEHAVIOR OF NORMAL MICE

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It is well known that individual consistency of laterality in turning behavior depends on endogenous cerebral asymmetries. Here we employed the free-swimming rotatory test to investigate the relationship between the time that the animals remained immobile and the total turning activity of normal Swiss mice (n = 149). The effects of sex and consistency of laterality on immobility were also investigated. Each animal was tested for 5 min on 3 different days and consistency of laterality was defined considering the persistence of the same preferred turning side in the three sessions. We found that immobility was not explained by total turning activity and, thus, immobility was analyzed independently from activity. There was an increase in immobility times as test progressed and upon repeated testing sessions. Along the first session, side-consistent males adopted a passive strategy more quickly than side-consistent females. In particular, consistent-right-turner males exhibited a significant higher immobility time than consistent-left-turner females. We conclude that laterality is an important source of individual variability for the analysis of the sex-differences in immobility time.

IMPORTANCE OF MAGNETIC RESONANCE IMAGING IN CONGENITAL BRAIN ANOMALIES

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Purpose: To emphasize the pathomorphology of congenital brain anomalies (CBA) revealed on magnetic resonance imaging (MRI) as a consequence of damaged stage of brain development (BD).
Methods: Retrospective study of 1423 child brain MRI examinations was performed and 171 CBA were revealed (92 female, 79 male), age 2 weeks – 16 years. CBA were divided according to the stage of BD. Clinical presentation and the distributed of CBA for particular period of childhood were analysed.
Results: Number of CBA, according to the damaged stage of BD were: dorsal induction 23, ventral induction 37, neuronal proliferation 50 (18 cases of neuro-cutaneous syndrome), neuronal migration 37, myelination 27, unclassified 69. Ranked distribution of CBA clinical appearance and consecutive MRI positive finding according to the childhood period: 82 school-age children, 41 preschool, 34 adolescents, 8 small children, 5 infants. Epilepsy, psychomotor retardation (PMR), form of pyramid deficit (PD) or its combination appeared in 62% of all cases.
Conclusion: The most frequent damaged BD stages revealed on MRI were neuronal proliferation (domination of aqueductal stenosis) afterwards ventral induction (brain partial atrophy/hypoplasia) and myelination (retarded myelination) equally. CBA were densely represented in school-age children but the expression of epilepsy, PMR or PD, should suggest clinician the possibility of CBA presence in any stage. This fact may induce the establishing of diagnosis in the earlier childhood. In spite of necessity for frequent sedation of small children, our results suggest that MRI should be performed as diagnostic modality of choice if CBA are suspected, regarding its known neuroimaging advantages comparing to ultrasound and computerized tomography.

HIGH RESOLUTION MAGNETIC RESONANCE IMAGING OF THALAMIC NUCLEI

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Purpose to obtain as higher spatial and contrast resolution of the thalami as possible, using optimized Magnetic resonance imaging (MRI) sequence.
Matherials and Methods: one healthy volunteer underwent MRI of the brain on 1.5T imager and optimised high-resolution inversion recovery (IR) in coronal plane of the thalami was performed (TR 3000, TE 30, TI 600, 314x512 matrix; 2mm effective slice thickness) in order to depict precise localisation of the thalmic nuclei comparing stained sections of the human brain in analogical cut plane. Optimised IR sequence was applied on twelve patients with different descrete pathology in thalamic region revealed on routine MRI images (ischemic lesions, multiple sclerosis, small AVM or tumors) and two neuroradiologists independently evaluated topographic anatomy and involvement of the thalamic nuclei.
Results: optimised IR sequence obtained high contrast between thalami and surrounding internal capsule and mesencephalon caudally. Considerably high confidence of thalamic nuclei projection and consecutive pathological involvement was achieved after comparison with stained sections.
Conclusion: MRI is the only imaging procedure for the in vivo high resolution imaging of the thalami with promising potentials of substructure visualisation in future. IR sequence may be applied in anatomical studies and for neuroatlases of other complex brain regions.

CONTRAST ADAPTATION AND ITS ORIENTATION SPECIFICITY IN CORTICAL PINWHEEL CENTERS AND ISO-ORIENTATION DOMAINS

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Adaptation of visual cortical neurons may be interpreted as a normalization of their responses with respect to recently experienced contrast (Heeger, 1992). If adaptation is the result of an integration of local inputs from a pool of neurons, then its characteristics should depend on the position of an adapted neuron within the functional architecture of the visual cortex. We tested this hypothesis by measuring adaptation of neurons situated either in iso-orientation domains or orientation pinwheel centers. Orientation preference maps of the primary visual cortex of adult cats were generated using optical imaging of intrinsic signals. Electrode penetrations were then targeted at iso-orientation domains or pinwheel centers. Neurons were adapted to drifting gratings of either their preferred or orthogonal-to-preferred orientation at different contrast levels. Responses to gratings of optimal orientation and the same, half or twice the contrast of the respective adapting grating were measured. We tested whether responses depended primarily on the absolute contrast of the test grating or on its contrast relative to that of the adapting grating (ANOVA). As expected, for the majority of cells (44 of 60), neuronal responses depended on relative stimulus contrast when adapting and test stimuli were of the same, optimal orientation (Ohzawa et al. 1985). When the adapting stimulus was of orthogonal orientation, adaptation was mostly weak or absent, such that 39 of 60 cells responded to absolute rather than relative contrast. The cells exhibiting adaptation in that condition were found both in pinwheels centers (12) and iso-orientation domains (9). We conclude that some adaptation is present even for orthogonal adapting and test stimuli; but this is not obviously dependent on the local functional architecture.
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NEUROTRANSMITTER & DISEASE OF BRAIN

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Cellular events of various Central Nervous system disease we resort to the frequent use of frustrating but honest terms "possibly" "may" "seems to be" "perhaps" "and so on. Diseases of Nervous system clearly involves and co-relates to the major changes in messenger, Neurotransmitter has been worked out for the availability of the two new biophysical techniques, fluctuation analysis and patch clamp, particularly of individual ion channels. Great variety of Biochemical approaches are also available now for studying Neurotransmitter actions. Pharmacological approach (such as agonist and antagonist) are also employed to define the receptors site and involvement. Clinical relevance of some major mental illness and in degenerative disorders of central nervous system (Parkinsons disease, Schizophrenia, Huntington disease, and Alzheimer type dementia Epilepsy due to abnormalities in central Neurotransmitter system has been experimented to clarify our doubts, present concepts, methods facts which considers future prospects of our understanding the mechanisms and clinical relevance of Neurotransmitter and Neuromodulator. Together with a broad range of transmitter-active drugs eg. (anticonvulsants, antidepressants, antipsychotics and tranquillizing agents) that are currently used to treat various CNS disease, transmitter-oriented research continues to provide one of the greater therapeutic hopes for the immediate future. Not only does this approach currently represent a huge investment by pharmaceutical companies in the realms of neurology and psychiatry but futuristically inclined physicians are contemplating brain grafting as a new means of transmitter therapy.

TITLE: STAUROSPORINE INDUCES NEURITOGENESIS IN NEURO 2A CELLS THROUGH A CALCIUM MEDIATED MECHANISM.

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Staurosporine (ST) is a microbial alkaloid known to possess antifungal and strong hypotensive effects. It is the most potent inhibitor of the phospholipid/ Ca2+- dependent protein kinase C, but inhibits cAMP- and cGMP- dependent protein kinases and protein tyrosine kinases as well. ST has been shown to cause morphological changes in cultured epidermal cells, PK2 epithelial cells, Swiss 3T3 cells, and PC 12 cells. The mechanism of action of ST is not yet understood. In our study, exposure of Neuro2a cells to ST caused dramatic flattening of the cells and extension of neurites within 3 hours of addition. ST-induced neuritogenesis was blocked by the calcium chelator, EGTA, suggesting a Ca2+ mediated mechanism. Incubation of the cells with ST along with nifedipine, L type calcium channel blocker, caused inhibition of neuritogenesis. Neomycin, a phospholipase C inhibitor and sodium vanadate, a protein phosphatase inhibitor did not have any effect on ST-induced neuritogenesis. The involvement of calcium-calmodulin kinase in the calcium-mediated pathway was studied. Our results suggest that ST-induced morphological changes in Neuro2a are mediated by Ca2+ influx through L-type calcium channels.

SENSITIVITY TO CROSSES, CORNERS AND Y-LIKE FIGURES IN THE CAT VISUAL CORTEX (AREA 17)

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Orientation tuning of 154/271 (57%) neurons of the cat primary visual cortex (area 17) was found to be bimodal (double) that stimulated the study of their sensitivity to crosses, corners and y-like figures flashing in the receptive field. About 40% (114/289) of neurons studied with these figures gave a larger response (by a factor of 3.06±0.32 times on average) to one or other figure than to a single light bar of optimal orientation. Most such cells (71%) were found to be highly selective both to figure's shape (angle between the lines) and orientation. In the studied selection we have found also all possible types of the sensitivity invariance to orientation or/and shape of the figures (29% of cases). Parameters of tuning to bars, crosses and corners were interrelated: units with high selectivity to orientation of bar were typically high-selective to orientation and shape of cross and corner. Separated and combined stimulation of receptive field center and surrounding area revealed summation, antagonism or absence of interaction of these zones by figure/bar response ratio. A substantial contribution of intracortical $GABA_A$ergic inhibition to cross-sensitivity was revealed by microiontophoretical application of bicuculline. The sensitivity is generated or enhanced in roughly one third of units, suppressed or diminished in another third of cells, while in remaining third of neurons cross-sensitivity was absent or not influenced by inhibition. Units with high sensitivity to figures seem to be ideally suitable for their detection, rather than to serve as classical orientation detectors only. The mechanisms of selective sensitivity to figures, as well as functional implication of the observed phenomena for a second-order feature extraction in the primary visual cortex are discussed.

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CONCEPTUAL REPRESENTATIONS IMPOSE A SPECIFIC REPRESENTATION OF SPATIAL AMBIGUITIES

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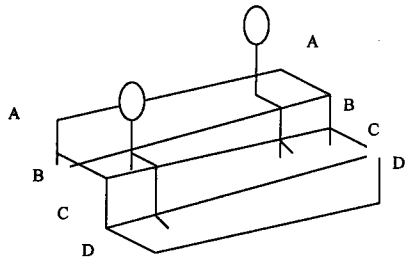
Abstract: This is a study that deals with spatial ambiguities and consists of the analysis of the 3-D significance of 2-D drawings bearing adjacent conceptual representations. Shigeo Fukuda's "Images of illusion" is analyzed because it is typical for the way a conceptual representation induces a spatial illusion.



The two sitting human bodies through the position of their concave and convex segments determine the corresponding interpretation of the space underneath. The dihedral angle AB is consequently perceived as concave or convex when related to the bottom left or top right body. The plane A is vertical from the point of view of the body bottom left and vertical when related to the top right body. The same applies for the BC angle or the rest of the planes. When this image is separated in two halves both are coherent, the contradiction of verticality-horizontality within each plane is induced by being adjoined. The figures are perceived in contradicting perspectives and an illusion is born because our perception is shaped exclusively around the natural angulation between the body segments. This spatial illusion is the result of the influence of the conceptual-cultural context (body segments) on surfaces which by itself have no spatial interpretation.

EFFECT OF NALOXONE INJECTION AT LATERAL AND VENTROMEDIAL HYPOTHALAMUS ON TOOTH PULP STIMULATION EVOKED NOCICEPTIVE JAW OPENING REFLEX RESPONSE IN RATS

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The present study investigates and compares the opiodergic involvement of lateral (LH) and ventromedial hypothalamus (VMN) in modulation of trigeminally mediated phasic pain in rats. In this study, lower incisor tooth-pulp was stimulated (Frequency: 0.5Hz; Duration: 0.3 msec;
Intensity: 1.5 times threshold) in anesthetized rats causing reflex opening of the lower jaw and the amplitude of digastric EMG was recorded. Thereafter, opioid antagonist naloxone was injected (10ug/0.5ul) at LH(NXLH) in one group (n=12) and at VMN(NXVMN) in another (n=9) and the percent change in dEMG amplitude in the post injection (Pi) periods (16 responses averaged over each 37 sec epoch) was recorded. NxLH increased the basal dEMG amplitude by 39.08±20.14% at Pi1 which became 33.66±17.38% at Pi5, the responses being marked but statistically insignificant. NxVMN increased the basal dEMG by 49.74±8.78% at Pi1 ($p<0.01$) and 51.16±7.36% at Pi3 ($p<0.01$). On comparison, NxVMN was observed to be significantly greater than NxLH at Pi2 and Pi3 ($p<0.05$). Therefore, increase in amplitude observed with both NxLH and NxVMN depicted a hyperalgesic response, suggesting
opoidergic involvement of these areas in modulation of phasic pain. However, the comparative significance of NxVMN over NxLH probably indicates a greater involvement of VMN in opiodirgic modulation of trigeminal pain, than LH.

PROTEIN SH-GROUPS CONTENTS IN THE OLFACTORY CORTEX SLICES AFTER POSTTETANIC POTENTIATION.

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Investigation have been directed first and foremost to the study of the macromolecular components of the cells of the nervous system. Before we have demonstrated to the activation of RNA and proteins metabolism under posttetanic potentiation in particular neurons and in satellite glial cells. It can be hypothesized that the neurochemical reaction of the cells of a surviving slices to LTP as a model of learning and memory will touch uppon the metabolism of the protein micromolecular components. The aim of this study was the determination of the total protein SH-group content. The experiments were carried out in male Wistar rats weighting 200 g. Tangential slices of the cortex olfactory region 400-500 μm in thickness, were prepared. It have been taken the micromethod of amperometrical registration that allows to measure the free SH-groups on the surface of protein moleculas. According to the data obtained, the non-bound free SH-groups of proteins content was decreased (about -31%). This reduction of the quantity of SH-groups during LTP was associated to some degree with a disturbance in total proteins, since the protein content in the neurons and glial cells has decreased (by 20 %). Thus induction fase of LTP is characterized by some structure-functional reorganization in protein molecules. It may be particulary molecules of the receptor membrain proteins, ion canals and protein kinazes.

PARALLEL PATHWAYS FOR PROCESSING SPATIAL AND TEMPORAL VIBRISSAL INFORMATION

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The rat vibrissal afferent system contains two parallel pathways: the 'lemniscal' pathway, which exhibits high spatial resolution and fast responses, and the 'paralemniscal' pathway, which exhibits low spatial resolution and slow responses. These two pathways involve different thalamic nuclei (VPM and POm, respectively) and different cortical structures (the 'lemniscal' involves layers 4 & 5b/6a within the barrel columns, and the 'paralemniscal' involves layers 1, 5a & 6 and the inter-barrel septae). While the potential role of the 'lemniscal' pathway in spatial processing is straightforward, the role of the 'paralemniscal' pathway was not clear. In order to reveal this role, we recorded in all major sites (in the brainstem, thalamus and cortex) of the two pathways. Using simultaneous recordings, we found a clear separation between the pathways, beginning at the thalamus. Neurons of the 'paralemniscal' pathway were sensitive to the input frequency (tested between 2 to 14 Hz), while those of the 'lemniscal' pathways were not. This segregation continued at the cortex, where neurons within the barrel's core (layer 4) were not sensitive to the tested frequencies, whereas neurons in layer 5a were. In the POm and layer 5a, the sensitivity to the input frequency was expressed in both the response strength (spike counts decreased with increasing frequencies) and the latency (which increased with increasing frequencies). The observed dynamics and steady-state behavior of the 'paralemniscal' thalamocortical circuits are consistent with an active temporal decoding in which thalamocortical neurons function as temporal comparators rather than relays. The result of such an active decoding is a re-coding of the temporally-encoded information by a population rate code, which can then be integrated with the spatially-encoded information (see Ahissar et. al. this meeting). Our findings indicate that spatially- and temporally-encoded information from the whiskers are processed in parallel via the 'lemniscal' and 'paralemniscal' pathways, respectively, and integrated in the barrel cortex.

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EFFECT OF ANDROGENS ON SEXUAL DIFFERENTIATION OF GABA-IMMUNOREACTIVE NEURONS IN THE RAT AMYGDALA

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GABA-immunoreactive (GABA-IR) neurons were studied in the rat amygdala to detect sex differences and possible mechanisms of sexual differentiation. Four experimental groups of Sprague-Dawley rats were examined: 1/ Males, castrated on the first day of life. 2/ Males, castrated at puberty (35th day of life). 3/ Males, injected with estrogen antagonist during the first 10 postnatal days. 4/ Males, injected with aromatase inhibitor during the first 10 postnatal days. Intact males and females were used for comparison. All animals were sacrificed at the 3rd month of life. Anti-GABA antibody (Sigma), anti-rabbit IgG conjugated to 5 nm gold particles (Sigma) and silver enhancement kit (Amersham) were used. GABA-IR neurons in the medial, cortical, central and basolateral amygdala were counted using image analysis system Olympus CUE2. Data were subjected to ANOVA and Student's t-test. Quantitative studies revealed that females had greater density of GABA-IR perikarya in the amygdaloid nuclei at 3 months of age than intact males ($P < 0.01$). In the medial and cortical amygdala, all experimental manipulations caused significant elevation of the density of GABA-IR neurons compared to that in intact males, but not reaching female levels in the medial amygdala. In the basolateral amygdala only castrations had effect on the expression of GABA in its neurons compared to intact males. In the central amygdala no change was observed after castrations or treatments with estrogen antagonist and aromatase inhibitor during the critical period of sexual differentiation of the brain. These results suggest that androgens have organizational and activational effects on amygdaloid GABA-IR neurons and may be other factors contribute to the formation of sex difference in the central amygdala.

INTERACTION BETWEEN ON-GOING AND EVOKED MEMBRANE POTENTIAL OF A SINGLE NEURON RESULTS IN A DECREASE OF CORRELATION WITH THE POPULATION ACTIVITY RECORDED OPTICALLY.

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There is a large discrepancy between the level of cortical synchronization revealed using different electrophysiological measurements: (1) Recording of action potentials (spikes) which reflects the output of single cortical neurons show low correlation between neurons recorded with different electrodes. (2) Recording of the population activity by EEG, LFP or real-time optical imaging (mostly synaptic potentials), shows highly synchronized activity over large cortical areas.

What is the degree of cortical synchronization in space and time as reflected from the relationship between individual neurons and the population activity? In order to address this question directly, we have examined the relationship between optical signals using voltage sensitive dyes and membrane potential changes recorded simultaneously with intracellular electrode. The visual cortex (areas 17 & 18) of anesthetized cats was stained with the dye RH-1692. Optical signals from a cortical area of 7x7 mm^2 were recorded simultaneously along with the membrane potential of a single neuron from various depths.

We found that:

One) The spontaneous intracellular fluctuations in membrane potential of a single neuron was highly correlated with optical signals over a large cortical region;

Two) During visual stimuli with orientation optimal for the recorded neuron the correlation coefficient between the optical signals and the intracellular membrane potential has spatial structure similar to the orientation map.

Three) During visual stimuli the evoked intracellular membrane potential changes had a strong influence on the on-going membrane potential. This influence resulted in a decrease of correlation with the population activity.

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EFFECT OF CASTRATION ON THE SEXUAL DIMORPHISM OF PARVALBUMIN-IMMUNOREACTIVE NEURONS IN THE RAT STRIATUM

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Effect of castration in the period of sexual differentiation of the brain was studied in rat striatum. One day old male rats were anaesthetized with ether and castrated. Three months after birth castrated males, normal male and female Sprague-Dawley rats were perfused transcardially with 4% paraformaldehyde in PBS, pH 7,4. Free floating sections were incubated in parvalbumin antibody (Sigma), 1:1000 for 24 h. Second antibody was anti-mouse IgG (Vector), 1:500. ABC peroxides method and DAB were used for visualization of the reaction. Numbers of parvalbumin-immunoreactive neurons in the striatum were counted using computer-assisted image analysis system. Comparison of males and females and detecting effect of castration in the critical period of sexual differentiation on the formation of sex differences in the striatum were done using Student's t-test. Dendritic fields of the Golgi-Rio-Hortega impregnated neurones were measured and compared using analysis system Olympus-CUE2. We found higher density of parvalbumin-immunoreactive in female than in male rats.

statistically significant differences in the density of parvalbumin-immunoreactive neurons in male, female and castrated rats.

NEURONAL AND ASTROGLIAL RESPONSE TO PRE- AND PERINATAL EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL IN THE RAT SUBSTANTIA NIGRA.

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The response of neurons and astroglial cells to pre- and perinatal exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) were evaluated in the substantia nigra (SN) in male and female rats at three postnatal ages (21, 30 and 70 days) by immunohistochemical detection of tyrosine hydroxylase (TH) in dopaminergic neurons and of glial fibrillary acidic protein (GFAP) in astrocytes. Δ^9-THC was administered to pregnant rats from day 5 of gestation until day 21 of lactation. Our results showed that the effects of pre- and perinatal exposure to Δ^9-THC on neuronal and astroglial immunoreactivities in the SN varied with sex, with male rats being more susceptible than females. Maternal exposure to Δ^9-THC decreased both TH and GFAP immunoreactivities in the SN of males, whereas significantly increased GFAP expression in the female SN. The increase in GFAP in Δ^9-THC-exposed females could be interpreted as indicative of significant Δ^9-THC-induced stimulation of astrocytes, resulting in a sexually dimorphic GFAP expression. These differences in glial development caused by prenatal Δ^9-THC exposure were associated with significant differences in TH expression by dopaminergic neurons in both sexes. Our observations show that pre- and perinatal exposure to Δ^9-THC can lead to long-term effects in both neurons and glial cells, which could suggest a tight structural and biochemical interdependence between neurons and astrocytes in the Substantia Nigra.

PROTECTIVE EFFECTS OF GRAPE POLYPHENOLS ON NEURO-DEGENERATIVE CHANGES INDUCED BY CHRONIC ETHANOL CONSUMPTION

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Many polyphenolic compounds from plants are known to possess potent antioxidant properties and dietary supplementation of these compounds can offer beneficial effects to human health. Polyphenols are enriched in grape skin and seeds and have been implicated as the active ingredients for the cardiac protective effect in red wine. However, whether these compounds may also offer neuroprotective effects has not been investigated in detail. Chronic ethanol consumption has been shown to increase oxidative stress in many organs including the brain. Excessive oxidative insult in brain has been linked to a number of neurodegenerative diseases. This study was designed to test whether dietary supplementation of grape polyphenols (GP) can ameliorate the neurodegenerative changes resulting from chronic ethanol consumption. Sprague-Dawley rats were fed a Leiber-DeCarli liquid diet with ethanol or isocaloric amount of maltose, and with or without GP for two months. Animals given the ethanol diet developed fatty liver and showed increased susceptibility of low density lipoproteins (LDL) to oxidation. Although supplementation of GP to the ethanol group did not alter the increase in hepatic triacylglycerols due to ethanol consumption, this dietary paradigm was able to reduce hepatic damages (based on morphological examination) and LDL-oxidation in the plasma. In the brain, frontal cortices were used to isolate synaptosomes and Na,K-ATPase and dopamine uptake activities were assayed. Chronic ethanol decreased both Na,K-ATPase (20.5%) and dopamine uptake (22.8%) activity as compared to controls. Although GP supplementation alone did not alter activities of these membrane-bound proteins, supplementation to the ethanol diet completely ameliorated the decrease in synaptic protein function elicited by chronic ethanol consumption. These results demonstrate the ability of GP to offer neuroprotection, especially when the brain is under oxidative insult. (Supported by AA 06661 from NIAAA).

THE ACUTE PHASE RESPONSE OF FETAL ALCOHOL-EXPOSED RATS: EFFECTS OF GENDER AND MATERNAL ADRENALECTOMY.

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Fetal alcohol exposure (FAE) is associated with impairments of immune functions and decreased resistance to infectious agents in children and animals. In order to assess the host's defense response to infection, we have used a rat model of FAE to examine aspects of the acute phase response to interleukin-1ß (IL-1), the cytokine mediator of antigen-induced responses. We have now extended our original observations in male rats (Yirmiya et al, Brain Behav. Immun. 10: 205-220, 1996) to female rats with the following results: (1) Febrile responses to IL-1 are significantly lower in male and female fetal alcohol-exposed (E) rats than in controls whether IL-1 is administered during the light (2 μg/kg, ip) or dark phase (10 μg/kg, ip) of the circadian cycle. (2) The initial hypothermia which precedes the IL-1-induced febrile response during the dark phase is of similar magnitude in E and control males, whereas it is significantly greater in E females than in controls. (3) IL-1 suppresses 24-h food consumption in E females but does not produce significant anorexia in E males. (4) IL-1-induced suppression of motor activity is unaffected in both E males and females. (5) Maternal adrenalectomy, which has been shown to prevent various postnatal effects of FAE, prevents the blunting of the febrile responses in both E males and females, as well as the enhanced hypothermia of E females, and restores IL-1-induced anorexia in E males while blunting anorexia in E females. These results indicate that FAE, possibly through ethanol's actions *in utero* on the maternal adrenal, has profound effects in both males and females on the neuroimmune interactions which mediate many of the host's defense responses to infection. (Supported by NIH/NIAAA-AA09850, Dept. Veterans Affairs Med. Res. Service, and US-Israel Binational Sci. Fdn.)

IMPROVEMENT OF PCR-SSCP ANALYSIS OF P53 MUTATIONS IN HUMAN ASTROCYTOMAS USING KLENOW TREATMENT

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Clarification of somatic mutations during the progression of human astrocytomas is important to understand the mechanisms underlying the development of these tumors. We analyzed surgical specimens of human astrocytomas for mutations in the p53 gene using single-strand conformation polymorphism analysis of polymerase chain reaction product (PCR-SSCP analysis) at a low pH. Klenow fragment treatment after PCR amplification was an effective means to get rid of some extra bands on the SSCP gel. Five mutations in 3 of 24 astrocytomas were identified by this improved SSCP method. The frequency of p53 gene mutations in astrocytomas examined was 12.5%. Further examination by direct sequencing showed that all five mutants had single-base substitutions resulting in missense mutations. The present studies revealed a loss of heterozygosity and two point mutations on the remaining allele in one of the fibrillary astrocytomas. Finally, the improvement of PCR-SSCP analysis using Klenow treatment and low pH showed a distinct pattern of electrophoresis gel and could be relevant for the prognosis of human astrocytomas.

MICROTOPOGRAPHY OF INTRINSIC CONNECTIONS IN VISUAL AREAS 17 AND 18 OF THE CAT

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Areal and laminar distributions of the population of cells sending axons to single cortical columns of areas 17 and 18 in the cat were investigated using microiontophoretic injections of horseradish peroxidase. 3-D reconstruction of retrogradely labelled cells' region was performed. In both areas the extent of horizontal connections of the column does not depend on its location in the visual field map. The regions with labelled cells in the supra- and infragranular layers lie in register. Areal distribution of labelling in a tangential plane was elongated in area 17 along the representation of horizontal meridian and in area 18 along the representation of vertical meridian. Thus spatial distributions of intrinsic connections in these areas are orthogonal. Labelled cells in area 17 were mainly arranged in two parallel rows running approximately perpendicular to the 17/18 border. Revealed architecture of intrinsic neuronal connections in areas 17 and 18 may be useful for the reconstruction of visual image in two hemispheres. We speculate that their role may be the fine control of horizontal and vertical displacements of image fragments' representations.

INFORMATION ABOUT LOCATION VERSUS CONTENT IN A NEOCORTICAL MODEL OF AUTOASSOCIATION MEMORY

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A model of storage and retrieval of autoassociative memory in the neocortex was studied using simulations. The model had two layers, with patterns from the lower layer been learnt on the forward and recurrent synapses of neurons in the upper layer. Neurons in each layer were arranged in a two dimensional sheet such that the forward and recurrent projections were made randomly with in a given radius. Training patterns in the lower layer were given both a centre and a pattern of activity such that activity outside a given radius was zero and activity inside was chosen independently for each neuron from some distribution. Since several patterns shared the same centre, the information in the response of upper layer neurons about which pattern was being presented was the sum of the information about its location ('where') and the information about which pattern it was, given the location ('what'). The information about 'where' remained roughly constant (in fact it went through a maximum) when the spread of the forward connections was trebled, but the information about 'what' increased in proportion to the spread, over the same three-fold range, suggesting that wide spread connections should be advantageous for information retrieval in associational cortices.

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SYNERGY AND REDUNDANCY IN THE ACTIVITY OF LARGE, UNSTRUCTURED POPULATIONS OF NEURONS

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We have derived a simple formula which enables separating out the information transmitted by individual spikes, emitted by single neurons within an ensemble, from positive or negative contributions due to correlations in firing activity among neurons (Panzeri et al, Proc. Roy. Soc. B., in press). The formula is applicable to short times, and it quantifies the corrections to the instantaneous information rate which result from correlations in spike emission between pairs of neurons. Positive corrections imply synergy, while negative corrections indicate redundancy. This analysis, which has previously been applied to recordings from small ensembles, is developed further by considering a model of a large ensemble, in which correlations among the signal and noise components of neuronal firing are small in absolute value and entirely random in origin. The main result is that even such small random correlations lead to large possible synergy or redundancy, as soon as the time window for extracting information from neuronal firing extends to the order of the mean interspike interval. This may indicate that the question of the nature of neural codes is intrinsically ill-posed, unless what is observable by the receiver (typically, a neuron downstream) is also taken into account.

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GEOMETRIC FIGURES IN VISUAL PERCEPTION AND BRAIN ASYMMETRY

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Object of study. The purpose of this study was to investigate the ability of mental construction of six geometric figures in the backward masking condition in healthy subjects and patients with chronic alcoholism. The influence of positive (word 'good') and negative (word 'mistake') verbal reinforcements was studied in this paradigm.
Methods. Two parts of simple geometric figures are consecutively presented in the left (LVF) and right visual fields (RVF); the subjects have to compare them mentally and decide whether these parts of figures formed standard geometric figures or not. The words 'good' and 'mistake' appeared in the centre of the screen 1500 ms after the second stimulus fragment of a task that corresponded to correct or incorrect performance respectively. The number of correct responses (NCR), the reaction time (RT), the latencies and amplitudes of the ERPs N200--P300 were analysed.
Results. The present investigation revealed that performance of the visual-spatial task was better if the figure-standard could be formed from the presented fragments. The RT was significantly longer and the NCR less in alcoholics than in healthy subjects. The RT was shorter if the stimuli were presented in LVF only for controls. In alcoholics the amplitude of ERPs was lesser and latencies were longer than in healthy subjects. It has been found that unlike a control group, in alcoholics the NCR was much greater and the RT lesser in tests following positive than negative reinforcements. The latter did not promote to learning of this task. In healthy persons the negative verbal reinforcement induced a reduction in the latency of N200 and augmentation of the amplitude of the N200--P300 components mainly in the right parietal area and vertex. Such changes were not observed in the patients. Worse performance of the visual-spatial task by the patients may be explained by the general suppression of the brain ERPs and disappearance of interhemispheric asymmetry that is characteristic for healthy subjects and play an important role in human visual-spatial recognition.
Conclusions. The findings support the view that in chronic alcoholism the hemispheric interaction is disturbed due to predominant deficiency of the visual-spatial function in the right hemisphere. Negative verbal reinforcement, unlike positive one produced a certain emotional reaction that may cause the worsening of recognition learning, especially, in the patients with chronic alcoholism. Thus verbal feedback mechanisms play an important role in the dynamic changes in the hemispheric lateralization of the human brain.

INTRACORTICAL ORIGIN OF VISUAL CORTICAL MAPS

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Neurons in primary visual cortex respond preferentially to moving elongated stimuli. The spike rate of a given cell depends on various features of the stimulus, such as position in visual space, its orientation and direction of motion. The response properties of cortical neurons change smoothly across the surface of the cortex, resulting in cortical maps. These maps appear early during development and exhibit characteristic pattern of mutual interrelations. Recent experiments indicated that, in contrast to a common belief, the shape of the visual maps does not depend on the visual experience and is stable throughout maturation. The mechanisms which determine the ultimate spatial shape of the maps are not known. We explore the possibility that the visual cortical maps could first appear spontaneously due to the intrinsic intra-cortical dynamics of activities in the network, mediated by lateral intracortical connections. To this end, we introduce and simulate a network of column-like units which demonstrates that the selectivity of individual columns and the structure of the feature maps may originate from stereotyped inter-unit interactions. Although the units in our model have rotationally symmetric input connectivity, their response to a slowly moving oriented grating depends on the direction of its motion due to the intrinsic dynamics of the network. The resulting maps of preferred orientation and preferred direction look surprisingly similar to the corresponding maps experimentally observed in the primary visual cortex of mammals. Stimulus-driven development of feed-forward and lateral connections could subsequently stabilize these initial maps.

A SELF-ORGANIZING OSCILLATORY MODEL OF PERCEPTION AND COORDINATION MECHANISMS

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The same mechanisms may underlie the system functions of perception and movement coordination. A conceptual model of these mechanisms as self-organizing lattices of relaxation oscillators with even cyclic inhibition is presented. The computational research based on this model shows the existence of several nonlinear phenomena: synchronization, phase transitions, multistability, frequency flutter, and others, similar to the neurodynamical ones and critically important for the implementation of perception and coordination functions. We postulate the use neuromodulatory (context) input for coordination of ongoing activity and formation of synchronized population codes. The modulatory input is not related to the sensory, i.e., informational, one; the former shapes the mechanism of selective choice, effectively changing the algorithm of information processing. On the other hand, the network architecture is designed in order to locate and amplify spatiotemporal correlations in the outer world by means of coding them with the specific patterns of neural activity. The dynamical repertoire of self-organizing lattices includes various types of oscillatory activity: train-like (spindle-like), continuous, and cluster ones; the current activity depends on the neuromodulatory inputs, relaxation parameters of the network oscillatory units, and external synaptic influence. The results of the computational experiments show that it is possible for regular activity waves to arise on the macrotemporal scale in spite of the frequency fluctuations and unstable behavior of the oscillatory units. This fact reflects the synchronization of the oscillatory activity on certain time intervals and points to a possible mechanism for solving the problem of binding segmented features of sensory images into the integral representations that formed and remembered in the brain. In our model the amplitude modulation and changes in relative phases arise in the spindle-like and cluster modes activity. In this case, the change in the amplitude of one of the oscillatory units can lead to the change of the effective degrees of freedom and transition to a new coordination pattern. Hence, the amplitude mechanism of pattern switching can be an effective means for the solution of coordination problems related to the tracking movements of a multicomponent performing organ.

H^+ NMR SPECTROSCOPY IN EPILEPSY.

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15 patients with the confirmed epileptic seizures have been examined. Localized spectra have been obtained by means of the unit Magnetom Vision (1,5 T, Siemens) with the volume 8 ml in medial section of temporal lobes with TE=135 mc. The following parameters have been evaluated: NAA (2,0 pmm), Cr (3,0 pmm) and Cho (3,2 pmm).

Investigation of temporal lobes in the patients revealed unilateral decrease of the NAA contents by 20 %, increase of Cr by 13-17% and increase of Cho - by 22-25%. Correlation NAA/Cho÷Cr was lower in the unilateral lesion. The side with the less NAA contents was considered to be more affected. The signal difference from both sides was equal to 10-15%. Left and right rates of other metabolites were symmetric, as a rule.

The H^+ NMR spectroscopy allows to determine the focus location and bilateral pathology which is very important in the treatment of epilepsy.

DIFFERENT SCALP CURRENT DENSITY DISTRIBUTIONS AND DIPOLE SOURCES FOR THE HUMAN AUDITORY N1 WAVES EVOKED BY INTERAURAL TIME AND INTENSITY SHIFTS

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We used two auditory directional stimuli which were designed to stimulate specifically the cortical binaural mechanisms presumably processing the two cues to sound lateralization (i.e., interaural time and intensity disparities; ITD and IID). Evoked responses of human subjects with normal hearing were recorded from 124 scalp electrodes. Scalp potential (SP) and current density (SCD) maps at N1 latency were obtained. A 3-sphere head model with bilateral dipoles was used for source analysis and a genetic algorithm was employed for solving the inverse problem. SP and SCD maps indicated significant differences between the N1 waves evoked by ITD- and IID-shifts, evidencing that the two stimuli are not processed by the same cortical mechanism. Dissociation of the two mechanisms was supported also by significant location and orientation differences between their estimated dipole sources. The results are discussed in connection with the psychoacoustical observations in the literature implying separate time and time-intensity images. It is concluded that, although the neural codes for ITD and IID may undergo considerable interaction in subcortical nuclei, they are not collapsed into a single lateralization code before reaching the cortex and that they are processed there in partially separate sites.
This work was supported by the Scientific and Technical Council of Turkey (TAG-1469) and by the Turkish Academy of Sciences.

HEPARIN-BINDING PROTEINS TAKE PART IN REGULATION OF BRAIN CELLS PROLIFERATION

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Endogenous carbohydrate-binding protein and related glycosaminoglycans play a key role in regulation of many important processes during CNS development. Specific protein-carbohydrate interaction may establish as hetero- and homophilic cell recognition system to provide signal transduction. Heparin-binding protein active specially during early steps only recently have been identified. The main idea of our work was to study the total heparin-binding activity during first steps of postnatal development of rat brain. We studied the distribution of the sites for the heparin-binding activity using histochemical procedure due to synthesized heparin-HRP conjugate. High level of the heparin-binding activity was shown in newborn cerebellum (in layer of external granule cells that active proliferated and migrated). Such activity quickly decreased after postnatal day 1. On postnatal day 5 heparin-binding activity was demonstrated only in extracellular matrix. The data showed rapid downregulation of heparin-binding activity during first stage of cerebellum maturation. Moreover the heparin-binding sites on the postnatal day 1 were coordinated with PCNA (proliferating cell nuclear antigen) staining. Obtained data allow us to suggest that heparin can induce the first steps of neuron proliferation.

SINGLE NEURON RESPONSE TO LASER RADIATION AND PHOTODYNAMIC EFFECT: PHENOMENOLOGY AND POSSIBLE MECHANISM

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The dynamics of an isolated crayfish stretch receptor neuron response to optical impact including laser microirradiation and photodynamic effect was studied. In these cases cell photodamage was mediated by endogenous or exogenous photosensitizers, respectively. Neuron responses included one or several alternating phases of firing acceleration or inhibition. Such dynamics depended on the cell metabolic and physiologic state, type and concentration of photosensitizers. Two main kinds of irreversible abolition of neuron firing were observed: (i) abrupt irreversible firing block resembling the depolarization block and (ii) gradual firing inhibition until its irreversible cessation. Action spectrum of non-stained neuron response to laser microirradiation showed a blue maximum at 460 nm that indicated to flavins as the endogenous photosensitizers. Cytological study demonstrated that mitochondria were the intracellular organelles most sensitive to blue laser light and that mitochondrial flavoenzyme succinate dehydrogenase was inactivated under 442 nm laser irradiation. The influence of 10 exogenous photosensitizers (hematoporphyrin derivatives, chlorins, phthalocyanines etc.) on the neuron activity was studied and compared. Physical and chemical properties of these dyes caused their specific intracellular localizations and consequently different neuron responses to photodynamic effect. Pharmacological analysis including the use of antioxidants, ion channel blockers and enzyme inhibitors showed free radicals participation in the both: acceleration and inhibition phases in the neuron responses. The possible reason of firing inhibition phase in the neuron response dynamics was Ca^{2+} release from photo-damaged mitochondria and endoplasmic reticulum and the presumable opening the Ca^{2+}-regulated K^+-channels. Enormous Ca^{2+}accumulation in cytosol promoted the cell death (along with other factors).
The work was supported by grants of RFBR and Competition Center for Fundamental Sciences at Sankt-Petersburg University.

EFFECT OF WEAK EXTRA LOW FREQUENCY MAGNETIC FIELDS ON A SINGLE NEURONAL CELL AND ITS POSSIBLE MECHANISM

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Responses of isolated crayfish stretch receptor neurons to weak extremely low frequency magnetic fields (ELF MF) were studied over the wide ranges of frequencies (from 0.001 to 100 Hz), and amplitudes (from 1 to 400 μT). Neuron spikes were amplified and their frequency was recorded by a chart-recorder. After a control recording of firing frequency during 1-2 hours cells were exposed to a sinusoidal ELF MF generated by a flat coil. Neuron firing shifts were weak and variable: slow firing frequency increase or decrease. Inhibitory neuron responses were more often than the excitatory ones. Such shifts were not destructive. Dependencies of neuron response probabilities on field frequency and amplitude: P(F) and P(B), respectively, were nonlinear and included several bands. P(F) was maximal at 0.001; 0.3; 3, and 60 Hz. P(B) was maximal at 5; 20; 50 and 300 μT. To explain our and others magneto-biological data we assume: (i) the validity of Liboff's ionic cyclotron or Lednev's parametric resonance mechanisms; (ii) geomagnetic field (~50 μT) serve as a steady component of the combined magnetic field (CMF) necessary for resonance mechanisms; (iii) various biochemical ions serve as the possible MF targets in cells; (iv) CMF affect electrostatic processes including phosphate group transfer, ligand-receptor association/dissociation, protein-protein and protein-nucleic acids interaction, etc; (v) effects important for the cell can be caused by the field influence on substances abundant in the cell and/or on molecules participating in intracellular signaling and membrane transport whose perturbations can be amplified by enzyme cascades or ion fluxes; (vi) amplification of weak ELF MF effects due to such non-linear mechanisms as behavior of non-equilibrium biological systems in the vicinity of bifurcation points, chaotic dynamics, stochastic resonance. The calculation of resonance conditions suggests biological ELF MF effects in the range 20-70 Hz due to cationic (mainly Ca^{2+}) resonances; near 20 Hz - due to phosphate group resonance; 1-6 Hz - due to non-specific influence on various metabolites; and <1 Hz - due to effect on weakly-charged proteins and their domains.

INFLUENCE OF LAMININ-2 (MEROSIN) ON THE DEVELOPMENT OF SCHWANN CELLS AND PERIPHERAL NERVE REPAIR IN THE *dy/dy* MOUSE

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The *dy/dy* mouse, a model for some forms of human congenital muscular dystrophy, has a severe deficiency of the laminin α2 chain, a subunit of laminin-2 (merosin). Laminin-2 is the only laminin isoform in peripheral nerve endoneurium. The *dy/dy* mouse also suffers from neural abnormalities including hypomyelination of the spinal roots and peripheral nerves. In this study we tested the possibility that laminin-2 is required for normal Schwann cell development, myelination and peripheral nerve regeneration. Schwann cells from the sciatic nerves of 2-3 week old *dy/dy* mice and their unaffected littermates were cultured on various ECM substrata. Antibodies to S-100 and the laminin α2 chain confirmed that whilst α2 chain was localised to the surface of S-100-positive Schwann cells from normal mice, *dy/dy* Schwann cells were α2-negative on all substrata. Under timelapse microscopy *dy/dy* Schwann cells were far more motile than their normal counterparts and extended many more processes with greater rapidity than wild type Schwann cells. These differences in the behaviour of mutant Schwann cells suggest that laminin-2 may be involved in regulating both motility and appropriate bipolar morphology which could perturb Schwann cell-axon interactions in vivo. We found that 13 days following a sciatic nerve crush in *dy/dy* mice, regeneration was severely delayed when compared with wild type nerves. Distal to the crush site, Schwann cells failed to ensheath regenerating axons appropriately, basal laminae were deficient and some axons appeared naked within the endoneurium. Cryoculture experiments have shown that the migration rate of control Scwhann cells is slowed on *dy/dy* nerve substrata. We propose that the deficiency of laminin α2 chain in the endoneurium of *dy/dy* peripheral nerves affects the ability of the nerve to repair normally; this deficiency may affect the polarity of the Schwann cells and render them unable to form normal one -to-one relationships with axons necessary for re-myelination. Our in vitro model systems will allow us to further test these possibilities.
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CHANGES IN CEREBRAL BLOOD OXYGENATION LEVEL AND NADH REDOX STATE IN RESPONSE TO SENSORY STIMULATION.

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What happens to the oxygen level in the brain after neuronal activation? Using PET measurements, Fox *et al.* (1986) detected a cerebral blood oxygenation increase following sensory stimulation. On the other hand, using imaging spectroscopy, Grinvald *et al.* (1986), Frostig *et al.*(1990) found this event to be preceded by a transient increase in the concentration of deoxy-hemoglobin, called the "initial dip". Recently, Malonek and Grinvald (1996) have confirmed this result by imaging spectroscopy studies. Because the interpretation interpretation of spectroscopic data obtained *in vivo* depends critically on the employed theoretical model (Mayhew *et al.*, 1998), and because only a small minority of f-MRI studies did confirm the initial dip, this important issue has remained controversial. Therefore, we decided to clarify this point by measuring blood oxygenation level directly, thus bypasses the difficulties affecting imaging spectroscopy. We used a technique developed by Wilson and colleagues (Wilson *et al.*, Science 1988), which is based on the oxygen dependent quenching of phosphorescence. The probe, Oxyphor R2, which does not leak out of the cerebral vasculature, was injected into the bloodstream of anesthetized cats. The changes in phosphorescence decay time resulting from visual stimulation were than measured in cortical area 18. These measurements validated the interpretation of our previous experiments, showing a fast de-oxygenation, starting earlier than 0.5s after stimulus onset, followed by a larger oxygenation increase. To relate those events to cellular metabolism, we also measured the stimulus-evoked changes in NADH fluorescence, directly. We found a fast decrease in NADH (less than 0.5s after onset of the visual stimulus). These results point towards an increase of aerobic cell metabolism after an increase in neuronal activity. This event is followed by a large response of the cerebral microvasculature, increasing the oxygen delivery to the tissue largely overcompensating for the newly arisen metabolic needs. [Supported by grants from GIF, Minerva, The Wolfson Foundation, and BMBF.]

SUBCELLULAR DISTRIBUTION OF TRH-DEGRADING ENZYMES IN THE DEVELOPING RAT BRAIN

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Pyroglutamyl-peptidase II and the prolyl endopeptidase are two neuronal peptidases involved in the inactivation of the thyrotropin-releasing hormone (TRH), a neurotransmisor/neuromodulador of the CNS with effects on the development of the brain. The significance of a proteolytic enzyme for the neuropeptide turnover is, at least in part, determined by its subcellular localization, so the study of the peptidase activity throughout the subcellular fractions could be important for understanding the regulatory mechanism controlling the activity of neuropeptides. In this work we analyze the activity of pyroglutamyl-peptidase II and prolyl endopeptidase in several subcellular fractions (synaptosomal, cytosolic, mitochondrial, microsomal, nuclear and myelinic) of the rat brain during the first postnatal month of life. The enzyme activity has been measured spectrofluorimetrically using TRH–β–naphtilamide and specific inhibitors for the pyroglutamyl-peptidase II and using Z-Gly-Pro–βNA for the prolyl endopeptidase. Results obtained in this research show that both enzymes are widely distributed in the studied subcellular fractions, but not homogeneously, and that the distribution of the enzyme changes with the development. These results could agree with the suggestion that those enzymes with the rest of the TRHergic system could have different functions in the distinct subcellular structures during the development of the rat brain.
This work has been supported by a grant from The Basque Government (EJ/GV 1999-2000).

Refractory period in Hanseri's disease.Detection of early nerve dysfunction

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Refractory period of nerve which measures the changes in accitability cycle of axon is a sensitive indicator of early nerve damage. Abnormalaties of Refractory period nerves have been reported with normal conduction velocities in peripheral nerve disorders due to toxic (Metabolic causes). Hansesi's disease is the single largest cause of peripheral neuropathy in developing countries. The objective of present study is to evaluate the sensibility of the refractory period of vinar sensory fibres in the detection of early nerve damage in Hansesi's disease and to compare with conventional conduction parameters, thirteen patients of Hensesi's disease comprising LL BB BT BL types were Assessment of absolute refractory period (ARP) and Relative refractory period (RRP) was sone by paired shock stimulus technique (Aldersons patajan(1987) in eighteen ulnar nerves, including fourteen nerves no evidence of clinical neuropathy. Refractory period of sensory fibres of ulnar nerve in 30 control subjects showed ARP 0.56 +0.15(Plus or minus); RRP 3.29+0.51(Plus or minus). 2m 18 ulcer nerves of. Hansesis's disease patients observed ARP is 0.92+0.43 (Plus or minus), RRP is 4.68+1.48(Plus or minus), among the eighteen nerves ARP was abnormal in six (3.33%) and RRP was abnormal in seven (38.9%) with state slicatly significant abnormality in both ARP & RRP as compared to controls. The analysis of the fourteen clinically normal nerves showed abnormally of motor conduction in 14%, sensory conduction in 28% and prolongation of refractory period in 35%. These findings suggest that the assessment of refractory period is more sensitive and complementary to routine conduction studies to detection of early neuropathy.

INHIBITORY TRANSMISSION AT SINGLE SYNAPSE IN CULTURED RAT HIPPOCAMPAL NEURONS.

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Miniature, spontaneous and evoked inhibitory postsynaptic currents were studied using whole-cell patch-clamp technique on synaptically connected cultured hippocampal neurons, at a holding potential of -75 mV. All experiments were done in TTX-containing solution to exclude an action potential generation. Spontaneously occurring whole-cell IPSCs (sIPSCs) were recorded in physiological solution. The average amplitude of spontaneously occurring currents depended on membrane potential and reversed at -18±5 mV. The amplitude distribution of sIPSC had one peak clearly detectable with the mean of 20.0±2.0 pA. Inhibitory postsynaptic stimulus-evoked currents (eIPSCs) arose in responses to gradual activation of neurotransmitter release by direct extracellular electrical stimulation of a single presynaptic bouton by short depolarizing pulses. The current voltage relation of the averaged amplitudes of eIPSCs was linear and reversed at potential predicted by the Nernst equation for corresponding intra- and extracellular Cl^- concentrations. The stimulus-evoked IPSCs fluctuated with regard to the discrete aliquot values of their peak amplitudes in all the investigated synapses from a measurable minimum of about 8 pA to 200 pA. The eIPSCs were assumed as superimposition of statistically independent quantal events. Fitting amplitude histograms of eIPSC with several Gaussian curves resulted in peaks that were equidistant with the mean space of 20±3 pA, which was assumed as one quantum (quantum size) to construct the Poisson's distribution. A possibility of simultaneous multiquantal release at single inhibitory synapses of rat hippocampal neurons was discussed.

EFFECTS OF MORPHINE ON THE F1 & F2 PROGENY OF MORPHINE-TREATED MALE WISTAR RATS

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We have examined the influence of chronic morphine administration to the male rats on the early development and sensitivity to morphine in their progeny. Male Wistar rats (8 weeks at the beginning of experiment) received chronic morphine injections for 5 weeks twice daily in increasing doses (5-60 mg/kg) during first week, then 60 mg/kg twice per day. Morphine-treated males were mated with naive females during last 5 days of morphine treatment. F2 progeny was obtained from breeding of untreated F1 animals. F1 and F2 progeny was compared with the progeny from untreated parents (control) in a number of tests. Analgesic effect of 1-st morphine injection (10 mg/kg, i.p.) was measured in "tail-withdrawal" and "hot-plate" tests. Physical dependence was investigated after 6 days of morphine treatment (increasing doses 10-60 mg/kg, i.p., twice daily) by naloxone-precipitated loss of weight. Results: (1) F1 males obtained from chronically morphine-treated males and intact females differed from control animals by increased analgesic effect of morphine in "tail-withdrawal" test and more pronounced withdrawal symptoms (increased naloxone-precipitated loss of weight) after being exposed to 6-days of morphine treatment; (2) F1 females didn't differ from the control animals; (3) only 1/4 (one quarter) of all F2 males from experimental group and only 1/4 of all F2 females from experimental group differed from their control groups by an increased analgesic effect of morphine in "tail-withdrawal" test. The obtained data suggest that offspring of morphine-treated males and intact females have enhanced sensitivity to morphine as a dominant character in F1 males and as a recessive character in F1 females, but in F2 progeny enhanced sensitivity to morphine exists as a recessive character in both sexes.

BIOTRANSFORMATION OF NOCICEPTIN/ORPHANIN FQ BY ENZYME ACTIVITY FROM MORPHINE-NAIVE AND MORPHINE-TREATED CELL CULTURES

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The biotransformation of Nociceptin/orphanin FQ (NOFQ) by enzyme activity isolated from U1690 human lung carcinoma and SH-SY5Y human neuroblastoma cell lines, and from rat brain cortex cells in primary culture was investigated. The identification and quantification of the cleavage products were performed using electrospray ionization mass spectrometry linked to size-exclusion chromatography. The effect of chronic morphine treatment of the cells (5 days) on NOFQ biotransformation was also studied. It was found that major products generated from NOFQ were the amino-terminal peptides N1-9 and N1-13. The pattern of NOFQ biotransformation was quite similar for all three cell cultures. However, different proportions of the formed peptides were noted. The cleavage was inhibited by EDTA, PMSF, Hg^{2+}, Cu^{2+} and Zn^{2+}. Dynorphin A2-13 inhibited NOFQ cleavage in a manner suggesting competition of the two peptides for the same enzyme. Chronic morphine treatment of the cell cultures resulted in a substantial increase in the enzyme activity, leading to higher levels of the major fragments and accumulation of the shorter peptides N1-5, N1-6 and N1-12. Since the effect of morphine treatment of the cells was blocked by naloxone it is likely that it was receptor specific. Taken together, the findings suggest that a metallosensitive endopeptidase, the activity of which is increased by chronic morphine treatment of the cells, is responsible for the biotransformation of NOFQ with fragments N1-9 and N1-13 being the major products. *Acknowledments:* This work was supported by the Swedish Medical Research Council and the National Institute of Drug Abuse, Rockville, MD, USA. The help of Prof. M. Schramm in discussion of the results is

THE ROLE OF NITROXIDERGIC MECHANISMS IN THE OPIOID CARDIOVASCULAR AND NOCICEPTIVE TOLERANCE

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The role of nitroxidergic mechanisms in the development of tolerance to cardiovascular and analgesic effects of opioids have been studied. Conscious, chronically instrumented with intravascular catheters for blood pressure and hearth rate measurements and i.v. drug administration rats were used. The acute effects of morphine (Mo) 5 mg/kg i.v. and NOS inhibitors (L-NAME and L-NNA 6 mg/kg i.v) or L-Arg, 300 mg/kg i.v. were studied. Mo alone or in combination with the NOS inhibitors or L-Arg were applied 24 h and 72 h after surgical instrumentation and cardiovascular changes recorded. Tolerance to Mo analgesic activity was evaluated by changes in EC_{50} (mg/kg) of Mo using tail flick and hot plate test. L-NAME and L-NNA (15 mg/kg s.c.) were applied prior to Mo. It was found that Mo evoked transient hypotension and bradycardia, which were abolished after second Mo-application. However pretreatment with L-NAME or L-NNA, but not L-Arg inhibited development of tolerance to the cardiovascular effects of Mo. It has also been found that repeated administration of Mo resulted in development of tolerance to the analgesic activity: EC_{50} for Mo increased from 4.9 ± 0.6 mg/kg (naïve) to 17.0 ± 1.6 mg/kg (tolerant). Co-administration of Mo with L-NAME or L-NNA decreased EC_{50} to 7.5 ± 0.6 mg/kg and 9.8 ± 0.9 mg/kg respectively. The data obtained show that nitroxidergic mechanisms are involved in the development of tolerance to Mo cardiovascular and analgesic effects in conscious rats.

XENOTRANSPLANTATION OF GANGLIA FROM NEWBORN MOLLUSK IN THE BRAIN OF ADULT RATS

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It is known that a system of histocompatibility is weakly developed in lower invertebrates in comparison with the vertebrate animals. We assumed that the xenografts from newborn invertebrate nervous system would not exert destructive effects on the brain of vertebrate recipient even without immunosuppressive therapy. In search of a brain xenograft (XG) capable to restitute the damaged brain of a recipient without intensive immunosuppression ganglia of a newborn terrestrial pulmonate snails (Helix sp.) were transplanted into the brain of adult Wistar rats.

Part of the snail XGs were stained by vital fluorescent dyes Bisbenzimid or Fast Blue. The snail XGs were implanted into the neocortex parenchyma in each hemisphere. Surgeries were performed under Nembutal anesthesia in aseptic conditions, using stereotaxic coordinates. Brains of the rats with the snail XGs were investigated 5 and 28 days after the transplantation. Serial frontal frozen sections were stained by Nissle and Spielmeyer methods. Nonstained sections with the XGs labeled with fluorescent dyes before transplantation were analyzed by fluorescent microscope and stained later with tionin and cresyl-violet. Quantitative videoimage analysis of lymphocyte aggregations, reactive gliosis, XG areas, and implantation trace was performed.

It was found that the snail XGs transplantation didn't elicit an intensive immunological conflict in the rat brain 5 and 28 days after the transplantation. Some snail neurons and numerous glial cells were found inside the host brain using fluorescence imaging 28 days after transplantation. Nerve fibers and vessels grew into the rat brain from the place of transplantation subserving humoral and nervous integration of the XG and the recipient brain. A migration of snail glial cells was observed in the host brain. Obtained results might be significant for veterinary and clinical applications.

AXONAL SWELLING IN THE CORPUS CALLOSUM AND INTERNAL CAPSULE IN TRAUMATIC BRAIN INJURY IN CHILDREN AND ADULTS

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As part of a multidisciplinary study comparing brain injury in children and adults following fatal traffic accidents, we have investigated axonal damage in 2 major tracts, the corpus callosum and internal capsule. The study involved 20 fatalities in pedestrians or vehicle occupants aged from 3 months - 20 years. Survival times ranged from 0 - 96 hours. Brains were removed at postmortem, immersion fixed and sliced in the coronal plane. Paraffin sections were immunoreacted with bAPP for axonal swelling. Axonal diameters in the corpus callosum and internal capsule were measured using an unbiased sampling technique and NIH image software. Axon damage could be visualised in both children and adult brains with survival times as short as 40 mins. Damaged axons were most commonly seen in the corpus callosum, and subcortical white matter. The mean diameter of damaged axons was found to increase with increasing survival
time (mean + SD for corpus callosum: after 1 hour, 2.7µm+0.8; after 96 hours, 4.6µm+2.2, $p < 0.001$). The time till clinical brain death correlated more closely with axon diameter than the actual survival time. In conclusion, bAPP has been found to be a useful marker of axonal injury, with major tracts showing a similar pattern of damage following traumatic injury in both child and adult brains.

MOLECULAR-CYTOGENETIC APPROACHES FOR STUDIES OF RETT SYNDROME: INTERPHASE ANALYSIS OF ASYNCHRONY IN DNA REPLICATION OF CHROMOSOME X.

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Rett syndrome (RS) is a developmental disorder, affecting girls. The aetiology of RS is unknown, but genetic factors are important. RS is commonly thought of as an X linked dominant disorder lethal to hemizygous males. However, at present time its biological and genetic basis remains obscure. Moreover, there are no laboratorial criteria for accurate preclinical and prenatal diagnosis of RS. There is one remarkable finding in genetic studies of RS, indicating on specific alterations in late chromosome X replication pattern in girls with RS. It was proposed that cytogenetic and molecular-cytogenetic investigations could be used as method for accurate diagnosis of RS. The aim of the present work is to combine classical and modern molecular-cytogenetic methods to the study of genetics of Rett syndrome and development of method for preclinical diagnosis . To perform cytogenetic investigations in RS, analysis of chromosomal DNA replication in chromosome X, accurate detalization of late X-chromosome replication pattern using BrdU + Hoechst staining was performed in 4 patients under analysis of 200 cells in each patient.. A pilot molecular-cytogenetic analysis of replication pattern in interphase chromosomes for specific loci of chromosomes X for identification of loci with altered order of replication in RS and development of new approach for preclinical diagnosis by FISH was also performed using telomeric PAC clones for Xptel and Xqtel, pseudoautosomal locus and centromeric alphoid DNA. Preliminary results indicate that interphase FISH analysis of DNA replication allows to detect the loci with altered order of replication (for example, the loci at telomeric region of the q-arm in chromosome X) in RS patients. A practical application, which is one of the aims of this study, will be to develop cytogenetic analysis of chromosome X replication, using cytogenetic and molecular- cytogenetic methods, as a test for preclinical diagnosis. Supported in parts by INTAS and IRSA grants.

IN THE TWINKLING OF AN EYE: PERCEIVED DURATION THROUGH THE MILLENNIA

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We gather in Jerusalem among many reminders of the common culture manifested in the Hebrew and Christian Bibles and the Koran. That culture linked the briefest moment with sensation. An exact estimate was given by Mar Samuel (d. 254) who divided the hour so that a twinkling of an eye *(heref 'ayyin)* equaled 63 msec and a moment or atom *(rega')* was twice as long (Gandz, 1952). Less exact characterizations were also given: The fusion seen in a spinning particolored wheel led Ptolemy (87-150) to say that a too rapid motion simply cannot be detected (Smith, 1996). Ibn al-Haytham (965-1040/41) expanded on this concept by saying that any point will "traverse, in the smallest moment of time, the whole circle on which it moves" (Sabra, 1989). Such ideas led Francis Bacon (1620) to reason that the investigation of nature required the competent measurement of the "degree in respect to duration" of "those actions which seem to be performed suddenly and (as we say) in the twinkling of an eye."

Three centuries later, such reasoning bred behavioral measurements of the perceived duration of sudden actions. Nisly & Wasserman (1989) reviewed over thirty recent studies of the relation of intensity to the perceived duration of brief flashes. They yielded both direct and inverse proportionalities. Hawkins & Shulman (1979) and Long (1979) had suggested that these conflicting trends were linked to different behavioral tasks mediated by different analyses of sensory signals (or candidate neural codes) with different intensity dependencies.

We have further investigated this hypothesis by measuring the response durations of intracellular receptor potentials (RPs) recorded from single photoreceptors in response to flashes whose intensity varied over 3.6 or more log units and in light adaptation states which differed in sensitivity by 3.5 log units. Six very different neural codes for response duration were applied to these RPs.

Intensity, adaptation, and candidate code all had large and statistically quite robust effects on neural response duration. Direct, inverse, invariant, and U-shaped functions of intensity resulted. While caution is necessary when generalizing from neural response duration to perceived duration, these data show that both task and stimulus variables must be exhaustively explored in far more complete behavioral experiments before a full understanding of perceived duration will be in hand. It is now highly likely that intensity dependent [Coltheart (1980); DiLollo (1984)] and task dependent models of perceived duration simply address different aspects of the same phenomenon.

For a ms. with complete references, see *http://www.psych.purdue.edu/~codelab/ibro.html*

VOLTAGE-SENSITIVE DYE IMAGING IN THE BEHAVING MONKEY

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Voltage-sensitive dye imaging (VSDI) *in-vivo* is a powerful technique for exploring cortical dynamics with a high spatial (~50-100μm) and temporal resolution (sub-millisecond). This method was proved to be valuable in anesthetized animals (Grinvald et al., 1984; Glaser et al. 1998 Soc. Neurosci. Abstr.) In pursuit of the spatial and temporal organization underlying higher cognitive functions in the primate neocortex, we have adapted standard VSDI methodology to study spatio-temporal patterns of cortical activation in the awake behaving monkey, repeatedly, over a long period of time.

A macaque monkey was trained on a task that required animal to fixate a small dot on a monitor during the presentation of high contrast drifting gratings of varying orientations. After implementation of artificial dura we performed optical imaging of intrinsic signal of the cortex and obtained ocular dominance and orientation domains in V1. In the following weeks, the brain was stained once a week when the monkey was awake. Immediately following the staining, the monkey started to perform the task, while its cortex was imaged. We found that the dye signal appeared about 60-100 ms following the stimulus onset and peaked about 100 ms later on as expected from electrical recordings. The ocular-dominance and orientation domains maps from the fast voltage-dye signal were thus visualized in real time. Since pharmacological side effects and/or photo damage to the cortical tissue are a major concern, we assessed the "condition" of the cortex repeatedly, by re-evaluation of its functional architecture . We were always able to obtain the intrinsic imaging maps of ocular dominance and of orientation domains, during or immediately after the voltage-sensitive dye imaging session. These results suggest that the population activity of the cortex was not significantly affected by the dye or the imaging sessions. We confirmed that the maps obtained by VSDI and those obtained by intrinsic signals had a similar spatial pattern as found for anesthetized animals (Shoham et al, 1993, Soc. Neurosci. Abstr.; Glaser et al., 1998, Soc. Neurosci. Abst.) We conclude that real-time optical imaging is now feasible also in the awake behaving primate.

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TACTILE PERCEPTION: FEATURE EXTRACTION IN THE SAI AFFERENT POPULATION

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We are amazingly dexterous in part because of the information transmitted from the mechanoreceptors in the skin of the hands about the objects we manipulate. This cutaneous information forms an essential part of the sensorimotor loop controlling hand function. In a number of quantitative psychophysics experiments, we have established that there is a monotonic relationship between perceived and actual magnitude for a range of tactile stimuli, such as shape and position, which are essential for successful manipulations. Furthermore humans can scale these features even in the presence of random changes in other stimulus parameters. So these characteristics are obviously mapped in the neural responses of mechanoreceptive afferents with sufficient precision to allow changes in a specific parameter to be detected independent of changes in other parameters. Our focus is on determining how this information is represented in the peripheral neural population. Because the information is multidimensional and much of the essential detail is spatial, the information content in individual fibre responses is ambiguous. It is only in the combined responses of populations of afferent fibres that this information is signalled. We record the responses of single slowly adapting type I afferent fibres (SAIs) from the median nerve in anaesthetised monkeys using a passive paradigm. Response characteristics are measured precisely to stimuli presented at various locations within individual SAI receptive fields. Given that the receptive field characteristics are similar for all fibres within a class, we can reconstruct the whole population response from those single fibre responses. For an ideal population with uniform sensitivity and infinite sampling density, the surface features of an object are represented as approximately isomorphic images in the SAI population response. Contact position and force are reflected respectively by a lateral translation and a scaling of those images. The images are blurred and distorted, however, when account is taken of real neural population parameters, such as innervation density, differences in fibre sensitivity, and the effects of random noise. Do higher centres in the neural pathway have prior knowledge of the characteristics of the signalling population which correct for these factors? Because in spite of the distortions produced by varying fibre sensitivity and the fact that stimulus information is sampled by a neural population with finite density, humans are able to discriminate small changes in a stimulus parameter. A more specific and immediate question to be resolved is: what are the critical elements within the population responses which are extracted for further processing?

PRE AND POSTSYNAPTIC AMPLIFICATION MECHANISMS ENHANCE SYNAPTIC COUPLING BETWEEN CORTICAL PYRAMIDAL NEURONS.

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The cellular mechanisms underlying burst firing and its postsynaptic consequences in layer 5 pyramidal neurons are largely unknown. Patch-clamp recordings from layer 5 neurons in a slice preparation from 3-5 week-old Wistar rats were used to investigate these mechanisms. Somatic cell-attached recordings revealed that synaptic stimulation could generate high frequency bursts of action potentials (2-6; 1st ISI 299±15 Hz; n=10); formation of whole-cell recordings did not modify action potential firing. The removal of extracellular Ca2+ resulted in a dramatic decrease in the average number of action potentials composing a burst (3.5±1 to 1.5±0.3; n=5), as did the bath application of the Ca2+ channel antagonist Ni2+ (0.25 or 0.5mM; 3.3±0.3 to 2 or 3±0.7 to 1; n=8). To investigate the contribution of dendrite mechanisms, we locally applied the Na channel blocker TTX to apical dendritic sites, resulting in a large decrease in the number of action potentials in a burst (4±1.1 to 1.3±0.3; n=5) without effecting the properties of the first action potential. Similarly, the local application f Ni2+ to apical dendritic sites resulted in a decrease of the number of action potential composing a burst (3.5±0.5 to 1.6±0.7; n=5). These results indicate that recruitment of dendritic Ni2+-sensitve Ca2+ channels by backpropagating action potentials generates burst firing. Simultaneous dendritic and somatic recordings were used to further explore dendritic mechanisms. In neurons that fired single action potentials to somatic current injection, the threshold response to dendritic current injection made >150 μm from the soma was a low frequency burst discharge (2-3; 1st ISI 157±26 Hz; n=6), whereas in neurons that fired burst discharges to somatic current injection, dendritic current injection evoked more powerful bursts. Each action potential in a burst arose first at the somatic site (n=10), and simultaneous axonal and somatic whole-cell recording showed that each action potential was axonally generated (n=4). To address the postsynaptic consequence of burst firing paired whole-cell recordings from pyramidal neurons were made (n=21). The postsynaptic response was a high frequency series of EPSCs that showed step-wise depression. Near firing threshold later EPSPs in a burst were preferentially amplified by the persistent Na current, providing a mechanism that converts synaptic depression to facilitation. A coupling of distinct pre- and postsynaptic amplification mechanisms, therefore, enhances the synaptic coupling of layer 5 pyramidal neurons and may underpin the synchronizing role of these neurons in the neocortex.

PRIMARY VISUAL CORTEX OF VISUALLY DEPRIVED HAMSTERS IS ACTIVATED BY AUDITORY STIMULI

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Auditory compensation in neonatally enucleated Syrian hamsters was explored electrophysiologically and behaviorally. Enucleation, performed under hypothermia within 12 hr. after birth, induced complete degeneration of the optic tract and optic chiasm. Gross morphology of the primary visual cortex (VC) appeared normal and no obvious cytoarchitectural malformation was discerned. Cellular spontaneous activity in the primary auditory cortex (AC) was not affected by enucleation. However, in the VC it induced a significant increase in firing rate. Responses to auditory stimuli in the AC of anesthetized blind hamsters were similar to those characterizing intact animals, as were the latencies and the selectivity to various auditory stimuli. No auditory responses could be elicited in the VC of intact hamsters. In blind animals, however, auditory stimuli evoked field potentials and single unit responses. About 63% of the 126 cells isolated in the VC of 16 blind hamsters responded to at least one of our auditory stimuli. This responsiveness was significantly lower as compared to the AC of either blind or intact animals. Most of the responses were less vigorous, less time-locked and more readily habituated than those of the AC and thresholds were typically higher. HRP applied to the VC disclosed reciprocal connections between the VC and the dLGN in both normal and blind animals. In addition, retrogradely labeled cells were found in the ipsilateral primary AC, the number of which was significantly larger in the blind animals. Retrogradely labeled cells were also found in the inferior colliculus, mainly ipsilaterally to the marker application side. Behavioral hearing range of both intact and blind hamsters extended from 96 Hz to 46.5 kHz with a best sensitivity at about 8.0 kHz, matching fairly well with electrophysiologically determined cellular maximum sensitivity. Minimum azimuthal audible angle, as determined by a conditioned avoidance task, ranged between 18 and 22 degrees. There was no significant difference, in this respect, between normal and blind animals. However, testing unconditioned head orienting response in blind hamsters and their sighted littermates disclosed an increased responsiveness to sudden noises in the blind animals, which might account for the increased 'sensitivity' to sound sometimes reported as evidence for compensatory enhancement of hearing in blind humans.

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INCREASED SENSITIVITY TO THE ANTI-IMMOBILITY EFFECTS OF IDAZOXAN FOLLOWING SOCIAL ISOLATION IN RATS

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The objective of the present investigation was to determine whether social isolation rearing alters the effects of the selective alpha 2-adrenoceptor antagonist, idazoxan in the forced swimming test. Methods: Male Wistar rats were raised from weaning either alone(isolation rearing) or in groups of five rats/cage (social rearing). Four weeks later, these rats were tested for their sensitivity to idazoxan using the forced swimming test. Results: The results showed that systemic administration of idazoxan (0.5, 1, 2 and 5 mg/kg i.p.) 24, 5, and 1 h to both isolation and socially reared rats produced a dose-related reduce immobility time and increase in struggling compared to the saline treated group. Low dose of idazoxan (0.5 mg/kg i.p.) significantly reduced immobility and increased struggling only in the isolation reared rats but had not significantly altered the forced swimming behaviour in the socially reared rats. However, higher doses of idazoxan (1, 2 and 5 mg/kg i.p.) significantly decreased immobility and enhanced struggling in both socially and isolation reared rats. The anti-immobility effect of idazoxan was more pronounced in isolation than socially reared rats. Conclusion: The results suggest that rearing rats in social isolation from weaning may produce some of its behavioural effects through central adrenergic mechanisms. The isolation reared rats are more sensitive to the anti-immobility effect of idazoxan than the socially reared rats. This abnormality may involve alterations in the responsiveness of alpha 2-adrenoceptors in the brain.

CORRELATION OF BETA AND GAMMA OSCILLATORY ACTIVITY IN THE CORTICO-THALAMIC SYSTEM OF CATS ATTENDING TO VISUAL STIMULI.

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We have previously shown[1] that Fourier transform of the local field potentials (LFP) recorded in the lateral geniculate nucleus (LGN) and primary visual cortex (VCx) of cats attending to visual stimuli contains more spectral power in beta (16-24 Hz) frequency band as compared to the auditory attentive situation. We were interested in the correlation between this activity and the gamma oscillations that were proposed to be an information carrier in the feature binding hypothesis. The LFPs, registered on the videotape during the experiment, were filtered off-line within 16-24 Hz and 30-45 Hz frequency ranges. The envelopes of both signals recorded at the given site were then obtained and the normalized temporal crosscorrelation between them calculated. The significant positive correlations were found in those LGN and VCx sites, which corresponded to the central representation of the visual field. All peaks of the correlation histograms were located within 50 ms of the central window. The correlation values obtained during the visually attentive trials were significantly stronger, as compared to those calculated from the data recorded in auditory trials.

These findings are in agreement with the hypothesis[1] that beta activity provides the necessary excitatory background for appearance of oscillations in gamma band.

1. M. Bekisz and A. Wróbel, Acta Neurobiol. Exp. (1993), 53: 175-182.

DIFFERENCES IN SCALP CURRENT DENSITY DISTRIBUTIONS AND DIPOLE SOURCES OF THE HUMAN AUDITORY N1 WAVES EVOKED BY ONSET, OFFSET, PITCH-SHIFT, LEVEL-SHIFT, AND SOUND PIP STIMULI

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Auditory evoked responses of humans to onset, offset, pitch-shift, level-shift, and pip stimuli were all recorded simultaneously by using a cycling sequence of these stimuli in a single session. Possible response waveform differences which normally occur due to changes in the subject's brain state could thus be avoided. Scalp potential (SP) and current density (SCD) maps at N1 latency (post-stimulus time the global field power peaked around 100 msec) were obtained from the recordings made with a 124-electrode cap. A 3-sphere head model with bilateral dipoles was used for source analysis and a genetic algorithm was employed for solving the inverse problem. SP and SCD maps of the N1 responses to these stimuli displayed differences at various significance levels. Significant location and orientation differences were also observed between their estimated dipole sources. These observations support the results of the magnetoencephalographic studies which indicate the presence of at least partially different auditory cortical areas processing different aspects of auditory events, certainly challenging the classical view which has considered all the N1-P2 wave complexes as nonspecific vertex-potentials or responses to any change in auditory environment.

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TIME-DEPENDENT BEHAVIORAL EFFECT OF KETAMINE ADMINISTRATION IN WISTAR RATS

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Ketamine is used for anesthesia in small animal surgery. Our studies have found a long-term, time-dependent effect of a high dose of ketamine (160 mg/kg, ip injections). Specifically, ketamine aggravates learned despair in rats (male Wistar rats) as measured by forced (Porsolt) swim tests 3 days after injection, but has a protective effect 10 days after injections. The present study investigated the effect of the same dose of ketamine injections on performance of male Wistar rats in open field and elevated plus-maze tests. Independent groups of male Wistar rats (6-8 per group) were injected either with ketamine (160 mg/kg ip; 50 mg/ml saline) or an equivalent volume of saline and tested either 3 or 10 days after injections. Open field and elevated plus-maze tests (5 min each) were run (in a counterbalanced fashion) the same day separated by 3 hr. Results showed a significant increase in the number of squares crossed and a reduction in immobility 10 days after ketamine injections compared to ketamine administration 3 days before the tests. There was no significant difference among the groups in the elevated plus-maze test. Results indicate a time-dependent effect of ketamine in rats that have implications for behavioral testing after its administration as an anesthetic. (Supported by Bogazici University Research Grant 97B0703 to RC).

CHRONIC AMPHETAMINE ADMINISTRATION UPREGULATES THE DENSITY OF GLUTAMIC ACID DECARBOXYLASE-BOUTONS IN THE DEVELOPING AND ADULT RAT BRAINS

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Amphetamine (AMPH) is a potent agonist of catechoamines and known to induce depletion of biogenic amines, and thereby produces behavioral abnormality in the animal model. However, the role the inhibitory neurotransmitter system plays in the pathogenesis of the disease is unknown. Thus this study examines the responses of the GABAergic neurons in the brain to chronic AMPH treatment. Male Wistar rats of postnatal day 60 and 21 were intraperitoneally injected with saline or 5 mg/kg of AMPH 3 times daily at 9 am, 1 pm and 5 pm for 6 days. After 1 day withdrawal from the drug, they were challenged on the 8th day 9 am with one dosage and perfused 4 h later with Bouin's fixative. Paraffin brain sections were obtained for immunocytochemistry to localize GABAergic neurons by an anti-glutamic acid decarboxylase (GAD_{67}) antiserum. Following each AMPH injection, the behavior of the animals was rated for 2-3 h. The drug treatment resulted in hyper-locomotion and stereotypies of both young and adult rats; shorter time to onset and longer duration of stereotypy were seen for the adult. The area of the lateral ventricle was increased to about 2.5-folds and 125% of the respective controls in the drug-treated juvenile and adult brains at the level of the rostral striatum. The GAD-immunoreactivity was present in the axonal terminal boutons and somata in the cerebral cortex and sub-cortical regions. AMPH treatment increased the density of the GAD-immunoreactive boutons by about 49%-90% and 34-151% in the layer IV of rostral to caudal somatosensory cotex of the juvenile and adult rats respectively. Variable levels of upregulation in the density of GAD-boutons were also seen in layers II-III and V of the somatosensory and motor cortices, and in the pyramidal cell layer of hippocampus of both ages. The data indicate that GABAergic neurons are significantly involved in the neuronal reorganization evoked by the chronic drug administration and reflect synaptic plasticity of the developing and adult brains. *Supported by NSC-88-2314-B-002-057 from NSC of Taiwan.*

THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION OF LAYER II/III AND IV LATERAL NETWORKS IN THE CAT VISUAL CORTEX (AREA 18)

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The functional specificity of long-range horizontal connections was studied in layers II/III and IV using optical imaging of intrinsic signals in combination with reconstruction of cortical connectivity after injections of fluorescent latex-beads and biocytin. Following histological reconstruction of all labelled somata and axon terminals the anatomical distributions were compared with the functional maps obtained with optical imaging. Injections into layers II/III resulted in a more extensive and patchy horizontal labelling than those into layer IV. Shart-range (<600 µm) and long-range projections (>600 µm) were distinguished with respect to their radial distance from the centre of the injections. Quantitative evaluation of the anatomical connectivity and orientation maps showed that long-range projections in layer II/III had a bias to connect with locations of similar orientations whereas the projections in layer IV had a weak bias to contact dissimilar orientations, including cross-orientations. For layers II/III and IV injections the short-range projections preferred similar orientations although the whole of connections spanned a broad range of orientations. The results suggest that the functional specificity of long-range lateral networks differs between the superficial and the granular layers indicating their involvement in different tasks of early image analysis.

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ANALYSIS OF INDIVIDUAL CHROMOSOMES IN BRAIN TISSUE SECTIONS BY MULTICOLOR FISH.

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The aim of this work was identification and analysis of interphase chromosomes in differentiated cells of human brain by multicolor FISH. Special rapid FISH protocol and software for studies of chromosomes in brain sections by computer image analyses were developed. Application of alphoid DNA probes, directly labeled by fluorophores, and fixation of brain sections in fixative with acetic acid allow to avoid technical restrictions of common FISH protocol due to preventing low penetration of probes to targets and decreasing of autofluorescence of brain sections in fluorescence microscopic analysis. FISH analysis of brain samples in four control samples and two samples of patients with schizophrenia was performed with DNA probes for chromosomes 13, 21, 18, X and Y. It was shown that in nuclei of neurons in all samples studied all of target chromosomes were positioned at Nucleolar Organizer Regions. However, in patients with schizophrenia, statistically significant level of association of nonhomologous chromosomes in intrephase nuclei of glial cells was detected. Cytogenetic studies indicated that glial cells in brain of schisophrenic patients show significantly high level of heterochromatinization of nuclear DNA in contrast to control nonschizophrenic individuals. FISH studies on brain sections with specially optimized DNA probes offers additional possibilities for investigation of interphase organization of chromosomes, for analysis of position and rearrangements of individual chromosomes and detection of low level of mosaizism or aneuploidies in interphase nucleus of brain cells in individuals with psychiatric diseases.(Supported, in parts, by grants from Russian National Human Genome Program)

SPECIFIC CONTROL OF OPIOID FUNCTIONS BY NEUROPEPTIDE FF.

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Neuropeptide FF (NPFF) is an endogenous peptide able to modulate opioid functions via specific receptors localized in the central nervous system. NPFF attenuates opioid analgesia in rodents and is very likely involved in the establishment of opioid tolerance. Putative interactions between NPFF and opioid receptors have been investigated by using nociceptin which triggers the same G-protein signalling pathways as do opioids. Intracellular Ca2+ concentration ([Ca2+]i) was measured in neurons, acutely dissociated from the dorsal raph nucleus of rats aged 8 to 20 days, with the fluorescent calcium probe Fluo3. Nociceptin had no effect on resting [Ca2+]i but reduced by 33 % the magnitude of the [Ca2+]i transient triggered by depolarization in 89 % of neurons having polygonal or fusiform perikarya. 5-HT (30 µM) also reduced the magnitude of the [Ca2+]i transient and this effect was blocked by the selective 5-HT1A antagonist p-MPPI. The neuropeptide FF analog [D-Tyr1, (N-Me)Phe3]NPFF did not change neither the resting [Ca2+]i nor the [Ca2+]i transient triggered by depolarization but decreased the response to nociceptin (EC50 = 1.8 nM) and had no effect on the response to 5-HT.
These features reveal the specificity of cellular interactions between opioid and Neuropeptide FF receptors.

NEUROMODULATION IN NETWORK FOR WITHDRAWAL BEHAVIOR OF TERRESTRIAL SNAIL

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The role of neuromodulation is described for many behaviors. We searched for identified neurons modulating synaptic inputs of giant premotor (command) interneurons triggering withdrawal behavior in terrestrial snail *Helix*.

Activation of serotonergic identifiable pedal neurons was shown to increase the amplitude of synaptic responses to noxious stimulation and to change the excitability in command neurons for withdrawal. Observed changes in the EPSP amplitude were mimicked by serotonin application.

Intracellular strong activation of one of 4 parietal command neurons for withdrawal elicited significant decrease in amplitude of synaptic responses to noxious stimulation in all parietal command neurons for withdrawal. This effect was mimicked by application of tetrapeptide FMRFamide known to be present in all 4 parietal command neurons for withdrawal. Therefore, down-regulation of investigated synaptic input at least partially is achieved by recurrent modulatory influence of postsynaptic cell onto its own synaptic input and synaptic inputs of other command cells of the withdrawal network.

Therefore, at the level of premotor interneurons of the withdrawal behavior network, up-regulation is achieved by modulatory influence of pedal serotonergic cells, while down-regulation is achieved by modulatory influence exerted by activity in the premotor interneurons themselves. Noxious stimuli activate serotonergic cells (up-regulation) which increase firing of premotor interneurons decreasing the synaptic input effectivity (down-regulation). Observed negative feedback at the level of premotor (command) interneurons is helpful in cessation of sensitization period in absence of dangerous stimuli.

THE ROLE OF THE CYTOSCELETON AND THE CALCIUM IONS IN THE SPATIAL ORGANISATION OF TRANSMITTER RELEASE AT THE SINGLE ACTIVE ZONES.

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The possible role of cytoskeleton and calcium ions in the spatial organisation of the single active zones (AZ) of the motor nerve endings was investigated using the three extracellular microelectrodes method (Zefirov et al., 1990, 1995). Experiments were performed on the nerve-muscle preparations of the frog Rana Ridibunda. The coordinates of the sites of release was determined using the analysis of several hundreds of miniature end-plate currents (MEPC) amplitude in normal and after the colchicine (10 mkmol) or EGTA (5mmol) adding. Results. In normal the sites of release, responsible for the MEPC generation, are arranged in the in groups and reflects the transmitter release profile at the single AZ (Zefirov et al., 1995). It is shown, that after the colchicine adding or the decreasing the extracellular calcium concentration to zero the pictures of transmitter release at the AZ disrupted and the sites of release are scattered along the whole nerve ending. The functional roles of microtubules and calcium ions in the supporting of the AZ structure are discussed.

RETROGRADE DENDRITIC RELEASE OF GABA INDUCES EPSP DEPRESSION IN GLUTAMATERGIC SYNAPSES IN NEOCORTEX.

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In GABA-ergic somatostatin-positive bitufted interneurons in L2/3 of rat neocortex synaptically connected to pyramidal cells, short bursts of backpropagating dendritic action potentials (APs) initiated depression of EPSPs. Synaptic depression approached a steady state level in 5-7 min during the conditioning AP train protocol and EPSPs recovered during a similar time period following the conditioning protocol. Synaptic depression was dependent on a rise in dendritic Ca2+ and was prevented by loading bitufted cells with BAPTA or EGTA (5 mM). The failure rate of EPSPs as well as paired pulse facilitation of EPSPs increased significantly following the AP bursts. Depression was blocked by the GABAb receptor (GBR) antagonist CGP55845A and was mimicked by the GBR agonist baclofen indicating that activation of GBRs underlies this form of synaptic modulation. These GBRs were expressed on the pyramidal cell axon terminals and were neither activated by somatostatin nor by GABA released from autaptic nerve terminals. We conclude that presynaptic GBRs are activated by a retrograde messenger, most likely GABA, released from bitufted cell dendrites following back-propagating APs. Dendritic vesicular exocytosis is likely to underly the Ca2+-dependent GABA release process since synaptic depression was completely prevented by the light chain of botulinum toxin-D while the inactive mutant of the same toxin did not affect the depression. GBRs induce inhibition of terminal Ca2+ channels via a G-protein dependent pathway leading to a reduction in glutamate release and synaptic depression.

NEUROACTIVE STEROIDS DIRECTLY REGULATE THE ACTIVITY OF Ca^{2+}-ATPase PURIFIED FROM RAT CORTEX.

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The prevailing classical hypotheses for the mechanism of steroid hormones regulation postulate their genomic action. Recently, the plasma membrane has been extensively studied as a possible site of steroid binding. The plasma membrane calcium pump is responsible for ATP-powered returning of $[Ca^{2+}]i$ to a basal level after depolarization of the neuronal cell. The aim of our study was to determine whether neuroactive steroids: dehydroepiandrosterone sulfate, pregnenolone sulfate, 17-β-estradiol and testosterone, are able to modulate directly the activity of purified rat cortical Ca^{2+}-ATPase. The sulfate derivatives of pregnenolone and dehydroepiandrosterone applied at concentrations 10^{-11}-10^{-6}M showed an inverted U-shape potency in the regulation of Ca^{2+}-ATPase activity. At physiologically relevant concentration (10^{-8}-10^{-9} M) the maximal enhancement of the activity reached 200%. Testosterone (10^{-11}-10^{-6} M) and 17-β-estradiol (10^{-12}-10^{-9} M) caused a dose-dependent increase in hydrolytic ability of enzyme, and the activity reached the highest values 470% and 200%, respectively. All examined steroids decreased the stimulatory effect of the naturally existing activator of calcium pump – calmodulin. Our results indicate that Ca^{2+}-ATPase isolated from cortical synaptosomal membranes appears to be a target site for neuroactive steroid action at biologically relevant concentrations, and structural differences of the steroids could be a critical determinant of the steroids-calcium pump interaction. The observed non-genomic effects of steroids may represent a new regulatory process involved in the maintenance of neuronal calcium homeostasis.

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PHYSIOLOGY OF PARA- AND ENDOKRINE SECREATION IN THE STOMACH OF BIRDS AND MAMMALIAN WITH DIFFERENT KIND FOOD

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The functional morphology locally regulating humoral gastric Apparatus of birds and mammalia with different kind of food was stuided in complex using histological , hostochemical , electron microscopic , quantitative and statistical methods of study . It was proved that this cjonsisted of specialized paraendrocrine cells - APUD-cells. It was stated that topography , structure and fuction of APUD-cells have toxonamic common character in birds and mammalia. Moreover , there were revealed common structural and functional teatures related to the character of food and regimen of its digestion in the stomach. Realcytovariants of APUD-cells for common character of their specialization were identified . It was proved that above said cells can predominantly secrete oligipeptide hormones , particularly biogenic amines both sinchronously . moreover , we first proved that same vertebrate have secretory cells with the parametrs of exocrine and apudocytic. We observed that in the stomach of birds and mammalia with animal food the more active were APUD-cells - stimulators of secretion of gastrik ferments and acidity and also muscular peristalsis. In birds and mammalia with vegetable food APUD-cells stimulated the secration of mucus and peristalsis .

THE SUBCOMMISSURAL ORGAN SECRETION COULD BE INVOLVED IN LIZARD'S KYPHOTIC MALFORMATION (*agama impalearis*)

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The subcommissural organ (SCO), one of the circumventricular organs, is formed by secretory ependymal cells and secretes a glycoproteine called Reissner's fiber (RF). It has been reported the probable implication of the SCO secretion in malformative syndromes such as lordosis, therefore the physiological mechanisms involved in such skeletal malformation are, to date, little understood. We studied normal and kyphotic lizards (*agama impalearis*) by immunohistochemical procedure, using RF and c-fos antibodies, to point out the possible involvement of the SCO secretion in this syndrome. Compared to normal *agama*, the kyphotic SCO ependymocytes display a different immunoreactive secretory material of basal round-shaped structures surrounding the SCO cells. A condensation of the immunoreactivity was observed also in the apical part of the kyphotic SCO. Numerous c-fos immunoreactive ependymal cells were observed in the basal pole of the SCO, whereas no c-fos activity was revealed in normal. We report in this study on the increase of the activity of ependymal secretory cells either in apical and basal parts of the kyphotic SCO, and the possible involvement of the SCO secretion in kyphosis malformation of the *agama*.

BASAL GANGLIA INFLUENCES ON THE CARDIORESPIRATORY FUNCTIONS IN CATS

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A vast amount of data has been accumulated in the literature to show the role of the basal ganglia circuitry in the control of somatomotor activity. Far fewer reports are known to talk about the involvement of the basal ganglia in altering cardiorespiratory functions. Previous studies from this laboratory showed that electrical stimulation in the basal ganglia circuitry caused locus-dependent cardiorespiratory changes in freely moving cats. This presentation compares the arterial blood pressure (BP), heart rate (HR) and respiratory rate (RR) effects elicited electrically from the globus pallidus (GP), the subthalamic nucleus (Sub) and the substantia nigra (SN). Though significant increases in BP, HR and RR were obtained from all of these loci, the patterns of the changes were different. The high amplitude increase in BP failed to occur during stimulations repeated under the blockade of adrenergic α_1-receptors by phentolamine. The cardiorespiratory responses might be elicited also in the cat anesthetized with ketamin. BP, HR and RR effects failed to appear after local microinjection of kainic acid. The fact that the basal ganglia are able to modify the cardiorespiratory functions simultaneously with the somatomotor behaviour, suggests the involvement of the basal ganglia circuitry in the cardiorespiratory adjustments to the somatomotor activity.

MECHANISMS INVOLVED IN SINDBIS VIRUS-INDUCED APOPTOSIS OF NEURONAL AND GLIAL CELLS

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Sindbis virus is an alpha virus used as a model for studying the pathogenesis of viral encephalitis. SV infects cells in either persistent infection or lytic infection that leads to apoptosis. In a recent study we found that virus strains which lead to apoptosis induce the expression of bax and reduce the expression of Bcl2, whereas persistent infection resulted in opposite effects. To examine the role Bcl2 in SV-induced apoptosis we overexpressed Bcl2 and Bcl2-antisense in neuroblastoma and glial cells and measured apoptosis in response to infection with the with the neurovirulent strain SVNI. We found that overexpression of Bcl2 did not protect neuroblastoma or C6 cells from apoptosis induced by SVNI and expression of antisense Bcl2 rendered the cells more sensitive to SVNI. The mechanisms involved in the effects of SVNI and another neurovirulent strain, SVN, on cell apoptosis were also studied. Although these two SV strains induced similar effects on the expression of Bcl2, they exerted different effects on cell apoptosis. Thus, SVN induced apoptosis only in mixed neuron-glia cultures whereas it did not affect isolated neuronal cultures. Significant differences were observed also in the induction of TNF-α by the viruses. SVNI induced a large increase in the expression of TNF-α mRNA protein, whereas SVN had no significant effects. Addition of exogenous TNF-α induced apoptosis in cells infected with SVN to a similar degree of that obtained with SVNI. Our results suggest an important role for TNF-α as a possible mediator in the apoptotic effect induced by SVNI. The role of bcl2 and other apoptotic-related genes is discussed.

THE INTERHEMISPERE SLEEP ASYMMETRIA, EPILEPSIA AND POSTEPILEPTIC SLEEP

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In the animals (cats) with «neurosurgical simplification» of projectional and comissural connections of the brain, during normal sleep, during penicillin epilepsy, and during the sleep after epileptical convulsion, we recorded TTG, TMG and EOG. In the investigations the analysis is given of penicillin epilepsy in animals in conditions of different forms of differentiation of one of the halves of the fore brain. It is shown, that in conditions of combined section of one half of the operculum of the midbrain and comissural systems of the endbrain, diencephalon and midbrain, under large doses of penicillin, unilateral convulsive activity is recorded in summate electrical activity of one of the brain halves at the side of the midbrain lesion. After the completion of unilateral organization of the epileptic process an asymmetrical sleep takes place: at the side, at which epileptiform activity was recorded, a more deep phase of the sleep appears than in the opposite brain half.

CELL COUPLING IN NORMAL AND AXOTOMIZED SENSORY GANGLIA

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Research in recent years has shown that dorsal root ganglia (DRG) serve not only as matabolic depots but also take part in the processing of sensory information in both normal and pathologic states. DRG neurons have been studied thoroughly, but very little is known about the satellite glial cells (SCs) surrounding them. We investigated coupling among SCs in DRG of mice and guinea-pigs by injecting them with the fluorescent dye Lucifer yellow (LY) from micropipettes. We found that under control conditions SCs were coupled only to other SCs around a given neuron. This coupling appeared to be mediated by gap junctions as it was blocked by octanol or acidic pH. We next asked whether coupling is altered after transection of the axons of DRG neurons. We transected mouse sciatic and saphenous nerves, and 7-14 days later removed the DRGs of segments L4-L5. LY was injected into single neurons or SCs. In 32 (65%) of 49 cases the injection of a single SC resulted in staining of up to 20 neighboring neuronal and SC's. Some of the SCs appeared to hypertrophy after axotomy, which correlated with upregulation of glial fibrilary acid protein (a glial marker). Fifty three neurons from axotomized mice were also dye-injected. In 53% of cases dye coupling of up to 20 neighboring neuronal and SCs was observed. No morphological changes was noticed in the neurons. In either contralateral side or in control group of animals abnormal coupling was not found (29 SCs, 31 neurons). Axotomy- induced dye coupling was blocked (in 70% of cases) by octanol (1mM), suggesting that coupling was mediated by gap junctions. In summary, after axotomy glia and neurons are mutually coupled, apparently by gap junctions. We propose that coupling is one of the cellular reactions in DRG to axotomy and can contribute to neuropathic pain that is associated with nerve injury.

INTERRELATION BETWEEN THE STATE OF THE IMMUNE SYSTEM, CENTRAL ADRENERGIC ACTIVITY AND BLOOD-BRAIN BARRIER IN PATIENTS WITH ISCHEMIC INSULT

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According to a contemporary concept of the unity of neuronal and immune systems, any changes in the immune system status must be accompanied with the changes in functional and structural state of the central nervous system. In the present work the possible interrelation between the changes of some immunologic parameters, central adrenergic activity and blood-brain barrier integrity has been studied in a group of patients with ischemic insult (n=20), in comparison to a healthy volunteer's group (n=30). The data obtained have shown the correlation between the increase of the concentration of soluble and insoluble circulating immune complexes (CIC) in the blood of the affected individuals and decrease in dopamine-β-monooxygenase activity. This enzyme is converting dopamine to noradrenaline and is considered as the blood indicator of the central adrenergic activity. The identification of the protein composition of the affected individuals' CIC by the use of SDS PAAG electrophoresis has revealed the presence of the specific protein bands, absent from the CIC of the healthy volunteers. In addition by the use of a double immune diffusion procedure in a blood serum of all the patients it has been shown the presence of the autoantigens to a brain specific proteins, absent from the serum of the healthy volunteers. The latest is suggesting about the dysfunction of the brain-blood barrier upon the ischemic insult pathology, thus presenting further evidence in favour of the unity of neuronal and immune systems.

REINFORCEMENT DRIVEN DIMENSIONALITY REDUCTION IN THE BASAL GANGLIA

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The basal ganglia are part of a loop connecting the entire cortex to the frontal cortex. Despite a large body of clinical and experimental data and the critical role they play pathogenesis of various movement disorders such as Parkinson's and Huntington's diseases, the processing they perform remains obscure. Anatomical data has shown two major aspects: Massive stepwise funneling architecture of the neural population in the different nuclei and an extensive network of lateral inhibitory within these nuclei. However, recent physiological data has shown that these lateral connections seem devoid of any functional significance and that no intra-nuclear correlation exists in striatal and pallidal firing. In this study we report on a new hypothesis that explains the above-mentioned discrepancies. The hypothesis is based on the proposal that the basal ganglia perform dimensionality reduction of the large and complex information space spanned by the activity of cortical neurons. We show that a neural network featuring key aspects of the known anatomical and physiological facts that characterize the basal ganglia is ideally suited to perform such a process. The network combines reinforcement driven and unsupervised learning to achieve optimal information extraction of the input that it receives. During the learning phase, the neurons within the same layer of the network have correlated activity and their lateral interactions are active. Both the correlations and the activity disappear once optimal information extraction is achieved. Finally, we use this computational model to provide explicit predictions for future in-vitro and in-vivo experiments.

NEGATIVE SIGNALS FROM THE GASTRO-INTESTINAL SYSTEM: A POSSIBLE KEY TO UNDERSTAND SATIETY, MOOD CHANGES AND SICKNESS BEHAVIOR

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Behavioral effects of intestinal signals are often neglected because of their special sensory characteristics. Unless being painful or very strong, this afferent information remains unconscious and therefore resists introspection. It doesn't necessary mean, however, that GI signals has no capacity to influence behavior: on the contrary, many features of the behavior are in fact strongly influenced by changes of the internal organs and vice-versa. By scanning human and animal experimental data one may arrive to an attracting hypothesis: behaviorally effective gastro-intestinal signals are (almost exclusively) unpleasant and negative. This presentation examines this possibility in an animal model.
The main features of this model are: repeatable and long available intestinal surface using an isolated (Thiry-Vella) intestinal loop in rats; differential and measurable stimulation, e.g. isometric versus volumetric distension by rubber balloon; independence of the alimentary functioning. In subsequent experimental series, different methods detecting and/or measuring aversiveness were applied with identical stimulus parameters providing a mean to compare their effects under different conditions and from different aspects (threshold and open-field experiments, free drinking with behavioral measurements, taste-aversion, taste-reactivity, etc.).
Results show that behavioral effects of the intestinal signals depends on the intensity of the stimulus as well as on its specificity in terms of the ongoing behavior and on the complexity of the environment, respectively. Isometric distension, for example, is only detected if the environment is simple and the situation is specific; otherwise remains undetected; its affect is termed discomfort. Volumetric distension, on the other hand is usually felt clearly unpleasant, frequently painful and has an evident capacity to immediately interrupt ongoing behavior. These studies may help to map the continuum of the visceral negative affects of which a significant range has only covert effects. Phenomena like alliesthesia, mood problems, functional GI diseases may be better understand and explained by this approach.

DOPAMINE UPTAKE BY MITOCHONDRIA: IMPLICATIONS FOR DOPAMINE NEUROTOXICITY.

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The mechanisms underlying dopaminergic abnormalities in neuronal disorders such as schizophrenia and Parkinson's disease are still unknown, yet in-vivo and in-vitro exposure to dopamine may result in neuronal death. Dopamine toxicity has been attributed to various mechanisms among them a direct inhibition of the mitochondrial respiratory chain by the dopamine. We have previously shown that dopamine inhibited mitochondrial complex I activity with high efficiency (IC_{50}=8μM). In order to be able to inhibit mitochondrial respiration in-vivo, dopamine has to be taken up through the selective inner membrane of the mitochondria. Indeed, our studies demonstrate that dopamine is taken up by intact mitochondria in a saturated manner with apparent K_m of 122.1±28.6 nM and V_{max} of 1.4±0.15 pmol/mg protein/min. Assuming a mitochondrial volume of 1μl/mg protein, dopamine is concentrated 45 times by mitochondria. Dopamine uptake process is dependent on membrane potential and the presence of Na^+ (plus ATP in non-respiring mitochondria), suggesting a Na^+ gradient as a source of energy for dopamine uptake. In addition, the pharmacological profile of mitochondrial dopamine uptake process was different from that of dopamine plasma membrane transporter and the vesicular amine transporter. In human neuroblstoma SH-SY5Y cells exposed to dopamine a high negative correlation (r^2=0.92; p=0.012) was found between intracellular dopamine and ATP levels. These results further support the hypothesis of dopamine interaction with mitochondrial respiration as a possible mechanism of dopamine neurotoxicity.

DEVELOPMENTAL REGULATION OF AMPHIPHYSIN EXPRESSION IN THE CHICK VISUAL SYSTEM

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The molecular machinery that orchestrates the process of clathrin-mediated endocytosis is important for the synaptic vesicle recycling in neurons. Recent data elucidated that amphiphysin is closely connected with dynamin-mediated vesicle budding at the synapse. So far only few data are available for the developmental transcription of mRNA or the onset of protein expression during these processes. To investigate the developmental changes in the expression of amphiphysin, a key player for the clathrin-mediated endocytosis in neurons, we used the retinotectal system of the chick, a highly ordered primary pathway.
RT-PCR of total RNA from chick retina and tectum revealed first transcripts at embryonic day 5 (E 5) for both regions. The amount of RNA was increasing until E 11 and kept the high level until adulthood. Surprisingly, western blots revealed protein expression only after E 11 for the retina or E 9 for the tectum noticing that not only retinal afferents are attended. Further on, a steady increase of the proteins was found until the first postnatal week.
Immunofluorescence staining for amphiphysin was not detected before E11 in the developing chick retina, while other presynaptic proteins, like syntaxin, showed signals soon after neuronal birth of retinal ganglion cells around E4. With the onset of synaptogenesis in the retina around E 13, amphiphysin was distributed predominantly in the inner plexiform layer and from E 16 on in the developing outer plexiform layer when first synaptic specialications such as synaptic ribbons and synaptic vesicles in photoreceptor pedicles were seen. Ultrastructural analysis exhibited that immunolabeling for amphiphysin using the preembedding peroxidase technique was localized on synaptic vesicles exclusively within presynaptic terminals of the inner and outer plexiform layer of the retina. As found in the retina, immunoreactivity in the retinorecipient layers of the tectum was found from E 13, e.g. at the time of synapse formation in these areas.
Taken together our data reveal (1) that there is a developmental delay between mRNA transcription and protein expression for key proteins involved in endocytosis, suggesting a posttranscriptional control of protein biosynthesis. (2) Proteins implicated in regulated membrane retrieval get upregulated after synapse formation in the investigated system, indicating their primary participation in endocytosis.

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LOW DOSE ANADAMIDE AFFECTS FOOD INTAKE, COGNITIVE FUNCTION AND NEUROTRANSMITTER LEVELS IN DIET-RESTRICTED MICE

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In order to study possible novel therapies for anorexia (AN), we have studied the effect of low dose anandamide (ANA, 0.001 mg/kg) administration on food intake, cognitive function, catecholaminergic and serotoninergic pathways in two murine brain areas concerned with appetite (hypothalamus) and learning (hippocampus). Eight week old female BALB/c mice were fed 2.5 hours each day. They consumed significantly more food daily ($p<0.05$) during one week of ANA treatment. In the hypothalamus, there were significantly increased concentrations of NE ($p<0.01$), DA ($p<0.05$) and 5-HT ($p<0.001$). In the hippocampus, ANA also increased significantly NE and DA, but decreased 5-HT (all at $p<0.001$).
Another group was fed diet restriction (DR) to 40% of the daily nutritional requirements. ANA administration reversed the DR-induced impairment in eight-arm maze performance. This was associated with a significant increase in NE turnover, and normalization of both DA and 5-HT concentrations, the later probably secondary to reduced turnover. The low dose ANA which improved food intake, cognitive function and reversed some of the neurotransmitter changes caused by DR, might have implications for the possible treatment of patients with AN.

APPROACHING THE MOLECULAR MECHANISMS UNDERLYING LATE-ONSET NEURODEGENERATION OF CHOLINERGIC NEURONS USING INDUCIBLE ANTISENSE AND RIBOZYME SUPRESSION OF ACETYLCHOLINESTERASE GENE EXPRESSION IN TRANSFECTED CELLS

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The function of acetylcholinesterase (AChE) in terminating cholinergic neurotransmission is well recognized. However, this enzyme has gained new importance since it has been found to be involved in other processes, such as cell motility, division, development, growth, and stress responses. These attribute to the AChE protein non-catalytic morphogenic activities that may be deleterious to the nervous system and suggest that interference with its accumulation may be therapeutically beneficial. Moreover, modulation of the cellular level of this protein would be instrumental for elucidating the mechanisms of its complex function. In particular, the role of AChE in late-onset neurodegeneration of cholinergic neurons is of utmost importance. To establish controlled downregulation of the ACHE gene, we use tetracycline controlled expression of antisense/ribozyme constructs targeted to the domain on AChE mRNA that we have found to be especially vulnerable to ribozyme degradation. Ribozyme and antisense expressing constructs were demonstrated to suppress human AChE in vitro and in CHO tet-off cells as well as to prevent the accumulation of endogenous rat AchE in PC12 tet-on cells treated with nerve frowth factor.

THE NEUROMUSCULAR JUNCTION: AGRIN - INDUCED CLUSTERING OF NITRIC OXIDE SYNTHASE (NOS) IN MOUSE SKELETAL MYOTUBES

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The neuromuscular junction (NMJ) is a highly specialized region with distinct molecular anatomical structures. Formation and maintenance of the molecular architecture of the NMJ appears to be regulated by a concert of proteins and modulators derived from presynaptic and postsynaptic partners i.e. the motoneuron and myofiber, as well as from extracellular matrix (ECM) molecules that build up a unique compartment at the synaptic cleft. One of these proteins is agrin which is synthetized and released by developing motoneurons to form synapses with their target cells in e.g. skeletal muscle. At the first formed NMJ agrin is involved in the accumulation of synaptic molecules such as the nicotinic acetylcholine receptor (AChR) that normally disperses over the myofiber sarcolemm. NOS which generates the gaseous messenger NO, appears to be accumulated at the NMJ in innervated skeletal myofibers. Both agrin and NOS bind to members of the transmembraneous dystroglycan-glycoprotein complex (DGC)-complex i.e., via extracellular dystroglycan (DG) and subsarcolemmal syntrophin-PDZ, respectively. The molecular interplay between agrin and NOS in NMJ-formation is not known. We therefore tested for the possible agrin-induced clustering of NOS in mouse C2C12 skeletal myotubes in vitro. C2-myotubes expressing diffuse cytosolic NOS-1 (neuronal NOS) were incubated in the presence of full-length s-agrin 4,19 (100pM) or one of its truncated isoforms (N-4) followed by detection of AChR by fluorochrome-labelled alpha-bungarotoxin (BGTx) or monoclonal anti-s-agrin (mab 131) or mab 30 (N-4). In agrin-stimulated myotubes BGTx-positive clusters were seen that co-immunostained for neuronal NOS (NOS-I). Co-clustering was absent in control cultures without agrin. The results suggest the presence of a co-clustering activity of s-agrin for both AChR and NOS-I in C2-myotubes in vitro supporting our hypothesis of common molecular and possibly functional interactions in NMJ formation and function, The molecular anatomical arrangement of other NMJ-related molecules is presently being investigated by colocalization studies. The cellular regulation of NOS-isoforms by neuron/muscle-derived molecules is being studied in motoneuron-muscle co-cultures. (Supported by DFG-grant to D.B.)

FAILURE OF PHOSPHORYLATION OF SYNAPSIN-I MAY CONTRIBUTE TO LONG LASTING SYNAPTIC TRANSMISSION DEFECT AFTER TRANSIENT FOCAL CEREBRAL ISCHEMIA

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We recently showed that transient ischemia caused a long lasting synaptic transmission defect in the penumbral motor cortex by evoked potential recordings (*Bolay and Dalkara, Stroke 29: 1988-1994, 1998*). In the present study, we extended these findings by recording intracellularly from cortical motor neurons in the ischemic penumbra, and by studying phosphorylation of synapsin-I to elucidate mechanisms of synaptic transmission failure. One-24 h after reperfusion following 1-h proximal middle cerebral artery occlusion in rats, spontaneous activity, direct excitability of neurons, post-synaptic potentials evoked by stimulation of premotor afferents to motor cortex were recorded in vivo by borosilicate glass microelectrodes filled with 3 M KCl and having tip resistances of 35-100 MΩ. Rat brain slices were stained with anti-synapsin-I, anti-phosphosynapsin-I and anti-synaptophysin antibodies 1-24 h after reperfusion. Neurons were partially depolarized and no post-synaptic activity could be evoked by either ortho- or antidromic stimulation for 24 h after 1-h transient ischemia. However, these neurons were able to generate action potentials, indicating that neuronal excitability was preserved but synaptic transmission was defective. There was no difference between ischemic and non-ischemic hemispheres after 1-h ischemia when brain slices were stained with synapsin-I or synaptophysin antibody. However, phosphosynapsin-I immunostaining was not detected in the densely ischemic core region and was significantly decreased in the penumbral cortex that received a low level of blood flow through collaterals during ischemia. In conclusion, these data indicate that transient cerebral ischemia can lead to a long lasting synaptic transmission defect and, failure of phosphorylation of synaptic proteins may be one of the contributing mechanisms. Additionally, phospho-synapsin staining could be a marker of functionally impaired but viable neurons in the ischemic penumbra.

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THE ROLE OF ENDOTHELIAL NITRIC OXIDE GENERATION AND PEROXYNITIRITE FORMATION IN REPERFUSION INJURY IN FOCAL CEREBRAL ISCHEMIA

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Reperfusion injury is one of the factors that unfavourably effect the stroke outcome and shortens the window of opportunity for thrombolysis. Surges of NO and superoxide generation upon reperfusion have been demonstrated. Concomitant generation of these radicals can lead to formation of the strong oxidant, peroxynitrite. We have examined the role of NO generation and peroxynitrite formation on reperfusion injury in a mouse model of middle cerebral artery occlusion (2 h) and reperfusion (22 h). Four groups of Swiss Albino mice received one of the following treatments a) the non-selective nitric oxide synthase (NOS) inhibitor N-ω-Nitro-L-Arginine (L-NA) just before reperfusion (n=6), b) a specific inhibitor of neuronal NOS, 7-Nitroindazole (7-NI) either before reperfusion (n=4) or induction of ischemia (n=4) c) saline (n=10). The infarct volume detected by TTC staining was significantly decreased (49%) in animals treated with L-NA at reperfusion. 7-NI given at reperfusion showed no protection although pre-ischemic treatment with 7-NI was protective (40 % decrease in infarct volume). L-NA treatment also significantly reduced brain edema and Evans blue extravasation (detected spectro-photometrically). Cerebral blood flow levels measured (by laser-Doppler flowmetry) during ischemia and first 30 minutes of reperfusion, and arterial blood gases were not significantly different between groups. Staining of brain sections with anti-nitrotyrosine antibody showed a decreased immunostaining in L-NA treated animals. These data support the hypothesis that concomitant generation of NO with superoxide at the onset of reperfusion plays a significant role in reperfusion injury possibly via peroxynitrite formation. Contrary to L-NA, failure of 7-NI to protect against reperfusion injury suggests that the source of NO is likely to be the cerebrovascular endothelium.
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ELECTROPHYSIOLOGICAL MAPPING OF THE PALLIDAL ACTIVITY LINKED TO PASSIVE LIMB MOVEMENT IN THE NORMAL MONKEY

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Comprehensive electrophysiological mapping of the pallidum (GP) and particularly of its internal part, the GPi, considered the main output structure of the whole basal ganglia network for the command of body movements, is becoming a prerequisite for the successful surgical treament of Parkinson's disease using techniques such as high frequency stimulation or pallidotomy. Extracellular unit recordings were carried out in two calm, awake and drug naive monkeys (Macaca fascicularis) to investigate any correlations between passive limb or oculomotor movements and modifications in the neuronal activity of the external pallidum (GPe) and the GPi. Existing anatomical data would have led us to expect a somatotopic organization in the pallidum. Our results show, on the contrary, that GPe and GPi cells are organized in clusters which present no somatotopic organization. In the GPe arm and leg related-neurones are located in clusters on the oral part and less oculomotor related-neurones located in a cluster in the medial part. In the GPi we only found limb clusters in the medial part. No occulomotor cell was recorded. With regard to the firing frequency, the vast majority (89%) of both GPe and GPi cells increased their firing during movement, but a reduction in firing frequency was observed for the remaining 11%. It would seem important to pursue the exploration of pallidal activity in experimental models since GP would appeared not to be somatotopically organized but in clusters. The implication for deep brain surgery in the treatment of Parkinson's disease are wide-reaching.

ELECTROPHYSIOLOGICAL INVESTIGATION OF DOPA-INDUCED DYSKINESIAS IN THE MPTP-TREATED MONKEYS : SINGLE UNIT RECORDING OF GLOBUS PALLIDUS NEURONAL ACTIVITY

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In order to study the physiopathology of dopaminergic-induced dyskinesias, we administred apomorphine (0.1 mg/kg, a dopaminergic mixt agonist), SKF-38393 (1.5 mg/kg, a D1 partial agonist) and piribedil (3mg/kg, a D2/D3 agonist) to two monkeys before and after MPTP induced parkinsonian syndrom. The effects were evaluated both by clinical quantification (motor and dyskinesia score) and electrophysiological single unit recording of the neuronal activity of the globus pallidus internalis (GPi), the principal basal ganglia output, and the globus pallidus externalis (GPe). Two parameters were studied : mean firing requency and firing pattern. No clinical changes were observed in the normal monkeys but both electrophysiological parameters were affected by drug administrations. In MPTP-monkeys, at GPi level, frequency modifications were correlated to clinical motor improvement and firing pattern changes were concomitant with the onset of dyskinesias. No correlation was found between modifications in GPe frequency and firing pattern and clinical changes. These results confirm that dopaminergic depletion is necessary for dyskinesias, but are at variance with the current functional model of the extrapyramidal motor loop. In the normal monkeys, modifications in firing pattern were not accompanied by dyskinesias but in the MPTP-treated monkeys a correlation was observed. This would indicate that other, so far unidentified factors are involved in the physiopathology of dyskinesias.

NEUROPEPTIDE-Y, AND PARVALBUMIN PARCELLATE THE HUMAN PRETECTAL COMPLEX

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Various oculomotor functions are processed in the mammalian pretectum. In subhuman species the different pretectal nuclei have been named by their position and function. The human pretectal nuclei - contrary to subhuman species - still bear their traditional nomenclature. Previously we reported that in the cat NPY characteristically labels the individual pretectal nuclei. The nucleus of the optic tract (NOT) and the anterior pretectal nucleus (APN) contain NPY perikarya. The posterior pretectal nucleus (PPN), the olivary pretectal nucleus (OPN) and the medial pretectal nucleus (MPN) contain NPY fibers only. In order to identify the human pretectal nuclei and relate them to the subhuman nomenclature NPY and parvalbumin immunohistochemistries were performed on sections of the human pretectum. Numerous NPY neurons were found in the nucleus lentiformis, which proved to be the equivalent to the NOT, and in the anterior bulge of the pretectum that we describe as the human APN. The nucleus sublentiformis is the human equivalent of the PPN, since both contain abundant NPY fiber plexuses, while the nucleus of the pretectal area is equivalent to the MPN. Parvalbumin immunolabeling in human pretectal nuclei delineates the OPN. Based on these equivalencies in the immunostaining patterns we have revealed the exact comparative chemoanatomical topography of the individual nuclei of the human and subhuman pretectum.

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LOCALIZATION OF NEUROPEPTIDERGIC CHANGES IN THE RAT TRIGEMINAL BRAINSTEM NUCLEAR COMPLEX FOLLOWING SUPRAORBITAL VIBRISSAL NERVES' TRANSECTION

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The changes of NPY and VIP immunoreactivity in neurons of the rat trigeminal brainstem nuclear complex following supraorbital vibrissal nerves' transection were related to the terminal arborization pattern of choleratoxin B subunit (CTb) labeled supraorbital primary afferents. After transection, NPY-immunoreactive fibers appeared in the trigeminal principal sensory nucleus, in the spinal trigeminal subnucleus oralis, and in the caudal part of the spinal trigeminal subnucleus interpolaris. CTb labeled supraorbital primary afferents showed identical termination fashion. While NPY-immunolabeling increased substantially in fibers of lamina II of the medullary dorsal horn, and VIP-immunolabeling appeared in the outer part of this lamina transganglionic CTb labeling from the two supraorbital vibrissae were found in the magnocellular layer of the spinal trigeminal subnucleus caudalis. Based upon our results different populations of vibrissal primary afferents are proposed, which exhibit differences both in the induction of peptidergic immunoreactivity and in their termination pattern.

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RESOTIALIZATION DEGREE IN PATIENTS AFTER STROKE

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As a part of investigations of Regional Model of Community Based Rehabilitation in Western Bačka District, about values of conditions and needs for people with disabilities the attention was concentrated upon the after stroke patients resocialization degree.
Thirty two patients (16 male and 16 female) with different consequencies after stroke were analysed.The questionaries for analyses the conditions and needs of people with disabilities in their physical and social disricts (Zamurović A. 1997) were used. To obtain better informations and evaluation of real situation in the data collection the family members of a disabled person also took part.
Most of the patients were over the age 60 years (84,4%), and 59,4% of them had the stroke over a year ago. More than 2/3 of the patients were retired, only 1 was working person, and others were social help users or had not-regulated status. In 84,4% hospital rehabilitation was carried out, and ambulatory rehabilitation in only 9,4% patients. At 59,4% patients psychological problems after hospital rehabilitation developed, and only 25% patients received professional psychological help. All the patients had many significant social problems (financeal, familyal, in marriage...), but only 31,3% received help from special servises. In activities of daily living more than 1/2 of the patients were totaly independent, except for personal hygiene activities (bath) in which only 1/4 of the patients was totally independent. 1/4 -1/3 of the patients were totaly independent in most activities of daily living. A bit less than the patients the duties which are part of there social role in family accomplisshed fully, but it is not so in according to community social role.
This research shows the problems of resotialization in patients after cerebrovascular insult, which must include the disabled persons, their family, special services and all other community resources.

MULTIPLE ROLES FOR THE ELECTROSTATIC PROPERTIES OF CHOLINESTERASES II: MOLECULAR TRAFFIC.

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In this second study, we focus on the contribution of the electrostatic properties of ChEs to the molecular traffic of substrates and products of catalysis. We present a model for the molecular traffic of ligands, substrates and products through the active site of ChEs. First, we describe a common treatment of the diffusion to a buried active site of cationic and neutral species. We then explain the specificity of ChEs for cationic ligands and substrates by introducing two additional components to this common treatment. The first module is a surface trap for cationic species at the entrance to the active-site gorge that operates through local, short-range electrostatic interactions and is independent of ionic strength. This hypotheses is being tested by constructing a series of acetylcholinesterase (AChE) mutants in which key negative residues in the cationic trap, such as D72 are mutated to tyrosine or phenylalanine. Preliminary results show that the K_M values of both D72Y and D72F mutants of *Tc*AChE are not significantly different from those of WT-*Tc*AChE, thus providing strong supportive evidence for our model.
The second module is an ionic-strength dependent steering mechanism generated by long-range electrostatic interactions arising from the global asymmetric distribution of charges in ChEs. Our calculations show that diffusion of charged ligands relative to neutral isosteric analogs is enhanced ~ 10-fold by the surface trap, while electrostatic steering contributes only a 1.5- to 2-fold rate enhancement at physiological salt concentration. We model clearance of cationic products from the active-site gorge as analogous to the escape of a particle from a one-dimensional well in the presence of a linear electrostatic potential. We evaluate the potential inside the gorge and provide evidence that while contributing to the steering of cationic species towards the active site, it does not appreciably retard their clearance. This optimal fine-tuning of global and local electrostatic interactions endows ChEs with maximal catalytic efficiency and specificity for a positively charged substrate, while at the same time not hindering clearance of the positively charged products. The role of the gorge potential is being investigated by introducing either a positive or a negative charge midway through the active-site gorge. Kinetic measurements are underway on the F330D and F330K mutants of *Tc*AChE. Preliminary results indicate that the K_M value for the F330D mutant is only five-fold larger than that of the wild-type, but the mutant enzyme displays marked substrate activation.

DIAGNOSTICS OF BRAIN INSULTS BY H^+ NMR SPECTROSCOPY

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H^+ NMR spectroscopy has been performed in 27 patients with various forms of insults by means of the unit Magnetom Vision (1,5 T, Siemens). Investigations with H^+ NMR spectroscopy reliably confirm the NAA reduction at the center of the focus in acute insult. At the peripheral zone of the insult the NAA contents is reduced by 25% relatively to healthy areas. The contents of Cho and Cr is increased. In hemorrhagic insult a double peak is appeared, in ischaemic insult - a singal peak of Lipid. In chronic zones of injury the contents of NAA and Cho have been restored only along the periphery. Diagnostic terms for a definition of the injury zone and its restoration in acute insults are equal to 2-3 days< prognostic terms to define the outcomes - 3 weeks from the beginning of the disease.

H^+ NMR spectroscopy gives an important diagnostic and prognostic information in the patient with insult.

HEAT-SENSITIVE NOCICEPTORS IN RAT DORSAL ROOT GANGLIA: ELECTROPHYSIOLOGICAL PROPERTIES OF THE SOMA

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Polymodal nociceptors in the skin respond to various painful stimuli. To investigate fast transduction mechanisms for noxious heat we used acutely isolated dorsal root ganglion (DRG) neurones as models for their own peripheral terminals. Adult rats were anaesthetised with diethylether and rapidly decapitated. DRGs were quickly dissected, enzymatically dissociated and investigated within 3-30 h electrophysiologically (whole-cell patch-clamp). Application of preheated extracellular solution resulting in up to 53°C within about 250 ms, was used as noxious heat stimulus. Whole-cell patch-clamp recordings were performed with borosilicate pipettes (5±2 MΩ, mean±SD). The resting membrane potential was -48±11 mV. Heat responses were elicited in voltage-clamp mode (holding potential -80 mV) by rapid application of preheated extracellular solution. Heat-evoked inward currents (330±320 pA) were found in 85/127 neurones. They were accompanied by an increase of membrane conductance (320±305%). Replacing extracellular [Na^+] by *N*-methyl-*D*-glucamine reduced the inward current to 41±19%; raising extracellular [Ca^{2+}] to 10 mM enhanced the inward current to 150±20% of control. The competitive vanilloid receptor antagonist capsazepine dose-dependently reduced the heat-evoked current with an IC_{50} of 13 µM. **Heat-sensitive DRG neurones were significantly smaller (27±2 mm) than heat-insensitive ones (31±6 mm). Action potential duration was significantly longer in heat-sensitive (10-90% decay time 4.8±2.1 ms) than in heat-insensitive neurones (2.2±1.0 ms).** The inward currents elicited by noxious heat can be explained by the opening of temperature-operated channels. These channels are permeable for sodium and calcium ions, and are inhibited by the vanilloid receptor antagonist capsazepine. Therefore heat-evoked inward currents may be mediated by native vanilloid receptors. The small cell size, capsaicin sensitivity and long action potential duration are typical for nociceptive DRG neurones. Thus, we conclude, that heat-evoked currents are a further criterion to identify nociceptive DRG neurones.

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BDNF GENE TRANSCRIPTS IN ADULT SUBSTANTIA NIGRA AND ITS DIFFERENTIAL REGULATION BY KAINIC ACID

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Brain-derived neurotrophic factor (BDNF) enhances survival and protects dopaminergic neurons from neurotoxicity. Thus, understanding the regulatory mechanisms governing BDNF gene expression in adult substantia nigra (SN), might contribute to the search of therapies for Parkinson's disease. Differential splicing and alternative usage of different promoters within the BDNF generates five BDNF mRNAs. We have recently shown that glutamate receptor stimulation may differentially up-regulate the expression of BDNF transcripts in fetal rat mesencephalon (NeuroReport 9: 1959-1962, 1998). Therefore, we have examined in adult SN the expression of BDNF transcripts after administration of kainic acid (KA), a glutamatergic agonist. As an appropiate control of specificity, we studied the hippocampus which is known to increase BDNF mRNA expression after KA administration.

EFFECTS OF HEMISPHERECTOMY ON IMMOBILITY TIME IN THE ROTATIONAL SWIMMING BEHAVIOR OF SWISS MICE

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Previously we reported that sex difference in immobility time on the rotatory swimming test (RSWT) depends on laterality. Here, we investigated sex-differences in immobility time during RSWT of unilateral hemispherectomized adult mice. The hemispherectomized group consisted of 25 adult mice. Fourteen animals received a sham operation. After 15 days of recovery from the surgical procedures, each mouse was placed in a container (diameter = 21cm) filled with water for 5 min on 3 different days (test-retest time interval = 48h). Vigorous movements from 30° to 30° were counted and consistency of laterality was defined considering the persistence of the same preferred turning side in three sessions. The time that the animals remained immobile was measured for each session. After the behavioral tests, the animals were anesthetized and perfused. The brains were coronally cut and stained with cresyl-violet. In all animals, the forebrains were entirely eliminated. In the hemispherectomized group 90% of the animals were classified as side-consistent rotators whereas in the control group only 50% were side-consistent rotators. Hemispherectomized males exhibited a significant higher immobility time than hemispherectomized females. Moreover, this sex-difference is greater than that exhibited for side-consistent normal animals. Therefore, hemispherectomy seems to exacerbate sex-differences.

CALCIUM CURRENT DEFICITS IN ATM-DEFICIENT MICE

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Neurologic deterioration is a major cause of death for patients with ataxia-telangiectasia (AT): in the cerebellum mainly Purkinje cells (PCs) are affected. The animal model of the human disease, the AT mutated mouse (Atm-/-), has neurologic dysfunction, but no neuronal degeneration (C. Barlow et al., Cell 86:159-171, 1996). We performed a detailed morphological analysis of cerebellar neurons of Atm-/-, and found no histological or immunohistochemical abnormalities compared with wild type (WT) cerebella. For an electrophysiological evaluation, PCs were whole-cell patch-clamp recorded in acute slices: in current clamp mode no alteration was found in resting membrane potential, input resistance, anomalous rectification or postsynaptic potentials. In response to depolarizing stimuli (0.5 nA for 1 sec) the duration of Na^+ firing was significantly shorter in Atm-/- PCs compared with WT. While in WT 90% of PCs (N= 11) produced Ca^{2+} spikes during injection of depolarizing current (1 nA for 10 sec), in Atm-/- 1.5 months old the value was reduced to 66% (N=9) and in Atm-/- older than 3 months it was only 35% (N=17). We also demonstrated that this impairment was due to smaller Ca^{2+} currents in Atm-/- (5.6±1.7 nA, N=21) compared with WT (8.2±2.8 nA, N=20) while there was no significant difference in K^+ currents between the two groups. Our results suggest that in Atm-/- mice an impairment of Ca^{2+} currents leads to a deficit in the generation of Ca^{2+}-dependent action potentials.

THE *IN VITRO* NEUROPROTECTIVE EFFECT OF GUANOSINE AND RELATED DRUGS INVOLVES ASTROCYTES.

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Astrocytes release guanine-based purines in amounts larger than their adenine counterparts both in basal condition and after hypoxia/hypoglycemia (Glia, 25:93-98, 1999). In particular, extracellular levels of guanosine (Guo) were found long elevated after insults either *in vitro* or *in vivo*. Guo was shown to exert trophic effects on neurons as well as a related drug, 4-[[3-(1,6-dihydro-6-oxo-9-purin-9-yl)-1-oxopropyl]amino] benzoic acid (AIT-082), that resulted neuroprotective also *in vivo*. In this study we wondered whether the two drugs involved astrocytes to elicit neuroprotection, by stimulating from these cells the production of neuro/pleiotrophins (N/PTs) able to prevent neuronal damages. We found that Guo (10-500 μM) and AIT-082 (10-200 μM) dose- and time-dependently increased the extracellular accumulation of N/PTs such as nerve growth factor (NGF) and S100β protein, measured by specific ELISA assays in the culture medium from rat cultured astrocytes. Drug-induced increase of NGF but not of S100β protein was prevented by the culture pretreatment with 0.5 μg/ml cycloheximide, thus indicating the requirement for NGF of *ex novo* protein synthesis. Selective Western blot analysis for NGF on cell lysate confirmed that a significant NGF increase induced by the two drugs occurred only after 4-6 hr of cell stimulation. This effect caused by either Guo or AIT-082 was linked to the activation of mitogen-activated protein (MAP) kinase cascade, as both drugs increased the phosphorylated form of the enzyme in astrocyte lysate after 5-10 min of stimulation; conversely, specific inhibitors of MAP kinase pathway, wortmannin or PD 098,059, significantly reduced NGF production from astrocytes caused by Guo or AIT-082. Finally, the conditioned medium (CM) of rat cultured astrocytes, pre-treated with 300 μM Guo or 100 μM AIT-082 and collected 6 or 24 hr after drug removal, prevented the death of cultured hippocampal neurons induced *in vitro* by a 10 min pulse with 100 μM NMDA. The neuroprotective effect of the CM was lost by adding to the medium neutralizing polyclonal antibodies specific for NGF. Therefore, Guo and AIT-082 resulted to be neuroprotective by increasing the local release of N/PTs from astrocytes and this may provide the basis for a novel therapeutic strategy in acute or chronic brain damages.

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ANTISENSE SUPPRESSION OF EXCESSIVE SOCIAL EXPLORATION BEHAVIOR IN ACHE TRANSGENIC MICE

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Neuronal circuits operating through the neurotransmitter acetylcholine (ACh) are notably involved in behavioral processes, including working and reference memory, attention, central control of movement, stimulus processing and behavioral inhibition. Brain AChE excess has been variably associated with psychological stress and cognitive deficiencies. To explore the behavioral and molecular mechanisms underlying the cognitive deficiencies induced by AChE overproduction, we employed transgenic (Tg) mice expressing human AChE in brain neurons. Tg-AChE mice and matched controls were subjected to a behavioral working memory task which involved cholinergic learning processes, the social recognition/exploration (SE) paradigm. Significant memory deficits were observed in this test in Tg AChE mice, which displayed excessive exploration behavior associated with deficient recognition capacity. Intraperitoneal administration of 1-1.5mg/kg of the AChE inhibitor tacrine retrieved short-term recognition to control levels for up to 40min. However, intracerebroventricular (icv) injection [2x25 ng/animal, 24 hr apart] of the 2-o-methylated AS3 antisense oligodeoxynucleotide targeted against AChE mRNA ameliorated the excessive exploration behavior for 24 hr at least. Immunodetection with antibodies targeted against the N-terminus, as well as the C-terminally alternative "readthrough" peptide of AChE, revealed that the improvement in behavioral performance coincided with reduction in the AChE protein in hippocampus of AS3 treated as compared to untreated mice. An AS agent targeted toward butyrylcholinesterase mRNA did not have any effects. Our findings demonstrate involvement of the AChE protein with recognition behavior and suggest that antisense suppression of AChE gene expression can facilitate short-term memory functions.

Sphingosine and sphingosine 1-phosphate modulate calcium signals in glioma C6 cells

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Sphingosine (SPH) and its derivative, sphingosine-1-phosphate (SPP), natural constituents of animal cells, attend considerable attraction as bioactive lipids that regulate cell physiology. They act as first and second messengers and may modulate the calcium signalling pathways. This study was aimed to investigate the effect of SPH and SPP on changes in intracellular Ca^{2+} concentration in glioma C6 cells and was studied with Fura-2 video imaging technique.

SPH, at high concentration of 100 μM caused a rise in intracellular calcium level, ranging from 50 to 200 nM. It also diminished the response for thapsigargin (TG – an inhibitor of calcium pump in endoplasmic reticulum) and ATP (acting via IP_3) when applied 5 min before. Preincubation with neomycin, which inhibits PLC partially eliminates this effect. This suggests the involvement of PLC in sphingosine-mediated calcium mobilisation.

However, SPH added 1 min. before ATP increases the calcium response for this agonist. This is due to inhibition of PKC. TPA has an opposite effect. It diminishes calcium response for ATP by activating PKC. SPH and TPA added simultaneously also cause a decrease in IP_3-mediated response for ATP.

SPP added extracellularly in nM concentrations caused an immediate and high rise in cytosolic Ca^{2+} level, in PTX-sensitive manner. It is therefore suggested that when SPH acts inside the cell and modulates Ca^{2+} signals in a pleiothropic manner, SPP has a property of the first messenger, acting on glioma C6 plasma membrane receptor.

NEUROPROTECTIVE ACTION OF NEUROSTEROIDS AND THE EFFECT OF PROGESTERONE ON [3H] MUSCIMOL BINDING

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Neurosteroids have been shown to modulate excitatory and inhibitory amino acid receptor function. They elicit anticonvulsant, anesthetic, neuroprotective and anxiolytic actions. Several lines of evidence suggest that such neurosteroid as 3alfa-hydroxy-5beta-pregnan-20-one (allopregnanolone, AP), 3alfa-21-dihydroksy-5beta-pregnan-20-one (tetrahydrodeoxycorticosterone, THDOC) and progesterone (PROG) can act as positive modulators at the GABA-A receptor, whereas 3alfa-hydroxy-5beta-pregnen-20-one sulfate (pregnenolone sulfate, PS) and 5-androsten-3beta-ol-17-one sulfate (dehydroepiandrosterone sulfate, DHEAS) as negative modulators. The present study was aimed to assess neuroprotective effects of some neurosteroids. We examined the action of PROG and its metabolite (5beta-pregnan-3,20-dione, pregnanedion, PREG) administered intraperitoneally on picrotoxin-, bicuculline- and NMDA-induced lethality in mice. The results indicated that PROG protected the mice against picrotoxin (ED50 142,36 mg/kg)-, bicuculline (ED50 116,68 mg/kg)- and NMDA (ED50 165,85 mg/kg)-induced mortality. PREG also decreased lethality produced by picrotoxin (ED50 149,10 mg/kg) and NMDA (ED50 160,38 mg/kg). Diazepam, as a reference compound, showed the protective effects on picrotoxin (ED50 4,98 mg/kg)- and bicuculline (ED50 0,78 mg/kg)-induced neurotoxicity in mice. It was also shown that intracerebroventricular injections of AP (ED50 8,39 ug/5ul) and 5B-THDOC (ED50 27,92 ug/5ul) attenuated the picrotoxin-induced neurotoxicity. Furthermore, 5B-THDOC (ED50 102,70 ug/5ul) also appeared protective in a model of NMDA-induced mortality. MK-801, as a reference compound, decreased lethality produced by NMDA (ED50 5,96 ug/5ul). In the autoradiographic study, progesterone produced no changes in [3H] muscimol binding in dentate gyrus, occipital and enthorinal cortex. In summary, this data suggested that neurosteroids may have neuroprotective effects. This action is accomplished via interaction with the GABA-A receptor complex and, to a lesser extent, the NMDA receptor complex. Moreover, the dissociation of behavioral and biochemical effects of progesterone is suggested.

ANALYSIS OF FOS PROTEIN IMMUNOREACTIVITY AFTER SPINAL CORD CONTUSION

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The objective of the present investigation was to examine mechanisms involved in acute lesion-induced cellular alterations, in a rat model of spinal cord injury, produced by dropping a 5-g weight from 20.0 cm onto the exposed dura at the T10-L1vertebral level. To examine these mechanisms immunoreactive (ir) labeling of Fos, the nuclear protein encoded by the *c-fos* gene and a marker of neuronal activation, was observed in the spinal cord and the midbrain of rats killed 2 hr after weight drop contusion. Behavioral alterations were analyzed by clinical observation. Results showed that there was a significant increase of Fos-Ir neurons in laminae I-II and X of spinal cord, reticular formation, area postrema and solitarius tract nuclei of lesionated rats. c-Fos immunoreactivity was detected in the laminectomy group indicating that some surgery microtraumas may occur during surgical procedures, and must be carefully considered. These results showed a local and remote effect of a distal contusion of the spinal cord and medulla oblongata, implicating sensorial neurons and brain stem centers related to autonomic control in the reaction to this kind of injury. These observations suggest that the detection of Fos-Ir may be a useful tool to study neuronal changes induced by spinal weight drop contusion.

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CONTRIBUTION OF VARIOUS CALCIUM CURRENTS TO CALCIUM INFLUX DURING ACTION POTENTIALS IN RAT SUPRAOPTIC NEURONES.

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Magnocellular hypothalamic neurones display specific electrical activities : oxytocin neurones discharge continuously whereas the activity of vasopressin neurones is phasic. These activities are differentially modulated by free intracellular Ca^{++} and thus depend on calcium influx and intracellular Ca^{++} buffering. During electrical activity, Ca^{++} enters into neurones via various voltage-operated channels, which differ in pharmacology, biophysical properties and localisation. To understand the mechanisms by which Ca^{++} controls electrical activity in these neurones, we analysed the respective contribution of these Ca^{++} channels to influx during a single or a train of action potentials.

Using patch clamp recordings from freshly dissociated rat supraoptic neurones, we compared the Ca^{++} currents evoked by i) a square pulse depolarisation, ii) an action potential waveform and iii) a train of action potentials. The use of specific blockers on square pulse-evoked currents confirmed the presence of L, N, P/Q and R currents in the soma of SON neurones. The various currents differed by their relative current density, rate of activation and inactivation and voltage dependency. Single action potential waveform revealed that the relative participation of each current type was not as expected from densities, but was more closely related to the rate and voltage dependency of activation. Indeed, the R current, that activates rapidly and at a relatively low voltage, participated more to Ca^{++} entry during an action potential than to square pulse-evoked currents. On the other hand, the slowly and high voltage activating N current had a minor participation to Ca^{++} entry during a single spike. During a train of action potentials, we observed a use-dependent modification of the role of the channels. Most noticeable is the marked increase in the N current participation during the train.

In conclusion, we show that the relative participation of the various Ca^{++} currents to activity-induced Ca^{++} influx depends more on the biophysical properties of the current than on their density. Furthermore, this contribution is subject to modulation during the course of a burst of action potentials.

THE HYPOXANTHINE-DERIVATIVE AIT-082 IS NEUROPROTECTIVE IN NMDA-INDUCED RAT STRIATAL AND HIPPOCAMPAL LESIONS *IN VIVO*.

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Excitotoxicity produced by overactivation of glutamatergic neurotransmission and the consequent stimulation of NMDA or non-NMDA receptors has been considered responsible for the cell death observed either in neurodegenerative or in acute disorders of CNS. High levels of extracellular adenosine have also been found in these pathological conditions and are considered to be neuroprotective by inhibiting the release of glutamate. However, antagonists of glutamate receptors or agonists of adenosine receptors are not currently used in the therapy of these disorders. A more recent therapeutic strategy is to use drugs able to produce neuroprotective substances from glial cells. The hypoxanthine analog AIT-082 has been shown to stimulate the release of adenosine, guanosine, and neurotrophic factors, e.g. NGF and TGFβ from rat cultured astrocytes (Rathbone et al. Alz. Dis. Assoc. Dis. 12:S36-S45, 1998). Moreover, AIT-082 exerted a potent protective activity in a *in vitro* model of NMDA-induced neuronal toxicity (Rathbone et al. Drug Dev. Res. 45:345-361, 1998). Based on this evidence, we evaluated whether AIT-082 was able to protect against the NMDA-induced neurotoxicity *in vivo*. The unilateral injection of NMDA either in striatum or in anterior hippocampus of rat caused neurotoxic lesions evaluated by T_2-weighted MRI and histological processing (cresyl violet staining). The hyperintensity at the NMDA-injected sites gradually decreased in time-dependent fashion, and it was related to volumes of the lesioned areas showing marked loss of neurons and gliosis. We measured glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) activity, as markers for the integrity of GABAergic and cholinergic neurons, in the NMDA-injected sites of striatum and hippocampus. The activity of both enzymes was reduced by 55±6% in the striatum and 30±5% in the hippocampus (compared to the respective controlateral site) by the injection of NMDA at the apparent EC_{50} of 200 or 350 nmoles for the striatum or hippocampus, respectively. AIT-082 locally co-injected with NMDA caused a significant time- and dose-dependent reduction of the lesioned areas, observed as a marked reduction of the brightness and the extent of neuronal damage at the site of the injection. Parallely, AIT-082 restored in dose-dependent fashion the GAD and ChAT activities in the lesioned areas. The NMDA-induced loss of GABAergic or cholinergic neurons was completely prevented by the co-infusion of 300 nmoles of AIT-082. Similar neuroprotective effects were obtained by the daily administration of AIT-082 (30-60 mg/kg) via i.p. for 7 days, starting from 1 hour prior to the NMDA injection.

NEURONAL COMPLICATIONS OF HIV-INFECTION IN VITRO

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The Acquired Immuno-Deficiency Syndrome (AIDS) is often associated with signs of neurological deficits such as loss of memory and progressive motor and cognitive neurological complications. Loss of cortical neurons has been reported in certain regions of post-mortem brains in symptomatic and also in HIV-infected asymptomatic patients. It seems that different mechanisms contribute to this neurodegeneration and suggests that HIV-induced neuronal death may occur via an indirect pathway requiring biochemical and cellular factors (microglia. macrophages, and macrophage-like giant cells). We used brain cell cultures of 18-days Wistar rat embryo and stress them with the HIV glycoprotein envelope (gp120). Different pharmacological agents were also applied to block this gp120 neurotocixity such as NMDA antagonist MK801, Tetrodotoxin, and Nifedipine. an anti-Ca^{++} molecule. Gp120 exposure *in vitro* in rodent cortical cell cultures demonstrated the presence of a clear-cut neurodegeneration. Cellular mechanisms involved in neuron death included the activation of NMDA glutamate receptors and hyperdepolarization, blocked by tetrodotoxin. The presence of two Ca^{++}-binding proteins such as Calbindin D_{28K} or Calretinin in neurons enhances their resistance to the gp120 stress. HIV envelope gp120 induces neuronal degeneration and apoptosis. In this study using both the TUNEL and DAPI staining methods, we report that gp120-induced apoptosis in neuronal cultures is attenuated by the interleukin converting enzyme (ICE) inhibitor YVAD-CHO in a dose-dependent and a time-dependent manners. suggesting an intracellular signaling pathway leading to caspases I. These results demonstrated that neuronal complications of HIV-infection occurs through various mechanisms including membrane and intraneuronal pathways and that tentative treatment should include different sites of action, associated with the anti-retroviral drugs.

CODING BIMANUAL MOVEMENTS: SINGLE UNIT ACTIVITY, LOCAL FIELD POTENTIALS, AND POPULATION VECTORS

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We have been studying the activity of neurons in motor cortical areas during unimanual and bimanual movements of the arms. Neurons in both primary motor area (MI) and supplementary motor area (SMA) are activated differently during bimanual movements than they are during unimanual movements. These differences reflect both increases and decreases in the amount of activation during bimanual movements relative to unimanual movements. In contrast, local field potentials (LFP) are always increased during bimanual movements. Surprisingly, despite the differences in the responses in different types of movement, preferred directions are largely preserved across movement type. For LFP, this is not the case. These similarities and differences raise the question of whether, as a population, the activity of cells represents the movement consistently in different types of movements. To address this question, we compare the population vectors calculated during different types of movement. Our results show that different methods generate a population vector that points in the direction of the movements. However, in many cases, the methods that took into account task-dependent modification of neural activity were significantly more accurate. We also show that population vectors in both hemispheres give a reasonable representation of the movement for unimanual and bimanual movements. Categorizing the cells according to their activities rather than their hemispheric affiliation gives more accurate results.

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MICROSTIMULATION-INDUCED INHIBITION OF NEURONAL ACTIVITY IN HUMAN GLOBUS PALLIDUS

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Chronic stimulation of the globus pallidus internus (GPi) in Parkinson's disease (PD) patients is currently an alternative therapy to posteroventral pallidotomy (PVP). The mechanism of action of these deep brain stimulation (DBS) effects is generally assumed to be depolarization block of GPi neurons. To test this hypothesis we examined the effect of microstimulation through a microelectrode located about 300um away from the recording electrode in a PD patient undergoing PVP. In all 5 GPi neurons tested we found that microstimulation at 5uA (0.1ms biphasic pulses at 5 Hz) produced a short lasting inhibition of neuronal activity recorded by the second electrode. This inhibition had a latency of onset of about 3 ms and a duration of 25ms. When the stimulation frequency was increased to 50Hz the firing was totally inhibited. These findings suggest that stimulation within GPi preferentially activates the GABAergic axon terminals of striatal and/or external pallidal neurons thus evoking the release of GABA and post-synaptic inhibition of GPi neurons. Thus stimulation-evoked release within GPi may explain the therapeutic effects of pallidal DBS and the similar clinical effect to pallidotomy.

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UBIQUITIN AS 2,4-DICHLOROPHENOXYACETIC ACID EXPOSURE RESPONSE ON CULTURED CEREBELLAR GRANULE NEURONS.

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2,4-Dichlorophenoxyacetic acid (2,4-D) is a herbicide widely used in agriculture in Argentine and other countries of the world. It has been reported that the central nervous system (CNS) is one of the targets for the toxic effects of phenoxyherbicides. A variety of toxic effects of 2,4-D have been reported in humans and experimental animals, including neurotoxic, embryotoxic and teratogenic effects. The basic mechanisms by which 2,4-D produces neuronal damage, however, have not been determined.

Ubiquitin (Ub) is a small and highly conserved protein present in all eukaryotes. A major pathway for the selective degradation of abnormal and short-lived proteins in the cytosol depends both on ATP and Ubiquitin.

In the present work, we study the possible participation of proteolic pathway Ub/proteosome on cellular response to toxicity produced by 2,4-D exposure. Cerebellar granule neurons of neonate rats of 6 days of age were used. Cells were cultured and exposed to several concentrations of the herbicide (1 and 2 mM) from 0 DIV (days *in vitro*) and during different periods of differentiation (24, 48 and 72 hours).

Changes in Ub genes expression were analyzed through *in situ* hybridization and conjugates levels was determined by inmunocytochemical methods and western blot.

We have determined an increase in mRNA of Ub an increment of Ub-protein conjugates in 2,4-D exposured neurons. These results could imply that Ub/proteosome system is involved in the cellular response to the toxic exposure to 2,4-D for metabolising damaged proteins.

DEGENERATION OF DOPAMINERGIC NIGRO-STRIATAL NEURONS IN BRAINS OF ATM-DEFICIENT MICE

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Ataxia-telangiectasia (AT) is a human disease caused by mutations in the *ATM* gene. The neural phenotype of AT includes progressive cerebellar neurodegeneration which results in ataxia and eventual motor dysfunction. Surprisingly, mice which lack the *Atm* gene do not share distinct behavioral or neurological symptoms with the human case. We compared brains from male Atm-deficient mice and age-matched controls and found that adult (4 month old) Atm-deficient mice exhibit severe loss of TH- positive, dopaminergic nigro-striatal neurons, down to about 26% of age-matched controls. This was accompanied by a large reduction in immunoreactivity for the dopamine transporter in the striatum. A reduction in dopaminergic neurons was also evident in the ventral tegmental area. This effect was selective in that the noradrenergic nucleus locus coeruleus was normal in these mice. The loss of TH-positive neurons was gradual; only a small reduction of 17% in the number of TH-positive cells was seen in one-month old Atm-deficient mice, compared to controls. At this age, the number of TH-positive cells in the substantia nigra of control mice was similar to the number of cells (5964 cells) in the adult mice. This means that the TH-positive cells are formed normally and disappear between one and four months of age in the Atm-deficient mice. Behaviorally, Atm-deficient mice expressed locomotor abnormalities manifested as stride length asymmetry, which could be corrected by peripheral application of the dopaminergic precursor, l-DOPA. In addition, these mice were hypersensitive to the dopamine releasing drug, d-amphetamine. These results indicate that ATM deficiency can severely affect dopaminergic neurons in the central nervous system and suggest possible strategies for treating this aspect of the disease.

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IN VITRO AND *IN VIVO* EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID HERBICIDE ON MONOAMINE OXIDASE ACTIVITY IN RAT BRAIN.

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Amine oxidases are ubiquitous enzymes, found in both micro-organisms and higher organisms. Among the various types of amine oxidase, the mitochondrial flavoenzyme monoamine oxidase (MAO) is of special interest for neuropsychiatry. MAO is involved in the biodegradation of aromatic monoamines such as serotonin (5-HT), norepinephrine, histamine and dopamine (DA), and appears to play a central role in several neurological disorders. In addition, there is evidence that MAO has an important function as scavenger of various other amines (e.g., tyramine, octopamine, tryptamine). Our laboratory have demonstrated, in distinct 2,4-Dichlorophenoxyacetic acid (2,4-D) exposure models to rats, behavioral alterations including depression in motor activity, 5-HT-syndrome, circling and catalepsy (Evangelista de Duffard et al., 1990, 1995; Bortolozzi et al., 1998). Recently, we have also reported increases in 5-HT, DA and their metabolites in different brain areas and we described peripheral serotonin levels alterations (Bortolozzi et al., 1998). The aim of this study was to investigate changes in brain MAO activity of adult male rats after both *in vitro* and *in vivo* 2,4-D exposures. Brain MAO activity was assayed spectrofluorometrically by the method of Krajl (1960) with some modifications (Chakrabarti et al., 1998) using kynuramine as substrate and monitoring the 4-hydroquinoline (4-OHQ) formation rate at 318 nm of excitation and 380 nm of emission wavelengths. *In vitro*, the formed 4-OHQ amount from total brain of adult male rats treated with different concentrations (10^{-8} to 10^{-3} M) of 2,4-D was significantly decreased. *In vivo*, repeated exposure to 2,4-D [70 mg/kg/day orally from gestation day (GD) 16 to postnatal day (PND) 90] showed an inhibition (24%, $p < 0.01$) of brain MAO activity in adult male rats with respect to matched controls. However, when 2,4-D exposure was only during a period of neonatal rat development (70 mg/kg/day orally to mothers from GD16 to PND 23 and then, fed with untreated diet until PND 90), no changes in MAO activity were observed. 2,4-D and propargylamine MAO inhibitors were employed to characterize the inhibition of the oxidative deamination of kynuramine. In conclusion, the present results suggest that the neurotoxic action of 2,4-D may be mediated, in part, through the alteration of brain MAO activity (directly or indirectly), increasing the endogenous levels of biogenic amines with concomitant behavioral disturbs, as they were described previously by us.

PROTEIN KINASE C IN THE CAROTID BODY.

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The carotid body is a chemosensory organ which, by sensing reduction in arterial blood oxygen tension, is responsible for the major part of the hyperventilation of hypoxia. We previously demonstrated that the cat carotid bodies exposed to hypoxia in vivo had higher phospholipase C (PLC) activity than those in normoxia (1). The PLC-derived signalling molecules are known to activate protein kinase C (PKC). This study was design to identify the expression of PKC isoforms in carotid body and to determine the effect of hypoxia on PKC isoform cellular compartmentation. To this end we found, by immunofluorescence microscopy, using the PKC-isoform-specific monoclonal antibodies, that only two isoforms α and λPKC were expressed in the clustered type I cells of the normoxic cat carotid body. Other PKC isoforms such as ι and δ were expressed in other than the type I cells parenchymal elements of the carotid body. The immunogold technique by transmission electron microscopy revealed that αPKC is not membrane bound and is distributed throughout the cytoplasm. Hypoxia induced partial redistribution of αPKC from the cytoplasm to the cell membrane and to intracellular organelle membranes. The results strongly suggest a role for this enzyme in transduction of the hypoxic signal in cat carotid body.

1. M. Pokorski and R. Strosznajder, 1993, Adv. Exp. Med. Biol. 337, 191.

A CLINICAL CONTROL STUDY: TREATING ACUTE CEREBRAL HEMORRHAGE WITH LIANG-XUE-TONG-YU ORAL SYRUP

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Background and Purpose~{_T~}he morbidity and mortality of acute cerebral hemorrhage are very high, and in the survived patients the disabled rate is also high in China. A clinical control study was carried out, Liang-Xue-Tong-Yu Oral Syrup (LXTY Syrup) and traditional neurointernal treatments (included deprivation, anti-infection and cerebral function recovery agents, etc.)-as Treating Group compared with simple traditional neurointernal treatments-as Control Group. The study compared 2 groups in the changes of the Stroke Impairment Scales, the Bathel Activities of Daily Living(ADL) Index, CT Hematoma Volume and CT Cerebral Edema Degree, and Blood Rheology, at 2 and 30 days after stroke. Methods~{_T~}he Participants in this study were 72 individuals who suffered from cerebral hemorrhage (acute state) and recruited for the Jiangsu Provincial Stroke Study (Traditional Chinese Medicine Item :9705).All patients were prospectively evaluated using standardized assessments at enrollment (within 2 days of stroke onset), and followed at 30 days after treatments. Two groups' curative effect were compared with statistic methods. Results~{_M~}eans and SDs of Stroke Impairment Scale measured at baseline were 16.91~{!@~}7.16 and 17.03~{!@~}7.36 (p>0.05), at post-treatment were 5.69~{!@~}5.10 and 10.56~{!@~}8.51(*p<0.01,comparied by pretreatment and post-treatment respectively, and 2 groups post-treatment) respectively; ADL measured at baseline were 45.43~{!@~}10.66 and 44.98~{!@~}9.51(p>0.05),at post-treatment were 90.12~{!@~}12.74 and 71.34~{!@~}15.04 (*p<0.01) respectively; CT Hematoma Volume at baseline were 19.22~{!@~}13.39 and 19.16~{!@~}9.82,at post-treatment were 2.83~{!@~}4.19 and 4.46~{!@~}4.01 respectively; CT Cerebral Edema Degree at baseline were A0,B6,C8,D18 and A0,B4,C8,D20 (Ridit, P>0.05), at post-treatment were A27,B4,C1,D0 and A11,B12,C6,D3 (Ridit, *P<0.01) respectively; Blood Rheology Exames at baseline were abnormal and at post-treatment they were improved more in treating group than in control group. Conclusion~{_O~}ur results demonstrate that in a sample of mostly mild and moderate Hemorrhagic Stroke(acute state),Liang-Xue-Tong-Yu Oral Syrup(LXTY Syrup) is helpful in decreasing cerebral hematoma volume and cerebral edema degree, speeding up the patients' improvement of nerve impairment and ADL index, improving the blood rheology.

NEUROPROTECTIVE EFFECTS OF FLAVONOIDES DURING CNS ISCHEMIA.

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The reduced oxygen supply of the brain results in the large quantity of acutely originated free radicals which may provoke serious disturbances in neuron functioning. Flavones, flavonoles, flavonoides and some other substances have been reported as free radical scavengers. Different plants contain the substances mentioned. Thus we investigated the effects of Proanthocyanidol-BP1, a sort of flavonoides isolated from the grape seed, on the overall bioelectrical activity (EEG) during transient brain ischemia in rabbits.

The experiments were performed on awake adult Chinchilla rabbits after electrode implantation in different cortical and subcortical brain structures. The EEG was recorded and analyzed by special programs by computer before and after carotid artery trunk was compressed unilateraly during 10 minutes. Then Proanthocyanidol BP1 (patented by Pekić and Kovač YU, Pat, P-205/931993), was applicated orally, intraperitoneally (i.p.) and intracerebrally (i.c.v) before carotid compression.

The results obtained showed that the EEG activity, recorded before cerebral ischemia was not affected significantly by BP1. EEG phenomena as a result of carotid compression, and mostly expressed as ipsilateraly brain waves frequency slowing down, was lowered or ceased after BP application. Rarely provoked irritative phenomena, were also lowered or ceased after BP1 application. The duration of BP1 effects depended on the dose and the way of drug application. The maximum effect was registered after i.c.v. BP1 application.

The data obtained confirm the neuroprotective effects of proanthocyanidol-BP1 in conditions of EEG transient brain ischemia.

KINETIC CHARACTERISTICS OF INTERACTIONS OF ω-CTx-GVIA TOXIN WITH N-TYPE Ca^{2+} CHANNEL IN RAT HIPPOCAMPAL NEURONS

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The binding and unbinding constants describing interaction of ω-CTx-GVIA with N-type Ca^{2+} channels have been obtained from the time course of the blocking action of the toxin. The experiments were carried out on pyramidal neurons freshly dissociated from the CA3 region of the rat hippocampus using patch-clamp technique in the whole-cell configuration. The binding k_1 and unbinding k_{-1} constants are 0.32 $(\mu M*sec)^{-1}$ and 0.004 sec^{-1} respectively. The dissociation constant K_D kinetically derived from the ratio k_{-1}/k_1 is 0.012 μM. These values allow to account for the apparent "irreversibility" of the toxin action.

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HYPOXIA DOES NOT AFFECT TRANSIENT AND PERSISITENT Na^+ CURRENTS IN LAYER V NEOCORTICAL NEURONS.

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We previously showed that in neocortical slice neurons, hypoxic episodes are associated with a decrease in the rate of rise of action potentials, which we attributed to a decrease in neuronal input resistance. In order to eliminate the possibility that the change in excitability reflects a direct affect on the Na^+ channels themselves, we examined the influence of hypoxia on transient (I_{NaT}) and persistent (I_{NaP}) Na^+ currents in Layer V neurons, using patch clamp techniques in 400 μm slices of mouse SM1 cortex maintained at 36-37°C. In cell-attached recordings from patches containing >15 Na^+ channels, I_{NaT} was studied by averaging 30-50 sweeps and subtracting leak and capacitive components. Hypoxic episodes, produced by switching the gas flow over the slice from 95% O_2-5% CO_2 to 95% N_2-5% CO_2 for 5 min, caused no obvious change in I_{NaT}. It also did not cause a change in frequency of late Na^+ channel openings. I_{NaP} was measured in whole-cell recordings by imposing depolarizing voltage ramps (-70 mV to 0 mV) with a rising rate slow enough to entirely inactivate I_{NaT}. For these recordings, Cs^+ replaced K^+ in the in the intracellular solution, and 200 μM Cd^{++} was added to the bath. When the main intracellular anion was Cl^- or gluconate, the hypoxic episode evoked no observable change in I_{NaP}. When the pipette contained fluoride, however, there was a three-fold increase in I_{NaP} which began 70-90 sec after the onset of the hypoxic episode. The dramatic effect of fluoride on the sensitivity of the Na^+ channel to hypoxia may be related to the known effects of this halogen on G-proteins and other membrane regulatory systems. Supported by the German-Israel Foundation for Scientific Research.

HUMAN BLOOD-BRAIN-BARRIER DISRUPTION IS ASSOCIATED WITH STRESS RESPONSES

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In search for the clinical and molecular parameters associated with Blood-Brain-Barrier (BBB) disruption, we developed a quantitative approach for analyzing human brain images derived by Computerized Tomography (CT), Magnetic Resonance Imaging (MRI) or Single Photon Emission CT (SPECT). BBB disruption was observed as >50% increase in brain penetrance of the corresponding contrast agents (i.e. omnipaque, gadolinium or DTPA, respectively) in 17 out of 34 patients with diverse central-nervous-system (CNS) related symptoms. Stress-related clinical indices such as heart rate, white-blood-cell counts and cortisol levels displayed significant correlation with BBB disruption for 19 patients. Other indices (e.g. blood pressure and body temperature) appeared unrelated. In several samples of cerebrospinal fluid (CSF) from patients with BBB disruption we further identified significant levels of blood albumin. Moreover, immunoblot analyses using antibodies raised against distinct domains in human acetylcholinesterase (AChE) revealed conspicuous levels of the stress-associated "readthrough" AChE isoform in these CSF samples. Our data demonstrate a minimally invasive approach for evaluating human BBB intactness and suggest the accumulation of "readthrough" AChE in the human CNS under diverse stress conditions.

REORGANIZATION OF THE BLOOD-BRAIN WITHIN THE EPENDYMA AND CHOROID PLEXUS BY APOPTOSIS AND PROLIFERATION INDUCED BY SEROTONINERGIC TERMINAL DEGENERATION

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Serotoninergic (5-HT) terminals form dense supra- and sub-ependymal plexus along the ventricles. We have previously shown that these 5-HT terminals are involved in the maturation and differentiation of the ependyma, while their disappearance induces transient changes in ependymocytes morphology, suggesting remodelling of the ependyma. In the present study, we have demonstrated by in-situ labelling of DNA breaks, that 5-HT terminal destruction by 5,7-dihydroxytryptamine triggers apoptotic cell death in the ependyma and choroid plexus epithelium's early as two days after treatment. The data were paralleled with hippocampus analysed as another brain structure innervated by 5-HT. Only the dentate gyrus contained a high level of apoptotic cells. In the ependyma and choroid plexus, a proliferate activity visualized by BrdU incorporation occurred concomitantly to this cell death transiently observed. In the dentate gyrus, cell proliferation was delayed relative to apoptosis. These data suggested that 5-HT depletion may induce rapid reorganization of periventricular components of the blood-brain barrier by apoptosis and renewal at the level of these structures. More generally, these results show the remarkable plasticity of blood-brain components in response to brain injury, insuring functional restoration.

PROOPIOMELANOCORTIN (POMC) PEPTIDE IN THE BRAIN OF A SEMI-DESERTIC RODENT, MERIONES SHAWI: EFFECTS OF HYDROUS STRESS.

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Recent works have shown that proopiomelanocortin (POMC) peptide has been implicated in neuroendocrine stress response and homeostatic balance. In this study, quantitative in situ hybridization and immunohistochemistry were carried out: 1- To localize the POMC neurones in the merione brain. 2- To determine whether the hydrous stress is accompanied by changes in biosynthesis and genetic expression of neuronal POMC. In control meriones brain, neurones positively hybridized for POMC mRNA and immunostained for POMC peptide were prominently expressed in the anterior (AL) and intermediate (IL) lobes of the pituitary gland (PG) and in arcuate nucleus (AN), whereas, POMC immunoreactive fibers are widely distributed in nervous system. In dehydrated meriones, the osmotic stress induced a decrease in both POMC mRNA and POMC peptide labelling in AN as well as in the AL of PG. It has been shown that serotonin exerts a positive control on POMC system. Taking into account our previous results demonstrating a developed serotoninergic system in the meriones brain and a resistance capacity of this species to water deprivation, it appears of great interest to investigate in the merione the possible involvement of POMC system and serotoninergic neurones in osmotic stress response.

ESSENTIAL ROLE OF BILATERAL ORGANIZATION DURING FORWARD STEPPING INITIATION IN PARKINSONIAN PATIENTS

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A proper initial posture, especially that, which is characterized with a goal-directed postural alignment and stability is critical for efficient motor control. As a consequence of the disease the postural instability is a common problem observed in Parkinson's disease. Taking into consideration the essential role of bilateral organization during forward stepping, seven Parkinsonian patients (PD) from mild to moderate stage and 7 healthy age-matched controls were instructed from quite unsupported stance on two separate force platforms, to perform FOS initiation. Based on the CP curves alternations beneath each leg in sagittal plane *'phase of pure initiation'* (PPI) starting with the onset of CP beneath each leg and finishing with the maximal backward shift under 'leading' leg was defined, followed by a *'phase of weight transfer'* (PWT) which ends, when the *'leading leg'* leaves the platform. The results have shown impairment of both phases of interest in PD. The shape of the PPI was preserved in general, whereas the shape of the PWT phase was completely reorganized. A considerable reduce of the initial backward shift of CP beneath both legs was observed during PPI. In healthy subjects the CP curve for the left side was characterized with second well defined peak however in PD patients it disappear For both CP curves the total time duration increased significantly. The initial inhibition of both GM muscles well observed in healthy controls was not preserved. It is more likely a tremor like burst activity was observed for one or both TA muscles in PD patients. Initial simultaneous activation for both TA was relatively preserved but at the later stage of the disease it was observed as a unilateral activity. The GMr was recruited before or later in respect to the PPI. The duration of both GM and TA muscles was significantly prolong.

The considerable reduce of the initial backward displacement of the CP beneath both legs during the PPI in fact represents a lower mechanical effectiveness of the subsequent forward propulsion necessary for FOS initiation. For the PD patients the smooth transfer of the body weight laterally, before unloading and advancing it, requires considerable efforts. The pattern of CP alternations during FOS initiation was considered to reflects the problems, PD patients experienced, resulting from their impaired initiation ability. The CP alternations during FOS initiation in both PPI and PWT in fact reflects the postural impairments of central program, which have to be transformed to specific program for the given muscle group. Failure to promote precise co-ordination between initial posture and subsequent FOS, nevertheless the motor task involves upper or lower extremities, it could be consider to be part of more general deficit in planning and programming voluntary movements in Parkinson disease.

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IMMUNOMODULATION MAY ALLEVIATE SYMPTOMS OF PARKINSON'S DISEASE IN A RAT MODEL

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Degeneration of the nigrostriatal dopaminergic neurons is characteristic of Parkinson's disease (PD). New treatment proposed for PD include transplantation of fetal dopamine-producing cells and administration of intracerebral glial-derived - neurotrophic factor (GDNF). In the present study we tested the effect of the immunomodulator AS101 on an astrocyte cell line, genetically modified to express the rate-limiting enzyme for l-dopa, tyrosine hydroxylase (SVG-TH). Our results show that AS101 stimulates IL-6 secretion in this cells in a dose- and time- dependent manner. Constitutive expression of GDNF mRNA was found, but no spontaneous secretion of the growth factor was observed. Stimulation with AS101 (0.2 mg/ml) enhanced mRNA production and induced secretion of GDNF as tested by ELISA. In vivo experiments showed that direct injection of AS101 (30 µg for 3 days, at 0.1 µg/hr) via cannula to the substantia nigra (SN) of 6-hydroxy dopamine partial lesion rats reduced apomorphine-induced rotation by 90%. Our results suggest that in partial loss of dopaminergic cells in the SN, local administration of AS101 may stimulate SN glia cells to secrete growth factors that may support the residual dopaminergic neurons. A combined treatment of AS101 and transplantation of SVG-TH cells may prove effective in relieving PD, the latter supplying dopamine, and the former supplying growth factors necessary for the regeneration of lost neurons.

A NOVEL TREATMENT FOR PARKINSON'S DISEASE USING AN IMMUNOMODULATOR

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Degeneration of the nigrostriatal pathway is characteristic of Parkinson's disease (PD). Current treatments are aimed at pharmacologically augmenting striatal dopamine (DA) but do not prevent continued neuron degeneration. A protective/restorative treatment that would slow, prevent or even reverse the degenerative process is therefore needed. Herein we propose a therapeutic approach for treating PD based on neuro-immunomodulation. We tested the effect of the immunomodulator AS101 on an astrocytes cell line (SVG). Our results show that AS101 (0.2 g/ml) stimulates the production and secretion of GDNF and IL-6 in these cells in a dose and time dependent manners. Administration of AS101 (30 g for 3 days, at 0.1 g/hr) into the diseased substantia nigra (SN) of partially 6-OHDA lesion rats reduced apomorphine-induced rotation by 90%. DA and DA metabolites levels were higher in AS101 treated rats compared to PBS treated rats in the striatum and the SN, as determined by HPLC analysis. These results were supported by increased immunohistochemically staining of tyrosine hydroxylase. Our findings suggest a novel treatment that may provide a dopaminergic supportive environment in the diseased SN.

AGMATINE NEUROPROTECTION AFTER SPINAL CORD ISCHEMIA

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Agmatine, a naturally occurring guanidino compound found in abundance in bacteria and plants, was recently identified in mammalian tissues. Agmatine is formed by decarboxylation of arginine, but our findings indicate that in mammals its synthesis may be catalyzed by ornithine decarboxylase, the enzyme catalyzing the first step in polyamine synthesis, rather than by arginine decarboxylase. In mammalian tissues, agmatine is principally metabolized into urea and putrescine, the diamine precursor of polyamine synthesis. The compound is present in the brain where its synthesis, which is normally very low, is greatly increased during brain development and after brain ischemia, in parallel to polyamine synthesis. Treatment with agmatine proved to be nontoxic and to exert potent neuroprotective effects in models of neurotoxic and ischemic brain injuries. We, therefore, sought to find out whether agmatine treatment would also prove beneficial in spinal cord ischemia. Spinal cord ischemia was produced in 4-month-old male Wistar rats under halothane anesthesia, by inserting a balloon catheter through the abdominal aorta, below the kidneys, and inflating the balloon bellow the branching point of the brachial arteries for 5 min. Injection of agmatine (100 mg/kg, ip) 5 min after beginning of re-perfusion and again once daily for the next 3 postoperative days, was found to accelerate recovery from motor deficits and to prevent the loss of motoneurons in the spinal cord. Together, the present and previous findings demonstrate the potent neuroprotective effects of agmatine and indicate that this naturally occurring nontoxic compound should be tried for potential therapeutic use after neurotrauma and in neurodegenerative diseases.
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KEY ROLE OF TNF-α AND ITS LIPID MESSENGER CERAMIDE IN STRESS ADAPTATION OF BRAIN CELLS

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Various sublethal stress conditions such as heat, oxidative stress, and ischemia induce an adaptive response which results in cell tolerance to a subsequent challenge that would otherwise be lethal. Ischemic preconditioning of brain has been extensively studied in search of mechanisms that could improve treatment or prevention of stroke. Many of the deleterious as well as the neuroprotective reactions in ischemic brain are mediated by the pleiotropic cytokines TNF-α and IL-1. Recent animal studies suggest that the state of tolerance induced by ischemic preconditioning also might involve these cytokines. To test this hypothesis in vitro we have developed a quantitative cellular model of ischemic tolerance using different types of brain cells. We demonstrated that preconditioning of cortical neurons with mild hypoxia protects them from hypoxia- and oxygen/glucose deprivation injury 24 hours later (50% protection). Hypoxic preconditioning could be substituted with TNF-α pretreatment and was attenuated by TNF-α-neutralizing antibody. The preconditioning effect of TNF-α was also demonstrated in astrocytes and brain microvascular endothelilal cells (BMEC), which when pretreated with TNF-α for only 4 hours became resistant to inflammatory effects of hypoxia and TNF-α 24 hours later. These latter stimuli caused significantly less expression of ICAM-1 mRNA (30% inhibition) and surface protein (80% inhibition) in preconditioned cells than in naïve cells. We have demonstrated for the first time that in all three types of brain cells, hypoxic or TNF-α preconditioning causes a delayed 2-3 fold increase of ceramide levels which occurs between 18 and 24 hours after preconditioning and coincides with the state of tolerance. The role of ceramide in signaling events leading to tolerance is supported by the following findings: 1) mimicking of preconditioning by addition of exogenous ceramide, and 2) attenuation of preconditioning by Fumonisin B1, an inhibitor of ceramide synthesis. In contrast to observations in transformed cell lines, the delayed ceramide increase in brain cells was transient and did not induce apoptosis.

FUNCTIONAL DEGENERATION OF ORGANOTYPIC STRIATAL SLICES AFTER TREATMENT WITH NEUROTOXINS

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Adult striatal organotypic slices from C57/Bl6 mice were used as a model system to study the degeneration of dopaminergic nerve endings after treatment with different neurotoxins. Neurotoxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP^+) and δ-carbolines were shown to accelerate functional loss as measured by activity of tyrosine hydroxylase (TH), dopamine content and oxidative metabolism. Organotypic slices (400μm) of striata were cultured on Millicell CM membranes for up to 120 h. Tyrosine hydroxylase-activity and dopamine content were measured by HPLC with electrochemical detection. Measurements of mitochondrial enzymes were determined by a microscope photometric method on 8μm sections of the original slices. Degenerating slices rapidly lose their dopamine content within 24 h. TH-activity decreases with a half-time of approx. 3 d. The addition of MPTP, MPP^+ or δ-carbolines (all 10-100 μM) aggravated TH-degeneration. Mitochondrial complexes were selectively inhibited depending on the neurotoxin applied. Adult striatal organotypic slices represent a useful model to study the degenerative effect of neurotoxins on dopaminergic nerve endings and the concomitant impairment of oxidative metabolism. They may also serve as an alternative to costly animal experiments or primary cell culture systems.

INTRACELLULAR Ca^{2+} ALTERATION IN LAYER II AND III PROJECTION NEURONS OF THE ENTORHINAL CORTEX DURING ACTIVATION OF MUSCARINIC RECEPTORS.

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The entorhinal cortex (EC) is a major gateway for sensory information into the hippocampus and receives a cholinergic input from the forebrain. Using combined electrophysiological and imaging techniques we studied muscarinic effects on excitability and intracellular Ca^{2+}-signaling in layer II stellate and layer III pyramidal projection neurons of the medial EC. CCh (10 mM) applied to deep layers or to layer II-III induced a large membrane depolarization associated with synaptic oscillations and epileptiform activity in both classes of neurons. Although CCh-induced epileptiform activity was associated with increases in intracellular free Ca^{2+} in both, layer II and layer III cells, the observed $[Ca^{2+}]_i$ accumulation was significantly larger in layer III than in layer II cells. In layer II stellate cells epileptiform activity and weak and strong direct depolarization evoked a small $[Ca^{2+}]_i$ increase mediated most likely by low and high voltage-activated Ca^{2+}-channels, whereas in layer III cells a large $[Ca^{2+}]_i$ accumulation is mediated apparently by high voltage-activated Ca^{2+}-channels. Given the different projection patterns of the EC layer II and III cells towards the dentate gyrus and hippocampus, respectively, their different control of excitability and Ca^{2+}-signaling may be of major importance for the interaction between the EC and the hippocampus.

THE DEVELOPMENTAL ABILITIES OF SCHOOL CHILDREN WHO WERE BORN PREMATURELY AND HAD ASPHYXIA AND ASPHYXIA WITH HEMORRHAGE AT BIRTH

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Prematurely born children with several risk factors at birth present an exceptionally high risk for the developmental abilities even at the school age.

The sample in this examination consisted of 20 prematurely born children, divided into two groups. The first group comprised 10 prematurely born children with asphyxia, whereas the second group consisted of 10 prematurely born children with asphyxia and hemorrhage at birth. The groups were levelled regarding the sex, age weight at birth (up to 1500 gr., 1501 - 2000 gr. and 2001 - 2500 gr.). There were 6 boys and 4 girls in each group, aged from 7.5 to 9.

The developmental abilities were tested by applying the Acadia Test, while the intellectual abilities were examined by using the WISC.

The results have shown that the children were remarkably unsuccessful in speech and language development tests (60-75%), and there were no differences between the groups studied. The greatest deviations were found in the subtests of auditive memory, visual discrimination, audio-visual association and sequence and coding. The intellectual abilities were tested by applying WISC, and were found to range in the first group from 90-112 (IQ), while in the second group from 93-105 (IQ).

A logopedic treatment was required by 12 children (60%) out of 20 children examined.

EFFECT OF CATIONS ON THE Ca^{2+}/H^+ ANTIPORT ACTIVITY OF SYNAPTIC VESICLES ISOLATED FROM SHEEP BRAIN CORTEX

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Synaptic vesicles isolated from sheep brain cortex contain a Ca^{2+}/H^+ antiport that permits Ca^{2+} accumulation inside the vesicles (~5 nmol/mg protein) at expenses of the ΔpH generated by the proton pump. This system associates Ca^{2+} influx to H^+ release and operates with low affinity ($K_{0.5}$=217 μM) for Ca^{2+}. The Ca^{2+}/H^+ antiport mediates exchange of protons with other cations such as Zn^{2+} and Cd^{2+}, suggesting that these cations and Ca^{2+} share the same transporter molecules to enter the intravesicular space. Zn^{2+} and Cd^{2+} induce H^+ release in a concentration-dependent manner and they inhibit the antiport-mediated Ca^{2+} uptake by the vesicles. In contrast, large cations such as Ba^{2+} and Cs^+ do not alter Ca^{2+} influx and they are unable to induce proton release from the vesicles. With respect to Sr^{2+}, which has an intermediary size relatively to the other groups of cations, it does not induce H^+ liberation from the vesicles, but it has an inhibitory effect on the Ca^{2+}-induced H^+ release and Ca^{2+} uptake by the vesicles. These results indicate that synaptic vesicle Ca^{2+}/H^+ antiport selects cations for proton exchanging according to their size in the dehydrated form.

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ACUTE ALCOHOL INTOXICATION ALTERS INFLAMMATORY CYTOKINES FOLLOWING TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is often complicated by infections that exacerbate the severity of the injury. The exacerbation may be explained in part by the finding that TBI in humans is associated with immunosuppression that correlates in magnitude with the poor outcome. Increasing evidence indicates that alcohol intoxication worsens the severity of TBI. The underlying mechanisms for this interaction remain to be examined. It is well recognized, however, that a variety of inflammatory cytokines play a fundamental role in the immune response to injury, infection and inflammation. Thus, we tested the hypothesis that alcohol intoxication alters levels of inflammatory cytokines following TBI.

Methods: Adult rats were administered saline or ethanol (3 mg/kg) 1h prior to a single moderate cortical impact (2 m/sec, 2.5 mm deformation) under anesthesia (4% isoflurane with a 2:1 N_2O:O_2 mixture). Blood and tissues were collected 4h later for the assessments of cytokines, including the pro-inflammatory interleukin-1β (IL-1β) and the anti-inflammatory immunosuppressor IL-10.

Results: In saline-treated rats, TBI elicited a marked increase in IL-1β levels in the ipsilateral cortex and hippocampus, compared to the contralateral side. In the same animals, IL-1β levels were detected in the intestinal (ileum) muscle and mucosa, as well as in spleen and serum. In the alcohol-intoxicated TBI rats (blood alcohol levels 220 mg/dl), IL-1β levels displayed a 3-fold decrease in both sides of the cerebral cortex, compared to the saline-treated. In contrast, IL-1β was increased in ileal muscle (3-fold), spleen (4-fold) as well as serum (2-fold). In addition, alcohol intoxication elicited an 80-fold increase in IL-10 levels in serum compared to the saline-treated TBI rats.

Conclusion: The alcohol-induced perturbation of normal cytokine responses following TBI may contribute to the impaired immunity that may result in multiple organ failure after TBI.

A DYNAMIC STUDY OF THE EVOLUTION OF THE MULTIUNIT ACTIVITY OF THE BASAL GANGLIA IN EXPERIMENTAL PARKINSONISM

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The electrophysiological activity of the basal ganglia network has been well documented for the normal and full parkinsonian states, but not for the long intermediate stages which characterize this pathology. To come closer to the real conditions of Parkinson's disease, we have recently developed a dynamic MPTP monkey model and have now used this to study the electrophysiological changes occuring over time in the globus pallidus pars externalis (GPe), the globus pallidus pars internalis (GPi) and the subthalamic nucleus (STN). The two monkeys in our study became parkinsonian from D14.5±0.5. However, already from D12 onwards, before the first appearance of clinical signs, although GPe activity remained unchanges, both STN and GPi activity increased. From D14.5, a clear correlation could be observed between the increase in STN and GPi activity and a degradation of motor performance as measured on a behavioural rating scale. These results confirm the lack of influence of the GPe on STN activity through the indirect pathway. They particularly underline the progressive augmentation of both STN and GPi activity in the course of parkinsonism and the parallel revelation and aggravation of motor disorders. Since the increase in electrophysiological activity precedes the first clinical signs manifestations of the disease, it would seem more than likely that previously described glutamatergic compensatory mechanisms which mask the disease are mediated by the STN.

RECOVERY FROM DESENSITIZATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IS AGONIST DEPENDENT

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Prolonged exposure of the nicotinic acetylcholine receptor (nAChR) to agonists results in desensitization--an inactive receptor whose channel is blocked to passage of ions. Desensitization of nAChRs in various tissues is likely to be an important modulatory mechanism. Furthermore, receptor desensitization may contribute to behavioral changes (in humans or animals) after prolonged exposure to nicotine (as in tobacco use). Using whole-cell voltage clamp current measurements, we investigated recovery from desensitization of the muscle-type nAChR in TE671/RD cells induced by exposure to acetylcholine or nicotine. Recovery from desensitization depended on the length of agonist exposure and on the identity of the agonist used to induce desensitization. Prolonging exposure, at a given concentration, increased the time constant for recovery for both agonists. Recovery from nicotine-induced desensitization was consistently faster than that from acetylcholine-induced desensitization, whether nicotine or acetylcholine was used to assess desensitization. These findings suggest the existence of multiple states of receptor desensitization and the fact that agonists differ in their efficiency of inducing the extent of desensitization.

ANALYSIS OF TREMOR IN DIFFERENT MPTP PRIMATE MODELS OF PARKINSONISM

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Tremor is the most commonly known manifestation of Parkinson's disease (P.D). Two primate models of Parkinsonism correspond to two main subclasses of patients: the vervet monkeys mimic the tremulous patients and the rhesus monkeys correspond to the rigid akinetic patients who exhibit almost no tremor. We present a previously unreported phenomenon of vigorous episodes of tremor in a non-tremulous Parkinsonian rhesus monkey, in response to Dopamine replacement treatment. We address two questions regarding the different expressions of tremor in P.D: Is the rhesus L-Dopa related tremor the same as the unprovoked tremor of the Parkinsonian vervet monkey? Does the Dopamine related tremor change with the appearance of L-Dopa induced dyskinesia? Two vervet monkeys and one rhesus monkey were rendered Parkinsonian by sequential intra-muscular injections of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The rhesus monkey was later treated with the standard medication for Parkinson's disease: L-Dopa and Bromocryptine, in increasing doses until the appearance of dyskinesia. The tremor was recorded with an accelerometer and analyzed with FFT spectral analysis. All three monkeys expressed akinesia, bradykinesia and flexed posture. In addition, the vervet monkeys exhibited numerous episodes of tremor in two main frequencies - 6Hz and 13Hz, while the rhesus monkey expressed only short infrequent tremor episodes mainly around 10Hz. After each dose of medication ('on' period), there was a dramatic improvement in the akinesia and bradykinesia, but at the same time the rhesus developed vigorous episodes of 12-14Hz tremor. Although this Dopa induced tremor persisted throughout late phases of treatment after the appearance of the dyskinesia, it's frequency was reduced to 8-10Hz. In summary, we show quantitative and qualitative differences between the tremor of the vervet monkey and the rhesus monkey. Moreover we also demonstrate quantitative and qualitative changes in the tremor of one monkey throughout different stages of the treatment. We suspect that these findings reflect changes in the activity of the neuronal network of the basal ganglia.

VAGAL AFFERENT SIGNALLING OF ACID INSULT IN RAT GASTRIC MUCOSA

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Gastric acid is a factor in upper abdominal pain. However, the afferent pathways whereby a chemical insult in the gastric mucosa is signalled centralwards are little known. This study examined which neurons in the brain respond to challenge of the gastric mucosa by a noxious concentration of HCl. Activation of central neurons was mapped via transcription of c-fos mRNA by in situ hybridization autoradiography. When examined 45 min after intragastric treatment of rats with HCl (0.5 M), many neurons in the nucleus tractus solitarii, lateral parabrachial nucleus, thalamic and hypothalamic paraventricular nucleus, supraoptic nucleus, central amygdala and habenula expressed c-fos mRNA as compared to intragastric treatment with saline. The c-fos mRNA response to intragastric HCl in all these regions was depressed by about 75 % five days after bilateral subdiaphragmatic vagotomy, whereas the number c-fos mRNA-positive cells in the dorsal raphe nucleus and central grey was enhanced. In contrast, pretreatment of rats with the sensory neurotoxin capsaicin failed to alter the acid-evoked expression of c-fos mRNA in the brainstem. These data show that it is capsaicin-resistant vagal afferents which signal an acid insult in the gastric mucosa to the brainstem, wherefrom the incoming information is transmitted to higher relay centres involved in the central processing of visceral afferent input.

MODULATION OF NEURONAL Na^+ CHANNELS ACTIVITIES BY CONOTOXIN

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The effects of crude venom and purified toxin of *Conus textile,* collected from the South China Sea, on the activities of neuronal Na^+ channels were studies. Hippocampal CA_1 pyramidal neurons were acutely isolated from 6 -11 days SD rats. The patch clamp whole cell recording was used. At a concentration of 0.2 mg/ml crude venom, the Na^+ currents were blocked 34% (n=5) at 10 min after bath application of venom. The blockade was reversible after washing with normal saline. The giant neurons in the suboesphageal ganglia of the snail *Achatina fulia* were used for qualitative study with conventional intracellular microelectrode method. Some cells exhibited spontaneous activity with a frequency of 0.5 - 1.0 Hz, others were silent but could be stimulated by intracellular stimulation. With bath application of 0.25 mg/ml crude venom, the frequency of spontaneous activity increased by 1.5 times, or the latency threshold of electrical stimulation lowered and the latency shortened. After HPLC purification 12 peaks can be obtained. Peak 11 was almost purified and tested. At the concentration of 0.1 mg/ml, the Na^+ currents of the rat hippocampal neurons were blocked 73% after 10 min of application (n=5). At the same concentration of this toxin, the giant cells of snail expressed excitatory effects as mentioned above. It is noteworthy, the neuronal Na^+ channels of vertebrate and invertebrate can be modulation in a different way by the same toxin. Acknowledgments: This work was supported by a Foundation of the National Joint Laboratory of Biomembrane and Membrane Biotechnology, Chinese Academy of Sinica.

COPPER-INDUCED INHIBITION OF THE IONIC CHANNEL GATED BY ATP $P2X_4$ RECEPTOR.

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To study whether the brain $P2X_4$ ATP receptor is a target of copper and may explain copper neurotoxicity, Xenopus Laevis oocytes were microinjected with a plasmid containing the $P2X_4$ cDNA. The two-electrode technique was used to record the currents evoked by a 20 sec ATP application. Co-application of 300 μM copper with ATP caused a non-parallel and concentration-dependent displacement of the ATP concentration-response curve without modifying the reverse ATP potential. Prolonging the exposure of copper substantially enhanced the inhibition. A 48±7 % inhibition of the 100 μM ATP current was attained with either 300 μM copper co-applied with ATP during 20 sec, or when 10 μM or 3 μM copper was pre-applied during 1 and 2 min respectively prior to ATP. Exposure of 10 μM copper during 60 sec 6 min prior to 100 μM ATP caused a 44±7% inhibition; 67±6 and 78±4 % inhibition was attained when 10 μM copper was applied 2 or 1 min prior to ATP. Recovery was proportional to the concentration and time of copper exposure. Alkylation with 0.5 mM DEPC, did not alter the copper-induced inhibition suggesting that histidine residues are not necessarily involved in the copper effect. Results suggest that copper interact at a metal site in the extracellular domain preferably in the unoccupied receptor conformation. This site is not easily accessible and takes time to washout. Zinc-copper competition experiments are underway.
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MECHANISM OF THE ZINC-INDUCED POTENTIATION OF THE IONIC CHANNEL GATED BY ATP $P2X_4$ RECEPTOR.

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The mechanism of the zinc-induced potentiation of the ATP $P2X_4$ receptor was assessed in Xenopus Laevis oocytes microinjected nuclearly with a plasmid containing the $P2X_4$ receptor. The oocytes were exposed to ATP during 20 sec; the current generated was recorded by the two-electrode technique. The zinc potentiation was concentration dependent; it did not modify the ATP reverse potential. Following washout of the metal, the effect easily reversed. 10 μM zinc co-applied with ATP displaced leftward the ATP concentration-response curve, without altering the maximal current. Furthermore, 10 μM zinc shifted the ADP and α, β methylene-ATP curves leftward, augmenting the maximal response. The 1 μM ATP potentiation was 2-fold increased by a 60 sec pre-exposure to 3 μM zinc, implying that the metal may be hindered in the close vicinity of its receptor locus. In contrast, pre-application of zinc 60 sec or more, before the ATP application, did not potentiate ATP. Chemical alkylation with 0.5 mM DEPC during 3 min decreased by 50 % the 10 μM ATP-induced current but did not inhibit the zinc potentiation, implying that zinc does not apparently require histidine residues for the potentiation. Our results allow hypothesizing that zinc binds to a metal site in the extracellular domain of the receptor increasing the affinity of the receptor for ATP. The metal does not necessarily bind to the triphosphate chain of the nucleotide stabilizing ATP to a preferred receptor conformation.
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IMMUNOCYTOCHEMICAL LOCALISATION OF SODIUM CHANNELS IN CENTRAL NEURONS

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Voltage-activated sodium channels are the primary proteins responsible for the generation and propagation of action potentials in the CNS. Here we describe studies on the location of these channels in layer 5 neocortical pyramidal neurons in 1 to 6 week old rats using a polyclonal antibody (from Prof. W. Caterall, Washington University Seattle, U.S.A.) specific for the alpha subunit of type 1, 2 and 3 sodium channels. The aim of the experiments was to determine the relative density of voltage-activated sodium channels on the dendrites, soma and axon initial segment during postnatal development. In particular, we were interested in determining the location of sodium channels in the axon which could be responsible for action potential initiation. Neurons were stained with an intracellular fluorescent dye (sulforhodamine 101; Molecular Probes) via a somatic whole-cell patch pipette or cell somata counterstained with propidium iodide. Slices were fixed, processed for immunocytochemistry, and staining detected using confocal and light microscopy. Cytoplasmic immunostaining was not observed, and no distinct staining could be found on cell membranes of the soma or proximal dendrites. Diffuse staining was detected in the region of the dendritic tree, and annular staining detected in fiber tracks consistent with staining of nodes of Ranvier. At 6 weeks, virtually all propidium iodide stained pyramidal neurons had a brightly stained axon with the staining starting at or close to the soma. At 3 weeks, a diversity of staining patterns was observed at the axon initial segment. In large layer 5 pyramidal neurons staining of medium density was observed from the axon hillock along the axon, while some smaller layer 5 pyramidal neurons showed a gap of about 10 or 15 μm between the soma and the start of sodium channel staining. This gap was more prevalent in younger animals and overall axonal staining was less intense, however, some cells had brightly stained axons even at one week. Studies at the electronmicroscopic level are currently being undertaken to determine the relationship of the observed axonal sodium channel staining to myelination, and immunocytochemistry is planned to be combined in individual neurons with axonal patch-clamp recordings estimate sodium channel density electrophysiologically.

PHATOLOGICAL VARIATION OF THE FUNCTIONAL STATE OF SOME STRUCTURES OF THE STRIOPALLIDUM, THALAMUS AND MOTOR CORTEX IN VARIUS FORMS OF PARKINSONISM.

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The results neurophysiological of investigations at 74 patients with akinetic-rigidity and tremulous-rigidity forms of parkinsonism (average age 59±4,4 years) are presented which with the medical purposes were implantation long-term gold electrodes (working surface 0,1-0,2 mm^2) in structures of the striopallidum (Cd, GP) and thalamus (VL). A functional state this structures investigated on parameters of Stable potential of millivolt range (SP). 19 the patients with the same forms of parkinsonism, registration SP carried out in projection zones (PZ) of motor cortex (projections of hand, foot, head, trunk) at removal of this parameter from a surface of a head and use liquid, nonepolaerisation, chlorsilvery of electrodes. In rest (position laying, the eyes are closed) for the patients with tremulous-rigidity by the form parkinsonism (at tremor of rest up to 2,5-3 mark) the variability of a functional state researched subcortical of structures was characteristic, that found reflection in variations of size SP over a wide range (from 0 up to 110 mV). Thus in Cd of Stable potential varied from -20,5±4,6 mV up to -70,0±6,8 mV; in GP from -5,6±3,3 mV up to -80,4 ±8,2 mV; in VL from -2,8 ±4,3 mV up to -60,7 ±9,1 mV. The time of retaining of steady meanings SP did not exceed 2-4 min. At the general tendency to positivation SP in motor cortex (5,9± 2,1 mV), at these patients the high negative meanings SP (-13,4 ± 2,2 mV) in the contralateral PZ of the hand segments in the motor cortex, in relation to a hand were found out, where tremor was more expressed. In the patients with akinetic-rigidity the form of parkinsonism (at akinesya up to 2-3 mark) found out stability (till 40-60 of min.) stability values SP at sharp narrowing of borders of its variations at repeated researches in Cd (up to -15,2± 2,5 mV) and GP (up to -8,2 ± 5,1 mV), with loss of their modulating influences. Thus the wide borders of variations of Stable potential in VL (from -5,4± 2,8 mV up to -110 mV) manifestation it hiperactivation and emotiogenic properties of structure are found out at performance of motoric tests. The infringement cortical-subcortical of mutual relation was shown at these patients in bilateral negativation of Stable potential in PZ of motor cortex (-6,75± 2,81 mV), that it is possible to consider as irradiations of activation talamical of influences at an oppression (exhaustion) of functions motor cortex at the patients with akinetic-rigidity by the form parkinsonism. The received results was exhibited, that of clinical symptoms (mental, autonomic-vasculare and motor) features of pathological changes of a condition of the investigated structures striopallidum, talamus and motor cortex at the different forms parkinsonism.

CHANGES IN REACTIVE BEHAVIOURS OF MACROPHAGES AND ASTROCYTES SHOW OPPOSITE TRENDS IN THE INJURED BRAIN OF 6-DAY-OLD RAT EXPOSED TO GAMMA-IRRADIATION AT DIFFERENT STAGES OF PRENATAL DEVELOPMENT

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Pregnant Wistar rats were exposed to a single 1.0 Gy dose of gamma rays on gestational days 13, 15, 17 or 19 (E13s, E15s, E17s and E19s, respectively). A mechanical injury was made in the cerebral hemisphere of their 6 day-old male offsprings. The injured rats were injected with [3H]thymidine on day 1 or 2 after injury and killed 4 hours after the injection. Brain sections were immunostained for glial fibrillary acidic protein (GFAP) or S-100beta protein or processed for BSI-B4 isolectin histochemistry, subjected to autoradiography and examined microscopically to record proliferating astrocytes and macrophages. The intensity of astrocyte proliferation in response to injury showed a gradual decrease from the level maximal in E13s to minimal in E19s. The total number of macrophages as well as number of their divisions were minimal in E13s then showed a regular increase in E15s and E17s, and reached their maximal levels in E19s. Thus, changes in the reactive behaviour of astrocytes and macrophages were regarded as being related to the stage of prenatal development whenirradiation of the brain was performed. Nevertheless, trends of changes showed by the two cell types were opposite. Therefore, the recruitment and proliferation of macrophages, and the astrocyte proliferation were considered as reactive processes occurring under control of different regulatory mechanisms acting within the region of injury.

INTRACELLULAR CALCIUM DYNAMICS IN HIPPOCAMPAL CELLS IN CULTURE IS REGULATED ALSO BY CALCIUM-GATED SK-CHANNELS

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In hippocampal cells in culture the intracellular calcium concentration is not constant but fluctuates. The waves are long, lasting more than 100 seconds and are highly correlated with smaller and localized calcium increases known as calcium sparks (see additional abstract by Kachalsky et. al.). Calcium waves in the cytosol may origin from the release of calcium of intracellular stores or by the influx of calcium from the extracellular medium. We have investigated the effect of the inhibition of the calcium-activated small-conductance K^+ channels (SK channels) by apamin, on calcium waves and sparks. Addition of apamin (200nM) abolished the appearance of calcium waves (n=9) without considerably affecting the number of sparks (before apamin n=44 pixels, 4 cells, m=78.4 ± 11.87(mean ± SD number of sparks in 98 seconds); after apamin n=57 pixels, 9 cells, m=83.54 ± 12.54). The effect of SK channel block is similar to the inhibition of the intracellular calcium store system (the ryanodine and IP3 dependent calcium channels). These results show that the inhibition of SK K^+ channels, inhibits calcium waves, suggesting that the intracellular calcium waves represent a complex phenomenon involving intracellular and membrane components.

POLARITY-TOLERANT SENSITIVITY TO INTERAURAL TIME DIFFERENCES IN THE INFERIOR COLLICULUS

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Many cells in the inferior colliculus (IC) are sensitive to interaural time delays (ITDs) of low-frequency broadband noise. This sensitivity is generally well-predicted by a linear superposition of responses to tones (Yin et al., J. Neurophysiol., 55, 280-300, 1986) and is consistent with a cross-correlation model. In a study of responses of IC cells to dynamically varying interaural correlation, it was noted that some cells show a peculiar form of ITD-sensitivity in which an increase in discharge rate occurs at phases of full correlation as well as full anti-correlation (Joris, Soc. for Neurosc Abstr., 22, 648, 1996). In the present experiments, these cells were studied with static ITDs. Responses were obtained in the IC of pentobarbital-anesthetized cats. Noise tokens (low-pass 4 or 8 kHz) were created digitally and presented over a calibrated, closed acoustic system. Noise-delay functions were obtained by presenting these noise tokens, or filtered versions thereof, over a range of interaural delays, in discrete steps. In a minority of phase-sensitive cells noise-delay functions were polarity-tolerant, i.e. the cells were excited over a similar ITD-range by correlated and anti-correlated waveforms, but were insensitive to uncorrelated noise tokens. In the most extreme cases, the noise-delay functions to correlated and anti-correlated signals were identical and showed a single central peak with an absence of periodicity in the ITD domain. More frequently, both functions showed a central mound of activity over a common ITD-range, superimposed by a periodic fine structure which was out-of-phase for the two conditions. The polarity-tolerant cells had characteristic frequencies mostly in the mid-frequency range (1-3 kHz) and were located in the central nucleus of the IC. These properties suggest that the polarity tolerant component of ITD-sensitivity is based on envelope correlation, but the sharpness of ITD-tuning is much higher than in previous studies of envelope ITD-sensitivity.
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CHARACTERIZATION OF INTRACELLULAR CALCIUM OSCILLATIONS IN HIPPOCAMPAL CELLS IN CULTURE.

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In neuronal cells, intracellular calcium oscillations affect diverse cell processes such as repetitive firing, transmitter release, and gene expression. We have shown the existence of calcium sparks in hippocampal cells in culture. Moreover, we have shown the existence of "calcium noise" as a more generalized of intracellular calcium fluctuations. Calcium "sparks" were suggested to be the elementary events underlying excitation-contraction coupling in heart muscle. We studied using confocal laser scanning microscopy, fluorescent calcium probes and stochastic analysis the correlation between calcium sparks and calcium waves in hippocampal cells and their regulation by the different systems affecting the intracellular calcium concentration. We report here that in hippocampal cells, calcium sparks are part of a more generalized process that spread throughout the cell forming calcium waves. The waves are slow (more than 100 seconds in duration , n=25) and spread throughout the cell. Calcium waves are not directly dependent of the extracellular calcium concentration or sodium dependent synaptic activity, since they exist in nominally calcium-free solution (n=9) and in the presence of TTX (30 M , n=5). However, calcium waves are directly dependent on intracellular calcium stores. Addition of Ryanodine (100nM, n=6 or 2 n=4) that activate or inhibit the ryanodine-dependent calcium release respectively or inhibition of SERCA pumps by thapsigargin (5 M, n=7) abolish the calcium waves. We suggest that these two intracellular calcium pools are involved in the regulation of calcium waves in hippocampal cells in culture.

SOLUBLE MEDIATORS PRODUCED BY SCHWANNOMAS IN VIVO: EFFECT ON BONE MARROW PROLIFERATION IN VITRO AND HEMATOLOGICAL CHANGES IN SCHWANNOMA BEARING HOSTS

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Objectives: Alike glioblastomas, Schwannoma cell tumors can secrete a spectrum of systemically active mediators, thus e. g. influencing immune function. The rat Schwannoma cell line NV2cd forms tumors with unlimited growth following inoculation onto syngeneic hosts, produces EGF, TGFβ1, TGFβ2, TGFβ3 and VEGF, partly enhanced by hypoxic conditions as they are found intratumorally near to fluid-filled pseudocystic regression areals, and induces in tumor hosts a significant spleen enlargement due to T cell proliferation. Since bone marrow depression is a prominent feature of neoplasia, we were interested to evaluate the effect of the secreted mediators in their entirety on BM.
Material and methods: Schwannoma pseudocyst content was added in various amounts to primary bone marrow cultures and their proliferation assayed by ^{3}H-Thymidin uptake. Conventional hematologic parameters were investigated on rats three weeks following subcutaneous NV2cd inoculation and animals receiving pseudocyst fluid three to seven times i. v. or i. p. during the same period.
Results: Increasing addition of pseudocyst fluid to culture medium up to 15% (v/v) results in a proliferation enhancement, further addition in a decrease. Neoplasia-induced anemia, leukocytosis and relative lymphocytopenia can not be reproduced solely by the applied mediator transfer.
Conclusion: Schwannomas serve as an example for nervous system neoplasias secreting several mediators which can induce synergistic and pleiotrophic systemic effects, their biological activity contributes to but not fully determines the complexity of a host-tumor-interrelation.

THE STRESS-RELATED "READTHROUGH" ACETYLCHOLINESTERASE UNDERGOES C-TERMINAL TRUNCATION *IN-VIVO* AND *EX-VIVO*

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The mammalian acetylcholinesterase (AChE) gene undergoes alternative splicing, yielding three 3'-terminally distinct splice variants. One of these mRNA variants reads through pseudointron 4 and translates into a catalytically active, soluble, monomeric isoform of the enzyme ("readthrough", AChE-R). We have identified AChE-R upregulation under exposure to various stressors including psychological, chemical and physical insults at both the mRNA and the enzyme activity levels. Moreover, the subcellular distribution of AChE-R mRNA was modified under variable stressors. While under control conditions AChE-R mRNA is observed exclusively in the soma of cortical neurons, following stress treatments AChE-R mRNA extends into the proximal domain of dendrites. To explore the biochemical properties of AChE-R we subjected extracts of *Xenopus laevis* oocytes expressing the different AChE isoforms to SDS-PAGE and immunoblotting. In extracts expressing AChE-R or the synaptic AChE isoform (AChE-S), a pool of monoclonal antibodies to the common AChE domain interacted not only with AChE-R and AChE-S but also with a faster-migrating band. This band corresponded in size to a recombinant C-terminally truncated AChE (AChE-E4) and may thus be a naturally truncated AChE-E4. This apparent cleavage prompted raising of a polyclonal antibody against the I4-encoded acetylcholinesterase "readthrough" peptide (ARP). This antibody recognized AChE-R in brain following stress. To test the long-term consequences of AChE-R overexpression, we generated two lines of transgenic mice overexpressing AChE-R, and compared them to mice transgenic to active and inactive synaptic AChE and to control FVB/N mice. In the cortex of all of these mice, the novel anti-readthrough antibody recognized AChE-R whereas monoclonal antibodies to the common AChE domain recognized the band corresponding to the C-terminally truncated AChE-E4. Therefore, AChE-R can be identified as a general stress marker which undergoes cleavage to yield the core AChE domain and the C-terminal peptide, ARP.

EFFECT OF NEURAL TRANSPLANTS IN THE MOTOR CORTEX AFTER CEREBRAL ISCHEMIA BY MIDDLE CEREBRAL ARTERY OCCLUSION IN BONNET MONKEY (Macaca radiata)

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Objectives : To assess the role of Human Embryonic Cortical Tissue (HECT) transplants in correcting the deficits in the host cortex after ischemia.

Methods : With sterile surgical procedures, craniotomy was done in 8 young male bonnet monkeys weighing 1.5 to 3.5 kg. After careful displacement of the brain, the cortical branches of the Middle Cerebral Artery (MCA) at the limen insula was permanently occluded by electrocauterisation. After an hour of vascular lesion the cortical area received HECT as transplants which served as vascular lesion and transplanted model. 15, 60, 120 and 240 days after surgery, gait, posture, stepping and placing action, contactual reflex and individual limb function were analysed to measure the neurological deficits produced. Paraffin method and Golgi cox preparation were carried out for histological analysis.

Results : Unilateral motor impairment of the contralateral fore and hind limbs were observed in all animals subjected to vascular lesion. Histological analysis showed extensive frontal cortical damage in all these animals. Differences between vascular lesioned animals and animals with lesion and transplants in behaviour were apparent by the 4th week onwards. The transplants helped in regaining movements in the contralateral side during feeding and locomotion. The histological analysis in vascular lesion and transplanted cortex revealed that HECT were able to survive and could make intrinsic connection with the host cortex.

After two months the number of cells implanted were not found to be retained at the injected area. The transplanted cells could have migrated from the grafted area into layer 2-3 and some cells into layer 4 of the host cortex which was evident by morphometric observation.

Conclusion : This study clearly shows that it is possible to produce quantifiable behavioural deficits in the animal following MCA occlusion. The results demonstrate the use of this primate species in focal ischemic research to assess the histopathological reduction of the infarct provided by transplantation.

CHANGES OF THE SYNAPTIC STRUCTURES IN THE MESENCEPHALIC TRIGEMINAL NUCLEUS OF RAT DURING AGING

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The purpose of this study was investigate the changes of the synaptic structures in the mesencephalic trigeminal nucleus of rat during aging by transmission electron microscope. The neurons of the mesencephalic trigeminal nucleus are concerned with proprioception from the masticatory muscles, periodontal ligament and temporomandibular joint. Sprague-Dawley rat 3, 12, 24 and 36 months of age were used in this study. Micrographs were taken with JEOL 1200 EX electron microscope at a constant magnification of ×10,000, enlarged photographically three times. An area of 48μm^2 real size per micrograph was considered. The number of synapses, the length of postsynaptic thickening, and the number and the area of axon terminal were measured using image analyzer(BMI PLUS). The number of synapses was 19.6, 17.2, 13.4 and 8.5 per 240μm^2 in the 3, 12, 24 and 36months of age, respectively. The length of postsynaptic densities was 8.1μm, 6.9μm, 5.1μm and 2.3μm per 240μm^2 in the 3, 12, 24 and 36 months of age, respectively. The number of axon terminals was 73.6, 64.2, 39.4 and 26.1 in the 3, 12, 24 and 36months per 240μm^2 of age, respectively. The area of axon terminals was 40.7μm^2, 31.3μm^2, 13.8μm^2 and 7.6μm^2 per 240μm^2 in the 3, 12, 24 and 36months of age, respectively. The results suggest that there are the changes of the synaptic structures in the mesencephalic trigeminal nucleus of rat during aging. These changes may be concerned to the decreased function of mediating proprioceptive sensation in the masticatory muscles, periodontal ligament and temporomandibular joint during aging.

Differential effect of SNARE proteins on neurite outgrowth

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VAMP2, Synaptosomal associated protein 25 (SNAP25) and syntaxin have been regarded as SNAP receptors (SNARE) essential for exocytosis of vesicles in synapses. VAMP2 has been regarded as a vesicle-associated SNARE. SNAP25 and syntaxin have been regarded as target-associated SNAREs. We have previously reported that cleavage of syntaxin or SNAP25 with botulinum neurotoxin C1 or A resulted in inhibition of neurite extension. (Igarashi M. et al. 1996, J.Cell.Biol., 134, 205-215. Morihara T. et al. 1999, Neuroscience, in press.) As an attempt to explore the mechanism of growth cone extension, we examined effect of the overexpression of VAMP2, SNAP25 and syntaxin on neurite extension in NGF-differentiated PC12 cell. Overexpression of VAMP2 induced increment of neurite length. Overexpression of SNAP25 didn't change neurite length, but increased neurites number per cell. However overexpression of syntaxin had no distinct effect on neurite length and neurite number.

These results suggest that VAMP2 plays a role in vesicle fusion at the neurite extension sites of neuron and that SNAP25 determine the sites of neurite outgrowth by determining the vesicle fusion sites of cell body. Further analysis of role for SNARE proteins in neurite outgrowth is now under investigation.

INCREASED INTRACELLULAR CALCIUM AND GDNF PRODUCTION IN HUMAN FETAL ASTROCYTES UPON STIMULATION OF D_1 RECEPTORS

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The use of fetal astrocytes for gene delivery into brains with neurodegenerative diseases has been suggested. Therefore, the effects of neurotransmitters in the brain on such cells is of interest. Herein, the presence of D_1 (D_{1A}) receptors and the effect of dopamine on a fetal human astrocyte cell line (SVG cells) *in vitro* was examined. SVG cells expressed D_1 (D_{1A}) but not D_5 (D_{1B}) receptors, as revealed by flow cytometry and RT-PCR. Exposure to dopamine and its agonist, apomorphine, increased intracellular free calcium and glial-derived neurotrophic factor (GDNF) production of SVG cells by ELISA. Exposure to the specific D_1 antagonist, SCH 23390, blocked these effects. These findings indicate that fetal astrocytes, if implanted into a brain region rich in dopamine or if transfected with the tyrosine hydroxylase gene, may serve as autocrine cells and be able to supply critical growth factors to diseased brain tissue.

CD39 IS A CAVEOLAR-ASSOCIATED ECTONUCLEOTIDASE

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CD39, a human lymphoid cell activation antigen, is a known ATPDase that hydrolyzes extracellular ATP and ADP. This ectonucleotidase is considered to play an important role in purinergic signaling, in thromboregulation, and in neuroprotective processes by converting ATP ultimately to adenosine. Caveolae are suggested to play a major role in coordinating the interaction of endothelial cells with environmental factors. These microdomains possess special structural proteins as caveolin-1,2 and 3, furthermore, they serve as docking places in the plasmalemma of several proteins involved in signal transduction (e.g. G-proteins, eNOS). Endothelial (HUVEC) and epithelial (renal fibroblast COS-7) cell cultures expressed CD39/ecto-ATPDase; activity was localized by enzyme and immunohistochemical methods to the caveolae of the cells. COS-7 cells, naturally possess very low native ecto-ATPase activity. Following transfection with wild type CD39cDNA, however, we obtained similar localization pattern of CD39 activity as with HUVEC cells, similarly in the caveolae. We hypothesize, that targeting of CD39 to caveolae is mediated by palmitoylation (as in the case of eNOS) or by similar process. The high level of CD39 just in these dynamic structures of the cell membrane may help the cell to optimize its responses according to the actual spatio-temporal signaling.

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MITOCHONDRIAL DYSFUNCTION AND CALCIUM SIGNALLING IN MICE PRIMARY SENSORY NEURONS

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Calcium transients triggered by membrane depolarization were measured in mice dorsal root ganglion neurons with the help of fluorescent indicator indo-1AM. The application of mitochondrial uncoupler CCCP and Na^+/Ca^{2+} exchanger inhibitor TPP^+ to these neurons has shown that mitochondria participate actively in the shaping of calcium transients triggered by neuronal activity - by diminishing their peak amplitude due to rapid uptake of Ca^{2+} and substantially prolonging their recovery due to subsequent slow Ca^{2+} release back into the cytosol. Supression of Ca^{2+} accumulation in mitochondria by CCCP eliminated both changes, while inhibition of the Na^+/Ca^{2+}exchanger selectively depressed the residual elevation of cytosolic Ca^{2+}. This participation appears to be a threshold phenomenon and starts with elevation of cytosolic Ca^{2+} above ≈230 nM in large-sized cells and about 500 nM in small-sized (predominantly nociceptive) ones. The small-sized cells differ from large ones also by the availability and properties of other calcium-storing mechanisms, in particular they do not reveal effective accumulation and release of Ca^{2+} by the endoplasmic reticulum. Similar measurements on neurons from mice with streptozotocin-induced or genetically-determined (*db/db* line) diabetes revealed an extreme prolongation of the recovery phase of calcium transients, predominantly in small-sized cells, without substantial changes in transients amplitude. Changes in kinetics developed in parallel with increasing hyperglycemia and demonstrated quantitative differences depending on the type of induced pathology. The described prolongation could be abolished by swithching-off Ca^{2+} accumulation in mitochondria by CCCP. A conclusion is made that mitochondrial dysfunction during diabetic pathology (in particular alterations of their ion-exchanger mechanisms) plays an important role in the modifications of calcium signalling and related functional changes of the corresponding sensory neurons. Such dysfunction was only partially reversible during compensation of hyperglycemia by treatment of the animals with insulin infusions, indicating the severity of alterations in the mitochondrial membrane.

CEREBRAL MICROCIRCULATION DURING EARLY REPERFUSION AFTER FOCAL CEREBRAL ISCHEMIA IN RATS, EFFECT OF SUPEROXIDE DISMUTASE.

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The aim of present study was to find out whether scavenging of superoxide radical which has been reported to protect the brain against ischemia/reperfusion injury has also beneficial effect on depressed regulation of cerebral microcirculation after transient, focal cerebral ischemia. The experiments were performed on 52 anesthetized and mechanically ventilated male Wistar rats. Cerebrocortical microflow (LDF) was continuously monitored using laser-Doppler probe. Focal cerebral ischemia (decrease of LDF below 30% of control) was induced for 30 minutes using suture occlusion of MCA and was followed by reperfusion. Following experimental groups were studied: 1) sham rats, 2) rats with ischemia/reperfusion, 3) rats with ischemia/reperfusion pretreated with polyethylene glycol-conjugated superoxide dismutase (PEG-SOD, 10,000 U/kg i.v.). Groups 1 and 2 received i.v. either L-NAME (NO inhibitor), or indomethacin (prostaglandins inhibitor), acetylcholine (ACh_i) or CO_2. Group 3 went through the same tests with exception of L-NAME. Plasma SOD activity was measured using a cytochrome c modified gold electrode. It was 11±3 U/ml under baseline conditions and 280±40 U/ml at the time of LDF reactivity tests after reperfusion in group 3. In group 2 LDF response to L-NAME was preserved but response to indomethacin was abolished. There was also no response to ACh and CO_2. PEG-SOD did not affect severity of ischemia or time course of reperfusion in group 3. In this group the increase of LDF during ACh and CO_2 tests was observed. Pretreatment with PEG-SOD did not, however, restore the normal response to indomethacin. Our results demonstrate that pretreatment with PEG-SOD in this model of focal ischemia exerts some protection on blood vessels.

PECULIARITIES OF DISTURBANCE OF A CNS FUNCTIONAL STATE AND CEREBRAL HEMODINAMIC AT THE CHILDREN WITH CONSEQUENCES PERINATAL ENCEPHALOPATHY

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Influence of natal cerebrospinal traumas on ischemia of a developing brain, especially under action press is known. The special importance is given to infringements circulation vertebrobasilar region. (VBR). Changes of CNS functional state (on data EEG) at infringements cerebral hemodynamic (on data rheoencephalographe) in VBR and carotid artery region was investigated. We investigated 66 children in the age from 5 till 14 years (mean 8,75±5,25) with the complaints to head pains, parasomnia, tiredness and difficulty in training. The 2 groups the surveyed children among with moderately expressed (I) and sharply expressed (II) by changes of bioelectrical activity, mainly in parietal-ocipital (P-O) and pasterior temparal (T5, T6) areas of a head brain are allocated. In the first group (10 person) in the specified areas dominated hypersinchronous alpha-rhythm (frequency 8-10 Hz, amplitude up to 100 mkV). For the present group the moderate decrease of amplitude intracerebral pulse waves (AIPW) in VBR on rotation of a head on 30-40 %, from reference values with 0,1 on 0,17 Om was characteristic at safe reactivity of microvessels of a channel on delay of breath at 90 % of the children. The second group (10 person) differed by instability of a functional state with alternation alpha-rhythm and theta-waves (4-6 Hz, 80-120 mkV). Such instability is found out at sharply expressed (up to 45-70 %) decrease AIPW in VBR on rotation of a head. In 67 % of cases was marked as non reaction of microvessels of a channel on delay of breath. This groups was united by relative safety AIPW in VBR in limits of norms for ages and increased tonus of vessels in carotid arteries region and VBR. Thus at 30 % of the children both groups were decreased AIPW up to 0,1-0,12 Om (N=0,15-0,17 Om). Received data alloed to connect a degree of changes of a cerebral cortex functional state and cortical-subcortical relations with depth of infringements cerebral hemodynamic for the account compression influences in system spinal and basic arteries at the children with consequences perinatal encephalopathy.

Analysis of the Interictal and Ictal Activity recorded in the basal ganglia during invasice videoEEG

Kuba R, Rektor, I Brázdil M

Analysis of the Interictal and Ictal EEG Activity Recorded in the Basal Ganglia in Epileptic Patients during Invasive Video-EEG

Kuba R., Rektor I., Brázdil M.

Purpose: The investigation of ictal and interictal EEG activity recorded in the basal ganglia and the relationship among the ictal EEG pattern in the basal ganglia, the spatial distribution of paroxysmal activity in other structures and the motor and behavioural activity.
Methods: We evaluated ictal and interictal EEG activity in basal ganglia in 8 intractable epilepsy patients investigated prior to epilepsy surgery by means of the diagonally implanted multilead depth electrodes . The activity from putamen was recorded in 7 patients and this one from pallidum and caudate head in 1 patient. Several orthogonally implanted depth electrodes were placed into various cerebral regions to confirm the localisation of the epileptogenic zone (amygdala, hippocampus, lateral temporal neocortex, SMA, anterior cingulate gyrus, orbito-frontal cortex etc.) at the same subjects.
Results: The common interictal EEG activity in the basal ganglia consisted of an irregular, non-modulated mixture of prominent beta (25-30Hz) and alpha-theta (5-9Hz) activity. This activity has not been changed during aura, motionless period of complex partial seizures as well as during isolated contralateral ictal discharges in most of the seizures.
We observed a clear-cut slowing and the amplitude changes of the basal ganglia activity related to the presence of pseudo-purposeful behaviour or ictal motor manifestations and during ipsilateral temporal lobe structures involvement.
Conclusion: The basal ganglia are not leading structures in epileptic seizures. They are active during motor and behavioural manifestations of complex partial seizure as well as during involvement of the ipsilateral temporal lobe structures.
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NEUROTROPIC ACTION OF NICOTINAMIDE ADENINE DINUCLEOTIDE ON NORADRENALINE UPTAKE AND SEROTONIN RELEASE BY BRAIN SYNAPTOSOMES

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Vitamine PP and its derivatives are widely and successfully used for the treatment of different etiology mental diseases. We previously established that the functioning of NAD system is deranged by PP-hypovitaminosis, parkinsonism and diabetes. Although the characteristics of NAD binding by isolated membranes and processes of neurotransmission in experimental pathologies are shown to be impaired, the molecular mechanisms of biological effect of vitamine PP and its derivatives are still uncleard. As little has been reported about the NAD effects on the molecular mechanism of serotonin release and noradrenaline uptake by rat brain synaptosomes , we designed the present study to determine the effects of NAD on these processes. Indeed, it is reported that NAD is involved in the regulation of the neurotransmissin in the brain.
Examination of neurotoxins and NAD effects on [2-^{14}C]serotonin release by rat brain synaptosomes demonstrated that NAD partially normalized neurotransmitter release that was deteriorated by such neurotoxins as tetrodotoxin, veratridine, latrotoxin and oubain. Thus, in case of associated action of veratridine and latrotoxin with 1 μM NAD, [2-^{14}C]serotonin release was decreased respectively on 24.1 and 21.3% as compared to toxins effects. Under the same conditions NAD caused the opposite inffluence on tetrodotoxin and oubain action. Release of [2-^{14}C]serotonin was increased respectively on 50.4 and 23%. The uptake of [^{3}H]noradrenaline by rat brain synaptosomes was inhibited by veratridin and insignificantly activated by tetrodotoxin. 1 μM NAD partially elemenated these effects normalizing [^{3}H]noradrenaline uptake. Thus, modulative effect of NAD which binds specifically with synaptic membranes supposes that NAD acts via ion channels. We conclude that NAD is involved in the regulation of brain dysfunctions.

DIFFERENTIAL EFFECTS OF OLANZAPINE AT DOPAMINE RECEPTORS AND PREFERENTIAL INHIBITION OF DIZOCILPINE-INDUCED HYPERLOCOMOTION

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The aim of the present study was to investigate the locomotor stimulant effects of olanzapine, an atypical antipsychotic drug and its interactions with various dopaminergic drugs in dopamine depleted mice and naïve mice. In studies on dopamine depleted mice, olanzapine (0.5, 1 and 2 mg/kg) dose-dependently increased locomotor activity which was completely blocked by pimozide (0.5 mg/kg) but not by SCH 23390 (0.5 and 1 mg/kg). Olanzapine (1 and 2 mg/kg) blocked hyperlocomotion and stereotypy induced by SKF 38393 (10 and 25 mg/kg) and B-HT 920 (1 and 2 mg/kg) whereas it blocked the hyperlocomotion but not stereotypy induced by apomorphine (0.5 and 1 mg/kg). In studies on naïve mice, pretreatment with olanzapine (0.25 and 0.5 mg/kg) inhibited dizocilpine (0.5 mg/kg)-induced hyperlocomotion but not the stereotypy. But at the higher doses (1, 2 and 4 mg/kg), olanzapine blocked both stereotypy and hyperlocomotion induced by dizocilpine. Similarly, olanzapine (0.25 and 0.5 mg/kg) neither inhibited apomorphine (3 mg/kg)-induced stereotypy nor decreased spontaneous locomotor activity but at the higher doses (1, 2 and 4 mg/kg) olanzapine showed significant decrease in spontaneous locomotor activity. Olanzapine (2 and 4 mg/kg) showed significant catalepsy which lasted for more than 4 h. Olanzapine exhibited properties consistent with those of a D_2 partial agonist having strong D_1 antagonist property. Further, olanzapine selectively inhibited behaviours mediated by mesolimbic/mesocortical system.

THE CEREBROPROTECTIVE EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ANALOGUES.

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Goal: To determine the cerebroprotective potential of 2 synthetic peptides (SNV and NAP) related to vasoactive intestinal peptide (VIP). **Background**: VIP is a peptidergic neurotransmitter that was shown to have anti-ischemic effects in a model of myocardial ischemia. At least some of its effects are mediated by an astrocytic-derived protein – activity dependent neuroprotective protein (ADNP). SNV and NAP are synthetic compounds related to VIP and ADNP respectively that readily cross the blood brain barrier. The cerebroprotective effects of VIP or ADNP after focal ischemia have not been tested before and we therefore used SNV and NAP to test whether they posses neuroprotective effects. **Materials and Methods**: Spontaneously hypertensive rats (SHR) underwent permanent middle cerebral artery occlusion (PMCAO) by craniotomy and electrocoagulation. The animals were injected with either SNV or NAP intravenously (3μg/kg) 1hr after stroke onset. Infarct volumes were measured 24hrs later by staining with TTC and using an image analysis system. Results were compared to those obtained in vehicle injected rats. **Results**: Both proteins significantly reduced infarct volumes as compared to vehicle injected rats. Infarct volume reduction was of similar magnitude for both compounds (10.36±3.8% hemispheric volume for SNV and 8.65±3.9% hemispheric volume for NAP versus 16.23±3.07% hemispheric volume for vehicle injected rats). **Conclusions**: VIP related proteins appear to be cerebroprotective in this animal model of focal cerebral ischemia. Further experiments are in progress in order to elucidate their exact mechanisms of action in cerebroprotection and to establish the optimal dosage and timing schedule in stroke.

BINDING OF THE AMYLOIDOGENIC PROTEINS CYSTATIN C AND AMYLOID ß-PROTEIN.

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Cerebral amyloidosis comprises a heterogeneous group of disorders of different etiology characterized by deposition of amyloid in the brain parenchyma and blood vessel walls. Amyloid ß-protein (Aß) is the major constituent of the amyloid fibrils in aged individuals and in patients with Alzheimer's disease (AD), Down's syndrome, cerebral amyloid angiopathy (CAA) and hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA–D). A familial type of HCHWA with a clinical picture and pathological findings similar to HCHWA-D was described in patients from Iceland (HCHWA-I). The amyloid protein that is restricted to the cerebral vasculature in HCHWA-I patients is a variant of cystatin C, a cysteine proteinase inhibitor.

Immunohistochemical analysis for Aß and cystatin C in brains of patients with CAA, AD and HCHWA-D demonstrated dual staining of amyloid deposits in the brain parenchyma and vessel walls. We hypothesize that the colocalization of Aß and cystatin C may reflect protein-protein interaction. We demonstrate binding of cystatin C to ß-amyloid precursor protein (ßAPP) in tissue culture cells by immunoblot analysis of immunoprecipitated cell lysate and medium proteins with anti-ßAPP and anti-cystatin C antibodies. The interaction of cystatin C with ßAPP was confirmed using GST fusion proteins containing full length cystatin C or various fragments of ßAPP. The cystatin C binding site within ßAPP was localized to $Aß_{1-16}$. Cystatin C association to Aß may suggest a role for cystatin C in the pathogenesis of cerebral amyloidosis. (Supported by the NIA AG13705)

8-OH-DPAT, A SELECTIVE 5-HT_{1A} RECEPTOR AGONIST, COUNTERACTS THE HALOPERIDOL-INDUCED MUSCLE RIGIDITY IN RATS.

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Some anatomical and behavioural data suggest that serotonin (5-HT) may be implicated in motor functions. Although neuroleptics are believed to cause extrapyramidal symptoms by blocking the striatal dopamine receptor, it has been shown that the cataleptic effect of these drugs is reduced by a lesion of serotonin-containing neurons, by a blockade of synthesis of 5-HT by specific inhibitors, or by treatment with 5-HT antagonists. All these results suggest that the 5-HT-mediated transmission is also involved in extrapyramidal symptoms. However, no information is available on the role of serotonin receptors in the neuroleptic-induced muscle rigidity in rats. We have recently demonstrated that muscle rigidity induced by haloperidol is characterized by both enhanced muscle resistance (MMG) and an increased electromyographic (EMG) reflex activity. 8-OH-DPAT (8-hydroxy-2(di-N-propylamino) tetralin), a selective 5-HT_{1A} receptor agonist, has been found to reduce the synthesis of 5-HT in the brain and to induce behavioural effects compatibile with the reduced central function of 5-HT. The aim of present study was to find out whether 8-OH-DPAT was capable of counteracting the haloperidol-induced muscle rigidity. The muscle tone was measured as mechanical resistance of the hind foot to passive movements in the ankle joint. The reflex EMG activity of the gastrocnemius and tibialis anterior muscles was simultaneously recorded. To increase the muscle tone, the animals were pretreated with haloperidol (1mg/kg). 8-OH-DPAT (0.125, 0.250 and 0.500 mg/kg), injected 1h later, caused a significant and dose-dependet decrease in both muscle resistance (MMG) and the EMG activity in examined antagonistic muscles. The obtained results suggest that 8-OH-DPAT is effective in relieving the haloperidol-induced muscle rigidity.

CALCIUM-DEPENDENT SECRETION FROM CHROMAFFIN CELLS INVOLVES DIFFERENT TYPE OF VESICLES.

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Adrenal chromaffin cells ontogenetically originated from precursor cells common with sympathetic neurones and share many properties with neurones. Therefore, they are the most well-characterized model system for calcium - regulated exocytosis. It is known that in chromaffin cells the events involved in calcium - dependent secretion demonstrate a two step dependence of secretion on intracellular calcium (Ca_i). Using whole-cell patch-clamp and capacitance techniques combined with Fura-2 fluorescence measurements for simultaneous monitoring of calcium current, secretion, and intracellular calcium concentration (Ca_i) in bovine chromaffin cells as well as electron microscopy methods we established that calcium - dependent secretion induced by depolarization, proceeds in two steps, each of which depends on Ca_i and is determined by fusion of two different size-types of vesicles . We established that 200 nM Ca_i is critical concentration for calcium - dependent secretion from chromaffin cells. At Ca_i below this level only small vesicles with mean diameter of 72 nm fused whereas at higher Ca_i, the large vesicles with mean diameter equals to 185 nm started to fuse. By using ultra-thin serial slices of cells and electron microscopy, we have identified a pool of small vesicles in chromaffin cells that was located in the definite cytopmasmic part and its vesicles selectively docked and fused with cell membrane after KCl depolarization, whereas the large vesicles showed no tendency to form the secretory pools and diffusely fused along the cytoplasmic membrane. The presented observations allow to explain the biphasic calcium dependence of secretion from chromaffin cells and to suppose that the cell can use the calcium - dependent regulatory mechanism to discriminate between transmitter classes which have to be released during different extent of cell excitation.

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AN INDIVIDUAL APPROACH TO VASOACTIVE MEDICINAL TREATMENT OF DIFFERENTLY AGED PATIENTS WITH CEREBROVASCULAR DISEASES (CVD)

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The purpose is to study the individual influence of vasoactive agents on arteriovenous cerebral circulation under the control of ultrasound diagnostics (USD) including ultrasound dopplerography and scanning with normal and pathological conditions. This included using such methods as non-invasive investigation of arteriovenous cerebral circulation in differently aged people, the control of the efficacy of medicines in treatment of arterial and venous cerebral channel pathologies under the control of USD and prognosis of the necessity of the surgical treatment. The above points have provided the basis for new scientific research (State Patent of Ukraine N 10262 dated from 19/07/95). The present work was done based on clinical and instrumental inspection of 324 patients that were taking a course of treatment in vascular neurosurgery department of Kyiv Emergency Hospital. The control group consisted 215 healthy people of the same age.The research revealed different influences of vasoactive agents on arterial and venous parts of cerebral circulation. Conclusions: euphyllin, aescusan, a group of Ginko Biloba agents have to be used with venous cerebral discirculation. Nootropil or pyracetam are harmful with such disorder because of increased arterial cerebral hyperemia on the background of venous disorders. We think, the classical approach to treatment has to be reconsidered in aspects of venous tension and venous brain outflow correction.

MULTIPARAMETRIC RESPONSES OF NORMAL AND EXPERIMENTALLY INJURED RAT BRAINS TO MANNITOL

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Traumatic brain injury results in various physiological disturbances. One of the common effects is damage to the normal homeostatic mechanisms controlling the pressure of the cerebrospinal fluid. This leads to a relentless increase of the Intracranial Pressure (ICP), reduced cerebral blood flow and oxygen delivery (ischemia) and eventual brain death. Treatments given in order to reduce ICP may lead also to a decrease in blood flow and energy availability. Neurosurgical practice widely uses hyperosmolar solutions to control elevated ICP. Infusion of Mannitol are known to reverse blood-brain osmotic gradient, thereby reducing extracellular fluid volume in both normal and damaged brain (Marmarou A. 1996, In: Narayan RK et al. eds., Neurotrauma, McGraw-Hill Inc., pp. 413-428). The aims of the present study were to test the possible improvement of Mannitol on the elevated ICP in a rat model and how does Mannitol affect the various parameters measured from the brain. In the present study, male Wistar rats (~280 gr.) were connected to a multiparametric measuring device (Mayevsky et al. 1995, J. Appl. Physiol. 78: 1188-1196, 1995) on the surface of the left parietal cortex. Fluid percussion (FP) injury was performed in the contralateral hemisphere (parietal bone craniotomy) and the.following parameters were recorded in real time: Cerebral blood flow (CBF) and volume, ICP, extracellular potassium, calcium and pH, DC potential, ECoG as well as mean arterial blood pressure, and arterial blood gases. A control group received no FP. An IV bolus of Mannitol (1 gr./Kg 20% solution) was given 2 hours after FP, and monitoring continued for additional 2 h. Mannitol was found to decrease ICP in both groups 10-30 min following injection. Extracellular pH decreased, extracellular potassium increased, CBF was slightly elevated in most of the animals as well as mean arterial pressure, all during the first hour after injection.

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PECULIARITIES OF CEREBRAL COLLATERAL CIRCULATION IN BRAIN ISCHEMIA

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Cerebral collateral blood circulation has determinative influence on outcome of the occlusive process in cerebral vessels. The aim of our investigation was quantitative characteristics of compensatory resources of collateral circulation in cerebral arteries in patients with occlusion processes by Transcranial Ultra Sound Dopplerography. There were investigated 25 healthy subject and 25 patients with cerebrovascular pathology, in different phases of brain ischemia (compensation, subcompensation, decompensation and destruction), indicated by Blood Flow Symmetry Index (BSI) in M1 segment of Middle Cerebral Artery (MCA). In healthy subjects BSI achieves 15% , and less than 30% in main collateral circulation arteries . Investigations reveal decreasing of the blood flow in same side collateral circulation in compensation phase. During subcompensation phase there were insignificant asymmetry in MCA and changes in blood flow direction by collaterals . Decompensation ph!
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ase is define by increasing asymmetry in MCA and blood flow velocity in damaged side, the blood supply was from Anterior Communicans Artery passing to Posterior Communicans Artery . In the case of completely thrombosis of MCA there were severe neurological deficiency increased blood flow in collateral circulation. The investigations reveal hemodynamic changes in cerebral blood circulation during brain ischemia to reconstruction of normal blood flow in main arteries , different phases of ischemia development are define by consecutive inclusion of the collateral circulation reserve .

SHIFT OF THE BRAINS VESSELS FUNCTIONAL CONDITION AND BLOOD INDICATOR IN STRESS CONDITIONS

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The histochemical non-injection calcium-adenosine triphosphates (Ca-ATP) method was used for the study of brain vessels functional condition at rabbits and rats. This method allows to reveal clearly all groups of microcirculatory bed on the thick transparent brain cuts. While animals were get up molybdenum (300 mg/kg and 500 mg/kg), a typical picture of the acute stress (molybdenal toxicous) is with developed shifts of the brain vessels condition and blood indicator. A significant decrease of the mean diameter of brain functional capillaries is revealed an increase in manpower stenotic capillaries on the second-fifth day of the stress. A considerable higher blood viscosity and packed cell volume, decline of the endogenic heparin level and shorting of the blood coagulation time are revealed too. Appeared changes are apparently connected with the rise of adrenergic effect in stress condition, that leads to infringement of balance in sympathoadrenal system and which reflects on the important "vessel-blood" system. We recommend to take in consideration the "brain vessel-bloood" system conditions test. The possibility of using histochemical non-injection method for revealing organs microvessels during the infringements of the brain functions or during the stress and other pathological conditions is discussed.

MELATONIN AND NICOTINIC RECEPTORS PLASTICITY

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Melatonin, the pineal hormone produced during the dark phase (DP) of the day, modulates nicotinic receptors (nAChRs) located presynaptically on nerve terminals of rat vas deferens. Recently, we have shown the presence of nicotine high affinity binding sites during the light phase (LP) and DP, and low and high affinity binding sites during the DP. The appearance of the low affinity binding sites was due to nocturnal melatonin surge and could be mimicked by *in vitro* melatonin, and blocked by cycloheximide. In this study the receptor subtypes responsible for contractile response at the LP and the DP were identified. Agonists rank order of potency was dimethylphenylpiperazinium (DMPP) = cytisine > nicotine > carbachol and DMPP = nicotine = cytisine > carbachol, at the LP and DP, respectively. DMPP response was equally blocked, at both phases, by mecamylamine, while the nicotine response was more efficiently blocked at the LP. On the other hand, methyllycaconitine inhibits nicotine-induced response only at DP. As α7-nAChRs have low affinity to nicotine binding sites (binding assays), it is suggested that at night a mixed population composed by $\alpha 3\beta 4$ – plus α7-bearing nAChR subtypes is present. This plasticity is probably driven by melatonin, as mecamylamine partially blocks nicotine-induced contraction in organs obtained at 15:00h and incubated with melatonin (100pg/ml, 4h). Thus melatonin, by acting directly on the short adrenergic neurons induces the appearance of the low affinity binding site, probably an α7 subtype nAChR.

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MODULATION OF DENDRITIC OUTGROWTH IN DEVELOPING RETINAL GANGLION CELLS BY EARLY NEURAL ACTIVITY.

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Receptive fields (RFs) of developing retinal ganglion cells (GCs) undergo activity-dependent modification. We have investigated whether these changes correlate with modification in dendritic arborisation. GCs were back-labelled with horseradish peroxidase. Dendritic growth was investigated at different stages. Following an initial phase of elongation and branching (from embryonic stage 21 to S24), dendritic outgrowth stopped and significant pruning was observed by S26 (hatching). Dark-rearing, a condition known to enhance immature retinal spontaneous bursting activity (SBA) and cause RFs to expand, led to an increase in the size of dendritic arbors. However, when retinas were exposed from S26 to curare, a cholinergic nicotinic antagonist known to block immature SBA, the effects of dark rearing were inhibited. Similarly, chronic exposure of embryonic retinas to curare from S21 significantly suppressed dendritic outgrowth. These findings suggest that embryonic SBA initially induces dendritic outgrowth during a critical period of development. Towards hatching and during the early period of visual experience, however, SBA seems critical for dendritic remodelling. Because the emergence of early SBA coincides with intense dendritic outgrowth, enhancement of SBA post-hatching leads to abnormal dendritic proliferation and cholinergic blockade of SBA suppresses dendritic growth, we conclude that immature cholinergic SBA is a critical factor for dendritic outgrowth in GCs. Supported by MRC, Newcastle University Hospitals Special Trustees and Newcastle University Research Committee.

EXTRAOCULAR MUSCLE REPRESENTATION WITHIN THE SUBDIVISIONS OF OCULOMOTOR NUCLEUS USING HORSE RADISH PEROXIDASE

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Isolated oculomotor palsies are more commonly reported. In such cases, the knowledge of location of lesions within the subdivisions of the oculo -motor nucleus is very much essential for the clinicians. Hence an attempt has been made by injecting horse radish peroxidase into each extraocul ar muscle supplied by oculomotor nerve. It was observed that the Inferior rectus is projected into dorsal subdivision; inferior oblique is projected into the intermediate subdivision; levator palpebrae superioris is projected into the caudal central subdivision; superior rectus is projected into the ventrimedial subdivision and nucleus of Perlia. Medial recus is projected into the two subgroups of ventral subdivision and medial part of dorsal subdivision. Notable new observati ons were the bilateral projections of medial rectus and its projections to dorsal subdivision. Labelling of HRP into two subgroups within the ventral subdivision. Superior rectus is projected to ventrimedial subdivision contralaterally. The projection of superior rectus into the nucleus of perlia is also another new finding. Earlier it was considered that the nucleus of Perlia is function ally connected to medial rectus for convergence movement, which has been proved by this study that the nucleus of Perlia is connected to superior rectus and not to the medial rectus.

KAINATE RECEPTOR-DEPENDENT MODULATION OF GABAERGIC INHIBITION BY SYNAPTICALLY RELEASED GLUTAMATE.

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Exogenous application of agonists at the kainate subtype of glutamate receptors depresses evoked monosynaptic inhibition in the hippocampus. This suggests that synaptic release of glutamate might disinhibit neuronal circuits. Does glutamate released from excitatory neurons have the same kainate receptor-mediated effect on monosynaptic inhibitory transmission as exogenous agonist application? We examined the effect of synaptically released glutamate on monosynaptic IPSCs in guinea pig hippocampal slices. We recorded from a CA1 pyramidal neuron, and delivered stimuli either via a 'proximal' electrode positioned nearby to recruit monosynaptically coupled inhibitory interneurons, or via a 'distal' electrode used to excite Schaffer collaterals. NMDA receptors were blocked with APV (100 microM) throughout. When AMPA receptors were blocked with the selective antagonist GYKI52466 (100 microM), the response to the distal stimulus disappeared, but the proximal stimulus continued to elicit a monosynaptic IPSC. Brief trains of stimuli delivered via the distal electrode consistently reduced the amplitude of the GABAergic IPSC by 13-20%. This depression of inhibition was completely abolished by adding the non-selective AMPA/kainate blocker DNQX (50 microM), the reversible ionotropic glutamate receptor blocker kynurenate (2.5-5 mM) or the kainate blocker gadolinium (10 microM). The disinhibition persisted when GABAB receptors were blocked with CGP35348 (100 microM). It was enhanced by blocking metabotropic glutamate receptors with MCPG (250 microM) and MSOP (200 microM). We conclude that synaptically released glutamate is reduces monosynaptic inhibition through an action at kainate receptors. Kainate receptors may constitute an important target for anticonvulsant drug development.

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NITRIC OXIDE SYNTHASE-POSITIVE NEURONS IN THE RAT SUPERIOR COLLICULUS: CO-LOCALIZATION OF NOS WITH GABA AND CALCIUM-BINDING PROTEINS

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The interneuronal messenger nitric oxide (NO) is involved in multiple biological processes in the brain, including synaptic plasticity, neuronal development, and glutamate excitotoxicity. NO is synthesized by nitric oxide synthases (NOS). In this study we analyzed the distribution and morphology of nitric NOS-containing neurons in the rat superior colliculus (SC) using NADPH-diaphorase histochemistry and NOS immunohistochemistry. In order to examine whether putative nitric oxide synthesizing neurons represent a different subpopulation of cells in the SC we also studied co-localization of NOS with the neurotransmiter GABA, the calcium-binding proteins parvalbumin, calbinding and calretinin and with neuropeptides such as somatostatin, substance P and neuropeptide Y. We found that most of the cells in the superficial layers of the rat SC expressing NOS are also positive for GABA. In addition, a few NOS positive cells express parvalbumin. However, we have not found co-localization of NOS with calbindin, somatostatin, substance P or neuropeptide Y. The results of this study show that the capacity for synthesizing NO in the rat SC is linked to a subset of neurons defined by their ability to synthesize GABA and the presence of the calcium binding protein parvalbumin.

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LOCOMOTOR ACTIVITY IS REDUCED IN NEUROPEPTIDE Y TRANSGENIC RATS.

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Neuropeptide Y (NPY) affects energy balance by increasing appetite correlated with an increased body weight. The present study determined the effect of chronic NPY overexpression on energy expenditure by measuring locomotor activity in NPY transgenic male and female rats. NPY transgenic Sprague Dawley rats were developed by pronuclear injection of fertilized oocytes with a 14 kbp clone of the rat structural NPY gene. Incorporation of additional (five) copies of the NPY gene resulted in an increased NPY concentrations in a number of tissues, including neuronal. Experiments were performed on six to seven months old NPY-transgenic hemizygotes (n=9-10, line No 400). Non-transgenic litter mates were used as controls. Rats were placed in standard wire cages with constant access to running wheels (1.15 m circumference), food, and water. Running-wheel activity was recorded for 11 days. NPY transgenic male rats ran significantly less than their non-transgenic litter mates (372.7± 69.2 vs. 892.2 ±228.3 meters per day, ($p<0.01$). Females had a higher running activity than males and in the NPY transgenic females the attenuation of running activity was similar to that in NPY^+ males (4185±522 vs. 2502±414 m/day, $p<0.01$). These data suggest that NPY signaling inhibits energy expenditure in rats. The NPY transgenic rats may provide a new animal model for the study of energy metabolism including development of NPY receptor antagonists. (Supported by AHA and NIH grants).

LEVELS OF AMINO ACID NEUROTRANSMITTERS DURING MOUSE CEREBELLAR NEUROGENESIS AND IN HISTOTYPIC CEREBELLAR CULTURES

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The variation in the levels of Glu, Asp, Gly, Tau and GABA neurotransmitters were analyzed by HPLC during mouse cerebellar cortex neurogenesis, from embryonic E15 until young adult stage. Our data confirmed that Glu and GABA are the dominant excitatory and inhibitory transmitters in the cerebellum. In the early cerebellar neurogenesis, between E15 and E21, high contents of GABA, Glu and Asp were detected, with the GABA levels about three-fold higher than those of Glu and Asp. After birth, the levels of GABA remained high during the first two postnatal weeks and then reached plateau to adult values by the third week. The levels of Glu and Asp increased gradually from birth to young adult stage, showing peak values at postnatal P3, P11 and in young adult stage. Gly and Tau were present at relatively low concentrations during the prenatal period, then rose significantly by about four-fold at P1, and decreased thereafter to moderate values by the end of the first postnatal week. Their levels incremented gradually during the second postnatal week until reaching adult values by the third week. To determine the endogenous neurotransmitter production, we analyzed their contents in primary histotypic cerebellar cultures prepared at P10 and compared the in vitro levels with those obtained in young adult animals. The comparative analysis showed that Glu and Asp both play an equally important role in the excitatory neurotransmission of the cerebellar cortex internal circuitry pathways. After 6 days in vitro, the cultures showed the same levels of Glu and Asp but their concentrations were half-fold lower than their in vivo contents, suggesting that granule cells use Glu as well as Asp as transmitter and that in mature cerebellar cortex, about 50% of the excitatory synaptic inputs come from afferent fibers. These results indicate that the climbing and mossy fibers likewise utilize Glu and Asp as transmitters in about equal proportion. In these cultures, the levels of GABA were three-fold higher than those of Gly and about six-fold higher than those of Tau. The in vitro GABA and Gly contents were homologable with the in vivo levels, whereas the Tau concentrations were about five-fold lower than the in vivo values. These findings indicate that most of the GABA and Gly are produced intrinsically while a high proportion of Tau in the cerebellum come from extra cerebellar afferents.

DELAYED TRANSPLANTATION OF HUMAN AMNIONTIC EPITHELIAL CELLS AFTER VASCULAR OCCLUSION IN BONNET MONKEY(*Macaca radiata*).

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Cerebro-Vascular Disorders (CVD) are difficult to treat and often the recovery from such disorders is incomplete. Various cell implantation techniques, anti-oxidant therapies and other pharmacological interventions were tried in vain in the past to alleviate the complications of such disorders. Recently, the Human Amniotic Epithelial Cells (HAEC) have been found to have the multi-potency to differentiate into various types neurons and glia. Intra-cerebral implantation of HAEC seems to be a promising tool for treating the cerebral disorders. Bonnet monkeys were used as non-human primate model. Left middle cerebral artery was occluded through retro orbital approach to simulate CVD. In the experimental group, HAEC were implanted by injecting a suspension of cells one week after occluding the artery. The results were analysed by behavioural, physiological and histological parameters. Both the control and experimental group animals exhibited behavioural changes typical to any cerebro vascular disorders. The functional deficits were restricted to contra lateral limbs. The deficits were more marked and long lasting in fore limbs when compared with hind limbs. After an initial deficit period, gradual recovery occur in both the groups. The recovery was complete by the end of third month, after which the functional deficit was hardly noticeable. Histologically, the transplanted cells survive in the implanted site, however anatomical integration of the cells with the host was variable. The present study reveals that HAEC when implanted intra-cerebrally can survive for long periods. Functional implications of such surviving cells needs to be analysed as both control and transplantation group showed recovery.

POLARIZED DISTRIBUTION OF ACETYLCHOLINESTERASE mRNA IN EMBRYONIC MODEL SYSTEMS

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The newly established morphogenic roles of acetylcholinesterase (AChE) have been confirmed in multiple *in vitro* and animal models. At the clinical level, AChE accumulation in the amniotic fluid is notabley associated with defects in neural tube closure. However, the mechanism(s) ensuring that AChE would be at the right place, time and amout for performing its morphogenic functions are yet obscure. Also, it is yet unknown whether AChE secretion in neural tube defects reflects a causal involvement with this developmental malfunction. To explore these issues, we examined 3 AChE producing developing systems. These include proliferating epithelium in the ventricular zone of the neural tube and in developing somites of mouse embryos as well as embryoid bodies derived from aggregates of mouse embryonic stem cells. High resolution *in situ* hybridization using AChEcRNA probes selective for the 3' variant AChEmRNA transcripts was followed by confocal microscopy quantification. In neuroepithelial and somitic cells from FVB/N embryos at day 9.5, which matches the time of neural tube closure, we observed polarized subcellular localization of both of the 3'-alternative transcripts. Thus, AChE mRNAs accumulate in those regions of the cell that face open space, either external or internal and are less abundant in regions of intercellular contact. Unilateral accumulation of AChE mRNA close to exposed areas of the cell surface was also found in epithelial-like cells from embryoid bodies, which display cellular differentiation and organization. Compartmentalization of AChE mRNA can play an important role in directing these mRNA chains towards specific sites of protein synthesis and thereby effecting the consequent localization of their membrane-bound and/or secreted protein products. Subcellular management of AChE gene expression may further explain the excessive amounts of AChE in the amniotic fluid of embryos with open neural tube birth defects.

MICE HETEROZYGOUS FOR A CONNEXIN43 NULL MUTATION EXHIBIT INCREASED INFARCT SIZE AFTER STROKE

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Glial-neuronal interactions have been implicated in both normal information processing and neuroprotection. One pathway of cellular interactions involves gap junctional intercellular communication (GJIC). In astrocytes, gap junctions are composed primarily of the channel protein, connexin43 (Cx43), and provide a substrate for formation of a functional syncytium implicated in the spatial buffering capacity of astrocytes. One approach to study the function of Cx43 in brain involves targeted gene knockout through homologous recombination. Astrocytes cultured from homozygous null mice (Cx43-/-) exhibit some differences compared to wild type astrocytes (Cx43+/+), including impaired GJIC and attenuation of intercellular Ca^{++} signaling. Although homozygous null mice die at birth, heterozygotes survive and have reduced Cx43 expression. To assess the effect of reduced GJIC on neuroprotection, we examined wildtype and heterozygote mice with respect to the response to middle cerebral artery occlusion (MCAO). Mice were anaesthetised, the temporalis muscle retracted, a small burr hole made to expose the overlying dura mater, which was retracted to expose the MCA for coagulation. Four days after surgery, mice were euthanized, the brains sectioned and infarct size determined. We observed a significant ($p < 0.002$) increase in the infarct size in Cx43 heterozygous null (14.4 ± 1.4 mm^3) versus wildtype mice (7.7 ± 0.82 mm^3). These results suggest that augmentation of GJIC in astrocytes may improve neuroprotection following ischemic injury. Supported by the Medical Research Council of Canada (CCGN) and Heart & Stroke Foundation of Ontario (DC).

CALCIUM CHANNEL BLOCKERS ATTENUATE ATHEROSCLEROSIS AND ITS CONCEQUENCES DEVELOPMENT IN CHOLESTEROL-FED RABBITS

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In the prevention of atherosclerosis and diseases secondary caused still persist the aim at lowering the cholesterol content of the plasma lipoproteins by dietary and pharmacological means. But, this approach has only proved successful to a limited extent. Recent evidence suggest that oxidation of low density lipoproteins (LDL) may play an important role in pathogenesis of blood vessels endothelial dysfunction and atherosclerosis. Previous studies have shown that calcium channel blockers may effectively inhibit oxidation of LDL.
In our experiments we have documented differences in the electroencephalographic (EEG) activity at rabbits (group A) which developed atherosclerosis after cholesterol-rich diet (2% during 8 weeks) and at rabbits which was treated simultaneously with cholesterol- rich diet and calcium channel blocker Dyhidropyridine (Group B). This type of calcium channel blockers is agent with high central nervous system affinity.
In rabbits brain the EEG activity was registered by chronically implanted cortical (sensomotor, temporal and occipital cortex) and subcortical (hippocampus, centrum medianum thalamy) electrodes. The EEG activity in A group of rabbits was slower irregular mixed activity, diffuse expressed, while in B group the EEG activity was almost like in control animals, which were on usual nutriment. The plasma cholesterol values in rabbits in the beginning were average 2,68 mMol/l , while after 8 weeks of cholesterol-diet the plasma cholesterol was 6,7 mMol/l in both groups (A and B).
So, this study showed that calcium channel blockers can be not just useful therapeutic antihypertensive agents, but also may prevent occurring and development of atherosclerosis and the consequent ischemia , without affecting the high level of plasma lipids. According to many literature data this can be provide by the antioxidative, antiatherogenic effects, and also neuroprotective effects of calcium channel blockers.

INTERACTION OF SERUM LEPTIN LEVELS TO HYPOTHALAMO-HYPOPHYSEAL-THYROID AXIS IN PATIENTS WITH ANOREXIA NERVOSA

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In order to understand the interactions between the circulating levels of leptin and hypothalamo-pituitary-thyroid axis (HPT) in severe malnutrition, in the present work 15 patients with anorexia nervosa (AN) were studied before and 2 months after partial weight recovery, and were compared with 10 normal women as controls. Leptin, T4, T3, rT3, IGF-1, neuropeptid Y (NPY), serotonin in the serum and melatonin-sulfat in the urine were measured by commercial radioimmunoassays. The mean serum leptin levels were 9.4±2.8 µg/l in control women with BMI of 20.2±9.7, higher than that in the AN patients at diagnosis (3.7±1.6µg/l, BMI 15.5±2.9). The differences were also observed in T4, T3, and rT3 values that were 136±5.8 µg/l, 2.4±0.1 µg/l, and 0.3±0.09µg/l resp. in controls and 86.3±1.5µg/l, 1.7±0.6µg/l, and 0.15µg/l resp. in AN. Serum values of IGF-1 and serotonin and urine content of melatonin-sulfat were lower in AN than in controls (210±55.7µg/l vs 447±97µg/l, 194±62.5µ/l vs 482µg/l and 7.3±3.4µg/l vs 16.1±7.6µg/l resp). No differences were observed in NPY. The partial recovery in weight of AN led to a an enhancement in leptin (4.8±2.1µg/l, P=0.01 vs before treatment), and to the decrease of serum concentration of T4 and rT3. IGF-1 was increased after partial weight recovery (265±76.3µg/l vs before treatment). Serum levels of serotonin and urine contents of melatonin remained decreased after treatment. Conclussions. We did not found correlation between serum levels of leptin and serum T4. However, the ratio of T3/T4 was increased, and the ratio of rT3/T4 on the contrary decreased after partial weight recovery. The low serum levels of T3 associated with chronic starvation were though to be result of impaired peripheral conversion of T4 to T3. However, we are observed besides lower T3 also the low serum levels of T4 and rT3 in AN, and even after the partial weight recovery in the compare with control group. On the basis these results we assumed that low serum levels of thyroid hormones reflect rather a dysfunction of the HPT axis in AN patients It is known that in man serum serotonin levels correlate positively with T3 levels. It is possible that the low serum levels of thyroid hormones in AN patients result in low serum serotonin and its product melatonin. While IGF-1 reflects the energy intake of the previous few weeks, the serum leptin concentration reflect the true status of the adipose stores, a fact that has useful clinical implications. Our results indicate that all folowed parameters could be one of many factors involved in the pathogenesis of AN. Supported by IGA MH CR No. 4204-3.

HAPTOGLOBIN IN CSF AS A MARKER OF CNS INFLAMMATORY PROCCESS.

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Haptoglobin is a transport protein and protect organism against iron lost and it should be involved in CNS inflammatory process. Simultaneous serum and CSF samples were obtained of 38 pediatric patients; 14 suffering from viral meningoencephalitis and 24 from bacterial meningoencephalitis. Five control cases were examined too. Haptoglobin, IgA,IgM, IgG and albumin were quantified in both biological fluids by immunodiffusion. Haptoglobin/serum ratio and haptoglobin and haptoglobin/IgG index were calculated. Local immunoglobulin synthesis were determined according to Reiber criteria. Mean viral meningoencephalitis haptoglobin index was higher but not statistically significant in comparisoon with bacterial disease but both was statistically significant in comparison to control group.Increased haptoglobulin/IgG index levels were statistically significant in bacterial meningoencephalitis in comparison with viral meningoencephalitis.. There were not association between haptoglobin and polimorphonuclear cells count and globular sediment volume. Local IgM synthesis had predominance over the other ones in viral meningoencephalitits while local IgG was preffered in bacterial CNS disease.In control group no local synthesis was found. Haptoglobin should be considered a relevant marker of CNS inflammatory proccess.

HYPEROSMOLARITY AFFECTS THE GATING OF L-TYPE CALCIUM CHANNELS IN PITUITARY CELLS: A STUDY AT THE SINGLE CHANNEL LEVEL.

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In a previous study we have demonstrated that increase in extracellular osmolarity causes suppression of L-type calcium currents in pituitary cells (Matzner, Ben-Tabou and Nussinovitch 1996, J. Neurophysiol. 75, 1894-1900). In this study we examined whether this hyperosmotic suppression of whole-cell calcium currents results from changes in the gating of L-type calcium channels. Experiments were performed on enriched population of pituitary somatotrophs and the activity of single L-type calcium channels was monitored with the patch-clamp technique. Our results show that exposure of pituitary cells to hyperosmotic media 'in the cell attached mode' reduced the open probability of L-type calcium channels inside the patch pipette (indirect exposure). Similarly, exposure of cell-detached membrane patches to hyperosmotic media 'in the outside-out mode' resulted with decrease in the open probability of L-type calcium channels (direct exposure). These hyperosmotic effects on the gating of L-type calcium channels were not associated with changes in single channel conductance. Thus it is reasonable to conclude that the hyperosmotic induced suppression of whole-cell L-type calcium currents in pituitary cells stems from a hyperosmotic induced reduction in the open probability of single L-type calcium channels. A similar mechanism may underlie the hyperosmotic suppression of calcium currents observed in other cell types such as neurons, smooth muscle cells and cardiac cells.

MODEL FOR NEURAL MECHANISMS OF TEMPORAL-DURATION CODING

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Despite that capability of human and animals to process temporal duration seems obvious as demonstrated by cognitive and behavioural studies, little is known about underlying neural mechanisms. We theoretically discussed possible neural mechanisms for temporal-duration coding. Our idea was inspired by remarkable membrane properties of a corticostriatal (CST) neuron. The membrane potential of a CST neuron fluctuates between two subthreshold states; the hyperpolarized state (down state) and the depolarized state (up state). The neuron can fire bursts of action potentials in the up state but never in the down state. Cognition and behaviour that require processing temporal duration characterised by the time scale ranging from hundreds of millisecond to seconds are likely to involve functions of the basal ganglia, a subcortical system to which CST neurons principally project. Interestingly, the period of the membrane fluctuation of a CST neuron resides within this time scale. Hence we expected that these membrane properties of a CST neuron would be involved in temporal-duration coding. We investigated a model for recurrent networks of stochastic neurons resembling CST neurons. The results obtained show that bursts of the neurons triggered by the cue signals continue for prolonged duration, followed by an abrupt self-termination. By adjusting the synaptic strength between the neurons, the duration is developed to self-terminate at the target time. This work was supported by CREST of JST(Japan Science and Technology).

OXIDATIVE STRESS AND BRAIN NITRIC OXIDE SYNTHASE ACTIVITY: EFFECTS IN VIVO AND IN VITRO.

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Our previous in vivo data suggest that nitric oxide synthase activity in the brain is often inversely related with the generation of active oxygen species in vivo. (Gulyaeva, Onufriev, Stepanichev, 1994-1998). The aim of the present study was to compare in vivo and in vitro effects of oxidative stress on brain constitutive nitric oxide synthase activity. Eight days after 10-min cardiac arrest the accumulation of material reacting with 2-thiobarbituric acid was revealed in the hippocampus (by 74%) and the cerebellum (by 47%) of male Wistar rats. The oxidative stress in brain tissue was accompanied by a twofold decrease in the nitric oxide synthase activity. In the cerebral cortex, neither 2-thiobarbituric acid-reactive material accumulation, nor nitric oxide synthase changes were revealed. In vitro experiments showed the dose-dependent decrease of nitric oxide synthase activity in brain homogenates as a result of oxidative stress induced by hydrogen peroxide or sodium hypochlorite. The data suggest that oxidative stress may decrease nitric oxide synthase activity as a result of the direct effects of active oxygen species on the enzyme.
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HIGH MOLECULAR WEIGHT DNA FRAGMENTS IN CEREBELLAR GRANULE CELLS UNDERGOING GLUTAMATE-INDUCED CELL DEATH

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The excitatory amino acid glutamate induces cell death when released during ischemia in the brain in vivo or when added to cultured neurons in vitro. In cerebellar granule cells a rapid necrotic death has been observed during and immediately after glutamate exposure, followed by a delayed apoptotic type of neuronal death in a subpopulation of the surviving neurons. Necrosis is a passive process characterised by cell and organelle swelling, with leakage of intracellular contents into the extracellular milieu. Apoptosis is typified by cell shrinkage, membrane blebbing, release of apoptotic bodies, nuclear condensation and DNA fragmentation. We present a sensitive and non-radioactive method for labelling and detection of high molecular weight DNA fragments (50-700 kbp) characteristic of apoptosis. The method is based on introduction of thymine dimers into DNA separated by pulse field gel electrophoresis, and detection with thymine dimer specific antibodies conjugated to the reporter enzyme alkaline phosphatase. We have detected DNA fragments characteristic of apoptosis as early as 4 hours after glutamate exposure (0.1 mM, 15 minutes) in cultured cerebellar granule cells. The NMDA receptor antagonist MK-801 (1 microM) was able to protect against cell death and fragmentation, whereas the nonNMDA receptor antagonist CNQX (1 microM) offered no protection against fragmentation despite a partial (25-30 %) protection against cell death.
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FUNCTIONAL DISSECTION OF THE HUMAN HOMOLOG OF THE AXONAL GLYCOPROTEIN TAG-1 IN THE PROMOTION OF NEURITE OUTGROWTH

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The glycoprotein TAG-1 (named TAX-1 in the human) is a cell adhesion molecule (CAM) belonging to the immunoglobulin (Ig) superfamily. TAG-1/TAX1 is expressed in many different neuronal populations in the central nervous system (CNS) during development and postnatal growth and can be considered as a good indicator of axonogenesis. It is expressed at the earliest stages of axon outgrowth. In vivo studies have implicated the molecule in the guidance of commissural axons to the floor plate in embryonic spinal cord and in the differentiation of granule cell precursors in the postnatal cerebellum. In vitro, TAG-1 promotes adhesion and neurite outgrowth via homophilic and heterophilic mechanisms, respectively. It has been shown previously that the fibronectin (FNIII) domains of TAX-1 are necessary and sufficient for the homophilic interaction of the molecule.
To investigate further the binding properties of the TAX-1 domains, as well as the interaction of TAX-1 with its ligand L1, we prepared whole TAX-1 protein as well as its domains as Fc chimeric molecules and coupled them to fluorospheres. These were used for binding studies on sensory neurons or CHO stable lines expressing different domains of TAX-1. The intact molecule as well as the deletion lacking the first four Ig domains display good binding in contrast to the Ig domains alone.
Further functional dissection of TAX-1 includes the investigation of the roleof different domains in promoting neurite outgrowth. Towards this goal CHO cell lines, stably expressing different portions of the TAX-1 molecule, are used as substrates for sensory neurons. Our data so far suggest that the intact molecule as well as the Ig domains of TAX-1 promote neurite outgrowth, whereas the FNIII portion does so to a much lesser extent. These data indicate that TAX-1 homophilic adhesion is not a prerequisite for axonal growth and that there is a functional seggregation between the Ig and the FNIII domains.
This work has been supported by the Greek Secretariat for Research and Technology (PENED) by the Greek-French collaborative grant PLATON and by the EU BIOTECHNOLOGY grant BIO4-CT98-0329.

ACTIVATION OF NICOTINIC RECEPTORS INDUCED LHRH RELEASE IN BULLFROG SYMPATHETIC GANGLIA VIA A Na+ -DEPENDENT MECHANISM

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Nicotine at very low doses (5 - 30 nM) induced large amounts of leuteinizing hormone-releasing hormone (LHRH) release, which was monitored as slow membrane depolarizations in the ganglionic neurons of bullfrog sympathetic ganglia. A nicotinic antagonist, d-tubocurarine chloride (dTC) completely and reversibly blocked the nicotine-induced LHRH release but it did not block the nerve-firing evoked LHRH release. Thus, nicotine activated nicotinic acetylcholine receptors (nAChRs) and produced LHRH release via a mechanism that is different from the mechanism for evoked release. Moreover, this release was not caused by Ca2+ influx through either the nicotinic receptors or the voltage-gated Ca2+ channels because the release was increased moderately when the extracellular solution was changed into a Ca2+-free solution that also contained Mg2+ (4 mM) and Cd2+ (200 micro-M). The release did not depend on Ca2+ release from the intraterminal Ca2+ stores either, because fura-2 fluorimetry showed extremely low Ca2+ elevation (~30 nM) in response to nicotine (30 nM). Moreover, nicotine evoked LHRH release when [Ca2+] elevation in the terminals was prevented by loading the terminals with 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and fura-2. Instead, the nicotine-induced release required extracellular Na+ because substitution of extracellular NaCl with N-methyl, D-glucamine chloride (NMDGCl) completely blocked the release. The Na+-dependent mechanism was not via Na+ influx through the voltage-gated Na+ channels because the release was not affected by tetrodotoxin (TTX) (1-50 uM) plus Cd2+ (200 uM). Thus, nicotine at very low concentrations induced LHRH release via a Na+-dependent Ca2+-independent mechanism. Supported by NIH (NS32429).

INCREASED STRESS-RELATED AChEmRNA LEVELS CORRELATE WITH ASTROCYTOMA PATHOLOGICAL STAGES AND POST IRRADIATION RESPONSE

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In spite of the high prevalence and grave prognosis associated with glioblastoma tumors.little is known about the proteins modulating their robust proliferation and metastatic capacity or on the molecular mechanism(s) involved in the multileveled adverse post-trearment reactions in glioblastoma patients. The acetylcholine hydrolyzing enzyme acetylcholinesterase(AChE) has long been known to be over expressed in glioblastomas. The recent establishment of an alternatively spliced rare AChE variant as a stress-responsive protein with morphogenic and proliferative capacities in cultured glioblastoma cells prompted us to re-examine the possibility that this enigmatic protein is actively involved in tumor development and/or in the post-treatment stressful responses. Using high resolution *in-situ* hybridization to paraffin embeded human tumor sections, we found intensive overexpression of the stress-associated AChEmRNA transcript in glioblastoma multiforme tumors and particularly in their highly proliferative margins, as compared to the benign brain tissue surrounding these surgical removed tumors. Moreover, AChE expression levels increased considerably in post-surgery brain sections, especially following irradiation. Pathologically-classified tumor specimens further revealed pronounced increases in the extent and incidence of gliblastoma AChE overproduction as related to tumor grading. These findings add to our understanding of the molecular biology of glioblastoma tumors and can lead to novel, less harmfull treatment paradigms.

ON THE ORIGIN OF THE DIFFERENCE BETWEEN THE EFFECTS OF EXTERNAL Na^+ REPLACEMENT WITH Li^+ OR N-METHYL-D-GLUCAMINE ON NEURONAL Ca^{2+} HOMEOSTASIS

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In experiments with Fura-2 loaded cultured rat cerebellar granule cells it has been revealed that both the increase in baseline $[Ca^{2+}]_i$ and the delay in $[Ca^{2+}]_i$ recovery following a glutamate (Glu; 100μM) pulse caused by replacement of external Na^+ with the organic cation N-methyl-D-glucamine (NMDG) can be effectively abolished by NMDA receptor agonists. This led us to suggest that the perturbations of $[Ca^{2+}]_i$ homeostasis resulted not from the reversed Na^+/Ca^{2+} exchange, but mainly from the Ca^{2+} influx through NMDA channels activated by the Na^+ dependent release of endogenous excitatory amino acids ("reversed Glu uptake"). In contrast to Na^+/NMDG replacement the substitution of external Na^+ with Li^+ did not usually induce any increase in baseline $[Ca^{2+}]_i$ and caused only a very small delay in $[Ca^{2+}]_i$ recovery. Moreover, addition of Li^+ (20 mM) to a NMDG-containing Na^+-free solution strongly decreased both the pertubation of $[Ca^{2+}]_i$ homeostasis and delayed neuronal death produced by Na^+/NMDG substitution. Finally we established that Li^+ can suppress the $[Ca^{2+}]_i$ response produced by PDC (200 μM) known by its ability to enhance reversed Glu uptake. In contrast to Na^+/Li^+ replacement, the Na^+/NMDG replacement enhanced the $[Ca^{2+}]_i$ increase caused by PDC. Control experiments showed that Na^+/Li^+ substitution does not decrease the ability of Glu to increase $[Ca^{2+}]_i$. We concluded therefore that the considerable difference between the effects of Na^+/NMDG and Na^+/Li^+ replacements on both $[Ca^{2+}]_i$ homeostasis and cell viability resulted mainly from the ability of Li^+ to attenuate the release of Glu in response to a removal of external Na^+. Supported by RFBR.

DENDRITIC AND SOMATIC MUSCARINIC MODULATION OF EXCITABILITY AND Ca^{2+}-SIGNALS IN CA1 NEURONS IN RAT HIPPOCAMPAL SLICE.

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The cholinergic system is critically involved in synaptic models of learning and memory by enhancing dendritic $[Ca^{2+}]_i$-increases. Using sharp microelectrode recordings from CA1 somata or apical dendrites and ratiometric Ca^{2+}-imaging we investigated muscarinic effects on $\Delta[Ca^{2+}]_i$ and on membrane potential responses to repetitive stimulation of Schaffer collaterals (SC). Suprathreshold stimulation of SC evoked a widespread dendritic Ca^{2+}-response with a concentration peak at ~70μm from the soma and a gradual decrease towards the distal sites. We show that focal application of the cholinergic agonist carbachol (CCh) to distal apical dendrites (~350 μm from the soma) enhances $[Ca^{2+}]_i$ -increases ($\Delta[Ca^{2+}]_i$) evoked by train stimulation (SC, 1s, 50Hz) only after diffusion to the proximal dendrites and soma. The time course and magnitude of the $\Delta[Ca^{2+}]_i$-potentiation, muscarinic suppression of the somatic slow afterhyperpolarization (sAHP) following synaptic train stimulation and augmentation of the slow depolarization during it were correlated with the CCh concentration, as measured with an ion-selective microelectrode at the base of the apical dendrite. Direct application of CCh to this area potentiated $\Delta[Ca^{2+}]_i$ and blocked the sAHP within 1s, rapidly followed by potentiation of the slow depolarization during repetitive synaptic input. Dendritic but not somatic sAHP was blocked within 1s after focal application of CCh to the dendritic recording site, followed by potentiation of the dendritic slow depolarization during repetitive synaptic input. Synaptically evoked trains of action potentials (APs) showed little decay of amplitudes at proximal sites. This decay became gradually stronger with distance from the soma (up to 50% at 280μm). At these sites, decay of AP amplitudes was enhanced. Enhancement of $\Delta[Ca^{2+}]_i$ was highly correlated with augmentation of the slow depolarization and slowing of action potentials. With direct depolarization, the number of dendritically evoked action potentials was increased but action potential amplitude was not modulated by CCh. Blockade of NMDA-receptors by bath application of APV (30μM) suppressed the slow depolarization during repetitive synaptic input by 70 to 90 %. The muscarinic modulation of this depolarization was strongly reduced in absolute, but not in relative augmentation. In conclusion, muscarinic activation locally affects sAHP and NMDA receptor-mediated slow depolarization while augmentation of $[Ca^{2+}]_i$-responses depends on local together with proximal muscarinic modulation.

MOLECULAR NEIGHBOURHOOD OF BASP1 PROTEIN IN SYNAPTIC MEMBRANE.

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BASP1 (CAP-23, NAP-22) is an abundant in presynaptic area myristoylated protein. Recently (Plekhanov et al., J.Neurochem., 1998, v.71, Suppl., S71C) we have revealed that BASP1 is present in synaptic membrane as a part of a native lipoprotein complex stable in alkali (pH 12). The lipid composition of this complex is different from that of synaptosomal membrane and is mainly constituted by triglycerides and cholesterol esters. Besides BASP1, the main protein components of this complex (as revealed by 12% PAGE according to Laemmli) are 75, 70, 45, 40 and 30 kDa proteins. Obviously, these proteins are the nearest BASP1 neighbours in synaptic membrane and therefore they could act jointly with BASP1 during synaptic transduction events. The identification of these proteins could indicate the precise biochemical function of BASP1 in nerve processes.

Other abundant presynaptic protein GAP-43 (B-50, neuromodulin) is absent from this complex and therefore the functions of GAP-43 and BASP1 are apparently not strictly cooperative, which corresponds to our conclusion based on immunohistochemical data (Polenova et al., J.Neurochem., 1998, v.71., Suppl., S71D).

PHARMACOLOGICAL CHARACTERIZATION OF CI-1030 (7-[[4-(4-CHLOROPHENYL)-1-PIPERAZINYL]METHYL]-2H-1,4-BENZOXAAZIN-3-3(4H)-ONE, A POTENT, SELECTIVE DOPAMINE (DA) D_4 RECEPTOR ANTAGONIST.

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The localization of DA D4 receptors as well as clozapine's DA D4 preference suggests that antagonists selective for D4 receptors may be efficacious as antipsychotics without unwanted side effects. Several D4 receptor antagonists have been discovered and have been shown to be relatively inactive in preclinical tests of antipsychotic efficacy. The novel agent CI-1030 possesses a greater than 100-fold selectivity for human (h) DA D4.2 receptors as compared to hDA D2L, hD3 and hD1 receptors and a number of other sites. In vitro, CI-1030 showed D4 receptor antagonist activity, reversing the quinpirole stimulation of [^{3}H]thymidine at hD4.2 receptors without exhibiting any significant intrinsic activity in this assay. Although standard antipsychotics increase DA metabolism in striatum or plasma prolactin levels in rodents, CI-1030 had no effect in these assays at behaviorally active doses. A significant increase in catecholamine synthesis was observed in rat and mouse hippocampus but not in hippocampus of DA D4 knockout mice. These findings indicate that D4 receptors may play a role in modulating catecholamine synthesis in brain regions expressing D4 receptors thought to be related to psychotic symptoms. CI-1030 reduced both spontaneous and amphetamine-stimulated locomotor activity in rodents. CI-1030 reversed the apomorphine-induced disruption of prepulse inhibition of acoustic startle in rats after both acute and subacute dosing. These tests are predictive of antipsychotic efficacy and indicate that CI-1030 possesses a superior profile as compared to known DA D4 receptor antagonists. CI-1030 was not active in a conditioned avoidance test in monkeys and lacked significant cataleptogenic and extrapyramidal side effects (EPS) in rats and monkeys, respectively. The latter actions together with the lack of effect on serum prolactin and DA metabolism argue against CI-1030 having significant DA D2 receptor antagonism in vivo. CI-1030 did not exert any noradrenergic and serotonergic actions either in vitro or in vivo. The compound had good oral bioavailability (F=63%) and half life (12 hr) and good penetration into the CNS (brain to plasma ratio 11.5) in rats. Thus, CI-1030 shows some, but not all, of the preclinical effects of known antipsychotic compounds. Supported by Warner-Lambert Co.

SEROTONIN MODULATES THE ACTION OF PIGMENT DISPERSING FACTOR ON A STRUCTURAL CIRCADIAN RHYTHM IN THE FLY'S VISUAL SYSTEM

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In the first optic neuropile (lamina) of the housefly (*Musca domestica*) first-order interneurons, monopolar cells L1 and L2, show daily changes in axon cross-sectional area. They swell during the day and shrink by night. For L2, this rhythm is also circadian. Although the function of such rhythmic size changes is not known, we are attempting to elucidate their mechanism. When injected into the optic lobe various neurotransmitters have previously been shown to change axon size in L1 & L2. Injected during the day, for example, serotonin (5-HT) and pigment dispersing factor (PDF) both increase, whereas glutamate decreases, axon size in both cells. Because L1 & L2 shrink during the night, PDF and 5-HT might possibly exert different effects on these cells' axon sizes when injected at night. To examine this we injected PDF or 5-HT into the fly's optic lobe at night, and measured axon size in L1 & L2 by planimetric methods on semithin plastic sections. We also tested whether PDF's effect on L1 & L2 is dependent on 5-HT, by injecting PDF in flies previously depleted of 5-HT by a prior injection of reserpine into the hemolymph.

PDF injected at night exerted a similar effect to that previously found after daytime injection, increasing axon size in both L1 & L2. 5-HT also increased axon size in the cells; but after night injection this occurred only in L2, whereas day injections increase L1 more than L2. After reserpinizing, PDF failed to increase axon sizes in L1 & L2, indicating that the action of PDF depends on 5-HT. When injected alone during the day, reserpine exerted an effect of its own, decreasing the sizes of both cells compared with control saline injections, presumably through the loss of 5-HT .

The results show both that 5-HT exerts different actions on axon size in L1 & L2 during the day and night, and that it modulates the action of PDF on these cells.

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INTERACTION OF INHIBITORS WITH INDIVIDUAL MOLECULAR FORMS OF CHOLINESTERASE IN NORMAL AND ALZHEIMER'S DISEASE BRAINS

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In Alzheimer's disease (AD), cholinesterase inhibitors are currently used to increase the available acetylcholine (ACh) by preventing the hydrolysis of released ACh by inhibiting the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). This therapeutic approach is based on attempts to correct the cognitive decline by manipulating the cholinergic neurotransmission. The main goal was to measure *in vitro* the inhibition of human brain AChE extracted from normal and AD brains by using pharmaceutically significant cholinesterase inhibitors. The results on AChE were compared with those on a homologous enzyme, BuChE, from normal plasma. In addition, we measured the effects of clinically significant inhibitors on the individual AChE molecular forms. Membrane-associated AChE was extracted from the normal and AD *post-mortem* brain cortex, striatum and hippocampus. Monomeric (G_1) and tetrameric (G_4) AChE molecular forms were separated by density gradient ultracentrifugation. AChE activity was determined by radiometric assay. In enzyme inhibition studies, AChE was preincubated with different inhibitors for 30 min. and the reaction was started with substrate. The IC_{50} values were calculated by linear regression of the log concentration vs. % inhibition (range 20-80% inhibition). IC_{50} values of the following inhibitors were determined: tacrine, bis-tacrine, TAK-147, metrifonate, eptastigmine and rivastigmine. Among these inhibitors, tacrine, bis-tacrine, TAK-147 and metrifonate equally well inhibited the G_1 and G_4 AChE forms. Eptastigmine and rivastigmine displayed preferential inhibition for the G_1 form in both the normal and the AD brain. These data suggest the use of eptastigmine and rivastigmine as molecular form-selective inhibitors for therapeutic application. Eptastigmine was from Mediolanum Farmaceutici (Milano, Italy), bis-tacrine was provided by Dr. Y.-P. Pang (Mayo Clinic, Jacksonville FL, USA), TAK-147 was from Takeda Chem. Ind. (Osaka, Japan) and rivastigmine was from Novartis Pharma AG (Basel, Switzerland). Supported by grants ETT T-04 584/96, 652/96 and OTKA T 22683 and T 26470.

INHIBITION OF CHOLINESTERASES - A POSSIBLE MODE OF BENZALKONIUM CHLORIDE INDUCED NEUROTOXICITY

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Benzalkonium chloride (BAC), a widely used pharaceutical preservative has been documented to be a neurotoxin. However, its mode of action on neurons is unclear. The structure of BAC resembles many cholinesterase (ChE) inhibitors and therefore we have analysed its effect on ChEs from several sources. ChEs from human erythrocyte, human plasma, horse serum and Torpedo electric organ were incubated with increasing concentrations of BAC for 10 min and then assayed for the residual activities. BAC inhibited the ChEs in a concentration dependent manner. The inhibition was found to be reversible and of the 'linear-mixed' type, with inhibition constants in the micro molar range. The order of sensitivity was, human serum BChE > horse serum BChE > Torpedo AChE > human erythrocyte AChE. Thus, the data indicate that the neurotoxic effect of BAC would be mediated through its capacity to inhibit ChEs.

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DYNAMICAL OSCILLATIONS OF NEURONAL ACTIVITY IN THE GLOBUS PALLIDUS OF TREMULOUS PARKINSONIAN MONKEYS

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To test the hypothesis that parkinsonian tremor is related to synchronized neuronal oscillations in the basal ganglia, we simultaneously recorded the activity of 2-8 single pallidal neurons of two vervet monkeys before and after systemic treatment with the dopaminergic neurotoxin - MPTP.

Following MPTP treatment the monkeys developed typical parkinsonian symptoms including akinesia and postural abnormalities. The monkeys had many prolonged episodes of 5-7 Hz tremor, and also episodes of 10-14 Hz tremor.

Before MPTP, 12% of the cells had oscillatory auto-correlograms, whereas after the treatment 41% of the cells had oscillatory activity. The oscillation frequencies of different cells were uniformly distributed before the treatment, but after the treatment they were clustered around 7 and 13 Hz.

Cross-correlations of these neurons revealed only 1% oscillatory correlograms before the treatment and 38% after the treatment. The oscillation frequencies after the treatment were clustered around 13 Hz. The phase shifts of the oscillations were clustered around 0^{o}, so that 49% of the oscillatory correlograms had phase shift less then 45^{o}. Preliminary analysis of the time course of the oscillations and phase shifts revealed complicated dynamics, and suggests no simple relation to the tremor. In spite of this, timing of oscillatory activity seems to be correlated with the timing of the tremor.

These results support the view that dopamine depletion in Parkinson's disease causes synchronized oscillatory neuronal activity in the basal ganglia, and that zero phase locking of these oscillations is correlated with the appearance of tremor in parkinsonian state.

COGNITIVE AND MOVEMENT RELATED POTENTIALS IN THE BASAL GANGLIA. A SEEG STUDY IN EPILEPTIC PATIENTS.

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Bereitschaftpotential or Readiness potential (RP), preceding the self paced voluntary movement, is generated in several cortical structures. In previous studies (Rektor et al. Electroencephal. clin. Neurophysiol. 1994, 90: 273-283 and 1998, 107:277-286, Lamarche et al. Electroencephal. clin. Neurophysiol. 1995, 95: 268-276), the RP generators were localised in the primary motor and sensory cortex contralateral to the moving limb and bilaterally in the SMA and in the anterior cingular cortex. RP, which also has been called „movement related cortical potential", is considered to be a cortical phenomenon linked to some kind of cognitive function. In this study 8 patients were implanted with intracerebral depth electrodes in order to explore intractable temporal lobe epilepsy. DIXI five to fifteen contact electrodes were used. In each patient several electrodes were implanted orthogonally in the temporal, fronto-orbital and prefrontal cortices. No contacts were placed in regions generating cortical RP. One diagonal electrode reached the amygdala and the hippocampus passing through basal ganglia. Th ne putamen was explored in 7 patients and the nucleus caudatus and the pallidum internum were studied in one patient. RP was observed in internal pallidum and caudatum and was also recorded in putamen in 6 of 7 patients. RP were displayed bilaterally, preceding the movement onset by 750- 1900 ms. The shape of RP resembled the RP shape in the cortex and on the scalp. The potentials accompanying the movement were also present in all three explored structures. They electrophysiological properties differed from RP indicating separate generators. The fact that RP are generated in basal ganglia indicates that basal ganglia participate on some covert functions related to the motor preparation and activity. The probable interpretation is that there is a parallel processing of covert activity linked with the movement in cortical and subcortical structures. This also has been supported by our further results from posterior thalamus.

ELECTRICAL RESISTIVITY AND CURRENT-COURCE-DENSITY ANALYSIS OF RAT CEREBRAL CORTEX

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Evoked field potentials are important methodological tool in neurophysiology of neuronal ensembels.For interpretation of field potentials in the cerebral cortex it is important to consider the fact that inhomogeneities and anisotropy may be present in the conducting medium. The structure of the layers in the cortex suggests that differences may exist between the electrical properties of these layers. A technique for measuring of biological tissue was elaborated and used. Precision (about 0.05% in the tissue volume of 0.03 cube mm) of measuring was tested at special artificial models. Resistivity of cortex decrease to 7% when frequency of electrical current increase to 1 kHz and reach value 15% at 5 kHz. Potential dependence of resistivity during single evoked potential is not detected, although its alterations about 0.5 - 4.5% accompany prolonged afferent stimulation. Anisotropy of cortex is not detect as field of artificial pointy source into the cortex is spherical. Inhomogeneity is a function of cortex thickness. Discovering layering inhomogeneity has specific electrical resistance about 450-1000 Ohm cm and distribute into the cortex not regular. The maximum resistivity was found in the area of IV and VI layers, minimum - I-III and V layers. So arrangement and thickness of resistance layers did not correlate with citoarchitectonical layers. These results were used to carry out a one-dimensional CSD analysis of field potentials evoked by skin afferent stimulation of leg. Despite the differences in resistivity, the homogeneous and the inhomogeneous CSD approximations did not lead to differences in the spatial distribution of sources and sinks and only gave some differences in the current density at the second phase of EP.

TRANSGENIC HUMAN ACHE VARIANTS DISPLAY C-TERMINUS DEPENDENT INHIBITOR SENSITIVITIES

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The use of acetylcholinesterase inhibitors (anti AChEs) as therapeutic or prophylactic drugs or agricultural insecticides calls for comparing inhibitor sensitivities of the 3 C-terminally distinct mammalian AChE variants produced through alternative splicing from the single ACHE gene. Here, we report such comparison for 4 enzymes: (1) soluble, secretory "readthrough" AChE-R monomers accumulated in the milk or muscle of transgenic mice; (2) recombinant "synaptic" AChE-S produced in cultured human 293 cells; (3) "erythrocytic" AChE-E purified from human red blood cells; and (4) "truncated" AChE-E4 produced in transgenic mice devoid of the C-terminal peptides S, E or R. All of these C-terminally distinct human AChE isoforms displayed similar inhibition curves and IC_{50} values to pyridostigmine, tetrahydroamino acridinium (Cognex™, Tacrine), and Aricept (Donepezil™). However, synaptic AChE-S was 5 and 10-fold more sensitive to the peripheral inhibitor propidium than the AChE-R and AChE-E isoforms, respectively. In contrast, the stress-associated AChE-R isoform was considerably more sensitive than AChE-S and AChE-E to the organophosphate inhibitors DFP and paraoxon and the carbamate inhibitor Rivastigmine (Exelon™). Parallel inhibition profiles were obtained for AChE-R and AChE-E_4, demonstrating that the E_6- and E_5-derived C-terminal peptides were responsible for the binding differences. These findings explain reports of excessive sensitivity of globular G_1 AChE soluble monomers to Exelon and suggest effective dose differences for specific anti-AChEs under conditions where the relative fractions of AChE-R are modified.

PERSONALITY, VULNERABILITY TO CEREBRAL ISCHEMIA AND NEUROPROTECTIVE EFFECTS OF SUBSTANCE P

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Vulnerability to cerebral ischemia induced by occlusion of both common carotid arteries and neuroprotective effects of the neuropeptide substance P_{1-11} were studied in rats with different behavior types (active, middle, passive) selected from the population according to behavioral differences in the open field and forced swimming tests. It has been found that there are individual differences in vulnerability to cerebral ischemia depending on type of animals' behavior. The least vulnerability to cerebral ischemia (the least mortality, neurological deficit, ischemic brain damage, behavioral and memory disturbances) was in individuals with active type of behavior. Vulnerability in individuals of the middle and passive types of behavior was the most and middle respectively. Single i.p. injection of substance P (250 μg/kg) protected the brain cells ultrastructure against ischemic damage and counteracted pathophysiological consequences of cerebral ischemia (reduced mortality, neurological deficit, behavioral and memory disturbances). The efficacy of neuroprotective effects of substance P was dependent on behavior type (on the degree of ischemic brain injury). The most efficient substance P was in rats with passive type of behavior (middle-damaged individuals). Neuroprotective effect of substance P was associated with the counteraction of ischemia-induced changes in the brain lipid peroxidation processes and their interhemispheric asymmetry. The results suggest that personality is of great importance in vulnerability to cerebral ischemia and in the benefit of neuroprotective approach and should be a factor for consideration in experimental research and clinical application.

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AGE-RELATED CHANGES IN THE MODULATORY ACTION OF CARBON MONOXIDE AND FREE RADICALS ON CEREBELLAR Na,K-ATPase ACTIVITY.

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Recent studies of cerebellum and hippocampus have shown that Na+ pump activity can be regulated by cGMP and PKG through activation of soluble guanylyl ciclase (GC) by carbon monoxide (CO) and R. The aim of this study was to verify if cerebellum Na+ pump isozyme activity is modified by aging and if the regulation of this enzyme by CO and R is age-dependent. Cerebellum slices from 4 and 24-month-old Wistar rats were incubated for 15 min (310C) with Superoxide dismutases (SOD) (50 and 100 U), CO (100 ?M), 8-bromo-cGMP (4 mM). After drug removal, slices were permeabilized and assayed for Na,K-ATPase. The Na,K-ATPase activity increased linearly with time (15, 30 and 60 min) yielding an activity of 120-150 nmol of Pi per mg of protein per min. Aging (4 to 24 months) reduced Na,K-ATPase activity by 46,7%. 8-bromo-cGMP was able to increase the Na pump activity either in young or old tissue. However, SOD, that reduces the availability of R, and CO only modulate Na pump activity in young animals. Considering that the action of R and CO are due to stimulation of GC and the 8-bromo-cGMP acts downstream from GC, it is concluded that in aged cerebellum a reduction in GC activity occurs.

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STRETCH-ACTIVATED CALCIUM SIGNALS IN CULTURED MOUSE PRIMARY SENSORY NEURONS

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Cellular and molecular mechanisms underlying sensory transduction in primary sensory neurons remain poorly understood. Neurons in the trigeminal ganglion (TG) form a mixed population of sensory neurons, with nerve terminals that respond to physical and/or chemical stimuli. We have investigated the effects of membrane stretch on the intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) of cultured mouse (age P3-P5) TG neurons. $[Ca^{2+}]_i$ levels were monitored with fura-2-based ratiofluorometric digital imaging. Membrane stretch was accomplished with the application of extracellular hypotonic solutions.
Hypotonic cell swelling led to reversible elevations in $[Ca^{2+}]_i$ in 76% of all TG neurons tested (n=80). The amplitude of these responses varied with the degree of hypotonicity (15-45%). Responses could not be evoked by hypertonic solutions. The $[Ca^{2+}]_i$ responses were variable in amplitude and time course, allowing the classification of neurons into distinct subgroups. In 28% of the neurons $[Ca^{2+}]_i$ increases had a fast time course (mean time to peak 34 ± 11 s), while in the remaining cells, responses were much slower (mean time to peak 164 ± 57 s). The amplitude of responses in both groups were significantly different (174 ± 159 nM *vs* 56 ± 34 nM respectively). $[Ca^{2+}]_i$ elevations were completely abolished in the absence of external Ca^{2+}. Further, they were blocked by 20 μM Gd^{3+}.
We conclude that mechanical stretch increases $[Ca^{2+}]_i$ in a large subpopulation of mouse TG neurons in culture. Both, the rapid and slow $[Ca^{2+}]_i$ responses are triggered by Ca^{2+} entry and are sensitive to a blocker of stretch-activated ion channels. Currently, we are trying to determine the relation between the characteristics of stretch-evoked $[Ca^{2+}]_i$ elevations and the chemical sensitivity of individual neurons.
Study supported by a grant from Biomatrix, Inc., Ridgefield, New Jersey, USA.

A SWEET AND SOUR NEUROPROTECTIVE RECIPE AGAINST DELAYED ISCHEMIC NEURONAL DAMAGE *IN VIVO*

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Lactic acidosis has been promoted as a major detrimental factor in delayed ischemic neuronal damage, a claim supported by the fact that hyperglycemia aggravates such damage. Recently, we have demonstrated *in vitro* that anaerobically-produced lactate is an obligatory aerobic energy substrate for recovery of neuronal function posthypoxia. Moreover, contrary to the *in vivo* observations, the higher the glucose concentration prehypoxia *in vitro*, the lesser the neuronal damage observed posthypoxia. Here we tested the *in vivo* roles of lactate and glucose in cerebral ischemia. The monocarboxylate transporter inhibitor α-cyano-4-hydroxycinnamate (4-CIN) was used to inhibit brain lactate utilization *in vivo*. To determined the blood-brain barrier (BBB) permeability to 4-CIN, rats were injected (i.p.) with a solution of the inhibitor (1- 90 mg/kg, pH 7.4) or its vehicle and decapitated 30-120 min postinjection. Hippocampal slices were prepared and immediately incubated in artificial CSF containing 5 mM lactate as the sole energy substrate. Ninety min after slice preparation, the presence or absence of neuronal function (CA1-evoked population spike of ≥3 mV in amplitude) in each slice was determined. Only 57% of slices prepared from rats 60 min after 4-CIN (90 mg/kg) administration were neuronally functional compared to 90% of slices prepared from control rats. We concluded that 4-CIN crosses the BBB as enough of it was still present in tissue slices to inhibit lactate-supported neuronal function. To test the role of lactate in recovery of function postischemia, 24 h-fasted rats were injected with 4-CIN (90 mg/kg) and 60 min later were exposed to 5 min of cardiac arrest-induced transient global cerebral ischemia (TGI), produced by chest compression, followed by cardiopulmonary resuscitation. These rats exhibited a significantly greater degree of hippocampal neuronal damage than control, vehicle-injected rats as measured 7 days postischemia by electrophysiologic and histologic means. Rats made hyperglycemic (2 g/kg glucose, i.p.) 2 h prior to 7-min TGI, exhibited a significantly less delayed neuronal damage than rats made hyperglycemic 15 min prior to TGI or than control, euglycemic rats as measured 7 days postischemia. The beneficial effect of glucose, when given 2 h prior to TGI, was abolished by 4-CIN (90 mg/kg, i.p.) given 1 h later. Thus, hyperglycemia, when induced 15 min before ischemia, worsened delayed neuronal damage postischemia, while induction of hyperglycemia 2 h prior to ischemia significantly reduced such damage. The 4-CIN data agree with our *in vitro* results, i.e., aerobic utilization of lactate is crucial for postichemic rescue of neurons.
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THE ERG-LIKE K⁺ CURRENT IN RAT LACTOTROPHS

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The *ether-à-go-go*-related gene (erg)-like K^+ current in rat lactotrophs from primary culture is described and compared with that in clonal rat pituitary cells (GH_3/B_6). The class III antiarrhythmic E-4031 known to block specifically erg K^+ channels was used to isolate the erg-like current as the E-4031-sensitive current. The experiments were performed in external 150 mM K^+ solution using the patch-clamp technique in the whole-cell and perforated-patch configuration. In the presence of E-4031 a non-inactivating outward-rectifying K^+ current was observed. This E-4031-insensitive K^+ current started to activate near -70 mV and was half-activated at about -45 mV. The E-4031-sensitive, erg-like K^+ current elicited with hyperpolarizing pulses negative to -100 mV consisted of a fast and a pronounced slowly deactivating current component. The contribution of the slow component to the total current amplitude was potential dependent and varied from cell to cell. At -100 mV it ranged from 50 to 85% and at −140 mV from 21 to 45%. The potential-dependent availability curves determined with 2 s prepulses were fitted with the sum of two Boltzmann functions. The function related to the slowly deactivating component of the erg-like current was shifted by more than 40 mV to more negative membrane potentials compared to that of the fast component. In contrast with native lactotrophs studied under identical conditions the erg-like K^+ current of GH_3/B_6 cells was characterized by a predominant fast deactivating current component with similar kinetic and steady-state properties as the fast deactivating current component of native lactotrophs. Thyrotrophin-releasing hormone reduced the erg-like current in native lactotrophs via an intracellular signal cascade which seems to involve a pathway independent from protein kinase A and protein kinase C. RT-PCR from cytoplasm of single lactotrophs revealed the presence of mRNA of the rat homologue of *HERG* (r-*erg*1) as well as mRNA of the two other cloned r-*erg* cDNAs (r-*erg*2 and r-*erg*3) in different combinations. In GH_3/B_6 cells, only the transcripts of r-*erg*1 and r-*erg*2 were found.
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OSTEOGENIC AND HEMATOPOIETIC HACHE GENE EXPRESSION, AND ANTI-CHOLINESTERASE RESPONSES INVOLVE 17 KB OF UPSTREAM PROMOTER SEQUENCE

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To delineate the boundaries of promoter regions regulating tissue- and condition-specific expression patterns of human acetylcholinesterase (hAChE), we studied the hACHE upstream sequence up to 17 kb from its transcription start site. Six clusters were found of putative binding sites, 4 of osteogenic factors (e.g. 17-β-etsradiol and vitamin D receptor) and 2 of hematopoietic factors (e.g. NFκB, Stat-5 and EGR-1) and stress-responsive elements (e.g. GRE half palindromic site). To explore the function(s) of AChE in non-neuronal tissues, we tested the contribution of these sites *ex vivo* and *in vivo*. In Saos-2 osteosarcoma cells, AChE gene expression was elevated by both 17-β-estradiol and vitamin D_3. Moreover, its antisense suppression increased Saos-2 cell proliferation. *In vivo*, AChE mRNA levels rose in normally differentiating, postproliferative fetal chondrocytes but not in the osteogenically impaired chondrocytes of dwarf fetuses with thanatophoric dysplasia, suggesting a morphogenic involvement of AChE in the proliferation-differentiation balance characteristic of human osteogenesis. Co-clustered binding sites for hematopoietic and stress factors further predicted *in vivo* regulation of blood AChE production under stress conditions. Elevated blood levels of the "readthrough" AChE variant were indeed observed in psychologically-stressed as compared to non-stressed mice. In 3 out of 189 screened human patients we found a 17 Kb upstream 4 bp deletion, abolishing a binding site for the stress-related HNF3 transcription factor. One carrier displayed increased AChE steady-state levels and acute hypersensitivity to the anti-ChE, pyridostygmine, manifested as a dramatic reduction in blood AChE levels under exposure. Transgenic mice studies suggested that parallel AChE overproduction impairs AChE's transcriptional activation response to toxicological stress. Thus, searching for clusters of putative binding sites in promoter regions can indicate new roles for well characterized genes.

ADAPTATION MECHANISMS IN THE VISUAL SYSTEM TO PERIODIC STIMULI

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Purpose. To establish a comprehensive computational model of intensity adaptation mechanisms, which predicts experimental responses to both periodic and aperiodic stimuli. **Model.** We present an elaborated adaptation model (Dahari & Spitzer, 1996), that also includes prediction of responses to periodic stimuli. The model suggests that the decline in the response time course of the retinal ganglion cells is a reflection of the adaptation mechanism (Curve Shifting). The model adjusts its changing temporal properties through a change in the saturation constant in the Naka-Rushton equation. The subtraction between the receptive-field (RF) regions is performed only after the adaptation of each RF region separately. The model was tested by simulation of various temporal sinusoidal fields, which varied in DC level (1E+3-1E+6 [quanta/sec], amplitude (modulation depth of 1%-100%) and frequency (0.1-16 Hz). In addition, the gain of the response to the periodic stimuli was calculated, to predict experimental data. **Results.** The model results are in agreement with various psychophysical and physiological findings, e.g. the correspondence of the phase shift of the response with the stimulus phase, at various temporal frequencies (Hood et al., 1997). **Conclusions.** The model's results demonstrate the importance of dual distinctive adaptation channels in a model, which should predict both periodic and aperiodic adaptation dynamics. Until now, no existing model has been able to predict responses to these two types of stimuli (Hood et al., 1997).

Liposome-Promoted Unfolding of Acetylcholinesterase

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The kinetics and thermodynamics of interaction of proteins with the lipid bilayer are important for understanding their mode(s) of insertion into and translocation across biological membranes. These, in turn, may be relevant to the mechanisms of both cotranslational and posttranslational cellular traffic of proteins. Involvement of the lipid bilayer may also be envisaged in the so-called 'conformational' diseases, such as prion diseases (e.g. Creutzfeld-Jacob disease) and amyloid diseases (e.g. Alzheimer's disease). In some such diseases, at least part of the protein is correctly folded in a 'native' conformation, and the disease condition arises from subsequent conformational changes leading to aggregation and deposition of the protein.

The effect of unilamellar dimyristoylphosphatidylcholine (DMPC) liposomes was investigated on unfolding of acetylcholinesterase from electric organ tissue of *Torpedo californica* (*Tc*AChE) and from the venom of the snake, *Bungarus fasciatus* (*Bf*AChE). The former is a disulfide-linked homodimeric GPI-anchored protein, purified after solubilization with PI-specific phospholipase C; the latter is a soluble monomer. The kinetics of their thermal inactivation were studied in the absence and presence of the DMPC liposomes. Arrhenius plots revealed activation energies for thermal inactivation of 145 kcal/mol and 113 kcal/mol for *Tc*AChE and *Bf*AChE, respectively, in good agreement with the values obtained independently by differential scanning calorimetry. The liposomes lowered the energy barriers for this transition to 47 kcal/ mol and 52 kcal/mol for *Tc*AChE and *Bf*AChE, respectively. Both thermally denatured enzymes display spectroscopic characteristics typical of partially unfolded states. Enhanced thermal inactivation in the presence of the liposomes was accompanied by concomitant incorporation of partially unfolded *Tc*AChE into the lipid bilayer. Tryptic digestion permitted isolation and characterization of a hydrophobic peptide responsible for this association. It contains the most extended hydrophobic sequence in the protein, 35 amino acids. In contrast to *Tc*AChE, thermally denatured *Bf*AChE does not remain bound to the liposomes; it is released after unfolding, and rapidly aggregates. Thus the lipid bilayer serves as a catalyst for unfolding of the enzyme. Indeed, the dependence of the rate of liposome-catalyzed unfolding of *Bf*AChE on protein concentration can be described by the Michaelis-Menten equation ($K_m = 2 \cdot 10^{-6}$ M). Our findings for both types of AChE support the possibility that the membrane itself, by lowering the energy barrier for transition to a partially unfolded state, plays an active role in insertion and translocation of proteins *in situ*. Membrane-driven protein unfolding also merits consideration as a factor in the etiology of 'conformational' diseases.

3D SPATIAL DISTRIBUTION OF SYNAPTIC VESICLES CAN BE QUANTIFIED IN RANDOM SECTIONS USING COMPUTER SIMULATION

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We propose a computer simulation technique that allows to evaluate 3D spatial arrangement of synaptic vesicles from 2D quantities extracted from random thin sections. According to the technique the topographical distribution of vesicle profiles is estimated in digital images of a presynaptic terminal with minimal spanning tree tool. Further, with the help of the original software the particular 3D spatial distribution of synaptic vesicles, sectioning of a presynaptic terminal and, in result, 2D distribution of vesicle profiles are simulated. To interpret experimental quantities the simulated 2D distribution best fit to experimental data is selected and corresponding 3D density and pattern of vesicle scattering are considered to explain the real situation. The technique was used to evaluate 3D spatial arrangement of vesicles in presynaptic terminals in the hippocampal CA1 region (stratum radiatum) of intact 14 days old rats. In the majority of terminals vesicles formed clusters associated with the active zones of a synapse. The technique allowed to assess 3D density of vesicle scattering, as well as the average size of vesicle clusters. Parallel simulations showed that minimal spanning tree parameters are good predictors of changes in 3D spatial arrangement of synaptic vesicles. The proposed technique may serve as a real and more effective alternative to 3D reconstruction in the study of 3D spatial arrangement of synaptic vesicles and dynamics of the latter.

PECULIARITIES OF AUTOANTIBODIES TO NMDA-RECEPTOR FRAGMENT LEVEL IN BLOOD OF PATIENTS WITH ACUTE BRAIN ISCHEMIA

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It was shown that NMDA-receptors play a key role in pathogenesis of brain ischemia. New test of detecting of autoantibodies (Aab) to NMDA-receptor (NMDA-R) fragment in patient's serum was developed (metod of S.A. Dambinova, 1989,1993). Aab to NMDA-R were detected in blood serum of 29 patients with acute ischemic stroke (IS) on 1st, 3rd, 5-8th, 10-31st days. Diagnosis were confirmed by CT scan, diagnostic ultrasound, EEG. Comparison age- and sex-matched group (33 patients) suffered from chronic cerebrovascular insufficiency. Aab to NMDA-R level differed signi-ficantly in patients with IS depending on premorbid state: cerebral atherosclerosis (3,16 ± 0,30 ng/ml), chronic alcohol abuse or non-insulin dependent diabetes mellitus (5,85 ± 1,71 ng/ml), p< 0,01 (t-test). Aab to NMDA-R level was 1,50 ± 0,10 ng/ml in comparison group. It were revealed 3 types of changes in Aab to NMDA-R level in patients with IS depending on premorbid state. Type 1 can be characterized by wave-form changes of Aab level during the acute period of stroke, with the pikes on 1st (4,17 ± 0,56 ng/ml) and 5-8th (4,03 ± 0,66 ng/ml) days; type 2 - by drastic increase in Aab level at the first day with subsequent slow decrease; type 3 - steadily increased values of Aab level during the acute period of stroke. We found significant differences in autoantibodies to NMDA-binding proteins level in patients with ischemic stroke depending on premorbid state. It seem to be autoantibidies to NMDA-receptor fragment is a hallmark of acute brain ischemia.

THE SEVERITY OF KAINIC ACID-INDUCED SEIZURES IN RATS CORRELATES WITH TRANSCRIPTIONAL ACTIVITY, OXIDATIVE STRESS AND CASPASE-3 ACTIVITY IN THE HIPPOCAMPUS

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Systemic administration of kainic acid (KA) produces limbic seizures in rats and neurodegeneration in the hippocampus. We examined the possibility that seizure severity is related to transcription, oxidative stress, and apoptosis in the hippocampus. Male Wistar rats were injected with KA (10 mg/kg/ip), which produced pronounced neurodegeneration in the CA1 and CA3 regions of the hippocampus after 3 days. Significant correlations were observed in the hippocampus, over the next 3 days, between the maximal seizure severity and a) the nuclear activity of the transcription factor AP-1 (positive, P<0.01), b) lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) (positive, P=0.02), c) total sulfhydryl and glutathione levels (negative, P<0.05), and d) caspase-3 activity (positive, P<0.00001). The increase in caspase-3 activity and the decrease in total sulfhydryl content became evident in rats with seizures of stages 3 or higher, whereas the changes in glutathione and TBARS became significant only with stages 4 or 5. These data suggest that the severity of KA-induced seizures may be closely related to caspase-3-mediated apoptosis and oxidative stress in the hippocampus.

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ROLE OF GLIAL CELLS IN ECS DIFFUSION AND VOLUME TRANSMISSION

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Astrogliosis and cell swelling evoked by neurological disorders and brain trauma may alter the diffusion properties of nervous tissue [1]. Cell swelling and astrogliosis *in vitro* were therefore evoked by the application of either 50 mM K^+ or hypotonic solution (235 mmol kg^{-1}) to isolated spinal cords of 4-21-day-old rats. As a model of reactive astrogliosis *in vivo*, we used a cortical stab wound. ECS diffusion parameters - volume fraction (α = ECS volume/total tissue volume), tortuosity λ (λ^2= free/apparent diffusion coefficient) and tetramethylammonium nonspecific uptake k' - were determined using the real-time iontophoretic method, ADC_W (apparent diffusion coefficient of water) by diffusion-weighted NMR. After the experiments, tissue sections were immunostained for glial fibrillary acidic protein (GFAP) and chondroitin-sulphate proteoglycans (CSPG). In spinal cord, both 50 mM K^+ and hypotonic stress caused a decrease in α and an increase in λ. These changes were blocked in Cl^--free solution and slowed down by furosemide and bumetanide. After 10-20 min of K^+ application, α started to return to control values due to active regulation of cell volume (regulatory volume decrease - RVD). RVD was blocked by the gliotoxin fluoroacetate. During washout in control solution, α returned to and subsequently increased above control values by 50-100% with a second rise in λ. This rise in λ correlated with an increase in GFAP staining and astrogliosis. Diffusion parameters in gliotic cortex were measured 3, 7, 21 and 35 days post-wounding (dpw) in the hemisphere ipsilateral and contralateral to the lesion. In the area 300-1000 μm around the wound, α was increased at 3 and 7 dpw and returned to control values at 21 dpw; λ was increased at 3, 7, 21 and 35 dpw, reaching a maximum at 7 dpw. Measurements made 1500-2000 μm from the wound revealed only an increase in λ at 7 dpw. No changes in α and λ were found in the contralateral hemisphere. The time course of the changes correlated closely with increased staining for GFAP around the wound and with an increase in CSPG in the whole hemisphere. The increase in diffusion barriers was confirmed by a decrease in ADC_W, which occurred in the whole hemisphere and might therefore be related to changes in the extracellular matrix. We conclude that astrogliosis produces a significant increase in diffusion barriers in the ECS and thus affects synaptic and extrasynaptic transmission and neuron-glia communication.

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[1] E. Syková, The Neuroscientist, 3: 28-41, 1997.

FASTING HYPOMETABOLISM AND RE-FEEDING HYPERPHAGIA IN RATS: ROLE OF GASTROINTESTINAL SIGNALS AND OF NPY

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The mechanisms of starvation-induced hypometabolism and re-feeding hyperphagia (and hypermetabolism) have not been clarified. Cold-adapted (CA) and non-adapted (NA) Wistar rats were used with or without small-dose (5 mg/kg) intraperitoneal capsaicin pretreatment. Capsaicin severed the afferent vagal fibers that carry chemo- and mechano-sensitive signals to the CNS. Food deprivation (48-h in CA, 120-h in NA rats) elicited about 15% loss of body weight, and (at thermoneutrality) a fall in resting metabolic rate and hypothermia. Hypometabolic starving CA rats, when acutely transferred from thermoneutrality to cold, exhibited an „overshoot" increase in metabolic rate and a „paradoxical" rise in body temperature, similar to those seen in control CA rats. Thus, at thermoneutrality, these rats still had some energy stored, but its utilization was suppressed, as an energy-saving mechanism. In this suppression fasting-induced activity of endogenous neuropeptide Y (NPY) is presumed to participate, and some of the initiating or modulating signals presumably originate from the *empty* gastrointestinal tract. Indeed, in the absence of such signals (in capsaicin desensitized rats) the rate of body weight loss during starvation was 20% greater than in controls. Conversely, return of food was followed by quick re-feeding that was always larger in CA than in NA rats, and again larger in desensitized than in control animals. On the one hand, cold-adaptation is likely to increase the central sensitivity to NPY (similarly to the enhanced thermal responsiveness to central prostaglandin E). On the other hand, negative feedback signals of chemical or mechanical nature, this time due to *filling* the gastrointestinal tract, appear to modulate the release (or the effect) of endogenous NPY. The effect of exogenous NPY (given through a pre-implanted cannula into the lateral cerebral ventricle) on enhancing food intake is, however, not modified in capsaicin desensitized rats, as compared with controls, probably because in this case feedback signals cannot alter the amount of NPY. (Supported by OTKA T020277 and OTKA T026511)

CHRONIC MILD PRENATAL STRESS EXACERBATES THE ALLERGEN-INDUCED AIRWAY INFLAMMATION IN RATS

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The effects of chronic mild prenatal stress on leucocyte infiltration into the airways were investigated in the rat offspring. The chronic prenatal stress consisted of transitory and variable changes in the rat's living conditions, using the approach of Willner *et al*, 1987 (Psychopharmacology **93**: 358-364). Pregnant female Wistar rats were divided into either stressed or non-stressed groups (N=10, each), during the last two weeks of pregnancy. Offspring at adult age were actively sensitised (day 0) and intratracheally challenged (day 14) with ovalbumin. Bronchoalveolar lavage was performed in the offspring at 48 h after intratracheal challenge with ovalbumin. A significant increase in total leucocyte infiltration was observed in the non-stressed offspring group and this was associated with a marked recruitment of eosinophils without a significant effect on the influx of neutrophils and mononuclear cells. In the prenatal stressed offspring, the counts of total leucocyte and eosinophils as well as mononuclear cells were increased by 50% compared to the non-stressed offspring. We provide here the first experimental evidence that chronic mild unpredictable prenatal stress produces a marked increase in the allergen-induced airway inflammation in the rat offspring.

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MODULATION OF PHOSPHORYLATION OF ECTO KINASES IN *SCHISTOSOMA MANSONI* BY ACh AND CHOLINESTERASE INHIBITORS

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Parasites infect a very large number of people and present a major medical problem, especially in underdeveloped countries. Some 200 million people are affected by schistosomiasis. The causative agent of schistosomiasis is a trematode with a complex life cycle, involving two hosts: a mammalian host and a fresh water snail. Upon penetration of the mammalian skin cercariae lose their tails, discharge the contents of the pre and post-acetabular glands, shed their glycocalyx, and become water-intolerant. The resultant schistosomula are resistant to complement-mediated lysis. The trilaminar surface membrane transforms into an heptalaminar membrane limiting a syncytial cell layer called the tegument. Signal transduction processes are involved in the transformation of the cercariae to schistosomula; phosphorylation of specific proteins is observed. Ecto-protein kinases (ecto-PKS), primarily of the serine/threonine type, have been described on the surface of adult worms of *Schistosoma mansoni* . We have found that schistosomula in tissue culture have externally oriented ecto-PKS that are capable of phosphorylating proteins of the parasite itself of 30, 45 and 56kDa. No difference in ecto-PK activity was seen upon cultivation of parasites for different times (6, 24h). However, an additional, high molecular weight phosphorylated protein (>200kDa) could be detected after 48 hours of schistosomula development. The degree of phosphorylation of all 4 proteins is modulated by ACh and by acetylcholinesterase (AChE) inhibitors such as BW284c51 and echothiophate. These agents increase the level of surface protein phosphorylation upon their addition to live parasites cultured in the presence of [γ^{32}P]ATP. The supernatant from cultured parasites could phosphorylate exogenous substrates, such as phosvitin, protamine, histones and casein. Phosphoaminoacid analysis indicated that phosvitin and protamine were phosphorylated on serine and threonine residues. Staurosporine (5mM), a kinase inhibitor, inhibited phosphorylation of these exogenous substrates. Our findings should contribute to elucidation of both host-parasite interactions and of the mechanisms underlying regulation of development of the various life stages of the parasite.

TNFα, BAX AND CASPASE 9 ARE IMPLICATED IN NEUROPROTECTION BY NITRIC OXIDE (NO) IN DORSAL ROOT GANGLION (DRG) NEURONES

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NO functions as double edged sword having either a neurotoxic or neuroprotective role depending on its concentration, the type of cell in which it is produced and the local redox milieu. It is neuroprotective via the production of cGMP in dissociated DRG neurones (Thippeswamy and Morris, 1997). A possible biochemical pathway for such a protective mechanism by NO is reported. Dissociated DRG cultures were prepared from postnatal 15-18 days rats (ether anaesthetised and decapitated) and grown on poly-D-lysine coated tissue culture slides by standard techniques for five days. The cultures were plated with serum rich media with nerve growth factor (NGF) for two days followed by defined media without NGF for further three days. On fifth day, cultures were treated with Caspase inhibitors (Set II, Cat. No. 218772, Calbiochem; 50μM each) one hour before L-NAME and D-NAME (200μM; 2h interval for first 6h) overnight. The cultures were fixed in 4% paraformaldehyde and stained for nNOS and a specific neuronal nuclei marker NeuN by immunocytochemistry. Some of the cultures not treated with caspase inhibitors were stained for TNFα or Bax. Amongst the different caspase inhibitors tested, caspase-9 was most effective in preventing the death of DRG neurones following L-NAME treatment. D-NAME treated cultures were used as control. Overall the cell density decreased and > 50% of nNOS positive neurones were lost in culture treated with L-NAME alone, while their number did not altered in caspase-9 pre-treated cultures compared with D-NAME. TNFα and Bax immunoreactivity increased in L-NAME treated cultures. These observations suggest that TNFα, Bax and caspase 9 are involved in neuroprotective mechanism by NO in dissociated DRG neurones.

Reference: T. Thippeswamy and R. Morris, 1997. *Brain Res.*, **774**: 116-122.
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THE ROLE OF CALCIUM-INDUCED CALCIUM RELEASE IN TRANSMITTER SECRETION BY CULTURED NEURONES.

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This work analyses the role of intracellular calcium pools in transmitter release from nerve terminals. Experiments were carried out in cultured leech Retzius neurones that synthesise and release serotonin. Retzius neurones were plated single or paired with pressure-sensitive neurones, upon which they form presynaptic endings. To test whether calcium release from the endoplasmic reticulum is able to evoke transmitter release, Retzius neurones were incubated with caffeine 10 mM (an activator of ryanodine receptors), in a Ringer solution in which calcium had been substituted with magnesium, and in the presence of the fluorescent dye FM1-43. This dye stains endocityc vesicles in active terminals. In these conditions, neurones had a staining pattern similar to that produced by intracellular current injection. Depletion of reticular stores by a 20 min pre-incubation with the Ca^{2+}-ATPase blocker thapsigargin (500 nM) reduced the staining by 50%. Confocal microscopy of Retzius neurones double stained with FM1-43 and fluorescent ryanodine showed a close association between intracellular calcium pools and transmitter release sites. This was confirmed by EM sections showing endoplasmic reticulum in close proximity to presynaptic terminals.
The role of intracellular calcium release was studied in facilitation. In cultured synapses facilitation was induced by pairs or trains of impulses in the Retzius cell. Thapsigargin 500 nM abolished facilitation after 15 minutes, without any apparent change in the postsynaptic membrane properties, thus suggesting a reduction in transmitter release by the presynaptic terminals. These results suggest that calcium-induced calcium release participates in a feedback mechanism that enhances transmitter release and provide a complementary explanation to the residual calcium hypothesis.
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ENVIRONMENTAL HUMIDITY REGULATES NEUROPEPTIDE MRNA EXPRESSION IN RAT DORSAL ROOT GANGLION CELLS

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Physiological influences of environmental humidity conditions remain unclear. We previously found that cutaneous functions, such as barrier homeostasis (Denda et al., J Invest Dermatol 111: 858, 1998) and DNA synthesis (Denda et al., J Invest Dermatol 111: 873, 1998) in the epidermis are influenced by environmental humidity. We examined here, whether or not environmental humidity influences neuropeptide mRNA expression in rat dorsal root ganglion (DRG) cells.

Animals were housed in cages (1 animal/cage) which automatically received either dry air (approximately 10%) or high-humidified air (approximately 80%) continuously as described previously (Denda et al., J Invest Dermatol 111: 858, 1998). The lumbar DRG was dissected, and calcitonin gene-related peptide (CGRP) and substance P (SP) mRNAs expressions were analyzed by RT-PCR.

CGRP mRNA expression in DRG in rats housed in dry air for 2h was significantly lower than in those housed in high-humidified air for 2h , but the expression in DRG in rats in dry air for 2 days was significantly higher than in those housed in high-humidified air for 2 days. SP mRNA expression in DRG in rats housed in dry air for 2h was significantly higher than in those housed in high-humidified air for 2h. However, no difference was found in SP mRNA expression in DRG between rats housed in dry and high-humidified air for 2 days.

The findings suggested that environmental humidity conditions influence the function of the primary afferent neuron similar to thermal and mechanical stimuli (Tsuchiya et al., Neuropeptides 30: 149, 1986).

TYROSINE HYDROXYLASE- OR AROMATIC L-AMINO ACID DECARBOXYLASE-EXPRESSING NON-DOPAMINERGIC NEURONS IN THE ARCUATE NUCLEUS: DIFFERENTIATION AND POSSIBLE CO-OPERATION IN DOPAMINE SYNTHESIS

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This study has evaluated the differentiation and possible functional significance of non-dopaminergic neurons of the arcuate nucleus (AN) expressing one of the enzymes of dopamine (DA) synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC) in rats at the 17th embryonic day (E), E18, E20, E21, the 9th postnatal day (P) and in adults. Immunocytochemistry (mono- and double-labelling), image analysis, confocal microscopy, HPLC, dissociated cell culture, perifusion system and radioimmunoassay were used to solve this problem. TH-containing neurons were observed as early as on E18 locating in the ventrolateral AN whereas AADC- and AADC&TH-expressing neurons first appeared at E20 in the dorsomedial portion of this nucleus. At E21, these neuron populations were accounted for 45.3-39.4%, 53.8- 59,8% and 0.9-0.8% , respectively. In spite of a small number of dopaminergic neurons (AADC&TH), the dissected mediobasal hypothalamus contained a relatively high concentration of L-DOPA and DA. The latter was released in vitro in response to membrane depolarization. Similar data were obtained in dissociated cell culture. The DA yield in the AN of the fetuses occurred to be sufficient to provide an inhibitory control of prolactin secretion. This was proved by an increased concentration of prolactin in plasma and decreased concentration in the pituitary of fetuses following an inhibition of D2 receptors on lactotropes with haloperidol. The data mentioned above suggest that DA could be synthetized, at least in the AN of fetuses, by non-dopaminergic neurons containing either TH or AADC in co-operation. This might be also the case for the AN of adults though the fractions of TH- or AADC-expressing neurons decreased significantly (10.6-23.2% and 32.7-39.7%) while that of dopaminergic neurons proportionally increased (37.1-56.7%).

BRX-211 A PROMISING NEW NEUROPROTECTIVE COMPOUND

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BRX-211 was synthesized for the neuroprotective project in Biorex R & D Co. This compound is one of the most effective neuroprotective molecules from derivatives of Bimoclomol, a new, effective cytoprotective compound in nephropathy and peripheral neuropathy, which is in Phase II clinical trial. Bimoclomol facilitates the formation of chaperone molecules in eukaryotic cells by inducing or amplifying expression of heat-shock genes (Vigh et al. Nature Medicine 1997, 3: 1150.). Neuroprotective properties of BRX-211 were revealed in several experimental models of global and focal cerebral ischaemia in mice, rats and gerbils. In this study the neuroprotective efficacy of the compound is demonstrated in cytotoxic hypoxia in mice (induced by i.v. administered KCN, cytotoxic hypoxia model), in total cerebral ischaemia evoked by i.v. $MgCl_2$ (acute neuroprotective model in NMRI mice), in the transient bilateral carotid occlusion (BCo) model in Mongolian gerbil, a widely used model of global forebrain ischaemia, as well as in transient focal cerebral ischaemia in rats (Middle Cerebral Artery occlusion, MCAo stroke model). BRX-211 prolonged the survival time in KCN test (PD_{50} value 19.7 mg/kg p.o.) in a dose-dependent fashion (10-25-50 mg/kg caused 132.6-186.5-282.2 % prolongation in survival time, respectively) and displayed significant protection against $MgCl_2$ induced global cerebral ischaemia in 30 mg/kg dose p.o. The BRX-compound (12.5-25-50 mg/kg i.p. 30 min postocc.) significantly and dose-dependently antagonized the 5 min bilateral carotid artery occlusion evoked ischaemic effect in the CA1 region of the hippocampus in gerbils (32.8-49.1-72.2 % protection, respectively). In Long Evans rats BRX-211 given in a 2 mg/kg i.v. single dose 30 min postocclusion induced a statistically significant reduction of the cerebrocortical infarct volume in MCAo model (4.63±1.69 vs. control: 8.09±0.72). Furthermore, BRX-211 did exert cell-type independent neuronal inducer/trophic actions *in vitro* on neuronal progenitor cells (E. Madarasz, Neural Cell Biology Lab., Institute for Experimental Medicine, Hungarian Academy of Sciences, Budapest) and showed a cholinoprotective potential in NMDA evoked excitotoxic rat model (T. Harkany CNS Research Center, Haynal Imre University of Health Sciences, Budapest).
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GLYCINE INHIBITS SYNAPTIC TRANSMISSION AT THE SQUID GIANT SYNAPSE.

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The giant synapse of the squid is glutamatergic with postsynaptic AMPA/Kainate receptors [1]. Bath application of 0.1-1 mM glycine caused a reversible decrease in the quantity of postsynaptic action potentials (AP)(40/s stimulation of presynaptic nerve) before the 1st failure (19.8±5.1 % of control in ASW, n=4). This action was accompanied by an increased K+ accumulation during synaptic transmission that was absent during antidromic stimulation of the giant axon. Application of NMDA was without effect, while a blocker of the glycine site of NMDA receptor CPP attenuated the effect of glycine. The glycine effect was insensitive to strychnine (0.03 mM), and reduced by d-tubocurarine (d-TC; 0.1 mM) and DIDS (0.5 mM). 10 mM EGTA in the postsynaptic recording microelectrode significantly increased the number of APs before failure, and application of glycine decreased the quantity of transmitted APs to 40±8.3 % (n=3) of the control. Changes in K+ accumulation were not detected. These data indicate multiple actions of glycine on the reliability of signal transmission in the giant synapse, including activation of d-TC sensitive channels [2] and Ca activated potassium channnels. A moderate contribution of NMDA-like and presynaptically located metabotropic glutamate receptors cannot be excluded at this stage.

1. Messenger J.B., DeSantis A, Ogden D.C.1995. In:*Cephalopod Neurobiology*. OUP. Eds.Abbott N.J., Williamson R., Maddock L.
2. Stanley E.F.1985. *J.Neurosci.*, vol.4, 1904-1911.

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PROTECTION OF DOPARMINERGIC NEURONS BY DNF cDNA ENGINEERED CELLS: IN VITRO AND IN VIVO STUDIES

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Glial cell line derived neurotrophic factor (GDNF) has been shown to protect dopaminergic neurons from natural death and neurotoxicant-induced cell lesions. Since the molecular size of GDNF is too large to pass the blood brain barrier, an ex vivo strategy is to consider the rationale for delivering the molecule to the target site of central nervous system for gene therapy of Parkinson's disease (PD). Two cell lines were chosen as the substrate of cell engineering. MN9D cell line is derived from mesencephalic dopaminergic neurons which carries genes encoding tyrosine hydroxylase (TH), whereas NIH 3T3 cell line is derived from fibroblast, which carries genes coding for GDNF. GDNF cDNA was cloned with a Kozac sequence and inserted into $pcDNA_3$ plasmid downstream of CMV promotor, and then transfected into MN9D or 3T3 cells. Subclones stably expressing GDNF with high efficiency were selected by G418.
In an in vitro study, GDNF cDNA engineered cells were co-cultured in serum free media with primary mid brain neurons obtained from E14-E16 rats. The number of TH positive neurons, characterised by immunocytochemistry (ICC) and counted in 40 fields (10 x 25) across the time of culture was taken as the index of cell survival. Engineered 3T3 cells significantly Prolonged the survival of DA neurons, especially in 12day culture when most DA cells died of senility, and protected the DA neurons against neural toxicant MPP^+-induced cell lesion. Note that naive 3T3 cells were effective, but GDNF engineered 3T3 cells were even more effective. The cell size and the differentiation of the neural processes can reflect the status of living of the cells. The DA neurons co-cultured with engineered cells showed larger size and better differentiation and the cell damage such as loss and breakage of neural process were largely prevented. Similar results were obtained when engineered MN9D cells were used for co-culture and the results compared with naive MN9D cells. In an in vivo study, GDNF engineered MN9D cells were transplanted into 6OHDA-lesioned striatum of the rat and the rotational behavior was observed as index of PD. SY5Y cell (used as control) had no protective effect. Naive MN9D cells significantly reduced rotational behavior for 2 weeks, and GDNF/MN9D cells improved rotational behavior for at least 10 weeks. In conclusion, the biological effectiveness of the GDNF cDNA engineered cells is obvious. These engineered cells may have Potential value for gene therapy of PD.
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AN EXPERIMENTAL STUDY ON INTRACEREBRAL TRANSPLANTATION OF MICROCAPSULED CHROMAFFIN CELLS IN MONKEY MODEL OF PARKINSON'S DISEASE

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The experimental therapy on Parkinson's disease (PD) was made by using a method of intracerebral grafting of the chromaffin cells into the monkey model of PD. The chromaffin cells were isolated from the adrenal medulla of the ox and cultured *in vitro*. The cultured cells were then microcupsuled by using a special equipment offered by Prof. A.M. Sun from University of Toronto. These microcapsuled chromaffin cells were grafted into the caudal nucleus of PD model of the *macaques*. The models were made by ipsi- or bilateral injections of I-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the carotid artery. Their symptoms (such as tremor, rigidity and bradykinesia), brain image of MRI, the striatal levels of dopamine and its metabolisms and the immunoreactive assay before- and after-grafting were compared by using different test methods. The results showed that the xenografting of chromaffin cells which were microcapsuled into the brain was a useful tool for improving the situation of PD.

EFFECTS OF SOFT-DIET FEEDING ON SYNAPTOPHYSIN EXPRESSION IN THE CORTEX OF THE SENESCENCE ACCELERATED MICE

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Several reports have suggested that a decrease in masticatory work during development influences later learning abilities. In this study, we examined whether soft-diet feeding influenced synaptophysin levels of the cortex, using P8 strain of the senescence accelerated mouse (SAM-P8). Male mice, weaned at 3 weeks after birth, were fed either solid or powdered diet containing the same ingredients. At 2-12 months, they were decapitated under anesthesia, and the brains rapidly removed. Fresh cortical tissue was homogenized and centrifuged for radioimmunoassay. Test samples (10 μg protein) were pipetted into wells of a 96-well multi-screen (Immobilon-P), blocked for 2 hr in 3% bovine serum albumin, incubated for overnight with a monoclonal mouse anti-synaptophysin antibody (SY38, 0.1 μg/ml), and incubated for 2 hr with ^{125}I-labeled rabbit anti-mouse IgG (0.85 μg/ml). Radioactivity (cpm/μg protein) of the samples was determined with a microplate scintillation counter. The radioactivity of the solid-diet group increased between 2 and 3 months after birth, and then gradually decreased to return to 2-month-old level at 12 months after birth. The radioactivity of the powdered-diet group also peaked at 3 months after birth, but their mean counts were lower than those of the solid-diet group at all ages investigated. Difference of the mean counts between two groups was largest (16.1%) at 9 months after birth and smallest (3.0%) at 2 months after birth. Results suggest that the synaptic formation progresses incompletely in the cortex of the SAM-P8 mice fed a soft-diet.

ACETHYLCHOLINE INTERACTION WITH RAT BRAIN THIAMINE BINDING PROTEIN

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Thiamine binding protein (ThBP) was isolated from rat brain synaptosomes by means of the methods of affinity chromatograph and gel filtration. Study of ThBP biological activity has shown that it selectively hydrolyses thiamine phosphoric esters. omparison of kinetic parameters of thiamine binding with isolated synaptosomal plasmic membranes preparation and ThBP indicates the localisation of ThBP in synaptosomal plasmic membrane. Characteristic of thiamine triphosphatase activity and some other characteristics of both preparations confirm belonging the protein to peripheral protein of synaptosomal plasmic membrane. It was investigated that acetylcholine (AcCh) inhibits binding of thiamine with ThBP (Ki = 4,7 mkM). Measurement of AcCh affinity to ThBP with use [^{14}C]acethylcholine has shown that K_d for AcCh is 13,0+2,0 mkM.Results we did receive confirm a possibility of ThBP participation In realization of neurotrophic thiamine function and in realization of relationship between the metabolism of thiamine and AcCh in particular.

CHARACTERIZATION OF THE DES-2/DEG-3 RECEPTOR, AN ANCIENT MEMBER OF THE nAChR FAMILY

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DEG-3 is a subunit of a nicotinic acetylcholine receptor (nAChR) that can mutate to cause neuronal degeneration. *deg-3* is part of an operon containing a second nAChR subunit, *des-2*. DES-2 is necessary for DEG-3 dependent channel formation in *Xenopus* oocytes, and for *deg-3(u662)* induced degenerations. Thus the two genes probably interact in forming a channel in-*vivo* (Treinin et al, PNAS 95:15492).

Sequence analysis of both *deg-3* and *des-2* show that they are highly divergent members of the nAChR family. Both show similar low (30%) identity to different branches of the nAChR family, suggesting that they branched early from the family tree. Indeed, electrophysiological analysis of the DES-2/DEG-3 receptor expressed in *Xenopus* oocytes shows that it is a highly divergent nAChR, as application of choline leads to much higher responses than the application of acetylcholine (ACh). Choline was already shown to be an agonist of α-7, also a nAChR, but ours is the first demonstration of a receptor which is more sensitive to choline then to ACh. Choline being a metabolite is unlikely to play a role in synaptic transmission. Accordingly, DEG-3 is distributed all over the cell (as visualized by antibody staining), showing no preference to synaptic regions.

As part of the analysis of this divergent nAChR we are also doing structure function analysis, using mutations in *deg*-3 which where identified in screens for suppressors of *deg-3(u662)* induced degeneration. Sequences of the mutations demonstrate structural conservation within the nAChR family, as all missense mutations affect conserved residues. Genetic analysis of the mutations has identified a small number of negative dominant suppressors. These mutations are likely to effect functional attributes of the receptors and will be further analyzed using electrophysiology.

ALTERED FUNCTIONS OF CHOLINESTERASES IN RAT BRAIN UNDER CADMIUM CHLORIDE STRESS

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The present study is intended to know the impact of acute and chronic doses of cadmium chloride on the cholinergic system in different areas of rat brain. The LD50 calculated by probit method was 275 mg/ kg body weight.1/10th of LD50 was chosen as the sublethal dose. In acute dose studies maximum inhibition in AChE was noticed after 12h in all brain areas(striatum 54.55%, hippocampus 47.39%, pons medulla 38.88%, cerebellum 38.84% and cerebral cortex 38.55%), while in chronic dose studies peak inhibition was noticed on 7 th day and thence recovery was observed from 11th day.on contrary, in acute dose studies striatum (44.285) and cerebral cortex (30.29%)showed maximum elevation in ACh content at 3 hr, hippocampus (19%)and cerebellum (30.51%)at 12hr.pons medulla at 24hr. But in chronic dose studies accumulation of ACh i various regions was in the order of hippocampus (72.36%)> striatum(65.78%)>cerebral cortex(51.91%) >pons medulla (30.34%) >cerebellum(14.03%). Thus the changes in cholinergic system testify the differential sensitivity of brain areas to cadmium chloride stress. Further alterations in cholinergic system were correlated with the changes in the behaviour of rats such as hyperactivity , tremors and convulsions.

STUDY OF THE ROLE OF ADP-RIBOSYLATION IN RAT BRAIN AND LIVER CELLS

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The influence of X-irradiation of rats on the brain and liver cell poly(ADP-ribose) polymerase and NMN adenylyltransferase activities was studied. It was revealed that X-irradiation increases brain and liver cell nuclear poly(ADP-ribose) polymerase activity in a dose-dependet manner. At the same time nuclear matrices poly(ADP-ribose) polymerase activity is increased to a greater extent than nuclear activity at a low dose of irradiation (1.7Gy), while at a high dose of irradiation (6.7Gy) it sharply decreases. These data and the detection of significant part of NMN adenylyltransferase activity associated with the nuclear matrices indicate the participation of protein modification by ADP-ribosylation taking place on a matrix level in eucaryotic cell DNA repair. The inhibition of DNA topoisomerase II activity of isolated brain and liver cell nuclear matrices after the X-irradiation of rats was shown as well as the involvement of ADP-ribosylation into the modulation of chromatin structure of nerve and liver tissues of both intact and X-irradiated rats. The data obtained indicates that in vivo the stimulation of ADP-ribosylation of DNA topoisomerase II by X-irradiation of rats is the reason for the inhibition of DNA topoisomerase II activity of the matrices.

ELECTRON-RADIOAUTOGRAPHIC ANALYSIS OF CEREBRAL CELLS CHANGES IN DEEP HYPOXIA MODELLING

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Ultrastructural changes demonstrated the deepest dystrophic changes in the cells of cerebral hemospheres cortex. The most stable to hypoxia are ganglia and glia where large amount of cells survived unfavourable influence were concentrated. The authors used the material received in the course of neurosurgical operations as well as autopsy material received in different times after death. The degree of such changes may indicate the level of cellular ultrastructural changes being very important for differencial diagnostics of cerebral tissue affections.
Number of mitochondria and canaliculus of endoplasmatic net was sharply reduced. In swelling mitochondria matrix was enlightened, most crista were destroyed. The number of some membranes and small vacuolus of lamellar complex was reduced. In such cells there were zones of enlightened cytoplasma, disturbance of cytoplasmatic membranes and sharp reducing of cytogranules. In nuclei there were enlightening of nucleoplasma and uneven exfoliation of external nuclear membrane. Besides cells with significant ultrastructural changes, there were cells preserved ultrastructural elements with H-uridin included testifing the cerebral cells ability to synthesize RNA for a long time.
The application of complex approach allowed to characterize both ultrastructural organization of cerebral cells and preservation of their nuclei ability to synthesize RNA. It proved the continuation of synthesis in cells exposed to prolonged hypoxia.

IMPAIRED RESPONSE OF β-ENDORPHIN TO SEROTONIN STIMULATION IN THE BRAIN OF A RAT MODEL OF DEPRESSION

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The endogenous opioid peptide, β-endorphin, acts as an endogenous analgesic factor and may cause hedonic effects. Moreover, 50% of the depressed patients suffer from chronic pain. Therefore, we hypothesized that β-endorphin release in the brain may be impaired in depressive disorder. In this study, we applied the *in-vivo* microdialysis technique to assess β-endorphin release in the nucleus accumbens (N.Acc) and arcuate nucleus (Arc.N.) of the Flinders Sensitive Line (FSL) rat, a proposed genetic model for depressive behavior, as compared to normal Sprague-Dawley rats. Since the serotonergic system is known to be involved in depressive disorder and in the mechanism of antidepressant action, the effect of local application of serotonin on β-endorphin release was studied. Our results show that the basal extracellular levels of β-endorphin were 58 % lower in N.Acc and 41 % lower in the Arc.N. of FSL rats than those of control rats. Perfusion of 5 μM serotonin into N.Acc or 1 μM serotonin into the Arc.N. of control rats significantly increased β-endorphin dialysate levels. In FSL rats, perfusion of serotonin only slightly affected the dialysate β-endorphin levels. Chronic treatment with desipramine, which normalized the behavioral deficiency of FSL rats, significantly affected the serotonin - β-endorphin interaction in the nucleus accumbens of FSL rats. These results, together with our previous findings, showing absence of serotonin-dopamine interaction in FSL rats, suggest that depressive disorder might be correlated with decreased sensitivity to serotonin in N.Acc. We further suggest that decreased ability of serotonin to stimulate β-endorphin and dopamine release in N.Acc may lead to anhedonia and increased sensitivity to pain which are observed in depressive disorders.

AGE RELATED ALTERATION OF BRAIN RESPONSES TO ISCHEMIA

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Stroke developed in the elderly may lead to death or to major disabilities. Most of the *in vivo* animal experiments have usually relied on models of cerebral ischemia induced in the adult brain. A very small number of studies were performed in old aged animals, where the known decline in energy metabolism, were studied under *in vivo* conditions. The aim of this study was to evaluate the influence of aging on the responses of the brain to partial ischemia. In order to evaluate brain function and activities in real time we used the multiparametric monitoring system. This system enables continuous, simultaneous, on-line measurement of electrical hemodynamic, ionic and metabolic parameters of the cerebral cortex (Mayevsky et al. J. Appl. Physiol. 78: 1188-1196, 1995). The carotid arteries were isolated and the MPA was placed and cemented on the cerebral cortex of adult (2-3 months, n=7) and old (25-30 months, n=10) rats. Bilateral occlusion artery was performed in both groups of animals after recovery from surgery and monitoring the baseline level of the various parameters. The responses of the aging brain partial ischemia (carotid occlusion) were markedly different from the responses of the adult brain to the same treatment. In the adult rat brain and transient decrease in CBF followed by transient increase of the mitochondrial NADH was recorded. No other changes were observed during or after the ischemic period. In contradistinction, bilateral carotid occlusion in the aging rats resulted in a non-transient change in CBF and NADH redox state. Furthermore, as the ischemia persisted, only in the aged rats, general cortical depolarization developed and potassium leakage and acidosis was recorded. Only when the carotids were opened and blood supply renewed most of the parameters returned to their initial levels. However the tissue pH did not recover even 30 minutes after carotids opening and restoration of blood flow, suggesting a possible deterioration of mitochondrial function. This study suggests that the adult brain tolerates CBF disorders significantly better than the aging brain.
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PARTIAL NEUROPROTECTION BY LIPOIC ACID TREATMENT ON THE EFFECTS OF NEONATAL X-IRRADIATION.

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Developing Nervous System is very sensitive to ionizing radiation. Neonatal X-rays induces biochemical, morphological and functional changes. Biological effects of ionizing radiation includes the generation of free radicals and the subsequent chain reactions produce more radicals. α–Lipoic acid, a thiol that can scavenge free radicals as well as chelate transition metals was administered s.c. at 50 μg/g in 5% NaOH solution in NaCl 0.9% to neonatal rats at cephalic end (5Gy up to 72 hours after birth). one hour prior irradiation. Cerebellar noradrenaline (NA) concentration was determined spectrofluorometrically, motor gait was quantified by two *ad-hoc* motor test and morphological changes was evaluated by histological procedures.The data show that lipoic acid can prevent the motor abnormalities induced by x-rays, characterized by a dystonic syndrome. Cerebellar cytoarchitecture damage of irradiated animals, was partially protected by lipoic acid administration. Cerebellar NA concentration increase induced by x- rays might be a compensatory effect of histological and functional alterations. In conclusion, acute lipoic acid treatment to irradiated rats is effective in preventing the long term effects induced by x-irradiatin on motor gait and cerebellar cytoarquitecture. A temporal correlation between motor and morphological changes was obsrved, suggesting a primary action of free radicals on cerebellar cytoarchitecture leading to motor syndrome.
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NEW EVIDENCE ON VISCEROCEPTION AS CONSTITUENT OF SPECIAL SENSES

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In a recent monograph (G.Ádám /1998/: Visceral Perception. Understanding Internal Cognition. Plenum Press, New York and London) it has been proposed that visceral sensitivity should be included among the classical special senses.Visceral afferent apparatus acts far beyond the boundaries of sensing some homeostatic changes: it forms a more-or-less homogeneous and consistent sensory system. Recently two additional sets of data have been obtained which seem to reinforce this view:

1). The demonstration of the validity of the basic Law of Psychophysics (Fechner-Stevens equation) in the domain of human visceral perception. The gradual distension of the sigmoid colon in six colonostomy patients has been undertaken by a computerized pneumatic system. The tracking of "gut-feelings" by the subjects had been made possible with the aid of a Békésy-type sliding potentiometer. The results demonstrate a power relation between intestinal distension (ϕ) and the feeling of mild pressure in the gut (ψ), proving the validity of the Fechner-Stevens equation in the viscerosensory domain.

2). The PET-tracing of unilateral cortical activation evoked by bilateral carotid stimulation in humans. Baroreceptor stimulation has been undertaken by rhythmical suction of the neck using a computer driven pneumatic chamber in eight right handed male subjects. As shown in detail elsewhere (Weisz J.,et al., this Congress), the regional cerebral blood flow, assessed by 15-O-butanol 3D positron emission tomography, seemed to be significantly ($p < 0.01$) activated by carotid stimulation in the frontal and insular areas of the cortex. This activation was much more expressed in the right hemisphere than in the left.

Both the validity of the Fechner-Stevens equation and the marked unilateral cortical representation seem to confirm the previously formulated view that visceral sensitivity obeys the same general laws and bears features identical with the other classical sense organs.

SYNTHESIS OF GLUTAMIC ACID IN MITOCHONDRIAS OF DIFFERENT BRAIN CORTEX AREAS AND HYPOTHALAMUS IN THREE-MONTHED RATS UNDER THE ACOUSTIC ANALYSATOR DAMAGE

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The conducted studies shout that in normal conditions in the three-monthed vats the glutamic acid synthesis is the most intensive by means of reductive amination in hypothalamus mitochondrias while by means of transamination it is the most intensive in mitochondrias of hypothalamus and orbital cortex in comparison to the glutamic acid synthesis in mitochondrias of sensorimotor, limbic and visual cortex. After 10 days sine acoustic analysator damage the glutamic acid synthesis by means of reductive amination and transamination in mitochondrias of all the studied areas(sensorimotor, limbic, orbital and visual) of brain cortex and hypothalamus decrease acutely from 20 to 51% in comparison to the norm. The characteristic changes of glutamic acid synthesis are revealed in the mitochondrias of the studied brain cortex areas and hypothalamus after 30 days since acoustic analysator damage in the three-mouthed rats. Besides, the most prominent decrease is noticed after 30 days since analysator damage in comparison to changes noticed after 10 lays, with the exception of visual cortex mitochondrias, where on 30-th day after analysator damage the glutamic acid synthesis is still on the control level. The obtained date shot that glutamic acid as a neurotransmitter plays significant role in sensory information transduction.

ROLE OF BIOLOGICALLY ACTIVE COMPONENTS OF MATERNAL MILK IN MORPHO-FUNCTIONAL MATURATION OF NEWBORN'S BRAIN

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Maternal milk is rich with biologically active substances such as growth factors, hormones, immunoglobulins and many others. Their secretion in the milk is an active process. Most of this components both in quantity and particularly, qualitatively possess of high species-specificity. In early postnatal ontogenesis they play an important role, particularly in CNS development.

The concentrations of the thyroid hormones in the breast milk is significantly higher than in the blood of postpartum women. These hormones regulate in baby's organism the enzymes activities supporting myelinization of nervous system structures and synaptogenesis. The artificially-fed babies, fed with cow milk or other maternal milk substitutes, receive insufficient amounts of thyroid hormones that leads to abnormalities of morphological and functional brain development. So, these children are more susceptible to psycho-emotional disorders, possess worse memory and have slower progress at school in comparison with breast-fed children.

The prolactin (PRL) concentration in the breast milk is higher than in the blood of lactating woman and besides, the PRL concentration in the baby's blood 10-times and more higher than in the middle-aged man blood. Apparently, this determines the important biological expediency of the high PRL content in the breast milk. It plays role of " chemical signal" from mother to baby. In the child's organism the milk PRL induces the feedback mechanism, that is responsible for secretion of its own PRL. The main role in this mechanism has the hypothalamic dopamine, being the physiologic inhibitor of the prolactin secretion. In artificially-fed newborns, receiving with the milk unfamiliar bovine PRL, the hypothalamic dopaminergic mechanism of PRL secretion forms faintly, consequently the predisposition to hyperprolactinemia syndrome, one of the reasons of endocrine infertility, develops.

So, the maternal milk is unreplaceable for child, particularly for the normal brain development.

FUNCTIONAL PLASTICITY OF NEURONS INDUCED BY EPILEPTIFORM ACTIVITY (BUCCAL GANGLIA, HELIX POMATIA)

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Seizures often show characteristic durations. However, mechanisms underlying their spontaneous termination are little understood. It is presently shown that the paroxysmal depolarizations of the neurons induce non-synaptic functional adaptations which result in a block of the epileptiform activity.

Neurons B3 in the buccal ganglia of Helix pomatia were recorded intracellularly. Epileptiform activity consisted of paroxysmal depolarization shifts (PDS) which appeared synchronized in both B3-neurons with 40 mM of the epileptogenic drug pentylenetetrazol (PTZ). Also etomidate (0.5 mM) and heptanol (2 mM) were used as epileptogenic drugs. $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ were varied and forskolin (50 µM) was added to the solutions.

During application of PTZ for 24 h, PDS decreased for ca. 10 h (PDS-pause) and reappeared after ca 15 h. PDS-pause also appeared when etomidate or heptanol were used. When PDS in one B3 neuron were suppressed by injection of hyperpolarizing currents, this neuron did not develop a pause during its hyperpolarization whereas the simultaneously recorded neuron B3 of the contralateral ganglion showed the PDS-pause. In zero Mg-solution the pause development is missing or an established pause is blocked. Ca^{2+} could replace Mg^{2+} in part. When forskolin was applied, the pause was suppressed, i.e. the original epileptiform activity was restored.

The observed functional plasticity is thought to result from primarily Mg-dependent channel de-phosphorylation induced from the PDS and counteracted with forskolin via protein kinase A.

ALLOSTERIC INTERACTIONS RESCUE THE IKS LOSS FUNCTION INDUCED BY AN LQT MUTATION

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Combined genetic and physiological studies have established a link between potassium channel dysfunction and well known neuromuscular and neurological disorders. Many 'channelopathies' are accounted for by a dominant-lethal suppression of potassium channel function. In the cardiac IKS channel complex comprising the a and b subunits, KvLQT1 and IsK, respectively, several mutations lead to a dominant-negative loss of channel function, that is responsible for the most severe forms of a human cardiovascular disease called long QT (LQT) syndrome. Here we show that binding of IKS channel activators such as stilbenes or fenamates to an extracellular N-terminal domain of human IsK (aa. 39-43), restores normal IKS channel gating in otherwise inactive IsK C-terminal mutants, including a naturally occurring LQT5 mutant (D76N). Our data support a model in which the extracellular and intracellular domains flanking the IsK transmembrane segment interact allosterically. Disruption of this allosteric interplay impedes slow activation gating, recruitment of active KvLQT1 a subunits and suppression of channel inactivation. Owing to such allosteric interactions, stilbene and fenamate compounds can relieve the dominant-negative suppression of IKS produced by IsK mutants, including the one responsible for the LQT5 syndrome.

GRAFTED CNS STEM CELLS FORM FUNCTIONAL SYNAPSES *IN VIVO*

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Morphological and immunocytochemical evidence indicates that transplanted CNS stem cells can integrate into the host brain and acquire properties characteristic of the area where they are found. However, there has been no evidence that these transplanted cells are functionally integrated. Here we report that expanded CNS neuroepithelial stem cells, upon transplantation, can form neurons in the host brain and, in turn, form functional synapses with host neurons. Embryonic cortical stem cells were expanded *in vitro* and genetically labeled using a retrovirus carrying the gene for GFP. Following infection, the gfp-labeled stem cells were grafted *in utero* into the developing hippocampus of E18 rat embryos. The embryos were carried to term and examined postnatally. The gfp positive cells in grafted hippocampi were analyzed in acute slices using a combination of electrophysiological and immunocytochemical techniques. Grafted gfp-labeled cells exhibited spontaneous activity and showed evoked postsynaptic responses. This is indicative of the grafted cells being synaptically integrated into the circuitry of the host hippocampus. Recorded cells were filled with biocytin and the tissue was fixed for later immunocytochemistry. Confocal microscopy of immunostained slices revealed that the grafted stem cells adopted either glial or neuronal morphology. Staining for early neuronal markers together with biocytin detection indicated that the recorded, gfp-positive cells were, in fact, neurons. These data have important implications for targeted brain repair based on cell replacement therapy using an expandable population of CNS stem cells.

PROJECTIONS FROM THE CUNEIFORM NUCLEUS TO NEURONS OF THE BRAINSTEM THAT ARE INVOLVED IN MODULATING NOCICEPTION

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Aim of Investigation: Stimulation of neurons in the cuneiform nucleus (CnF) produces antinociception that is mediated by several neuronal systems including descending noradrenergic neurons. These studies determined the projections to noradrenergic and non-noradrenergic neurons in the brainstem.
Methods: The anterograde tracer, biotinylated dextran amine (BDA) combined with tyrosine hydroxylase immunocytochemistry was used to determine the projections of neurons in the CnF to spinally projecting noradrenergic cells and non-noradrenergic neurons in the A7, A5, and A6 (Locus Coeruleus) cell groups that may modulate nociception.
Results: The BDA deposit in the CnF produced anterograde labeling that was most dense on the ipsilateral side. Highly varicose, anterogradely labeled terminals were apposed to noradrenergic somata and dendrites, as well as non-tyrosine hydroxylase-immunoreactive neurons in the A7 and A5 cell groups, but not the A6 group. The appositions are highly suggestive of synaptic contacts.
Conclusions: These results provide presumptive evidence for direct projections from neurons in the CnF to noradrenergic and non-noradrenergic neurons in and near the A7 and A5 cell groups. These results suggest that the antinociception produced by stimulation of sites in the CnF is mediated in part by the descending noradrenergic cells of the lateral A7 and A5 cell groups and also by non-noradrenergic neurons.
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INFLUENCE OF THE BASOLATERAL AMYGDALOID COMPLEX ON BULBAR MECHANISMS OF REGULATION OF RESPIRATORY FUNCTION AND VASOMOTOR ACTIVITY

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In a series of microelectrophysiological investigations effects of stimulation of basolateral nuclei of amygdala on the impulse activity of functionally identified single respiratory neurons of the medulla oblongata were studied in anesthetized cats. It was found a marked predominance of inhibitory influence of lateral nucleus and medial part of basal nuclei of amygdaloid complex on the activity of inspiratory and expiratory neurons of bulbar respiratory center. It was shown that the prevailing effects in case of stimulation of the lateral part of the basal nuclei is excitatory for bulbar respiratory neurons.
In a series of chronic experiments effect of bilateral electrolytic lesion of basolateral nuclei of amygdala in development of chronic neurogenic hypertension were studied in rats. In control animals chronic four weeks overloading of the highest nervous activity induced a stable and well pronounced rise in arterial blood pressure. It was shown that after bilateral lesion of structures of basolateral amygdala the neurogenic stress induce a fulminant increase of arterial blood pressure. Possible mechanisms of regulation of activity of bulbar respiratory neurons and vasomotor reactions by structures of basolateral amygdala are discussed.

QUANTITIVE ASSESSMENT OF POSTURAL ASYNERGY IN PATIENTS WITH ATAXIA

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The main task of our investigation was to show the diagnostic value of posturography as a method of quantification of postural ataxia. Posturography was performed in 48 patients with various formes of ataxia and in control group of 20 healthy subjects. The method was performed, while the subject stood on forse-measuring platform. Strain gauges at corners of platform mesured the displacement of the center of foot pressure in anteroposterior and lateral directions and the sway area histogramm was plotted on a X-Y printer-plotter. The date presents in our investigations suggest that in the future, such quantitative study of ataxia might be used for differential diagnosis and for the effect of drugs.

CAN DL-α-LIPOIC ACID ACT AS AN ANTIDOTE FOR NEUROPOISONING?

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Occupation related toxic neuropathy monitoring has so far received little attention, mostly due to its intricate pathophysiology. In the present study, DL-α-Lipoic acid, a reputed dithiol (35mg/kg b.wt/day, ip) was used to mitigate the toxic effects of two occupational pollutants namely, mercury (1mg/kg b.wt/day, im) and acrylamide (35mg/kg b.wt/day, ip) in rat models. Prophylactic and curative therapies with lipoate were designed for 10 days against both the toxins. Release of biogenic amines is governed by the ionic regulation of the neurons. Therefore, the ascent in the levels of biogenic amines in the nervous tissue could be a possible explanation. Acetylcholine esterase activity was also reduced in both the toxic groups. The urinary excretion of 5-hydroxy indole acetic acid (5-HIAA) showed a rise during mercury toxicity and vice versa during acrylamide poisoning. The degree of perturbances seen accentuates the severity and specificity of acrylamide to mercury induced neurotoxicity. Adverse changes in the levels of biogenic amines, urinary 5-HIAA and activity of acetylcholine esterase were reversed to near normal levels on lipoic acid administration. Redemption of lipoic acid from mercury was found to be more distinguished compared to acrylamide poisoning.

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Kinetic and X-ray Crystallographic Studies on the Interaction of the Anti-Alzheimer Drug, ENA-713, with Acetylcholinesterase

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(+)S-*N*-ethyl-3-[(1-dimethylamino)ethyl]-*N*-methylphenylcarbamate (ENA-713; Exelon™), a miotine derivative, inhibits acetylcholinesterase (AChE) both *in vivo* and *in vitro*. The carbamate ENA-713 is undergoing clinical trails for Alzheimer disease treatment because it has long lasting activity *in vivo*, and preferentially inhibits AChE of the brain cortex and hippocampus. In order to understand the basic mechanism of the interaction of ENA-713 with AChE we studied its reaction with *Torpedo californica* (*Tc*) AChE and human (h) AChE *in vitro* by kinetic and X-ray crystallography methods. ENA-713 slowly (k_i = 6 $M^{-1}min^{-1}$) carbamylated *Tc*AChE; whereas the reaction rate for hAChE under the same conditions was 540-fold higher (k_i = 3257 $M^{-1}min^{-1}$). The kinetics of reactivation were slow and complex, and displayed a substantial irreversible component using gel filtration, oximes, or dialysis techniques. Irreversible inhibition probably depended upon the reaction product 3-[(1-di-methylamino)ethyl]phenol (NAP), because, in contrast to enzyme inhibited by ENA-713, AChE inhibited by ethylmethylcarbamyl chloride underwent relatively rapid decarbamylation with a $t_{1/2}$ of 26 or 4 min for *Tc*AChE or hAChE, respectively. NAP was a competitive reversible inhibitor of *Tc*AChE (K_i = 0.5 μM) and of hAChE (K_i = 36 μM), and bound much more tightly to AChE than did the intact carbamate in the reversible complex with *Tc*AChE (K_i = 200 μM). The 3D structure of trigonal *Tc*AChE crystals inhibited with ENA-713 was solved and refined (2.2 Å resolution). The refinement showed a binary complex of the ethylmethylcarbamylated enzyme and of NAP bound noncovalently in the active-site. The structure also revealed an unexpected movement of the active site histidine (H440) away from its normal H-bond partner, E327. Thus, we conclude that the irreversible inhibition by ENA-713 displays a novel mechanism that involves the leaving group, NAP, and results in disruption of the catalytic triad.

PRIMARY TRANSMISSION OF FELINE AND BOVINE SPONGIFORM ENCEPHALOPATHIES INTO MICE: COMPARATIVE ANALYSIS OF SPONGIFORM LESION AND PRP ACCUMULATION IN THE INFECTED BRAINS

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Transmissible spongiform encephalopathies (TSEs) or prion diseases are associated with the accumulation of abnormal prion protein (PrPSc) in the central nervous system of diseased individuals. Experimental transmission of natural TSEs into mice allows the study of the pathological processes involved in these diseases (of which the accumulation of PrPSc) and make possible the characterization of the strain of TSE agent involved in the disease. Three different cases of French bovine spongiform encephalopathy (BSE) compared to one case of feline TSE (FSE) from a cheetah were used for transmission studies to mice. Thirty to fifty C57Bl/6 mice were given an intracerebral injection of a 10% brain homogenate (BSE or FSE). The mice started to show symptoms of neurological disorders after 378 days on average for BSE and 302 days for FSE. The shape of survival curves are similar in each case but FSE inoculated mice were dying earlier. Spongiform lesions are studied by an image analysis method and abnormal PrP is investigated by western blot method. interestingly, while abnormal isoform of PrP was detectable in every FSE mice, only 57% of BSE-mice presents detectable levels of abnormal PrP. In the case of undetectable accumulation of abnormal PrP the mice brain were used to infect a second group of mice in which abnormal prion protein became detectable.

INTERHEMISPHERIC TRANSFER IN A CASE OF CHILDREN WITH CALLOSAL AGENESIS: SPATIAL INFORMATION IN THE TACTILE-KINAESTHETIC MODALITY, AND MOTOR TRANSFER

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Interhemispheric transfer difficulties in callosal agenesis appear to be related to motor control and spatial skills (Silver and Jeeves, 1991). We studied spatial information transfer in the tactile-kinaesthetic modality using tasks involving proximal space or subject body space (shape-orientation recognition tasks, a localization task, and a mental rotation task) and two motor tasks (a bimanual task and a motor learning task). The spatial tasks involved the distal parts of the body (fingers), so information reached the contralateral hemisphere. We hypothesized interhemispheric transfer difficulties and a simultaneous bilateral situation advantage in simple tasks. The subject was a non-right-handed, 11-year-old boy. A scan showed complete agenesis of the corpus callosum. The results indicated slightly impaired interhemispheric transfer but only on some tasks: (1) For the spatial tasks, there was a significant transfer deficit in the shape-orientation matching task compared to the intramanual situation, and asymmetry favoring the right hand. In tactile stimulation of the body (single lateralized or simultaneous bilateral), the subject's results were similar to those reported for split-brain patients in literacy tasks and many mirror responses. In localization of an object in proximal space, he failed to show impaired transfer. Response time on the mental rotation task provided evidence of transfer difficulties, but the mental rotation score did not. (2) The motor tasks indicated difficulty in bimanual coordination with the left hand in particular, but the sequential motor learning task failed to show interhemispheric differences between LH/RH and RH/LH transfers. The results are discussed with regard to both hypotheses: a transfer deficit and an advantage in simultaneous bilateral tasks. We suggest a compensation mechanism

THE CIRCUITS LINKING THE MIDLINE THALAMUS WITH THE CIRCADIAN TIMING SYSTEM

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With the aim of unraveling whether the midline thalamus is involved in the processing of photic and circadian information, we investigated with tract tracing the connections of the midline thalamus, and in particular the thalamic paraventricular nucleus (PVT), with the circadian timing system in the rat at the light and electronic microscopic levels. In our study we wished: *i*) to identify retinal ganglion cells projecting to PVT; *ii*) to investigate the PVT connections with the suprachiasmatic nucleus of the hypothalamus (SCN) and the thalamic intergeniculate leaflet (IGL), which are main relay centers of the circadian timing system; *iii*) to determine whether the information processed in the SCN has direct access to the limbic system through a relay in PVT. With retrograde tracing, the anterior portion of PVT was found to receive direct input from a limited number of ganglion cells in the peripheral retina, whereas input to the posterior PVT derived predominantly from the central retina. The IGL was found to project mainly to the posterior PVT. Anterograde and retrograde tracing revealed that the SCN innervates the entire extent of PVT. After anterograde tracing of SCN efferents combined with retrograde tracing of thalamo-amygdaloid neurons, many terminal-like puncta labeled from the SCN were seen close to PVT cell bodies labeled from the amygdala. Ultrastructural investigation demonstrated that SCN terminals established synaptic contacts with dendrites of some PVT neurons projecting to the amygdala. These data demonstrate that PVT receives photic input directly from the retina and indirectly through the SCN and the IGL. In addition, these findings indicate that the midline thalamus may represent a crucial site in the transfer of circadian and photic information to the limbic system.

STRUCTURAL CHANGES IN THE THALAMUS IN CHRONIC LIMBIC EPILEPSY IN THE RAT

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Systemic injection of the cholinergic muscarinic agonist pilocarpine elicits chronic recurrent seizures in rats. On the basis of the wealth of literature data pointing to the involvement of the thalamus in epilepsy, we investigated the thalamic changes occurring in this model of chronic epilepsy. We examined adult rats surviving up to one year after the initial pilocarpine-induced status epilepticus, followed by a seizure-free interval of about two weeks, and then by spontaneous recurrent generalized seizures. The study was based on Nissl staining, immunohistochemical visualization of the calcium binding proteins parvalbumin, calbindin and calretinin, and of the astrocyte marker glial fibrillary acidic protein. The expression of nitric oxide synthase (NOS) was investigated with immunohistochemistry and NADPH-diaphorase histochemistry. Focal areas of degeneration and cell loss were detected in the thalamic reticular nucleus of the chronic epileptic animals, and especially in the anterodorsal pole of the nucleus, which represents the "limbic" portion of this structure. Gliosis, and loss of calbindin- or calretinin-immunoreactive neurons were well evident in medial thalamic domains. In contrast, the density of NOS-positive neurons was unchanged or increased in the thalamic midline. These data point out a selective damage of thalamic cell populations in the pilocarpine model of chronic epilepsy, raising questions on the role played by thalamic circuits in epileptic phenomena.

SIMULATION OF THE BACK-PROPAGATING ACTION POTENTIAL IN LAYER 5 PYRAMIDAL NEURONS.

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Layer 5 (L5) pyramidal neurons of the rat neocortex have a complex dendritic tree that contains several voltage-gated channels. These channels modulate synaptic input, dendritic spikes, and back-propagation of axonally initiated action-potentials into the dendritic tree. Experimental investigations of these neurons were assisted by numerical simulations. These simulations were, however, based on incomplete description of the voltage-gated channels expressed in these neurons. We present here a new model of L5 neocortical pyramidal neurons. The model was based on a new description of the kinetics of voltage-gated potassium channels form L5 pyramidal neurons (Korngreen, A., S. Bergling & B. Sakmann. Biophysical Journal 76, A327, 1999), on the spatial distribution of these channels along the apical dendrite, on recent kinetic data obtained from the literature, and on morphological reconstruction. The model reproduced the shape of the action potential as it propagated back into the dendritic tree. Furthermore, it was possible to simulate dendritic sodium and calcium spikes. It was found that dendritic potassium channels, along the apical trunk, did not effect the amplitude of the back-propagating action potential. The simulations are presented in comparison to experimental data.

THE STUDY OF A POTENTIALLY SIGNIFICANT PROLINE-SPECIFIC PEPTIDASE FROM HUMAN AND BOVINE SERUM

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Accumulated evidence appears to suggest that prolyl oligopeptidase (EC 3.4.21.26) is implicated in the neuropathogenesis of Alzheimer's Disease, specifically through its role in the processing of the amyloid precursor protein. Reduced levels of activity have been frequently reported in AD patients and attributed to a generalised process of neurodegeneration. However, we question the validity of assigning such attributes to PO alone, based on the identification of a new proline-specific peptidase. Using the highly sensitive and reportedly specific fluorimetric substrate for PO, namely Z-Gly-Pro-NH-Mec, we have recently reported the detection and initial characterisation of a second and distinct Z-Gly-Pro-NH-Mec hydrolysing peptidase in bovine and human serum. This second activity is completely insensitive to the classical PO inhibitors; Z-Pro-prolinal and Fmoc-Ala-Pro-nitrile, and has thus been designated by us as Z-Pro-prolinal-Insensitive-Peptidase (ZIP). Activity represents 40% of the apparent PO levels in human serum. Both hydrophobic interaction and cation-exchange chromatography have successfully separated the two Z-Gly-Pro-NH-Mec degrading enzymes. ZIP has been further purified to homogeneity and preliminary investigation of bovine ZIP by HPLC analysis indicates its ability to cleave a number of bioactive neuropeptides. Future work aims at identifying a possible link between ZIP and the amyloid A4 peptide$_{1-42}$. The very presence of this enzyme questions the significance of the role played by prolyl oligopeptidase alone in neurodegenerative disorders such as Huntington's, Parkinson's and Alzheimer's Diseases.

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FALL-OFFS IN PERCEPTUAL PERFORMANCE AT RELATIVELY 'EASIER' DEMAND-PAYOFF FOR 'EFFORT PERFORMANCE' ON 'HARDER' TASK

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Evidence accumulated on lack of attention as cause for errors & limitation in attention resources as performance bottleneck. In this context we studied (12 subjects, Ss) heavy tasking identification of incomplete geometric forms, in Ss' brief in experience with the stimuli. Ss had to make 2-AFC on 'confusion stimuli' that at sites nodal to decision, had bilaterally missing segments inductive of illusory contours, to promote completion mechanisms. The "Stimulus pair", one frame different in the illusory contours, in 'pent' slanted in 'hex' straight line, were computer presented in equal # per experiment, orientations (upright Vs. rotated), display time (100, 160, 200, 260, 300, 360 ms), distance, 1.25 & 2.5 m, 5-9 acuity demand grades, 40 (hyperacuity) – 890 arcsec in terms of subtended tilt in 'pent'. Head angles & side arms' were equalized, to prevent additional cues. Four hybrid paradigms were used: uA (u=upright; A=local viewing), uB (B=global viewing), rA (r=rotated 10 deg right or left), rB. The display was in iterative bundle randomization to confound learning (item size n=6, n=12). Results show performance fall-offs at 'easy' ($P<0.01$); errors – non-specific mental blink, & mental lapse, either one, heavy-tasking-based deviations from log-linearity, identifiable with mismatches between 'observed' & mathematically 'expected' ($P<0.01$). It is proposed that higher error rates at 'easy' may be relatable ($P<0.01$) to attention/effort 'lapses'; a significant linkage ($P\leq0.001$) is shown between this and a seemingly preferred allocation of resources to the 'harder', at expense of the 'easier' task.

CEREBROVASCULAR EFFECTS OF CHRONIC ANTIHIPERTENSIVE TREATMENT WITH PERINDOPRIL IN HYPERTENSIVE RATS

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It is well know, that chronic hypertension causes the lower and upper limits of cerebral blood flow (CBF) autoregulation to reset to higher blood pressure levels. In the present study we examined whether a moderate lowering of blood pressure, following perindopril administration in renal hypertensive (RHR) and spontaneously hypertensive rats (SHR) would influence CBF autoregulation. *Methods.* Perindopril was administered daily (10 mg/kg, p.o.) for 4 weeks in RHR and SHR rats. The tail systolic pressure was measured weekly in the treated groups and in a control groups (RHR and SHR rats). At the and of the treatment period CBF autoregulation was studied. CBF was determined using the intracarotid 133-xenon injection method in halothane/nitrous oxide anaesthetised animals. CBF autoregulation was tested as follows: in one subgroup blood pressure was lowered stepwise by means of controlled bleeding in order to determine the lower limit of autoregulation (LLA), and in a second subgroup was raised stepwise by controlled norepinephrine infusion in order to determine the upper limit of autoregulation (ULA). *Results.* In the perindopril treated RHR and SHR groups, blood pressure significantly decreased, in the control RHR and SHR groups blood pressure increased slightly. In RHR rats, chronic antihypertensive treatment with perindopril lowered blood pressure to the same level as in normotensive rats and restored the LLA of CBF to normal. In SHR rats the lower parts of the autoregulation curve was shifted towards lower pressure in the treated group, although the LLA was no significantly different from that in controls. The ULA was above 180 mmHg in all groups. It seems, that the different effects of perindopril on LLA in RHR and SHR rats depend on antyhypertensive activity (angiotensin converting enzyme inhibitor).

MUTANT HUMAN PRESENILIN-1 GENE SENSITIZES PC12 CELLS TO APOPTOSIS

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Effects of nerve growth factor withdrawal and oxidative stress induced by hydrogen peroxide on apoptosis were studied in neuronal differenciated rat pheochromocytoma PC12 cells. Fore cultures have been analyzed: parent PC12 cells and three stable transfected polyclonal PC12 cell cultures: with pcDNA3 vector, with pcDNA3 carrying wild type human presenilin-1 (PS1) cDNA, and with pcDNA3 containing the same fragment of human PS1 with point mutation in 12-th exon, which couses Alzheimer's diseas. The presence of human PS1 in transfected cells was detected by PCR analyses and PS1 expression - by Nothern blotting. The cell culture, expressed mutant human presenilin-1 showed increased susceptibility to hydrogenperoxide as compared with other cultures without transfected genes or transfected with normal human presenilin-1 gene. It is suggested that presenilin-1 mutation in autosomal dominant early onset Alzheimer's disease may induce similar changes in neurons increasing their susceptibility to apoptotic stimuli.

NEUROANATOMICAL APPROVAL OF THE ASCENDING THERMOREGULATORY PATHWAY FROM BRAINSTEM IN RATS

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The central regulatory area for thermoregulation is located in the medial preoptic nucleus (POM) in the rostral hypothalamus. Since there are no direct peripheral inputs, the signal reaches the POM via pathways through the medulla and pons. It is known that the following areas respond to cold stress: in the medulla:

1. peritrigeminal nucleus
2. paratrigeminal nucleus in the pons:
3. lateral parabrachial nucleus
4. pontine thermosensitive area in the hypothalamus:
5. 5. medial preoptic nucleus

The previous trials do not give description concerning a direct connection between the hypothalamus and the brainstem thermosensitive nuclei. Among the above mentioned areas we studied the possible direct connection between the peritrigeminal nucleus and the POM. We used a combination of anterograde neuronal tracing and c-fos immunocytochemistry (a known marker of neuronal activation) in rats.Following PHA-L injection into the peritrigeminal nucleus PHA-L positive fibers were found in the area of the medial preoptic nucleus, ipsilateral to the injection site. Terminals of these fibers make synaptic contacts with POM cells.In case of the combination of PHA-L injection and 3 hour-long cold stress the double labelling showed that the fibers containing PHA-L, originating in the thermosensitive cells of the peritrigeminal nucleus, engulf the c-fos positive cells of the medial preoptic area.

ANTISENSE APPROACH FOR AMELIORATING NEUROMUSCULAR IMPAIRMENTS IN RODENT ANIMAL MODELS.

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Myasthenia Gravis (MG) is an autoimmune disease caused by autoantibody-mediated attack at the neuromuscular junction (NMJ) acetylcholine receptor. NMJ dysfunction can be transiently alleviated by carbamate acetylcholinesterase (AChE) inhibitors (i.e. pyridostigmine). However, anti-AChE treatment is short-lasting and does not slow the disease progression. Moreover, both stress and AChE inhibitors have recently been shown to elicit pronounced and persistent neuronal overexpression of AChE. Considering that activation of the AChE feedback loop may aggravate both the symptoms and progression of MG, we studied muscle AChE expression in rats with experimental autoimmune MG (EAMG). Naive EAMG rats displayed elevated levels of muscle AChE in a manner correlated with the severity of disease. Furthermore, in transgenic mice overexpressing AChE, chronic AChE excess causes in transgenic mice, progressive neuromotor deterioration. The pathological muscle fatigue was indicated by decrement response to repetitive nerve stimulation (RNS), similar to that observed in MG and EAMG. Intravenous administration of antisense oligodeoxynucleotides (AS-ODNs) suppressing AChE biosynthesis to the EAMG rats and to 5 month old transgenic mice restored normal responses to RNS and improved the overall well-being of the animals for up to 3 days, in a sequence and dose dependent manner. These data suggest a previously unrecognized role for overexpressed AChE in EAMG and demonstrate long-lasting therapeutic activity for AS-ODNs against AChEmRNA.

PERSYN IN DEVELOPING, AGEING AND DEGENERATING NERVOUS SYSTEM

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Synucleins comprise a group of unique small intracellular proteinsexpressed in the nervous system and localised in synaptic terminals. Alpha-synuclein has been implicated in at least one form of learning and memory and is believed to be involved in the pathogenesis of Alzheimer's disease and familial Parkinson's disease. However, the functions of synucleins remain elusive. We cloned mouse, human and chicken genes encoding persyn, a new member of the synuclein family. Although amino acid sequence and some physico-chemical properties of persyn and other synucleins are very similar there are substantial differences in their expression patterns. Persyn displays distinctive patterns of expression in subsets of neurons and their axons in both the developing and ageing nervous system. Furthermore it was shown that persyn is concentrated in cortical axonal lesions seen in neurodegenerative conditions. Overexpression of persyn in cultured sensory neurons by the microinjection of an expression plasmid into nuclei of these cells showed that persyn is involved in regulating the integrity of the neurofilament protein network. These findings suggest that persyn plays a role in modulating axonal architecture in the developing and mature nervous system and implicate persyn in the axonal pathology of degenerative conditions. Interestingly, persyn expression was also found in skin epidermis and in several infiltrating breast tumours, suggesting the involvement of persyn in modulating not only neurofilament, but also keratin network. Further functional studies of persyn involves generation of mice with targeted deletion of persyn gene. Supported by Grants from The Wellcome Trust and The Royal Society.

LOSS OF GLYCINERGIC INHIBITION REVEALS PRINCIPLES OF RESPIRATORY RHYTHM GENERATION.

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The neuronal network within the pre-Bötzinger complex generates the respiratory rhythm. This rhythm depends on synaptic inhibition between different types of respiratory neurones (pre-inspiratory, early-inspiratory, augmenting-inspiratory, late-inspiratory, post-inspiratory and expiratory neurones). To test the consequences of a specific loss of glycinergic inhibition, wild type and oscillator mice (spd^{ot}/spd^{ot}; P17 - P21) were decerebrated at the pre-collicular level under halothane anaesthesia and the brainstem was perfused intra-arterially with artificial cerebrospinal fluid. Respiratory activity recorded from a phrenic nerve was rectified and integrated. Membrane potentials of respiratory neurones were recorded with intracellular microelectrodes (40-80 MΩ). Strychnine (0.03-0.3 μM; Jonas et al., 1998) was added to the perfusate for specific block of glycine receptors. The three phase respiratory cycle (inspiration, post-inspiration and expiration) was reduced to a two phase cycle (inspiration, expiration). Simultaneously the discharge activity of post-inspiratory neurones was shifted towards the inspiratory phase, ramp-inspiratory neurones had prolonged (apneustic) bursts and pre-inspiratory neurones discharged in the expiratory, as well as in the inspiratory phase. While pre-inspiratory, late-inspiratory and post-inspiratory neurones are disconnected from the network, a kernel structure is left. Under these conditions early-inspiratory burster neurones start to pace the core network. Synaptic interaction within the network dominates under normal *in vivo* situations and glycinergic inhibition plays a key function. Genetic or acute loss of inhibitory glycine receptors, triggers a fundamental self-reorganisation of the network to retain its capacity for rhythm generation. Therefore, respiratory rhythm generation in mammals results from network interactions or pacemaker neurones depending on the efficacy of synaptic inhibition. The use of transgenic animals elucidated this process of self-reorganisation to a primordial network structure and the principle mechanisms of rhythm generation that control breathing under normal conditions.

Jonas P., Bischofberger J., Sandkühler J. *Science* 281: 419-424
Oscillator mice were bred at the Biochemistry Institute at the University of Erlangen (C.M. Becker). Supported by DFG.

CA-DEPENDENT K-CHANNELS ARE THE SENSITIVE TARGETS FOR PIRACETAM AND ITS NOVEL PEPTIDE ANALOGUE, GVS-111

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A question of interest in the field of investigation of the mechanisms of action of the nootropic drugs is their possible interaction with potential-operated ionic channels of neuronal membranes (for review, see Beneshova, 1994; Gouliaev et al, 1994). In the present work, we studied the effects of piracetam and its novel peptide analogue, ethyl ester of N-phenyl-acetyl-L-prolyl-glycine (GVS-111), on the high threshold Ca-current and high threshold K-currents of neuronal membrane. GVS-111 was designed and synthesized at the Institute of Pharmacology of the Russian Academy of Medical Sciences (Gudasheva et al., 1996). This compound was found to be four-five orders stronger as a cognitive enhancer than the prototype. Threshold doses for GVS-111 and piracetam in behavioral experiments were revealed to be 0.1-0.5 mg/kg and 200-300 mg/kg, accordingly (Ostrovskaya et al., 1994; Seredenin et al., 1995). The experiments of present work were conducted on the isolated neurones of land snail Helix. Using two microelectrodes voltage clamp method, high threshold Ca-current (I.Ca) and three types of high threshold K-current were recorded: 1) Ca-dependent K-current (I.K(Ca)), 2) delayed rectifier K-current (I.KD), 3) high threshold A-type of K-current (I.Ahth). It was found that all four types of ionic current were inhibited by both piracetam and GVS-111. The effective concentrations were 0.1-2 mM for piracetam and 0.001-2 mkM for GVS-111. The nootropics inhibited Ca-current and K-currents with different efficacy: the I.Ca suppression required an in order higher drugs concentration than K-current inhibition. Nootropics effects on K-currents depended on the K-current type. The strongest inhibition was observed on I.K(Ca) (40%-90%), and the weakest - on I.Ahth (0%-30%). This mode of action was the same for both piracetam and GVS-111 although concentrations required for GVS-111 were three-four orders less than those for piracetam. A downregulation of I.K(Ca) by nootropic drugs cannot be explained by the inhibition of I.Ca because no correlation was found between nootropics effects on I.K(Ca) and on I.Ca registrated in the same cells. The results suggest that the mechanisms of pharmacological effects of piracetam and GVS-111 are similar and include the I.K(Ca)-channels blockade. This work was supported by a grant (No. 98-04-48904) from Russian Fund for Fundamental Investigation.

NEUROPHARMACOLOGICAL PROFILE OF *AMBROSIA PANICULATA* (WILLD.) O.E. SCHULZ (MUG-WORT).

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In previous works the acute administration of the aqueous exctract the dry leaves of *A. paniculata* shown anticonvulsant effect in the penicillin focus and electroshock models. However, it is unknown the general neuropharmacological profile of the plant. The aim of the present work is to examine the acute administration of the decoctions, at different doses, of the dry leaves, on the Irwin test, exploratory behaviour and the analgesic tests in mice and on stereotypes induced by anfetamine in rats. The decoctions produced an increase of the passivity and sporadics contorsion in the Irwin test. The exploratory behaviour was significantly reduced. The decoctions were not effective on the hot plate test and stereotypes induced by anfetamine. Moreover, they shown an analgesic effect on the writhing test induced by acetic acid in a dose-related manner, compared with the saline group. Therefore, the most important result obtained with the aqueous extract of *A. paniculata* was the peripherical analgesic activity found.

NEUROPHARMACOLOGICAL PROFILE OF *ECHINODORUS BERTEROI* (SPRENG.) FASSET VAR. BERTEROI (WATER PLANTAIN).

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In previous works the acute administration of the decoction of the dry roots of *E. echinodorus,* significantly decreased the spike amplitude induced by topical application of penicillin, in the sensory motor cortex of the curarized rats. However, it is unknown the general neuropharmacological profile of the plant. The aim of the present work is to evaluate the acute administration of the decoction , at different doses, of the dry roots, on the Irwin test, exploratory behaviour and stereotypes induced by anfetamine in mice, and on the analgesic tests in rats. The decoctions produced an increase of the passivity and an attenuation of the pain response in the Irwin test. Moreover, it was observed a muscular relaxation. The highest doses produced a significant diminish of the exploratory behaviour, and the lowest one a decrease in the stereotypes induced by anfetamine. The decoctions produced an attenuation of the nociceptive response in the acetic acid test. The results shown that the decoctions of *E. berteroi* have sedative, peripherical analgesic and neuroleptic effects.

NEUROPSYCHOLOGY AND THE CLINICAL CASE STUDY

PSIC. GLADYS C. DE CAROZZO

This theorical study reviews the topics mentioned above in relation to one another and some whirling developments in sciences. Neuropsychology (N) is based on Neuroscience which receive contributions from multiple disciplines (Nuclear Physics and Imaging Technics (SPECT), Neuropsychological Tests among others and finds its aplication in context of the Clinical Case Study (C.C.S.) which has been enriched with Postmodernity promotions through its holistic, contextualized and echological approaches. The C.C.S. is a systematic procedure to obtain information about the patient in order to elaborate adequately the formulations of hypothesis and Clinical inferences to get to a descriptive diagnosis whose aim is to plan the best possible therapeutical help for the patient (pharmacological or other kind) Interdisciplinary interrelations are discussed as well. We have developed the following topics: 1. Revision and definition of the C.C.S. and N. 2. Postmodernity contributions to the methodology of the C.C.S. and N. 3. Diagnostic Process and Following Conclusions are brought to discussion: a) Multidisciplinary approaches are very important in N. to obtain a diagnosis when using the C.C.S. b) Interdisciplinary interrelations are fundamental to plan therapeutic interventions. c) The conjunction of Neuroscience contributions as well as those of Postmodernity innovating the Clinical Method and its C.C.S. demands a critical revision of Theorical statements in N.

GLUTAMATE-TRIGGERED EVENTS INDUCING CORTICOSTRIATAL LTD

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Repetitive activation of corticostriatal fibers produces long-term depression (LTD) of excitatory synaptic potentials recorded from striatal spiny neurons. In the present study, intracellular recordings were performed from rat corticostriatal slice preparations to study the role of glutamate and other critical factors underlying striatal LTD. In current-clamp, but not in voltage-clamp experiments, brief applications of glutamate, as well as high-frequency stimulation (HFS) of corticostriatal fibers, induced LTD. The glutamate-induced and the HFS-induced LTDs were mutually occlusive, suggesting that they both share common induction mechanisms. Isolated activation of either nonNMDA-ionotropic glutamate receptors (iGluRs) or metabotropic glutamate receptors (mGluRs) respectively by AMPA and *t*-ACPD failed to produce LTD. Conversely, LTD was obtained following the simultaneous application of AMPA plus *t*-ACPD or quisqualate alone. Electrical depolarization of the recorded neuron either alone or in the presence of *t*-ACPD and DA failed to induce LTD; however, it was able to produce LTD when preceded by co-administration of *t*-ACPD, DA and low-dose of hydroxylamine, a NO-generating drug. None of these compounds alone produced LTD. Glutamate-induced LTD, as well as the HFS-induced LTD, was blocked by L-sulpiride, a D2 dopamine (DA) receptor antagonist, and by 7-NINA, a NO synthase inhibitor. The present study indicates that four main factors are required to induce corticostriatal LTD: i) membrane depolarization of the postsynaptic neuron; ii) activation of mGluRs; iii) activation of DA receptors and iv) release of NO from striatal interneurons.

PARTICIPATION OF SPINAL METABOTROPIC GLUTAMATE RECEPTORS IN THE REGULATION OF THE BLOOD PRESSURE IN ANAESTHETIZED RATS.

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It has been reported that sympathoexcitatory cardiovascular responses induced by excitatory amino acids in the spinal cord involve the activation of ionotropic glutamate receptors. The aim of the present study was to evaluate the participation of spinal metabotropic glutamate receptors (mGluRs) in the regulation of the cardiovascular function. For that purpose it was analized whether the activation of spinal Group 1 and Group 2 mGluRs elicited cardiovascular responses in anaesthetized rats. Female Wistar rats (220-240g) were anaesthetized with sodium pentobarbital (40mg/kg, i.p.). A femoral artery was cannulated for recording of the blood pressure (BP). A PE-10 catheter was placed in the spinal subarachnoid space (T_{12}-L_1) for intrathecal injection of drugs. The resting mean BP (MBP) was 103.9 $\pm$2.2 mmHg and the heart rate (HR) was 420$\pm$6 beats/min (n=69). Intrathecal injection of saline (10µl) did not modify either the MBP (ΔMBP 1.9$\pm$1.6 mmHg; n=8) or the HR (ΔHR -3+3 beats/min; n=8). The Group 1/Group 2 mGluR agonist *trans*-ACPD (300 nmol; n=7) and the selective Group 1 mGluR agonist, 3,5-DHPG (300 nmol; n=8), induced increases in the MBP (ΔMBP 19.2$\pm$2.1 mmHg and 16.0$\pm$2.9 mmHg, respectively; $p<0.005$ compared with saline). The pressor response to *trans*-ACPD was antagonized by the Group 1 mGluR antagonists L-AP3 (300 nmol; ΔMBP 4.3$\pm$1.9mmHg; n=5) and CPG (300 nmol; ΔMBP 7.6$\pm$4.2 mmHg; n=9). CPG also prevented the pressor response induced by 3,5-DHPG (ΔMBP 4.3$\pm$3.1 mmHg; n=5). The Group 2 mGluR agonist DCG-IV (1.5 nmol) did not modify the MBP in control animals (ΔMBP 8.9$\pm$3.6 mmHg; n=16) but induced a hypotensive response (ΔMBP -16.0$\pm$3.9 mmHg; n=6; $p<0.005$ compared with saline) when the stimulant action of the drug on NMDA receptors was blocked by 30 nmol APV. The hypotensive effect of DCG-IV was abolished by the Group 2 mGluR antagonist MCCG (50 nmol; ΔMBP 4.0$\pm$2.2 mmHg; n=5). It is suggested that activation of Group 1 mGluRs in the spinal cord enhances preganglionic sympathetic nerve activity related to the control of the BP whereas spinal Group 2 mGluRs mediates inhibitory effects. Supported by Grant 4041/96 CONICET.

AN INSULIN-DEPENDENT HYPOGLYCEMIA INDUCED BY ELECTROACUPUNCTURE AT THE ZHONGWAN (CV12) ACUPOINT IN DIABETIC RATS

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Acupuncture at the Zhongwan acupoint has been widely used in traditional Chinese medicine to relieve symptoms of *diabetes mellitus*. The present study investigated the effect on plasma glucose of electroacupuncture (EA), applied at the Zhongwan acupoint in rats with diabetic models. Also, plasma levels of insulin, glucagon and β-endorphin were determined using radioimmunoassay (RIA). A decrease of plasma glucose was observed in rats after an application of EA (15 Hz, 10 mA) for 30 min at the Zhongwan acupoint. Lowering of plasma glucose by this EA stimulation was observed in normal rats and the rats with non-insulin dependent diabetes (NIDDM)-like model. However, no significant effect on plasma glucose was observed in rats with insulin dependent diabetes (IDDM)-like model; either the streptozotocin (STZ)-induced diabetic rats or the genetic one (BB/W rats). Also, the hypoglycemic action of EA stimulation was disappeared in rats with insulin-resistance that was induced by a daily repeated injection of human long-acting insulin to result in the loss of tolbutamide-induced hypoglycemia. An insulin-related action can thus be considered. Increase of plasma insulin-like immunoreactivity after EA stimulation in normal rats supported this view in addition. Otherwise, no change of plasma glucagon-like immunoreactivity by this EA stimulation ruled out the participation of glucagon. In addition to an elevation of plasma β-endorphin-like immunoreactivity by this EA stimulation, plasma glucose lowering action of EA stimulation at Zhongwan acupoint was abolished by naloxone at dose sufficient to block opioid receptors. We thus suggest that EA stimulation at Zhongwan acupoint induced secretion of endogenous β-endorphin to reduce plasma glucose level via an insulin-dependent manner.

POLYADP-RIBOSYLATION: A NOVEL TARGET FOR SIGNAL TRANSDUCTION MECHANISMS IN BRAIN CORTICAL NEURONS.

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We present the first evidence for activation of polyADP-ribose-polymerase (PARP) by signals evoked in the cell membrane. PARP is an abundant nuclear protein in eukaryotes, that exclusively catalyzes polyADP-ribosylation of DNA-binding proteins, and thereby modulates their activity. PolyADP-ribosylation is involved in regulation of DNA repair, transcription and replication. Activation of PARP is reportedly induced by DNA-nicks formation. Our recent experiments indicate, however, that activation of PARP is instantaneously evoked in cortical neurons and cardiomyocytes under physiological conditions. No DNA-damage involved. Thus, depolarizing stimulations of brain cortical neurons or treatment by nerve growth factor induce within minutes PARP activation and polyADP-ribosylation of one of its substrates, topoisomerase I, mediated by activation of phospholipase C and inositol 1,4,5,-trisphosphate (IP_3)-dependent Ca^{+2}-release. This implies that either membrane depolarization or stimulation of seven trans-membrane-domain receptors, activating Go-, Gi-, Gq- trimeric G-proteins and the activation of receptor tyrosine-kinases may induce PARP activation in brain cortical neurons, and thereby modulate the activity of transcription factors, RNA-polymerases, topoisomerases, histons, or DNA-repair enzymes. PARP activation is therefore a novel target for signaling by inositides enabling fast regulation of DNA repair and transcription in cortical neurons via polyADP-ribosylation. These findings imply a vital role of depolarizing stimulation in preserving the viability of cortical neurons.

METABOLIC EFFECTS OF DIAZEPAM IN STREPTOZOCIN-INDUCED DIABETIC RATS

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Previous studies suggest that benzodiazepines acting on central or peripheral receptors may modify the carbohydrate or lipid metabolism. Low doses (2.5 mg/kg body wt) could stimulate the central GABA receptor and result in hypoglycemia. Higher doses are needed to stimulate the peripheral GABA receptors and the effect is the decrease in plasma lipids. In our study, we assessed the effect of Diazepam a dministration (10 mg/kg body wt) on the lipid profile in streptozocin-induced diabetic rats (40 mg/kg body wt i.p.). Our results reveal that total cholesterol levels and LDL-cholesterol levels decreased in diabetic rats treated with Diazepam, comparative to untreated diabetic rats. By contrast, triglycerides levels were unchanged in diabetic conditions with or without diazepam administration. The Diazepam high doses used in our experiment caused the increase of glycemia values in treated animals as compared with untreated diabetic rats. Previous researches (Cuparencu et al., 1991) reported that administration of peripheral GABA receptor antagonists (PK11195) annihilate the effect of Diazepam on lipid metabolism. In conclusion, the efficient Diazepam doses to obtain hypolipidemia have had, in the same time, hyperglycemic action. Nevertheless, the study of peripheral GABA receptors involvement in lipid metabolism can offer interesting pathways in diabetic dyslipidemia therapy.

ACTIVATION OF LARGE CONDUCTANCE K_{Ca} CHANNELS BY SUPEROXIDE ANIONS INDUCES RELAXATION OF THE RAT BASILAR ARTERY

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The present study was undertaken to study the possible involvement of oxygen-derived free radicals and potassium channels on the relaxation elicited by electrical field stimulation (EFS). Basilar arteries were isolated from male Wistar rats (12-week-old), and segments, 2 mm length and 287±32 μm lumen diameter, were mounted in a wire myograph. Each segment was set to a normalized internal circunference equivalent to 90 % of it would have when relaxed in vivo under a transmural pressure of 100 mm Hg. EFS (a single pulse, 0.2 ms, 200 mA) was carried out using two platinum electrodes. EFS caused a rapid relaxation of segments precontracted with 10 μM $PGF_{2\alpha}$ which was abolished when the tone was induced by 30 mM KCl. The EFS-caused relaxation was not modified by endothelium removal or 10 μM TTX (an inhibitor of propagation of nerve impulses). This dilator response was significantly reduced by 50 U/ml superoxide dismutase (a superoxide anion scavenger). Charybdotoxin (ChTx, 0.2 μM), a blocker of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), significantly decreased the relaxation elicited by EFS. However, 1 μM glibenclamide or 1 μM apamine, blockers of K_{ATP} or small-conductance K_{Ca} channels, respectively, did not modify the relaxation to EFS. Thapsigargin (0.01 μM), inhibitor of the Ca^{2+}-ATPasa-mediated Ca^{2+} uptake into the sarcoplasmic reticulum, significantly increased the EFS-evoked relaxation; this increased response was abolished by ChTx. In conclusion, these results show that a single electrical pulse evokes marked endothelium-independent and non-neurogenic relaxations in the rat basilar artery. These myogenic responses appear to involve superoxide anion generation, increase of cytosolic free-Ca^{2+} concentration and subsequent activation of BK_{Ca} channels of smooth muscle cells.

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COMPETITIVE ELIMINATION AT INACTIVE NEUROMUSCULAR SYNAPSES.

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During reinnervation of adult skeletal muscle, fibres are contacted by more than one motor axon. Subsequently, many muscle fibres lose all but one input in a process of synapse elimination that resembles neuromuscular development. Several studies suggest that without activity, inputs are not eliminated. In contrast, a study from our lab has shown that synapse elimination can take place in the absence of evoked neurotransmitter release (R.R. Ribchester, *J. Physiol.* **466**:421-441, 1993). However, in that study spontaneous release was not abolished, and the possibility remained that spontaneous activity contributed to or accounted for the observed synapse elimination. In order to resolve this issue, we have now presented innervated fourth deep lumbrical (4DL) muscles with regenerating axons and abolished all activity. We blocked evoked activity by delivering TTX to the sciatic nerve, and spontaneous activity by daily injections of α-bungarotoxin (2.5μg) over the 4DL. Using the vital styryl dyes FM1-43 and RH414, we detected polyneuronally innervated endplates (96/803 fibres; n=5 muscles) as well as endplates solely supplied by either the regenerating (31/803) or intact (676/803) nerves. These results extend our earlier findings, suggesting that neither strong nor weak endplate depolarisations are required for elimination of multiple inputs. Thus, the timing of transmitter-receptor activation is not the only mechanism by which inputs are removed during neuromuscular synapse elimination. However, the data are consistent with a role for mismatch withdrawal and/or trophic factors during competitive reinnervation of skeletal muscle.

THE SULPHUR-CONTAINING AMINO ACIDS CYSTEIC ACID AND CYSTEINE SULPHINIC ACID INCREASE SYNAPTIC GLUTAMATE RELEASE BY AN ACTION AT PRESYNAPTIC GROUP I METABOTROPIC GLUTAMATE RECEPTORS

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Group I metabotropic glutamate receptors ($mGlu_{1+5}$) are positively coupled to phospholipase C. We have recently shown that presynaptic group I mGlu autoreceptors mediate a positive modulatory control on synaptic glutamate release in the rat forebrain both in vitro (Croucher et al. (1997) The Pharmacologist 39(1): 215) and in vivo (Patel & Croucher (1998) Br. J. Pharmacol. 123: 207P). Recently, certain sulphur-containing amino acids (SCAAs) have also been shown to stimulate phosphoinositide hydrolysis. We now report that the SCAAs L-cysteic acid (CA) and L-cysteine sulphinic acid (CSA) also enhance neuronal glutamate release by activation of presynaptic group I mGlu receptors. Glutamate release was studied using [^{3}H]D-aspartate ([^{3}H]D-asp) as a non-metabolisable marker for glutamate. Serial forebrain slices were cut and incubated as previously described (Patel & Croucher (1997) Eur. J. Pharmacol. 332: 143). After loading with [^{3}H]D-asp slices were superfused with oxygenated Krebs buffer and the influence of drugs on basal and electrically stimulated release of label was examined. Results are means of 3-6 independent observations. CSA, 1-100μM, dose-dependently enhanced electrically stimulated efflux (max. response 28.05-fold enhancement at 100μM; P<0.01) without influencing basal efflux. The mGlu receptor antagonist (±)-MCPG, 200μM, decreased the response to CSA, 10μM, by 78.4% (P<0.05 compared to CSA alone). However, the $mGlu_1$ receptor antagonist AIDA, 100μM, was inactive in this respect. CA, 3-100μM, also significantly potentiated electrically stimulated efflux with a maximum 6.1-fold enhancement seen following CA, 3μM (P<0.01). The somewhat lower responses seen at higher concentrations of CA were cyclothiazide-sensitive and probably resulted from mGlu receptor desensitization. As with CSA, (±)-MCPG, 200μM, significantly inhibited the response to CA, 3μM (79.0% reduction; P<0.05) whilst AIDA, 100μM, was without effect. Interestingly, CA, 1μM, caused a significant *reduction* in electrically stimulated efflux of label (to 39.1% of control values; P<0.01). The inhibition of this response by (±)-MCPG suggests an action at inhibitory presynaptic group II mGlu receptors. The present results demonstrate a predominant positive modulatory action of SCAAs on synaptic glutamate release mediated by activation of presynaptic group I mGlu receptors, probably of the $mGlu_5$ sub-type.

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GENERATION OF TRANSGENIC MICE EXPRESSING SHEEP PrP PROTEIN IN BRAIN TISSUE

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To achieve a better knowledge of spongiform encephalopathies of sheep, we have developed transgenic mice expressing sheep prion protein PrP. We have cloned the complete open reading frame (ORF) of the ovine PrP gene by PCR amplification from genomic DNA of sheep brain. This ORF, with alanine, arginine and glutamine at susceptibility codons (136, 154, 171), has been used for two constructs : one with the total ORF inserted downstream to the neuron specific enolase promoter, the other contains the same promoter but a fourteen amino acid sequence (FLAG peptide) inserted after the sequence of the signal peptide, in order to obtain an amino-terminal tagged protein facilitating immunodetection. These constructs have been microinjected to generate ovine PrP transgenic mice. Six of the seven founders obtained have been able to generate transgenic offspring. Transgenes expression in different tissues have been studied by RT-PCR, western blotting, and immunohistochemical analysis. The sheep prion protein was expressed at different levels depending on the mice lineage. Such mice have been crossed with *Prn-P*$^{-/-}$ mice to obtain transgenic mice devoid of the endogenous murine PrP gene, by crossing them with PrP "knock-out" mice (Ch. Weissmann, Zürich).The infectability of these mice will be assessed. Such mice would provide new tools for detection, characterisation of scrapie strains, and for studying the possible transmission of BSE agent to sheep.

CORRELATIVE ULTRASTRUCTURAL DISTRIBUTION OF A DOPAMINE RECEPTOR-RELATED PHOSPHOPROTEIN (DARPP-32) AND L-GLUTAMATE IN DOPAMINOCEPTIVE REGIONS OF CHICK BRAIN

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The phosphoprotein DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, Mr=32,000) is a marker for neurons expressing D1 dopamine receptor. DARPP-32 is phosphorylated by dopamine/cAMP/PKA and dephosphorylated by Ca^{2+} /calcineurin, enabling interaction with Ca^{2+} dependent signalling by other transmitters such as glutamate. The aim of the present study was to seek ultrastructural evidence for glutamate - dopamine interaction in avian brain regions with a high dopamine input: the medial striatum (lobus parolfactorius, LPO), posterolateral telencephalon, ventral subdivision (PLTv) and hippocampus (Hp). DARPP-32 was studied by preembedding immunocytochemistry on coronal brain slices of 7-day-old domestic chicks. From selected slices, blocks were embedded for EM and ultrathin sections were reacted for glutamate using the postembedding immunogold method. Enrichment of glutamate-like immunoreactivity (Glu-LI) associated with specific axon terminals was ca. 2.5-3 *vs* the rest of tissue, and 3-5 *vs* dendrites. DARPP-32 was primarily associated with somata and dendrites. The immunodeposit was observed in postsynaptic position as well as in extrasynaptic sites. In the LPO, which is characterised by numerous gutamate immunoreactive (Glu+) axon terminals, a relatively small proportion of such terminals was found to synapse with DARPP-32+ dendrites. Ultrastructurally these were similar to other Glu+ synapses in that they formed asymmetric junctions. In the PLTv region DARPP-32 was often observed in medium-sized somata, some of which received Glu+ asymmetric axosomatic synapses. Occasionally, DARPP-32+ perikarya displayed also Glu-LI, though less pronounced than non-DARPP-32+ ones. Such perikarya displaying both Glu-LI and DARPP-32 immunoreactivity were also found in the Hp. These results indicate that glutamatergic transmission may play a role in the postsynaptic regulation of dopamine (predominantly D1) receptive neurons in the avian brain. The findings are relevant to learning mechanisms, since LPO has been implicated in passive avoidance training, Hp is known to participate in spatial memory tasks, and PLTv belongs to those regions that are thought to be potential equivalents of the prefrontal cortex of mammals.

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Effects of oral administration of the competitive *N*-methyl-D-aspartate antagonist, CGP 40116, on passive avoidance, spatial learning, and neuromotor abilities in mice

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The effects were investigated of the potent competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist CGP 40116 [D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid] on the performance of mice in water maze and passive avoidance tasks, and in wire suspension, rotarod and cage activity tests. The drug was administered *per os* (p.o.) in its anticonvulsant dose range. CGP 40116 dose-dependently impaired passive avoidance learning when given before, but not when given after training. The antagonist (5, 10 and 20 mg/kg, administered 4 h before each training session) dose-dependently affected water maze acquisition, and impaired retention test performance in both hidden- and visible-platform water maze tasks. In addition, the drug had a dose-dependently decreased swimming speed during water maze acquisition. Repeated administration of CGP 40116 (20 mg/kg, p.o.) persistently decreased cage activity and wire suspension test performance, whereas motor coordination and equilibrium on the rotarod apparatus remained unimpaired. In our administration protocol, no tolerance was found to the effects of the drug on passive avoidance learning and neuromotor abilities. The parallel effects of CGP 40116 on memory and motor performance are discussed, and it was concluded that the antagonist impairs neuromotor abilities and also induces memory impairments which cannot be entirely reduced to motor interference.

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CO-OCCURRENCE OF TRANSFERRIN C2 AND APO E4 PREDICTS EARLY ONSET ALZHEIMER'S DISEASE

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Several studies have implicated free radical-induced damage as the cause of neurodegeneration in diseases such as Alzheimer's Disease (AD). These free radicals can easily be formed in the body especially when free iron is available. Transferrin is the major iron-binding molecule in the plasma and therefore abnormal iron-binding by transferrin has been proposed to be a possible etiological factor in AD. There are at least 3 variants of transferrin in the body, namely C1, C2 and C3.

The present study investigated the prevalence of transferrin C2 variant in AD. We also investigated the frequency of apolipoprotein E4 as it has also been implicated in AD. Finally, we determined the occurrence of both transferrin C2 and Apo E4 in our AD patient group.

In our study of 27 AD patients and 27 controls, the transferrin C2 allele had a frequency of 24% as opposed to the 13% in controls. The frequency of Apo E4 in the AD group was 44% vs. 17% in controls. Of the 27 AD patients, 8 had both transferrin C2 and Apo E4. It was further observed that the average age of onset of the disease in these patients was significantly earlier than the rest of the patient group i.e. 60.3 vs. 66.9 years.

These findings confirm a significantly high prevalence of both transferrin C2 and Apo E4 in AD respectively. The data furthermore suggest a strong association between the simultaneous occurrence of ApoE4 and transferrin C2 and early onset AD.

EVIDENCE FOR STATE-DEPENDENT REGULATION OF STRIATAL COMT BY MELATONIN

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Catechol-O-methyltransferase (EC 2.1.1.6; COMT) catalyses the O-methylation of catecholamines and their precursors. By regulating dopaminergic neurotransmission, the modulation of COMT activity may have clinical importance in diseases such as Parkinson's. This study investigated the ability of the pineal hormone melatonin (MEL) to modulate COMT activity of the corpus striatum. Experiments were performed using male Wistar rats acclimatized to a 12L:12D (LD) cycle. Mg^{2+}-dependent COMT activity was assayed radioenzymatically with the substrate dopamine. MEL (1 mg/kg i.p.) or vehicle was administered at either early photophase (08h00), mid-photophase (12h00), late photophase (16h00) or mid-scotophase (24h00) for 3 days. Vehicle-treated COMT activity showed clear day-night variations ($P<0.001$) with a peak at 24h00 and a trough at 08h00. MEL only significantly reduced activity in the 08h00-group ($P<0.001$). In a second study, rats were maintained under a LD cycle, constant darkness (DD) or constant light (LL) for 7 days. No difference in 12h00-COMT activity was seen between the groups. In another study, animals were maintained under LD or LL conditions for 3 weeks. The LL animals received vehicle or MEL (25 µg/animal s.c.) at 16h00 for 3 weeks. MEL significantly reversed an increase in 16h00-COMT activity observed in the LL group ($P<0.001$). Finally, MEL *in vitro* had no effect on Mg^{2+}-induced or basal COMT activity. Thus striatal COMT activity appears to be state-dependent and modulated chronotypically by exogenous and endogenous MEL *in vivo*. The extent of inhibition is strongly dependent on the magnitude and duration of the experimental manipulation of MEL levels. This effect does not appear to be due to direct inhibition of the enzyme.

EFFECTS OF 8-BROMO-cGMP MICROINJECTED INTO THE DORSAL CENTRAL GREY

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SIN-1, a nitric oxide (NO) donor, induces flight behaviour when microinjected into the dorsal periaqueductal grey matter (DPAG). Some physiological effects of NO are related to an increase of intra-cellular levels of cyclic GMP. (The purpose of the present study was to evaluate behavioural effects produced by the administration of 8-Bromo-cGMP, a membrane-permeable analogue of cGMP, into the DPAG. We also employed the detection of Fos-like immunoreactivity (FLI) to reveal brain areas activated by the drug (50 µg) and the NADPH-diaphorase reaction to detect the presence of NO synthase (NOS). Male Wistar rats (10-12/group) received intra-DPAG injection of 8-Bromo-cGMP (25-100 µg) or saline (0.5 µl) and locomotor behaviour was evaluated during 10 min using the ETHOVISION software. The animals were sacrificed 2:00 h later for c-Fos immunohistochemistry and NADPH-diaphorase reaction. 8-Bromo-cGMP increased locomotion, dose-dependently ($p<0.05$). Flight reactions were induced in 42% (50 µg) and 10% (25 or 100 µg) of the animals. FLI was induced by 8-Bromo-cGMP in several regions related to defensive reactions, including the periaqueductal grey, hypothalamic nuclei, medial amygdala and cingulate cortex ($p<0.05$). NADPH-diaphorase positive neurons with FLI were detected in the medial amygdala and hypothalamic paraventricular nucleus. In the DPAG most of the NADPH-diaphorase positive neurons did not show FLI. These results suggest that an increase in intraneuronal levels of cGMP in the DPAG activates defensive reactions. They also indicate that most of these neurons do not contain NOS.

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CALCIUM-ACTIVATED K+ CHANNELS IN HUMAN LEUKEMIC JURKAT T CELLS: MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION

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Small conductance calcium-activated potassium (SK) channels are present in central synapses and peripheral tissues. Physiological studies demonstrated the presence of apamin-sensitive SK channels in the human leukemic Jurkat T cell line. In this work, we employed biochemical, electrophysiological and PCR cloning techniques to characterize SK channels in Jurkat T cells. Using anti-apamin antibodies, we immunoprecipitated radiolabeled apamin cross-linked to its receptor. We found that SK channels are composed of at least two different subunits, a high molecular weight (~57 kDa) alpha subunit and a low molecular weight (~31 kDa) beta subunit. Our data indicate that apamin binds at the interface of these two subunits. Using a degenerate PCR cloning strategy , we identified a Jurkat cDNA sequence encoding hSK2 , the human version of the rat apamin-sensitive SK channel alpha subunit, rSK2. The hSK2 clone appears to be highly homologous to rSK2. Hydrophobicity analysis reveals the presence of six putative transmembrane domains with a P region. The hSK2 channel sequence contains several consensus sites for phosphorylation by protein kinase C and Ca2+/calmodulin-dependent protein kinases II. Northern blot analysis reveals that hSK2 is encoded by a transcript of about 2.3 kb in Jurkat T cells. The physiological characterization of the hSK2 channel is being currently investigated.

NORADRENERGIC MODULATION OF NEURONAL ACTIVITY IN CEREBELLAR DEEP NUCLEI OF THE RAT.

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The cerebellum receives a conspicuous noradrenergic input from the locus coeruleus. The presence of these afferent fibers is substantiated by a high level of noradrenaline (NA) and a high number of noradrenergic receptors. The aim of this study was to test whether NA is able to modulate the background neuronal firing of the various cerebellar nuclei as well as their neuronal responses to γ-amino-butyric acid (GABA), an inhibitory neurotransmitter strongly effective on nucleo-cerebellar cells.The electrical activity of single nucleo-cerebellar neurons was extracellularly recorded in deep anesthetized rats (urethane,1.3 g/kg) during microiontophoretic application of one or more drugs: NA, its agonists and GABA. In the majority of cases the response to NA application (10-80 nA, 30s) was a depression of the background firing rate in all the cerebellar nuclei. The effects had however higher intensity in the medial (MN) than in interposital anterior (IAN) and posterior (IPN) nuclei and were weak in the lateral nucleus (LN). Few excitatory responses to NA application, although weaker than the inhibitory ones, were recorded in IAN and LN. In all the nuclei, inhibitory responses were mimicked by noradrenergic α_2-receptor agonist clonidine and, at least in MN, by noradrenergic β-receptor agonist isoproterenol. Isoproterenol application induced either excitatory or inhibitory responses in IPN and LN, only excitatory responses in IAN. All the responses to NA were dose-dependent. Furthermore, NA ejection depressed inhibitory responses to GABA in MN neurons, enhanced them in IAN and had mixed effects in IPN and LN. It is concluded that NA applications mostly depress the background firing rate and exert a different modulation of GABA responses in the various cerebellar nuclei. Both types of actions involve α_2 and β receptors.

ORIGAMI AND MENTAL CONTROL

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The aim of our investigation was to reveal if there is any correlation between the art of origami practise and mental control in elderly people.

We considered the possibility of improvement and development of mental control as cognition aspect and the estimation of origami tehnique applitation for rehabilitation purposes.

We applied the method of experimental investigation in equivalent groups.

The sample consisted of 100 older persons 61-96 year old residents of Gerontolgic Centre and Zrenjanin Gerontologic Club members.Experimental and control group had 50 members each.

The folloving tehniques were applied: Luria` s Test mental control modified for our population,dementia scales Hasegawa-Hachinski systematic-strustured observation and shorter
qustionnare.

This research confirmed that origami is systematic factor which developed mental control with older persons who practised it.

Within the context of correlation obtained between the art of origami and mental control,it was established that the experimental group performed greater success in performing gestural activities comparing to the control group, as well as better immediate memorizing of a sequence of numbers and a sequance of the numeric operation of addition.

On the basis of statistically significant difference (at the level of 0.01) in the degree of development of mental control in demented members of both experimental and control group we can conclude that origami influenced significant development of mental control in persons with slight and moderate dementia who practised it.

Key words: origami,mental control,work therapy,rehabilitation.

AMYLOID PRECURSOR PROTEINS ASSOCIATE WITH HEME OXYGENASE TO INHIBIT ITS CATALYTIC ACTIVITY: INCREASED NEURONAL VULNERABILITY TO OXIDATIVE STRESS IN ALZHEIMER'S DISEASE

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Amyloid precursor protein (APP) is a member of a large family of proteins that includes of amyloid precursor like proteins-1 (APLP1) and –2 (APLP2). While the function of the APLP proteins is still puzzling; APP has been well characterized to be the precursor of the β-amyloid fragment (Aβ), known to be enriched in plaques in brains of patients with Alzheimer's disease (AD). The role of Aβ in neurotoxicity is not fully established. However, it has been reported that neuronal death in AD may be a consequence of oxidative stress mediated by production of reactive oxygen radicals. Here, we report the possible functional implication of an anti-stress enzyme, heme oxygenase (HO). Two HO active isozymes are well known: HO1, an inducible heat shock protein, and HO2 which is constitutive and highly concentrated in neurons. HO catalyzes the conversion of heme to carbon monoxide, iron and biliverdin, which is immediately reduced to bilirubin. We have previously shown that bilirubin formed by activation of HO2 protects cultured neurons against H_2O_2-induced injury. We recently have found that APP/APLPs directly interact with both HO1 and HO2 to inhibit HO enzymatic activity. APPs harboring mutations linked to the familial AD provide substantially greater inhibition of HO activity than wildtype APP. Induced oxidative neurotoxicity is markedly greater in cultures of cortical neurons from APP Swedish (ΔAPPswe) mutant transgenic mice than controls. We have also submitted wildtype and HO2 knockout animals to an in vivo ischemia model, using the middle cerebral artery occlusion paradigm and noticed that the damage in the $HO2^{-/-}$ animals is about twice that of the wildtype. This result is somewhat comparable to ischemic damage observed in the transgenic animals overexpressing ΔAPPswe. These findings suggest that diminished neuroprotective activities of HO by APP-HO interactions may play an important role in neurotoxicity in AD.

X-RD STUDIES ON DRIED WHOLE BLOOD SMEAR IN MUSCULAR DYSTROPHY (MD)

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When we take into account all the cases of MD and compare it with Parkinson's disease and normals,we arrive at a significant note that the X-RD can be gainfully used to understand the basic crystallo graphy involved in neuro-muscular disorders.X-RD study of whole blood smear shows that the small angle neutrons scattering (SANS) will further enhance our knowledge about the mechanisms involved in the progression of the disorder,it can also be used for monitoring the impact of a particular drug design.An ideal and effective drug should be successful in making the X-RD of the whole blood smear of patient almost identical to that of healthy subjects,if this drug therapy is effective particularly then we shall have partial change in the X-RD spectrum.These studies open the new vistas of thinking in the field of muscular physiology and pharmacology using the principle of X-RD,for blood smears which are easily available from the patient. The present technique is easier,economic and rapid in monitoring the stage of the disease.These studies also reveals distinct differences between different types of MD.

EFFECT OF LATERALITY ON SEX-DIFFERENCES IN IMMOBILITY TIME DURING THE ROTATORY SWIMMING BEHAVIOR OF NORMAL MICE.

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It is well known that individual consistency of laterality in turning behavior depends on endogenous cerebral asymmetries. Here we employed the free-swimming rotatory test to investigate the relationship between the time that the animals remained immobile and the total turning activity of normal Swiss mice (n = 149). The effects of sex and consistency of laterality on immobility were also investigated. Each animal was tested for 5 min on 3 different days and consistency of laterality was defined considering the persistence of the same preferred turning side in the three sessions. We found that immobility was not explained by total turning activity and, thus, immobility was analyzed independently from activity. There was an increase in immobility times as test progressed and upon repeated testing sessions. Along the first session, side-consistent males adopted a passive strategy more quickly than side-consistent females. In particular, consistent-right-turner males exhibited a significant higher immobility time than consistent-left-turner females. We conclude that laterality is an important source of individual variability for the analysis of the sex-differences in immobility time.

RESPIRATORY CENTRAL CHEMOSENSITIVITY IN THE NEWBORN MOUSE *IN VITRO*.

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The aim of the present work was to characterize the respiratory responses induced by pH stimulation of the brainstem of newborn mice *in vitro*. Isolated CNS of newborn mice *Swiss Rockefeller* (1-2 days old) were superfused at continuos flow of 0.6 –1.2 ml /min with BME (Basal Medium of Eagle's, Gibco) equilibrated with O_2: CO_2 (95% : 5%, pH 7.37 –7.40) at room temperature (19-24 °C). Spontaneous activity from C3-C5 ventral roots was recorded using glass suction microelectrodes. Electrical signals were amplified, integrated, displayed on an oscilloscope, and analyzed with AD data acquisition system. The pH of the brainstem superfusion was obtained by gassing BME in presence of different final concentrations of bicarbonate (13, 26, and 40 mM, giving pH of 7.1, 7.4, and 7.6, respectively). Steady-state recordings during acidification of the medium (from pH 7.4 to pH 7.1) increased the frequency of respiration in 50% and decreased its amplitude in 27%. Increase of pH from 7.4 to 7.6 decreased frequency in 46% with no significant change in amplitude. Changes in frequency depended on changes in expiratory duration, but not on inspiratory duration. This results indicate that in newborn mice fictive respiration is under control of brainstem chemosensitive structures. They also suggest that respiratory timing is controlled by "expiratory – off switch" events.
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Electrophysiological responses of DA-ergic neurons to systemic haloperidol: role of experimental model and anesthesia

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It has previously shown that general anesthesia attenuates the responses of DA-ergic neurons to systemic neuroleptics. We used different experimental preparations to further characterize this interaction. Briefly, extracellular single unit recording of DA-ergic neurons was carried in: (**a**) awake non-paralyzed, (**b**) awake paralyzed (locally anesthetized), (**c**) single-bolus chloral hydrate (**CH**) anesthetized, and (**d**) deeply **CH**-anesthetized rats, as well in midbrain slices. Haloperidol, administered i.v. at increasing doses starting from 5 μg/kg, induced a different response depending of the preparation. At the dose of 20 μg/kg, the drug causes a percent increase of discharge rate of 95 ± 7.5, 53 ± 3.4, 36 ± 9, and 0, in preparation **a** through **d**, respectively. Also burst firing percentage drastically depended on the preparation, being 750 ± 93, 345 ± 40, 258 ± 27, 38 ± 6, in preparation **a**, **b**, **c**, and **d**, respectively. Interesting, in **d** preparation, even high doses of haloperidol (up to 320 μg/kg) were ineffective. Moreover, increasing anesthesia dosage progressively modified firing pattern, pushing it to highly regular "pacemaker-like" mode. On the contrary, inhibition of DA-ergic firing by iv apomorphine (1-32 μg/kg) was unrelated to the type or the deep of anesthesia or by the experimental model. In midbrain slice, haloperidol perfusion (10-100 μM) was completely devoid of action, but quickly reverted apomorphine (1-10 μM) -induced inhibition. This finding extends previous data showing that the DA-ergic responsiveness to neuroleptics requires the functional integrity of feedback loop. When this integrity is lost, the excitatory responses to neuroleptics (in terms of rate and pattern) are only partially manifested (as in single bolus anesthesia) or completely absent (as after deep anesthesia or in slices). In appropriate dosage, **CH** silences GABA-ergic neurons (SN reticulata, pallidum, VTA interneurons) critical for the functioning of the feed-back circuitry to DA-ergic cells. Generalizing, GABA-ergic anesthetics prevent the action of neuroleptics on DA-ergic neurons by induction of a functional deafferentation of the feed-back circuitry that mimics an anatomical transection or brain slice preparation. However, the changes of firing rate and pattern suggests that anesthesia also affects glutamatergic afferences to DA neurons.

HISTAMINE RECEPTORS IN THE INFERIOR COLLICULUS DIFFERENTIALLY MODULATE AUDIOGENIC SEIZURE SUSCEPTIBILITY IN GENETICALLY-EPILEPSY PRONE RATS AS COMPARED TO ETHANOL-WITHDRAWN RATS

Carl L. Faingold and Hua-Jun Feng, Department of Pharmacology, Southern Illinois University Sch. Med., P.O. Box 19629, Springfield, IL 62794-9629 USA **RATIONALE:** Audiogenic seizures (AGS) are a common form of rodent epilepsy, occurring in genetically epilepsy-prone rats (GEPRs) and in normal rats during ethanol withdrawal (ETX-Rs). In both forms, AGS are initiated in the inferior colliculus (IC) from which they propagate to the other nuclei of the seizure network. Histamine is a putative neurotransmitter in IC neurons, and systemically administered agents that enhance the action of histamine block AGS. However, the role of histamine receptors in the IC in AGS modulation is unknown. **METHODS:** The present study examined AGS in GEPRs or ETX-Rs, which had been given ethanol (9-15 g/kg/day) intragastrically every 8 hr to maintain moderate intoxication. On day 4, ethanol was withdrawn and AGS were examined starting 19 hr later. Histamine (40, 60, or 120 nmol/side) was infused bilaterally into the IC at 0.25 μl/min for 2 min through chronically implanted cannulae. Susceptibility to AGS was tested at 0.5-216 hr post-infusion. **RESULTS:** AGS in GEPRs were significantly reduced at 40 and 60 nmol, while in ETX-Rs no effect of histamine was observed in doses up to 120 nmol. In GEPRs the anticonvulsant effect began at 0.5 hr, reached maximum at 24 hr, and recovered completely by 120-216 hr. **CONCLUSIONS:** These data support a modulatory role of histamine receptors in the IC of GEPRs that is absent in ETX-Rs. The differential effect of histamine in the IC of GEPRs versus ETX-Rs contrasts to findings with microinjection, into IC, of NMDA antagonists or GABA-A agonists, which suppressed seizures similarly in both AGS forms. (Support NIH AA 11628, NS 21281)

TRANSFORMATION OF SOMATIC POTASSIUM CURRENTS MODULATION BY ACTIVATION OF mGluR IN CULTURED HIPPOCAMPAL NEURONS.

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The modulation of potassium currents by endogenious GTPγS was studied by measuring whole cell potassium currents in the somatic membrane of cultured hippocampal neurons. The effect of application GTPγS was measured by successive using of two patch pipette with control extracellular solution and solution contained 50 μM GTPγS, correspondingly, for the same investigated neuron.
In majority of hippocampal neurons we defined two types of potassium currents, which were separated of the basis of their different voltage-dependence of activation. The GTPγS effect developed during 15 min after establishment a whole-cell configuration by patch pipette with solution contained GTPγS. The GTPγS effect on fast inactivating potassium current was suppression of peak current amplitude. The decrease of slow inactivating potassium currents was evident at the membrane potential more positive than +10 mV and was dominant for peak current amplitude in comparison with steady state current.
The mGluR agonist 1*S*,3*R*-ACPD (50 μM, 1*S*,3*R*-aminocyclopentane-1,3-dicarboxylic acid) was included after GTPγS effect accomplished. The fast inactivating current peak amplitude began to increase whereas the slow inactivating current amplitude persisted to decrease after extracellular application of agonist. That additional suppression of slow inactivating current by 1*S*,3*R*-ACPD also depended on membrane potential and explained by acceleration of its inactivation.
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FUNCTIONALITY OF K^+_{ATP} CHANNELS IN MESENTERIC BEDS ISOLATED FROM STREPTOZOTOCIN DIABETIC RATS

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The functionality of the K_{ATP} channels and its modulation by endothelial factors was studied in mesenteric beds isolated from diabetic rats 8 weeks after streptozotocin administration (60 mg/kg, i.p.). Rats with blood glucose concentrations higher than 300 mg/dl under fasting conditions were considered as diabetic. Controls were injected with vehicle (0.05M citrate buffer). Differences in the perfusion pressure were measured as a parameter of vascular resistance. The relaxant effects of the potassium channel openers (KCOS) were studied on the contractions elicited by 10 nmol noradrenaline (46.67±3.76 mmHg, n=5, in the controls and 41.76±3.18 mmHg, n=7, in the STZ-diabetic rats) and were expressed as the percentual reductions of the contractile responses. Acethylcoline and sodium nitroprusside were employed as indicators of endothelium-dependent and endothelium-independent relaxations, respectively. In mesenteric beds from STZ-diabetic rats, the relaxations produced by the KCOS, 1 µM cromakalim (CRK) and 30 µM diazoxide (DZX), were significantly smaller at $p<0.05$ (45.6±2.3 %, n=8 for CRK and 35.0±5.2%, n= 7, for DZX) than those caused in the control rats (80.0±6.0%, n=5 for CRK and 65.0±3.0%, n=5, for DZX). The relaxations were not modified by the pretreatment with the prostanoid synthesis inhibitor 10 µM indomethacin but were significantly attenuated ($p<0.05$) by pretreatment with the K^+ channel blocker 0.1 µM glibenclamide both in the controls: 13.5±1.8%, n=5, for CRK and 19.3±2.3%, n=5, for DZX and in the STZ-diabetic rats: 17.0±3.3 %, n=8, for CRK and 15.0±3.4 %, n=7, for DZX. On the other hand, the removal of the endothelium with 45 sec perfusion with 0.1% saponin as well as the pretreatment with the inhibitor of the nitric oxide synthase 100 µM L-NAME significantly decreased at $p<0.05$ the relaxation induced by the KCOS in control mesenteric beds but not those produced in the mesenteric beds from diabetic rats. These results suggest that, from a pharmacological point of view, the functionality of the K_{ATP} channels in the rat mesenteric bed is diminished in the diabetic status and independent on the presence of an intact endothelium.

EARLY DETECTION OF MOVEMENT DISORDERS IN NEUROLOGICAL MUTANT MICE

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The specific objective of this study was to explore the use of neurological mutant mice as a tool for tracing normal and disordered development of mammalian movement patterns. Individual weaver, staggerer, and jimpy mice were videotaped from postnatal day one during supported swimming and grooming tasks. The video records were scored by movement notation and computer methods to dissect individual movement parameters and their various combinations in time. In particular, the goal was to dissect parameters of normal and disordered movement at the levels of individual limb kinematics through complex sequences of integrated behavior. The hypothesis was that basal ganglia disorders (dopamine deficiencies in weaver mice) would be manifest primarily at the level of movement initiation and sequencing. Cerebellar disorders (weaver and staggerer mice) should be manifest primarily at the level of "lower order" movement coordination, and myelin deficiencies (jimpy) should be manifest in disordered phase relations among limbs. Since these are degenerative disorders, time-locked departures in the developmental trajectories based on cellular data for mutant and control animals were predicted. Overall the predictions were confirmed. However, it proved necessary to separate "activation" from "coordination" and "sequencing" parameters to attain unambiguous interpretation. Contextual (environmental) factors also proved important. Phasic perturbations during specific phases of ongoing movement were thus employed to clarify movement system integrity and autonomy at various levels. The data suggest that selected mutations may partially and selectively degrade movement parameters in a manner that makes them much more sensitive to contextual (extrinsic) perturbations. This leads to a perspective that emphasizes shifts in the dynamic balance (relative importance) of both interactive plus self-organizing properties of movement control networks. A "dynamic focus" model is proposed. Specifically, one can conceptualize normal operation of "movement fields" consisting of relatively low threshold excitatory cores and higher threshold inhibitory surrounds (cf. sensory receptive fields). Neurologically targeted mutations can affect relevant signal and noise parameters in these fields through selective cellular disruptions. Future studies that combine developmental observations with experimental (e.g. pharmacological) methods that modify phenotypic expression in mutant mice are needed.
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IMPAIRMENT OF MEMORY FUNCTION IN HUNTINGTON'S MODEL: TROPHIC INFLUENCE.

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Huntington's disease (HD) is an inherited neurodegenerative disorder in which the striatum undergoes a marked atrophy, and one of its hallmarks is the impairment of cognitive function., including deficient visuospatial skills, lack of cognitive flexibility and poor recall of memories. In order to evaluate the possible trophic influence of NGF and the nootropic drug Cerebrolysinr on the memory function, three month-old rats were divided randomly into four groups: (NGF) rats which received unilateral striatal lesion with quinolinic acid (225 nmol/2µl) and the single injection of NGF (586 µg/ml); (CER) rats that received striatal lesion and daily i.p. injection of Cerebrolysin (2,5 mg/kg) for 15 days, and the (LES) lesion and (INT) intact animals used as control groups. The animals were tested before surgery in Morris water maze in acquisition testr (8 trials/day). Fifteen days after surgery and treatment the animals were tested in the retention test (1 day; 4 trials/day). The next 3 days the transfer test was performed using the new platform position. The results show ed that good recall of memories was only observed in INT groups which was significantly different from LES group, while NGF and CER groups exhibited intermediate performances. However, when position of the platform was changed only INT and partially CER animals used efficiently previous knowledge to solve the task. NGF group was almost comparable in all three days with LES animals.

BEHAVIORAL CHARACTERIZATION OF STRIATAL LESION INDUCED BY QUINOLINIC ACID IN RATS. RELEVANCE FOR HUNTINGTON'S DISEASE.

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Huntington's disease (HD) is a progressive neurodegenerative disorder, characterized by severe degeneration of basal ganglia neurons. Behavioral symptoms of HD include abnormal, uncontrollable and constant choreiform movements, and impaired cognitive function. In order to explore this issue, we studied the behavior of rats with unilateral quinolinate induced lesions of the medial striatum. Intact 3 months old male rats (n=23) were trained in the Morris Water Maze during 3 consecutive days, 8 trials/day (acquisition), and before surgery they were randomly assigned either to intact or lesion groups. Fifteen days after the lesion the rats were tested using retention test (1 day/4 trials), on the next 3 days the rats were tested in the transfer test (3 days/8 trials-day). The asymmetrical rotational behavior test in respond to amphetamine and the Paw reaching test were also tested in these rats. Lesioned animals exhibited deficient retrieval of stored memories of visuospatial skills and impaired transfer of learning. In relation with motor activity the lesioned rats showed a profound impairment in the skill of the left forelimb for reaching food compared with its right forelimb as well as with the left forelimb of intact rats. The performance of the Paw reaching test exhibited a significant correlation with the observed amphetamine induced rotational behavior. These results are consistent with the notion that the striatal degeneration could sufficiently account for the cognitive abnormalities associated with HD and with the key role played by basal ganglia in enabling voluntary and postural adjustment of the movements.

CEREBROID GANGLION IS THE PRESUMPTIVE CIRCADIAN PACEMAKER OF THE ELECTRICAL RESPONSE TO LIGHT (ERG) CIRCADIAN RHYTHM IN THE CRAYFISH. Beatriz Fuentes-Pardo, Oscar H. Hernández. Facultad de Medicina, UNAM Apdo Postal 70-250 México, D.F. CP 04510. MÉXICO

Many experiments have proposed a periodic (circadian) release of distal dispersing hormone (DPDH) in the sinus gland of crayfish which is essential in the expression of the ERG circadian rhythm. However, it has not been established if the sinus gland has one (ore more than one) autonomous oscillator or if only displays a circadian rhythm generated in other place. The aim of this work is to establish the role of the protocerebrum in the expression of the sinus gland circadian activity. Unrestrained crayfish *Procambarus clarkii* kept in free-running and entrainment conditions were recorded by periods up to 15 days. Extracellular electrodes were cronically impaled in different sites of the cerebroid ganglion, particularly the protocerebrum, in order to obtain the temporal patterns of both spontaneous and light-evoked potentials. The electrical activity was filtered, amplified and digitezed. The spontaneous electrical activity from any region of the cerebroid ganglion displayed a clear circadian rhythm. When the electrode was impaled in the protocerebrum, both spontaneous and light-evoked potentials showed a circadian rhythm but almost 180° out of phase each of the other. The circadian rhythmicity was better defined in free-running. These results suggest that the cerebroid ganglion has an important role in the generation of rhythmic behaviors and that cellular elements of the protocerebrum involved in the visual integration are, at least, part of the pacemaker responsible of the ERG circadian rhythm of crayfish.

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PERTUSSIS TOXIN-SENSITIVE G-PROTEIN AND PROTEIN KINASE C ACTIVITY ARE NECESSARY FOR NORMAL SYNAPSE ELIMINATION IN THE neonatal RAT MUSCLE.

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Individual skeletal muscle fibres in new-born vertebrates are innervated at a single endplate by several motor axons. During the first postnatal weeks, the polyneuronal innervation decreases in a process of synaptic elimination. It has been shown recently that the naturally occurring serine-protease thrombin (THR) can be a mediator of activity-dependent synapse reduction at the neuromuscular junction (NMJ) in vitro. We hypotethise that Thr-receptor activation may modulate nerve terminal consolidation through a protein kinase mechanism. We applied external THR and several substances affecting G protein-protein kinase C system (GP-PC) directly over the external surface of the neonatal rat Levator auris longus muscle in vivo. Muscles were processed for immunocytochemistry to simultaneously detect AChRs and axons for counting the percentage of polyinnervated NMJ. We found that externally applied exogenous THR accelerated synapse loss both, in the percentage of polyinnervated junctions and in the number of the axonal endings per junction. Phorbol 12-myristate 13-acetate (TPA), a potent PKC activator, had a similar effect as THR whereas the PKC inhibitor staurosporine (STP) reduced axonal removal. Also, STP blocked axonal removal in the presence of exogenous THR. Pertussis toxin (PTX), the potent blocker of GP function, blocked synapse elimination and this powerful effect occurred also when THR was administered with PTX. These findings suggest that the normal synapse elimination in the neonatal rat muscle is mediated by the pertussis-sensitive G-protein and PKC activity and that THR could play a role in the postnatal synaptic maturation in vivo.

THE SEMANTIC DEVELOPMENT OF CHILDREN WITH DYSGRAPHIA

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The paper presents the results obtained by an examination of the semantic development of children with dysgraphia. The sample consisted of 89 children with dysgraphia, 65 boys and 24 girls, aged 8, 9, 10 and 11. In children with dysgraphia, the neurological findings were found to be standard, the intellectual abilities average, and there were no sight and hearing deficiencies. For the assessment of results The Semantic Test was used. By a qualitative analysis of the results, we have established that the children with dysgraphia show the highest degree of acquirement of antonyms (56.1%), followed by homonyms (45.5%), metonyms (42.7%), and, finally, synonyms (37.3%). Using a statistical processing of the results obtained, we have established that in the children with dysgraphia, the semantic development is lower than expected for the age, and that there are very high statistically significant deviations ($X2>9.210$, at the level of significance 0.001). It has also been established that, as far as the semantic development is concerned, there are not great statistically significant differences between boys and girls ($X2<6.635$, at the level of significance 0.001).

EVOKED LOCAL TEMPERATURE GRADIENTS IN THE HUMAN BRAIN

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Although brain temperature is important for neural function, basic information on the quantitative aspects of this physiological relationship is lacking. We address this fundamental issue by mapping brain temperature gradients intraoperatively. Using a highly sensitive infrared camera, we recorded in 27 patients the emission of infrared radiation from the exposed human cerebral cortex at baseline, during language and motor tasks performed by the patient, and during stimulation of the contralateral median nerve. The location of language and sensorimotor cortex was identified by standard mapping methods (electrical cortical stimulation, median nerve SSEP, and fMRI), which were compared to infrared functional localization. Infrared imaging accurately identifies functional cerebral cortex as defined by the standard methods of brain mapping. During functional activation, the temperature of functional cortical areas selectively increased, whereas preoperative fMRI signal change was centered over draining veins adjacent to, or overlapping, the functional cortex. We provide evidence that measurable cerebral temperature gradients can identify discreet cortical foci functionally activated by spontaneous and/or evoked behavior.Because during surgery arterial blood from the body core is warmer than the surface of the exposed brain cooled by room air, functionally evoked changes in local CBF affect cortical temperature gradients to a greater extent than heat produced by metabolic activity. Although heat is generated by cellular metabolism and electrical activity of neurons and glia, our findings indicate that the temperature gradients measured during surgery are dominated by changes in local CBF associated with evoked functional activation.

NADPH-DIAPHORASE ACTIVITY IN THE FRONTAL ORGAN AND THE HABENULAR ASYMMETRY IN DEVELOPING AND ADULT FROG.

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The role of nitric oxide in the developmental shaping of the epithalamus in the frog during the establishment of differences between the right and left side of the brain has been investigated in the pineal complex and habenular nuclei of *Rana esculenta*.
The histochemical NADPH-diaphorase (ND) method has been used on the developing animal (from tadpole to metamorphosis) and on adult. The pineal complex of the frog is a photoneuroendocrine structure organized into two portions, the extracranic frontal organ and the intracranic pineal organ. The frontal nerve arises from the frontal organ and connects this with the pineal organ and the brain. In the frog, a root of fibers, running in the frontal nerve, projects asymmetrically to thalamic regions crossing within the left habenular regions. The habenular nuclei are epithalamic structures which, in the frog, display a striking asymmetry: the left dorsal habenular nucleus develops in two portions - the medial and lateral subnucleus, while a unique nucleus appears on the right side of the encephalon. During development, an intense ND staining was detected in cell bodies of the frontal organ and, subsequently, in fibers of the frontal nerve arising from the frontal organ. Similar findings were also detected in the adult frog. The ND positivity allowed to follow the course of fibers of the frontal nerve in its extra-encephalic course. These stained fibers entered the brain at the level of the habenular commissure and crossed the pineal organ to reach the pineal tract. An intense ND activity has been detected, selectively, in the neuropil of the medial subnucleus of the left dorsal habenula within a lateral compartment of the medial subnucleus from the early stages of development until metamorphosis. In the latter stage some stained fibers were detected in the lateral compartment. In mature frog, staining was still present in the above location, but much less intense in the neuropil, indicating that the neurochemical pattern observed during development was at least in part transient. The pattern of transient histochemical reactivity observed in the neuropil of the left habenula of the developing frog may be related to the establishment of a distinct pattern of connectivity within the left medial subnucleus. The temporal coincidence of the ND expression found in the frontal organ and left habenular nucleus suggests that the enzyme nitric oxide synthase could be involved in the maturation of the asymmetric projections of the frontal organ. Thus, our data indicate that during frog development, nitric oxide could subserve a role in the differentiation of the structural arrangement in epithalamus leading to an asymmetrical specialization of the habenular nuclei.

N-METHYL-D-ASPARTATE RECEPTOR BLOCKADE BY MK-801 AND RADICAL SCAVENGERS PROTECT CHOLINERGIC NUCLEUS BASALIS NEURONS AGAINST β-AMYLOID NEUROTOXICITY

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Previous experimental data indicate the involvement of Ca^{2+}-related excitotoxic processes, possibly mediated by N-Methyl-D-Aspartate (NMDA) receptors, in β-amyloid (βA) neurotoxicity. On the other hand, another lines of evidence support the view that free radical generation is a critical step in the βA-induced neurodegenerative cascade. In the present study, therefore a neuroprotective strategy was applied to explore the contributions of each of these pathways in βA toxicity.

$βA_{(1-42)}$ was injected into the *magnocellular nucleus basalis* of rats (0.2 nmol/1 µl), while neuroprotection was achieved by either *single* or *combined* administration of the NMDA receptor antagonist MK-801 (2.5 mg/kg, 2 hrs prior to the neurotoxic lesion), and/or a vitamin E and C complex (150 mg/kg, administered from 48 hrs pre- up to 48 hrs post-lesion). The degree of neurodegeneration was determined by testing the animals in consecutive series of behavioral tasks including elevated plus maze (d5, d7), passive avoidance learning (d10-12), small open-field (d14) and open-field (d14) paradigms. These were followed by determinations of acetylcholinesterase (AChE), choline-acetyltransferase (ChAT) and superoxide dismutase (SOD) activities in the somatosensory cortex by means of biochemical methods.

βA injected in the *nucleus basalis* elicited significant anxiety in the elevated plus maze, derangement of passive avoidance learning, and altered spontaneous behaviors in both open-field tasks. A significant decrease in both AChE and ChAT accompanied by a similar decrement of MnSOD, but not of Cu/ZnSOD provided neurochemical substrates for the behavioral changes. Each of the *single* drug administrations protected against the neurotoxic events, whereas the *combined* treatment failed to ameliorate βA toxicity.

In conclusion, our studies demonstrate that βA toxicity is mediated by a complex excitotoxic cascade involving both NMDA receptor-mediated enhanced Ca^{2+}-entry and free radical generation. *Combined* drug administration exerts only limited neuroprotective effects that might be attributed to altered feed-back regulation of the NMDA receptor channel *via* its redox modulatory site.

PROPIONYL-IIGL TETRAPEPTIDE PREVENTS β-AMYLOID EXCITOTOXICITY IN RAT NUCLEUS BASALIS

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Experimental evidence indicates the involvement of common excitotoxic cascade mechanisms in β-amyloid(1-42) (Aβ(1-42)) neurotoxicity. A putative tetrapeptide Aβ antagonist (**Propionyl-Ile-Ile-Gly-Leu** [Pr-IIGL]) based on the [31-34] sequence of Aβ was previously shown to rescue neurons from Aβ-induced long-term elevations of the intracellular Ca^{2+} concentration *in vitro*. Here we provide further *in vitro* and *in vivo* evidence that the Pr-IIGL tetrapeptide effectively attenuates Aβ(1-42)-induced membrane depolarization of cultured rat astrocytes, and inhibits the excitotoxic action of Aβ(1-42) on cholinergic neurons of the rat *magnocellular nucleus basalis* (MBN).

The neuroprotective potential of Pr-IIGL was evaluated by means of correlative *in vitro* and *in vivo* approaches. Changes of astroglial membrane potential were traced by means of fluorescent dye-loading (DiS-C3(3)) of cultured glial cells. *In vivo* effects of Pr-IIGL in diminishing Aβ(1-42) excitotoxicity were determined by means of retrograde microdialysis of Aβ(1-42) and/or Pr-IIGL with simultaneous measurement of extracellular excitatory amino acid concentrations in the MBN of freely moving rats. Subsequently, microdialysed animals were tested in an open-field (d1) and in a one-way step-through passive avoidance task (d12-d14) to assess functional recovery following Pr-IIGL + Aβ(1-42) administration. Quantitative acetylcholinesterase (AChE, EC 3.1.1.7) histochemistry was employed to demonstrate the protective action of Pr-IIGL on cholinergic projection neurons of the rat MBN 14 days post-surgery.

Administration of Pr-IIGL abolished Aβ(1-42)-induced increases in extracellular aspartate and glutamate concentrations in the MBN which coincide with a significant preservation of cholinergic MBN neurons and their cortical projections as measured by means of quantitative AChE histochemistry. This neuroprotective effect was associated with increased exploratory behavior in the open-field and improved memory retention in a step-through passive avoidance task.

Our data presented here indicate for the first time the excitotoxic nature of Aβ(1-42) in the rat brain and the efficacy of short, modified Aβ antagonists (Pr-IIGL) in ameliorating Aβ toxicity *in vivo*.

Co-localization of neurons immunoreactive for the potassium channel Kv3.1b subunit and perineuronal nets presumed as a spatial buffering system for cations

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Chondroitin sulphate-rich, lattice-like coatings of the extracellular matrix are known as perineuronal nets (PNs) [Brückner et al. 1993, Glia 8:183; Celio et al. 1998, Trends Neurosci. 21:510]. The previously revealed map of PNs [Seeger et al. 1994, Neuroscience 58:371] appears to be largely similar to the distribution patterns of the calcium-binding protein parvalbumin and the Kv3.1b-subunit mainly co-occurring in fast-firing neurons in the rat brain [Weiser et al. 1995, J. Neurosci. 15:4298]. Subserving the idea that the highly anionic proteoglycans are involved in regulating extracellular cation mobility, the possibility of a generalized morphological relationship between PNs and Kv3.1b-expression was investigated in the present study focussed on rat and monkey cortex. We applied triple fluorescence labelling for the simultaneous demonstration of PNs with the N-acetylgalactosamine-specific *Wisteria floribunda* agglutinin (WFA), parvalbumin-immunoreactivity (ir) with a monoclonal antibody and of Kv3.1b-ir with different rabbit antibodies. In the rat and monkey neocortex and hippocampus, a large portion of non-pyramidal neurons co-expressing parvalbumin and Kv3.1b were found to be ensheathed by PNs. Some Kv3.1b- and parvalbumin-immunopositive cells devoid of PNs and nets around neurons lacking both immunoreactivities were also observed. By confocal laser scanning and electron microscopy, Kv3.1b-ir and WFA-binding sites were detected adjoining at the soma and proximal dendritic surface, while lectin-binding sites usually extended on more distal dendritic segments and the axon initial segments which failed to express detectable Kv3.1b-ir. The present data and a critical examination of current hypotheses lead to the conclusion that PNs may serve as rapid local buffers of excess cation changes in the extracellular space.

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NEURONAL PATHWAY OF THE AUDIOGENIC STRESS

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Extreme noises and sounds are manifested as audiogenic stresses by stimulatory inputs to the hypothalamo-pituitary adrenal axis. We used two neuroanatomical techniques to localize neuronal connections between acoustic system and the neuroendocrine hypothalamus, particularly the paraventricular nucleus (PVN): 1) Fos-immunostaining following acute audiogenic stress. The c-fos has been reported to be a marker of neurons that are activated by various types of stimuli. Rats were exposed to extreme: 105 dB SPL white noise for 3 min. Sixty min after the stimulus, rats were sacrificed by perfusion with Bouin fixative solution. Then the brains were removed, frozen, sliced by 30 mm thick coronal sections and immunohistochemically stained for Fos peptide by using the avidin-biotin procedure. The audiogenic stimuli induced intense expression of Fos-immunoreactivity in all of the components of the auditory pathway (cochlear nuclei, superior olivary complex, lateral lemniscal nuclei, inferior collicle, medial geniculate body, auditory cortical area), as well as in several thalamic and hypothalamic nuclei including the PVN and relay neurons in the lateral hypothalamus 2) Trans-neuronal tract-tracing technique was used by injecting neurogenic viruses (Barha strain pseudorabies) into the cochlear and the PVN. Five days after inoculation, labelled cells were found in all above listed brain areas and nuclei. These observations indicate that direct and indirect (relayed by lateral hypothalamic neurons) bidirectional neuronal connections exist between cochlear nuclei and the PVN which may serve as a neuroanatomical pathway of audiogenic stress stimuli to the hypothalamo-pituitary-adrenal axis.

OPIOID RECEPTOR BINDING IS ALTERED IN DISTINCT AREAS OF POST-MORTEM BRAIN OF ALZHEIMER'S DISEASE PATIENTS: A QUANTITATIVE AUTORADIOGRAPHIC STUDY.

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A similarity exists between the limbic system distribution of opioid receptors and the pathologic markers for Alzheimer's Disease (AD). This possible linkage between AD and the endogenous opioid system is further strengthened by recognition that the deficits in cognitive and memory functions apparent in the course of this disease may have an overlay with the putative role the opioid system plays in these functions. This information prompted us to initiate studies comparing opioid receptor populations in limbic structures from AD patients (n=11) to those present in the same brain areas from disease free age-matched individuals(n=10). Criteria for control subjects included normal physiological and neurological testing, the absence of more than occasional signs of focal degeneration in the cerebral cortex and the absence of senile plaques and/or neurofibrillary tangles. Mu, delta and kappa opioid receptors were labeled respectively with [^{3}H]-[D-Ala^2,N-Me-Phe^4,Gly-ol^5]-enkephalin (DAGO), [^{3}H]-[D-Pen^{2}D-Pen^5]-enkephalin (DPDPE) and [^{3}H]bremazocine (in the presence of DAGO and DPDPE). Nonspecific binding was determined in the presence of μM concentrations of naloxone. Data from image analysis of autoradiographs showed, that compared to control brain areas, statistically significant reductions in μ-opioid receptor binding occurred in the subiculum and hippocampus of AD brains. Binding of δ-opioid receptors was similarly decreased in the amygdaloid complex of AD brains. In contrast, large increases over the levels in control brains of κ-opioid receptor binding were found in the dorsal and ventral putamen as well as the cerebellum of AD brains. Levels of μ, δ, and κ opioid receptor binding were unaltered in the caudate, parahippocampal gyrus and occipito-temporal gyrus. These results confirm and expand results published by us previously on binding studies in homogenates of brain areas from control and AD individuals and suggests a role for the endogenous opioid system either in the etiology of this neurodegenerative process or in the multitude of effects that accompany this disease.

PHOSPHOLIPID COMPOSITION OF LIPIDS OF SUBCELLULAR STRUCTURE OF THE BRAIN IN GANGLIOSYMPATHECTOMY AND ACOUSTIC STRESS

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The phospholipid (PL) composition of the subcellular fraction of the brain has been studied in unilateral gangliosympathectomy (removal of right upper cervical sympathic ganglium) and its combination with acute acoustic stress (the noise level 91 dBA with maximal energy of average and high frequency during 2 hours). The data obtained testify to qualitative and quantitative changes in PL composition of nuclei, chromatine and mitochondria of cerebral tissue, the character and intensity of which depend on the observing fraction and the hemisphere (of right, ectomised side, and left). As a rule, the changes in the right hemisphere are more expressed, though the changes in the left side are also marked. An expressed increase lysophosphatidylcholine (LPLCh) content was more marked in lipid composition of chromatin., the decrease of phosphatidylcholine (PLCh) is more expressed in mitochondria, the level changes of sphingomielines (SM) have an opposite character in studied fractions. As a result of the changes of PL composition the total content of neutral and acid representatives of PL and their ratio are changed. Changes was estimated also in quantitative and qualitative content of fatty acids, supporting the physico-chemical status and the exchange of the very PL.The fact that the observed changes have asimmetric character, is of a special interest. They diminish in the following succession: chromatin, nuclei, mitochondrial fractions, indicating the important role in the function of the genetic apparatus of its minor component, PL.Smoothing out of asimmetry is estimated on the background of ganglioectomy in conditions of noise effect (acoustic stress): the changes in the left hemisphere are less expressed compared with the gangliosympathectomised animals (LPCh, PLCh). Simultaneously sphingomieline changes obtain contrary character, their level decreases in mitochondria of the both hemispheres. The carried out studies revealed the important role of the sympathic nervous system in the structural organisation of lipids of subcellular formations, the functioning of the central system of adaptation, particularly, in conditions of acoustic stress.

PRINCIPLES OF SYMPATHETIC CO-TRANSMISSION: STUDIES WITH ISOLATED RINGS FROM HUMAN VASCULAR BIOPSIES.

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The role of adenosine 5'-triphosphate (ATP) neuropeptide Y (NPY) and noradrenaline (NA) in the control of human vasomotor tone was assessed in isolated blood vessels. Rings were prepared from recently dissected vascular biopsies and mounted to record isometric contractions of the circular muscular layer. The rings were stimulated with agonist drugs or electrically depolarized (70 V, 0.5 msec, 0.5-40 Hz). The joint application of 5nM NA plus 100nM α,β m-ATP to rings from the saphenous vein, resulted in a 3-fold synergism, i.e., the vasomotor effect was significantly larger than that anticipated from the simple addition of each response. 10 nM NPY, which per-se did not elicit a vasomotor response, potentiates the contraction elicited by either NA or α,β m-ATP, or that elicited by of both agonists simultaneously. The synergism evoked by NPY is concentration dependent; NPY displaced leftward the corresponding agonist concentration-response curves. The synergism was significantly attenuated with 1 μM BIBP 3226, the Y_1 receptor antagonist. Electrical stimulation of the perivascular nerve fibers elicited frequency-dependent vasomotor contractions only antagonized by the triple combination of 30 nM prazosin plus 30 μM suramin plus 1 μM BIBP. Parallel protocols performed with internal mammary arteries and veins from the same patients revealed a more robust synergism of NA with α,β m-ATP in veins than arteries, suggesting territorial differences in sympathetic co-transmission.
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DEVELOPMENTAL CHANGES IN PROPERTIES OF $GABA_A$ RECEPTORS AND SYNAPSES.

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We examined the maturation of $GABA_A$ receptors and synapses in developing cortical neurons from primary cultures. Whole-cell voltage clamp recordings of spontaneous $GABA_A$ergic PSCs revealed that half of the cells form at least one functional $GABA_A$ergic synapse by the 4th day *in vitro* (DIV). Before this time, $GABA_A$ receptors are abundant, largely extrasynaptic, and probably have a neurotrophic role. The decay kinetics of even the earliest occurring (2 DIV) PSCs had a fast component. This component grew in relative prominence from approximately 20 to 45% of total decay as neurons matured over the first three weeks of culturing. During this time, the value of the decay time constant (mean $\approx$ 3 ms) was developmentally stable. In contrast, fast perfusion of 1 or 10 mM GABA on membrane patches evoked currents with different desensitizing time constants in young and old neurons. During the first week of culturing, patches lacked the fast component identified in PSCs. After the first week, a fast-desensitizing component appeared and then grew in prominence to levels comparable to those in PSCs. Immunofluorescent staining using antibodies to $GABA_A$ receptor subunits and synaptophysin (a presynaptic marker) predict that most patches excised from the soma during the first three weeks *in vitro* should contain mainly extrasynaptic receptors. Thus, the discrepancy between the decay kinetics of PSCs and patches during early development probably reflects differences between the functional characteristics of synaptic and extrasynaptic $GABA_A$ receptors during this time. In support of this SB-205384, a positive modulator of $GABA_A$ receptors that is selective for the α3-subunit, had no effect on PSCs at any time *in vitro* but potentiated extrasynaptic activity. Overall, these results suggest that synapse maturation does not proceed by a gradual exchange of early embryonic $GABA_A$ receptor subforms for adults ones. Instead, during all stages of development, inhibitory synapses appear capable of selectively capturing $GABA_A$ receptors having fast desensitization kinetics.

EFFECT OF THE CHOLINESTERASE INHIBITOR GALANTHAMINE ON THE ACTIVE AVOIDANCE BEHAVIOUR IN RATS AFTER TRANSIENT CEREBRAL ISCHEMIA

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Cerebral ischemia is one of the main reasons for death and invalidity in elderly people, as well as in newborns. The effect of the cholinesterase inhibitor galanthamine, widely used in conditions of peripheral paresis, cognitive deficits, anaesthesiology, etc., was tested on rat model of transient forebrain ischemia. Both carotid arteries of 12 male Sprague-Dawley rats (150-180 g.) were occluded for 20 minutes and 25 minutes after the removal of the ligatures galanthamine was administered to 6 of the animals (2 mg/kg i.p.). The speed of learning and the short-term memory were tested using Shuttle-box test starting 24 hours after the operation. Each animal underwent a complex of conditioned (sound and light) and unconditioned (electric shock) stimuli. The parameters checked were latency time and saved crossing time (maximum stimuli duration minus latency time). Both of them integrate the ability for conditioned as well as unconditioned stimuli avoidances. The speed of learning of the ischemic saline-injected animals was significantly impaired in comparison with the non-ischemic control and with the ischemic animals with galanthamine administration ($p<0.05$). At the days of 2,3,4,5,6 after the operation the memory abilities of the ischemic saline-injected rats also differed significantly from the other groups ($p<0.05$). After one week pause the animals were tested for their short-term memory. There was not significant change in the results of any group except the ischemic saline-injected one, which significantly improved its performance and equalized its results with all other groups. Our data show that galanthamine speeds up the recovery of the cognitive abilities in the first 7 days after transient forebrain ischemia when administered right after it.

DEVELOPMENTAL CHANGES IN FUNCTIONAL PEPTIDES INVOLVED IN CARDIO-REPIRATORY BRAIN CONTROL. POSSIBLE IMPLICATIONS IN SIDS

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Among the causes that lead to sudden death during infancy two main classes implicate central neurotransmitter as regulator of vegetative controls. Congenital abnormalities genetically or incidentally inherited facilitate the break-down of neuronal firing or detailed loops of escape which are present to avoid apnea. The second class of critical factors is the maturation changes in brain. The behavioural necessities and the final neuron network organization are the inducing factors of major change in the central nervous system. In mammalian species, the neuropeptides and their receptors increase dramatically during the perinatal period. Nevertheless, in most cases, there is no parallelism or succession in time between the transient overexpressions of a kind of neuropeptide and its specific receptor. Frequently, there are clear discrepancies between the rostral and caudal timings in maturation profile. This has been established in rodents for opiate ligands and receptors and partly in human brainstem. Independently of opioid effect, both neurotensin (NT) and substance P (SP) have been reported involved in cardio-respiratory control. We focus our studies on main brainstrem structures involved in cardio-respiratory control (i.e. nucleus solitarius and locus coeruleus). Opiates, NT and SP binding sites have been quantified by autoradiography in postmortem brainstem sections. We detailed developmental changes in the first year of the life and compared normal and SIDS cases.

BEHAVIOURAL EFFECTS OF RHOMBENCEPHALIC CELL SUSPENSION TRANSPLANTS INTO THE RAT AFTER CEREBELLAR LESIONING WITH KAINIC ACID: COMPARISON BETWEEN OPEN-FIELD TESTING AND NEUROLOGICAL EXAMINATION.

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The embryonic rhombencephalic tissue suspensions isolated from E15 stage rat embryos were injected into the cerebellar vermis of adult rats which had been lesioned with kainic acid 10 nM one week before transplantation. The result from the histological studies indicated that the grafted neural cells grow and differentiate into adult Purkinje cells, migrate into the host molecular layer and organize into a single cell layer to replace the Purkinje cell lost after kainic acid lesioning. The result from the behavioural studies indicated that the transplantation of the rhombencephalic cell suspension into the lesioned area in the cerebellar cortex can improve the motor deficits as shown by a decrease in the frequency of dysmetria and an increase in the frequency of normal rearing behaviour. At 3 months after transplantation, there was no significant difference in the frequencies of dysmetria and normal rearing behaviour between transplantation and control groups. However, when observed with neurological tests, the percent of correct response of the transplantation group was lager than the lesioned groups but still had a significance difference ($p<0.05$) from the control group. In summary, the embryonic rhombencephalic cell suspension can be functionally Abstractintegrated into a host brain and restore the motor deficits caused by the lesioning in the cerebellum, and although this restoration does not reach normal levels, it is significant different from the lesioned animals. The results from this experiment suggest that the open-field testing and the neurological examination are useful for evaluation of functional recovery as effects of the grafted tissues, with the neurological examination being more sensitive for detection the function effects of transplanted tissue than open-field testing.

EVOLUTIONAL MORPHOLOGY OF AMYGDALOID ANTERIOR CORTICAL NUCLEUS

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In our previously conducted researches on rats we investigated two levels (rostral and caudal) in the structure of anterior cortical nucleus (COa).

The cytoarchitecture of the cats COa was studied using Nissl techniques. The results suggests that the rostral level of COa is located between the nucleus of the lateral olfactory tract and the piriform cortex. In contrast to the last one the territory engaged by the nucleus is characterized by less cell density and its boundaries with depth structures are vague. But still the cells forming deep zone of COa differ from the neurons of anterior amegdaloid area by greater degree of polymorphism. There is a lateral part of surface cellular zone characterized by tendency to space arrangement of its containing neurons on the boundary with piriform cortex.

The nucleus on the caudal level of the anterior part is bordering on the medial nucleus neurons. Being the part of it they are characterized by dispersion arrangement. The devision into four zones on caudal level is being reserved.

The conducted cytoarchitectonical analysis of the anterior cortical nucleus of amygdala of rodents and predatory animals reveals the availability of their structural arrangement mutual plan. It gives a possibility to conjecture the existence of universal biological natural phenomena in the construction of this brain formation of the neighbouring vertebrates' orders.

STRUCTURAL-FUNCTIONAL ORGANIZATION OF AMYGDALOID POSTERIOR CORTICAL NUCLEUS IN THE RAT

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The purpose of this research was an investigation of the structural-functional organization of the amygdaloid posterior cortical nucleus (COp) with the help of morphological and electrophysiological techniques. The cytoarchitecture and neuronal morphology of the rats COp was studied using Nissl and Golgi techniques.

These studies have demonstrated that on the territory of the posterior part of the amygdala there are three levels (rostral, caudal and transition to hippocampus (COp_{III}). In rostral and caudal levels we have described three zones: surface, medial and lateral cellular zones. In contrast to the last two in territory of COp_{III} there are different three zones: surface, cellular surface and deep.

The electrophysiological characteristics of the COp we studied using chronically implanted stimulation and recording monopolar electrodes in different cellular layers of the posterior cortical nucleus. In animals spontaneous spiking and sharp waves was seen in prestimulation recordings, particularly those with electrodes in medial part of the posterior cortical nucleus.

BEHAVIOURAL COMPARISONS BETWEEN INBRED AND OUTBRED STRAINS OF SOCIALLY REARED RATS.

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Prepulse inhibition (PPI) of the acoustic startle response, an operational measure of sensorimotor gating, is impaired in schizophrenics. Similar impairment can be induced in rats by isolation rearing from weaning. Comparisons between isolation reared and socially reared outbred strains of rats have shown that PPI levels within rearing condition varied to such an extent that effects of isolation rearing were sometimes masked. It is therefore desirable to identify an experimental subject with less inter-animal variation. Five strains of weanling rats were socially housed for eight weeks, after which time they were tested for their levels of prepulse inhibition (PPI). Two outbred strains (Wistar and Lister Hooded) and three inbred strains (PVG, Fischer 344 and Lewis) rats were used. All rats were tested under the same conditions and the data analysed using two-way ANOVA of repeated measures. From the data it was found that there was both a significant strain and noise difference ($F_{(4,35)}$=3.169, P>0.03 and $F_{(2,70)}$=119.452, P>0.01, respectively) and there was also a significant strain x noise interaction ($F_{(8,70)}$=3.391, P>0.02). Analysing the startle reponses of each strain, a significant difference was found ($F_{(4,35)}$=31.552, P>0.01), a *post hoc* Duncan test found that there was a significant difference between the PVG rats versus all other strains used (P>0.01). Examining the habituation over the session, there were significant strain and noise differences ($F_{(4,35)}$=23.783, P>0.01 and $F_{(1,35)}$=40.245, P>0.01, respectively) however there was no strain x noise interaction. Overall there was a significant strain weight difference ($F_{(4,35)}$=50.511, P>0.01), with *post hoc* Newman-Keuls revealing a significant difference between the Lister Hooded rats versus the PVG and Fischer 344 rats (P>0.05), however there was no significant difference between either outbred strain and the Lewis rats. Further examination of the raw data, showed that Lewis rats resulted in a narrower spread of data when compared to all other strains. In conclusion, it would appear that the Lewis rats should be considered as an inbred strain worth investigating further in the isolation rearing paradigm.

SCRAPIE INFECTED MICE AND PrP KNOCKOUT MICE SHARE ABNORMAL LOCALIZATION AND ACTIVITY OF NEURONAL NITRIC OXIDE.

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PrP^{Sc}, the only identified component of the scrapie prion, is a conformational isoform of PrP^{c}. The physiological role of PrP^{c} , a glycolipid anchored glycoprotein is still unknown. We have shown previously that neuronal nitric oxide synthase (nNOS) activity is impaired in the brains of mice sick with experimental scrapie as well as in scrapie infected neuroblastoma cells. The aim of this study was to investigate why nNOS activity in scrapie infected mice is reduced, and thereby understand the relation of nNOS to PrP. To this aim we tested the subcellular localization of nNOS in brains of wt and scrapie infected mice as well as in mice in which the PrP gene was ablated. We now report that while in wt mice, nNOS, as PrP^{C}, is associated with detergent insoluble cholesterol-rich membranal microdomains (rafts), this is not the case in brains of scrapie infected or in those of adult $PrP^{0/0}$ mice. Also, adult $PrP^{0/0}$, as scrapie infected mice, present with reduced nNOS activity. We suggest that PrP^{c} may play a role in the targeting of nNOS to its proper subcellular localization. The similarities of nNOS properties in $PrP^{0/0}$ as compared to scrapie infected mice suggest that at least this role of PrP^{c} may be impaired in scrapie infected brains. We also found that nNOS immunoprecipitates with an anti-PrP antibody and that PrP immunoprecipitates with an anti-nNOS antibody, therefore we can conclude that nNOS and PrP reside in proximity. This study suggests that PrP and nNOS belong to the same functional complex.

We also found that nNOS immunoprecipitates with an anti-PrP antibody and that PrP immunoprecipitates with an anti-nNOS antibody, therefor we can conclud that nNOS and PrP reside in proximity.

PLATELET INOSITOL 1,4,5 TRIPHOSPHATE IN OBSESSIVE COMPULSIVE DISORDER

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Serotonin(5-HT) has been implicated in the pathogenesis of Obsessive Compulsive Disorder (OCD). Inositol 1,4,5-triphosphate (IP3) has been established as the second messenger linked to the activation of 5-HT2 receptor which mediates the effect of receptor activation by releasing intracellular calcium. The current study aims at examining the IP3 levels by a radioreceptor assay system in platelets obtained from patients with Obsessive Compulsive disorder and compared to that of normal healthy controls. 28 subjects with OCD and 12 normal subjects were recruited for this study. 40ml blood was collected in a fasting state, and the platelet rich plasma was separated. The platelet pellet was isolated and IP3 level assayed by using a commercially available kit (Biotrak, Amersham Inc). There were no significant differences between the two groups. Other markers of serotonergic function will have to be studied to determine the site of dysfunction in the neurobiology of OCD.

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TIME AND VOLTAGE-DEPENDENT BLOCK OF THE DELAY RECTIFIER K^+ CURRENT IN HIPPOCAMPAL PYRAMIDAL NEURONS BY THERAPEUTIC CONCENTRATION OF NIMODIPINE.

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Nimodipine is a high affinity blocker of L-type Ca channels which is widely used for therapeutic treatment of some central nervous system disorders, including focal brain ischemia, epilepsy and brain aging. Effects of nimodipine on delayed-rectifier potassium currents (I_k) were studied in acutely isolated rat CA1 and CA3 hippocampal neurons using the patch-clamp technique in the whole-cell configuration. Externally applied nimodipine produced a strong change in the kinetics of I_k. Block of I_k by nimodipine was accompanied by a dose-dependent acceleration of the current decay.The latter effect became noticeable at 100nM of nimodipine. The IC50 value of 3.4μM was obtained for peak amplitude of I_k and 0.71μM for I_k measured at 130ms and at testing potential (V_T)=+30 mV. Inhibition of I_k by nimodipine depends on the V_T. The 2μM of the drug depressed I_k by about 30% at V_T=+20mV and by 65% at V_T=-40 mV. The voltage-dependence of steady-state inactivation of I_k remained unaffected . Analyses of voltage and time-dependent block of I_k allow to suggest that nimodipine blocks open state of delayed rectifier potassium channels. It has been also found out that the strength of nimodipine blockade depend on $[Ca^{2+}]_{in}$. As a whole our results demonstrate that nimodipine is a voltage-dependent, reversible blocker of I_k. The delayed rectifier channels are the main pathway for the leak of extracellular potassium from the neurons, depolarized by ischaemia . The selective inhibition of this pathway by nimodipine may contribute to the neuroprotective action of nimodipine.

AMPA AND NMDA RECEPTOR MEDIATED COMPONENTS OF "MINIMAL" EPSPs RECORDED FROM THE SAME SYNAPTIC TERMINALS SHOW EQUAL POSTTETANIC LTP IN THE CA1 HIPPOCAMPAL REGION IN VITRO.

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AMPA and NMDA receptor mediated components of EPSPs (EPSP-A and EPSP-N) could exhibit, depending on the experimental parameters, equal or unequal degree of posttetanic LTP. Such variability may be explained either by differences in the mechanisms of LTP of these components or by differential distribution of the AMPA and NMDA receptors in synapses with different abilities to express LTP. To clarify this matter, we studied LTP of EPSP-A and EPSP-N generated at the same synaptic terminals. Whole-cell voltage clamp of the CA1 pyramidal neurons and "minimal" afferent stimulation were used. Pharmacological (application of NMDA antagonist APV, 25 mkm/l) and mathematical ("main components") analysis were employed to verify whether EPSP-A and EPSP-N were generated at the same synaptic terminals. We found that in cases when trial-to trial magnitudes of "minimal" EPSP-A and EPSP-N were highly correlated (indicating co-localization of the correpondent receptors), they exhibited equal degree and time course of LTP: at 5-15 min after tetanization 306+-24% and 343+-82%; at 40-60 min 232+-35 and 235+-44% for EPSP-A and EPSP-N respectively (N=10). Thus, co-localized at the same synapses, AMPA and NMDA receptors equally contribute to maintenance of posttetanic LTP; the difference in LTP of relatively big ("compound") EPSP-A and EPSP-N could be attributed to differential expression of the correspondent receptors in synapses with different abilities to express LTP.
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INHIBITORY ACTION OF AMBOCARB ON VOLTAGE-OPERATED SODIUM CHANNELS IN ISOLATED RAT HIPPOCAMPAL PYRAMIDAL NEURONES

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To determine whether antihypoxic actions of nootropic drugs (cognitive enhancers) could be mediated via interaction with voltage-operated sodium channels, we performed a whole-cell patch clamp study of the effects of four structurally unrelated nootrops on sodium currents in acutely isolated rat hippocampal pyramidal neurones. Piracetam (3 mM), nooglutil (100 μM) and etimizol (100 μM) did not affect the amplitude of sodium currents. At the same time, ambocarb, a novel β-carboline, potently and reversibly supressed sodium currents in the range of concentrations 3-300 μM. The amount of block was dependent on the holding potential with half-maximal inhibition values comprising 26 and 94 μM at −80 and −120 mV respectively. Ambocarb induced a hyperpolarising shift in the steady-state availability curve that indicates the increase in the proportion of inactivated sodium channels. This action is presumably mediated by promoting development of inactivation and slowing the recovery from inactivation of sodium channels.
Since many neuroprotective drugs are shown to inhibit sodium currents, down-modulation of voltage-operated sodium channels that complements known positive interaction of ambocarb and other 3,4-tetramethyleneharmanes with $GABA_A$ receptors, may provide a promising strategy in the treatment of brain disorders associated with trauma and ischaemia.
This study was supported by Swiss National Science Foundation grant # 7UKPJ048607 and INTAS grants # 96-1493 and 97-0382.

TYPES, DISTRIBUTION, AND FUNCTION OF VOLTAGE GATED K^+ CHANNELS IN LAYER 5 NEOCORTICAL PYRAMIDAL NEURONS FROM YOUNG RATS.

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We investigated the role of voltage gated potassium channels in the propagation of the action potential back into the apical dendrite. A slow outward K^+ current and a fast outward K^+ current were detected in nucleated outside-out patches under conditions minimizing the contribution of calcium activated potassium channels. The slow K^+ current was blocked by Tetraethylammonium (TEA) with an IC_{50} of 5±1 mM and only slightly blocked by 4-Aminopyridine (4-AP). The fast K^+ current was blocked by 4-AP with an IC_{50} of 0.30±0.05 mM and was not blocked by TEA. The current density of the fast K^+ current in cell-attached patches decreased moderately along the apical dendrite. The current density of the slow K^+ current did not change appreciably distal to the soma. Block of the slow potassium current with 1 mM TEA prolonged the duration of the back-propagating action potential and facilitated the generation of calcium spikes in the apical dendrite. Block of the fast potassium current with 50 μM 4-AP caused a marked increase in the firing frequency of the neuron at a given level of stimulation. It is concluded that in neocortical pyramidal neurons of layer 5 the fast, 4-AP sensitive, potassium current counteracts the depolarizing action of the sodium current while the slow, TEA sensitive, potassium current counteracts the depolarizing action of both the sodium and calcium currents.

ALZHEIMER'S SOLUBLE AMYLOID BETA PROTEIN IS NOT SECRETED BY INTESTINAL CACO-2 CELLS

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Amyloid β (Aβ) is an important constituent of Alzheimer's and Down's syndrome brain amyloid and is a normal soluble human protein (sAβ). Recently we reported that sAβ in plasma and CSF is associated with high density lipoprotein (LP) and that sAβ is secreted by hepatic cells complexed to the LP (*FASEB J* (1998) **12,** 1097-99) . However, the major sources of the LP in humans are hepatic and intestinal cells, but whether the latter cells are secreting sAβ and if so whether sAβ is apolipoprotein (apo) and/or lipid associated upon secretion or not was not known. We chose and tested intestinal Caco-2 cell line, widely used for different metabolic and LP syntheses studies. The results have shown, that although Caco-2 cells secreted into the media a significant amount of LP-lipid and apo A-I, A-II, A-IV, E, J and SAA (assessed by size exclusion HPLC, immunoblot analysis, immunoprecipitation with corresponding antibodies and by analysis of [14C]-acetate metabolically labeled lipids), they did not produce and secrete sAβ. Taken together with our report on sAβ secretion by hepatic cells current study suggests that hepatic (and not intestinal) cells may be an important source of the LP-associated systemic Aβ. Supported by RAMS, WIS, The Sir Charles Clore fellowship and Senetek, PLC.

ADVERSE EFFECTS OF PARATHYROID HORMONE ON RAT HIPPOCAMPAL NEURONS AS ONE OF RISK FACTORS FOR SENILE DEMENTIA

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Adverse effects of parathyroid hormone (PTH) were examined using rat hippocampal slices in organotypic culture. Exposure of cultured slice preparations to 0.1 mM PTH for 60 min resulted in a gradual increase in the intracellular Ca2+ concentration ([Ca2+]i); this effect was most obvious in the CA1 region. When PTH (1 nM) was added to the culture medium and its toxic effects examined using a propidium iodide intercalation method, significant toxicity was observed three days after exposure, the toxicity increasing with time. Cells in the CA1 region seemed more vulnerable to the hormone than cells in other regions. The toxic effects were dose-dependent over the range of 0.1 pM to 0.1 mM. The adverse effects were also induced by an active fragment PTH1-34, but not by an inactive fragment, PTH 39-84 , nor by an active fragment of PTH-related peptide (PTHrP 1-34), an intrinsic ligand of the brain PTH receptor. The PTH-induced adverse effects were significantly inhibited by 10 mM nifedipine. The present study demonstrates that sustained high levels of PTH in the brain might cause degeneration of specific brain regions due to Ca2+ overloading , and suggests that PTH may be a risk factor for senile dementia.

THE NEUROPATHOLOGY OF TRANSGENIC MICE CARRYING MUTANT APP AND PS-1 TRANSGENES; AN EM STUDY

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APP AND PS-1 MUTATIONS LEAD TO AN INCREASE IN BETA AMYLOID (Aβ) PRODUCTION. DESPITE THE FACT THAT A NUMBER OF TRANSGENIC MICE DEVELOP CEREBRAL Aβ PLAQUES, FEW HAVE BEEN SUBJECTED TO ULTRASTRUCTURAL INVESTIGATION. WE THEREFORE, INVESTIGATED THE DOUBLY TRANSGENIC (MUTANT $APP_{K670N,M671L}$ - MUTANT $PS1_{M146L}$) MOUSE WHICH DEVELOPS Aβ DEPOSITS MUCH EARLIER THAN SINGLY TRANSGENIC LITTERMATES. WIDESPREAD Aβ PLAQUES WITH OR WITHOUT A DISTINCT CORE WERE FOUND IN GREY MATTER. Aβ PLAQUES WERE ALSO PRESENT IN WHITE MATTER AS WERE CEREBROVASCULAR Aβ DEPOSITS. ASTROCYTOSIS WAS GREATER AROUND GREY MATTER THAN WHITE MATTER PLAQUES. IN SOME PLAQUES, Aβ CORES WERE ASSOCIATED WITH CELLULAR PROFILES CONTAINING PROMINENT ENDOPLASMIC RETICULUM AND A HOMOGENOUS CYTOPLASM THAT APPEARED TO BE NEURONAL. SOME OF THESE PROFILES IN GREY MATTER CONTAINED LARGE DENSE VESICLES. THE MORPHOLOGY AND LOCATION OF OTHER PROFILES INDICATED THEM TO BE MICROGLIA OR OLIGODENDOCYTES. SOME Aβ FIBRILS APPEARED TO LIE WITHIN THESE PROFILES BUT THEY MAY HAVE BEEN SIMPLY SURROUNDED BY THE CELL PROFILE SINCE THE PROFILE MEMBRANE WAS NOT ALWAYS VISIBLE. DARK ATROPHIC NEURONES WERE PRESENT AROUND GREY MATTER PLAQUES. INTERESTINGLY, FILAMENTOUS STRUCTURES REMINISCENT OF THE PHFS WERE FOUND INSIDE ONE ATROPHIC NEURON. THUS, THE NEUROPATHOLOGY OBSERVED IN PS1/APP MOUSE BRAIN IS SIMILAR TO THAT IN AD AND THEY APPEAR TO BE THE BEST MODEL OF AD PATHOLOGY CURRENTLY AVAILABLE.

ADENOSINE A1 RECEPTOR (A1R) ANTAGONIST KF15372 ENHANCES COGNITIVE FUNCTIONS VIA THE INCREASE OF ACETYLCHOLINE RELEASE IN CEREBRAL CORTEX

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Adenosine A1 receptor may play a significant role in the process of cognition, since it is abundantly expressed in both hippocampus and cerebral cortex. Therefore, we have first evaluated the effect of KF15372 on disturbance of passive avoidance task in rodents. KF15372 (1.25 - 5 mg/kg, p.o.) prevented N^6-(L-phenylisopropyl) adenosine (R-PIA; A1 agonist)-induced passive avoidance response failure in rats. In addition, repeated administration of KF15372 (0.08 - 5 mg/kg, p.o. x 7 days)ameliorated the impairment of learning induced by basal forebrain lesion in rats.

We further investigated the mechanism of cognitive enhancing effect of KF15372 in rat cerebral cortex using brain microdialysis method. Oral administration of KF15372 at doses of 1.25, 5 and 20 mg/kg significantly increased the extracellular levels of acetylcholine in rat cerebral cortex. A1 agonist R-PIA did not affect the extracellular level of acetylcholine by both oral and intracortical administrations via dialysis probe. These results suggest that extracellular level of acetylcholine is under tonic inhibitory control by endogenous adenosine via the A1 receptor and that adenosine A1 antagonist such as KF15372 may enhance the cognitive function via the increase of extracellular acetylcholine in cerebral cortex.

DISTRIBUTION AND CHEMICAL CODING OF NEURONS IN PREVERTEBRAL GANGLIA SUPPLYING THE VAS DEFERENS AND SEMINAL VESICLE IN THE PIG.

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The present study deals with the distribution and neurochemical characteristics of neurons in the inferior mesenteric and anterior pelvic ganglion (IMG and APG, respectively) supplying the vas deferens (VD) and seminal vesicle (SV) in the pig. Investigations were performed on 6 juvenile boars using combined retrograde tracing (retrograde tracer Fast Blue (FB) was injected into the wall of the left VD (n=3) or into the left SV) and double-labelling immunohistochemistry. The porcine IMG was found to contain many neurons projecting to both the organs studied. The Fast Blue-positive (FB^+) VD or SV projecting neurons occurred in both the left and right IMG with a distinct predominance in the ipsilateral one. In the ipsilateral ganglion, most of them were located close to the caudal pole. Some neurons formed also a narrow strip distributed along the dorso-lateral border of the ganglion extending from the caudal „centre" to the cranial pole. In the contralateral IMG, the FB^+ neurons were more dispersed, however, most of them were also located caudally. Immunohistochemical features of IMG neurons projecting to the VD and SV were very similar. The vast majority of them (≈90%) were tyrosine hydroxylase-immunoreactive (TH-IR) and many neurons (≈30%) contained immunoreactivity to TH and neuropeptide Y (NPY). The porcine APG was found to contain numerous neurons projecting to the VD and SV. The VD or SV projecting neurons occurred in both the left and right APG but most of them were found in the ipsilateral one. In the ipsilateral ganglion, many VD projecting neurons formed a cluster located close to the VD nerve output. In the contralateral APG, the neurons were scattered. Immunohistochemical features of pelvic neurons projecting to the VD and SV were very similar. About 50% of the neurons were TH-IR. These neurons contained also immunoreactivities to NPY, Met^5-enkephalin-Arg^6-Gly^7-Leu^8 or galanin. The majority of the TH-negative neurons displayed immunoreactivity to vasoactive intestinal polypeptide, somatostatin or NPY. The pelvic FB^+ neurons were intensely supplied with nerve fibres immunoreactive to substance P and calcitonin gene-related peptide. We conclude that the IMG, in addition to the pelvic ganglia, should be considered as a prominent source of innervation for the porcine male reproductive tract, and that the adrenergic (i.e. TH-IR) neurons in this two locations seem to constitute two distinct subpopulations with respect to their neuropeptide content.

A QUALITATIVE MATHEMATICAL MODEL FOR THE SIMULATION OF THE MOTOR CIRCADIAN RHYTHM OF CRAYFISH

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The different motor behaviors underlying the motor circadian system, necessarily produce a complex and, many times, irregular pattern in the overt motor rhythm. Crayfish, for example, display a wide range of rhythmic motor patterns many of them under the influence of a biological clock which, however, has been not located yet. To obtain information about the organization of oscillators underlying the motor circadian rhythm in crayfish, we have analyzed the motor activity patterns during several developmental stages, from very early after hatching to adult stage.

From comparison of these patterns, we have concluded that in crayfish there are, at least, two groups of oscillators involved in the generation and expression of the motor circadian rhythm. The first one which appears very early in ontogeny is located in the cerebroid ganglion, and would be the responsible of the generation of circadian motor rhythm, and a second oscillator, appearing later in ontogeny, is located in the eyestalk, i.e. in the sinus gland, and would be the responsible of the synchronization of the motor circadian rhythm to external signals. On the basis of these experimental results, we have built a qualitative mathematical model which simulates them and that let us to understand the main process involved in the generation and expression of the motor circadian rhythm of crayfish. Supported by 278-A1N CONACyT grant.

UNINVASIVE TRANSCRANIAL ELECTROSTIMULATION (TES) OF THE BRAIN ANTINOCICEPTIVE SYSTEM AS A POSSIBLE SUBSTITUTE OF THE ACUPUNTURE

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Aim of investigation. It is well know that in the effects of acupuncture the activation of brain antinociceptive system with its endorphinergic and serotoninergic mechanisms is involved. To use acupunture as a method of treatment rightly and effectively it is necessary to be educated and experienced for a long period of time. Simple method of TES was elaborated for direct activation of antinociceptive system based on experimental and clinical and clinical data. The experience of the results of 15-years experimental and clinical studies will be reviewed.

Methods. In screening experiments on different species of animals the effective regimen of TES for reduction of acute pain and increase of pain tolerance was elaborated. Quasiresonance activation namely of the brain antinociceptive system in with endorphinergic and serotoninergic mechanisms was demonstrated by study of current density and pathways in the brain (MRI), location of activated neuronal groups ($[^3H]$-deoxyglucose autoradiography, C-fos expression), β-endorphin release (radioimmunoassay) and usage of effects of agonists-antagonists of endorphinergic and serotoninergic neurotransmission. Besides the analgesia the very positive TES effects were proved in several experimental models of pathological processes (acceleration of healing of the damaged skin and gastric epithelium, connective tissue, afferent and efferent nerve fibers, reduction of alcohol abstinence syndrome, inhibition of implanted tumor growths). Special devices for clinical usage were developed.

Results. In randomized placebo-controlled (passive and active placebo were used) clinical studies in correspondence with experimental data the deep analgesic effect of TES was demonstrated in surgery and in treatment the pain syndromes of different types and ethiology (postoperative, oncological, low back, headache, trigeminitis etc). TES was effectively used for treatment of wounds and thermal burns, gastric and duodenal ulcers, acute myocardial infarction, sensory-neural deafness. In alcoholics TES reduces withdrawal syndrome, affective disturbances in remission and crawing. In several cases the curative effects of TES were higher in some respects in comparison with acupunture especially in acute period of diseases.

Conclusions. As it was demonstrated in experimental models and in clinical observations the central neurophysiological mechanisms and curative spectrum of TES effects are close to the acupunture ones. Technically the TES practical application is very simple and uninvasive. Taking into account the clinical effectiveness of TES this method could be recommended as the simple substitute of acupuncture.

COEXISTENCE OF PRION AND ß AMYLOID IMMUNOPOSITIVE SENILE PLAQUES IN A FAMILIAL ALZHEIMER'S DISEASE WITHOUT MUTATIONS IN THE AD GENES.

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The genetic profile of a family with autosomal dominant transmission of early-onset Alzheimer type dementia has been studied. No known mutations, either in the ß amyloid precursor protein or in the presenilin-1 and -2 genes have been found (Savioz et al., Dementia Cog Ger, in press). We describe here the brain pathology of five deceased members, studied with methenamin and Gallyas methods as well as with various antibodies (anti-ßA4, anti-prion PrP 106-126, anti-tau, anti-ubiquitin). For all five cases, data reveal in several cortical and subcortical regions a heavy typical Alzheimer's disease pathology with numerous methenamin and/or ßA4 positive senile plaques, together with a high number of Gallyas and tau positive neurofibrillary tangles and threads. Part of those are equally marked by ubiquitin. In addition, frequent plaques are also stained by the monoclonal antibody against PrP 106-126. They are more abundant in the superficial layers of the cerebral cortex, which show also some degree of spongiosity. Double-bind or alternate sections stained with ßA4 and PrP antibodies demonstrate that there are three populations of plaques, only ßA4 or PrP positive, or positive for both antibodies. Thus, a subtype of familial early-onset Alzheimer's disease can coexist with a prion pathology. As all studied members are similarly affected, the chance that it is purely coincident is very low. The prion gene is currently under investigation.

THE MANIPULATION OF LIGHT INDUCED RHYTHMIC OSCILLATION OF THE INTERGENICULATE LEAFLET NEURONS IN THE RAT

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The intergeniculate leaflet (IGL) is a subdivision of the thalamic lateral geniculate complex and is an important component of the mammalian circadian timekeeping system. The IGL integrate photic and nonphotic information to modify the suprachiasmatic nucleus (SCN) activation. The aim of the present study was to explain whether the light manipulation modulated the activity of neurons. We recorded multiple unit neuronal activity (MUA) from dorsal and ventral part of lateral geniculate nuclei and from IGL in anaesthetized rats. In all subdivision of lateral geniculate complex we observed spontaneous firing rates of cells but only in IGL after light ON the neurons activity was significant increase. But just after light OFF, the responses in IGL stabilized and exhibited surprisingly a highly regular oscillatory patterning. However, this effect was only observed in the area of the anatomical localization of the IGL. The results show that light is a most important stimulus for IGL neurons but by oneself can't evoked rhythmically oscillation in the rat IGL neurons. To evoked such rhythmically modulation, the IGL need additional nonphotic inputs. According to the others investigation we suggested that light -OFF influences on the IGL activity like nonphotic inputs.

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EFFECT OF SECTION OF TRACHEOSYRINGEAL NERVE ON CALL IN *CARDUELIS SINICA*

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4 healthy adult male *Carduelis sinica* were obtained during April and May, 1997. Birds were anaesthetized with 25% urethane(0.5g/kg weight) injecting to thoracic muscles and was put on the back. Surgery was performed in the neck and NXIIts is separated from either side of the trachea, and about 5mm of nerve was removed unilaterally or bilaterally. Calls were recorded with SHARP-GF-6060 tape recorder. Recordings were examined using a Kay sonagraph set (Model 662B). Frequency properties were measured by sonagram of filter and power spectra.

After unilateral section of tracheosyringeal nerve (NXIIts), *Carduelis sinica* may repeat the normal call,and has no effect on temporal properties. It suggested that either side of syrinx may produce sound alone and ipsilateral innervation of NXIIts for the syringeal muscles. Section of left NXIIts,the bird produce the vocal pattern of individual sound increase,and the effect on sound density and syllable number averages 1.4 and 1.7 times than that of section of right NXIIts ,suggested that the innervation of NXIIts has left side dominance. After bilateral section of NXIIts ,the call rhythm accompanied by expiratory motion is 98-146/min,and lost all sentence types and syllable structure of normal call.The call spectra produced by tympaniform membrane vibration without innervation is still reserved ,and with the similar frequency composition of main frequency and harmonic frequency of normal call. Compared call pre-and post NXIIts section in *Carduelis sinica*, we provide more knowledge about syringeal nerve function in vocal control.

THE ROLE OF CATECHOLAMINE IN MEMORY RFORMANCE ON THE PARTIALLY BAITED EIGHT-ARM MAZE IN THE RAT

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A variety of studies shows that catecholaminergic systems may be involved in the memory performance in the radial arm maze (RAM). To clarify the catecholaminergic function in spatial cognition of the rat, the present work investigated the effects dopaminergic and noradrenergic receptor antagonists on the memory performance using the partially baited eight-arm RAM. Food-deprived rats were first trained to enter the arms baited with chocolate in an eight-arm baited RAM. Each subject was randomly assigned to receive further training in either place or cue task. Specific four arms were baited throughout experimentation as the procedure for the place task, whereas four randomly-chosen arms each cued with a piece of sand paper on the arm entrance were baited in every trial as for that of the cue task. For the drug evaluation, the well-trained subjects were challenged with systemic injections of SCH23390 (SCH), spiperone (SPI), haloperidol (HAL), prazosin (PRA), yohimbine (YOH), and propranolol (PRO). In regarding the place task, SCH and PRO significantly impaired behavioral performance of the place task by increasing the number of arm entries as well as the time to complete task. The accurate performance on the cue task was not significantly affected by any of these drugs. However, the times to complete the cue task were significantly increased by SCH, SPI, HAL, PRA, and PRO. These data clearly indicate that the distinct catecholaminergic receptor subtypes differently involved in the memory retention of spatial and non-spatial on the partially baited eight-arm RAM.

ASTROGLIAL CELL TRANSPLANTATION IMPROVE PERFORMANCE IN A DELAYED RESPONSE TASK IN *Cebus apella* MONKEYS.

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Resolution of tasks involving motor-cognitive functions require prefrontal cortex-basal ganglia neural circuits. Cognitive function is affected in patients with idiopathic Parkinson's disease as well as in monkeys with experimental parkinsonism. In order to optimize the functional recovery of the striatal neuropil in monkeys with parkinsonism induced by systemic administration of the MPTP neurotoxin, transplantation of fetal astroglial cells was performed into the striatum of three groups of *Cebus apella* monkeys [Group A (N=3): unilateral transplant and sham operation on the opposite side; Group B (N=2): bilateral transplants; Group C (N=2): bilateral sham operations]. Before and after each procedure, a classical Delayed Response task was administered with delays ranging from 0 to 10 seconds. It was observed that: a) the efficiency was severely affected after the MPTP administration (succesfull attempts were attained at the following delays for each monkey: pre MPTP: Group A: 6, 10 and 6 seconds' delay; Group B: 10 and 10; Group C: 6 and 10; post MPTP: Groups A, B and C: 0 seconds' delay); b) the reaction time to open a well (p between <0.0001 and 0.0002, T Test) and reach the reward (p between <0.0001 and 0.0001, T Test) increased significantly after the induction of parkinsonism in every group; c) after carrying out the surgical procedures which included or not astroglial cell transplantation, groups A and B showed recovers (Group A: 6, 10 and 4 seconds' delay; Group B: 6 and 8) whereas Group C did not (Group C: 0 and 2 seconds' delay); d) after surgical procedures were completed every group showed only a partial improvement in the reaction time to open and reach. Transplantation of fetal astroglial cells proved to be effective to recover the performance in a classical Delayed Response task in adult monkeys with post MPTP experimental parkinsonism. Dissociation between cognitive and motor performance is also suggested. Acknowledgments: Corpo Médica, Fundación Conectar, CONICET, Fundación Bunge & Born, CEMIC.

ENVIRONMENTAL EFFECTS ON THE IMMUNOREACTIVE ASTROGLIA EXPRESSION IN FRONTAL AND CINGULATE CORTICES IN RATS: A LAMINAR STUDY.

Gayol SB, Lipina SJ, Colombo JA. Programa Unidad de Neurobiología Aplicada (PRUNA)(CEMIC, CONICET). Buenos Aires, Argentina.

Early environmental enrichment or deprivation alter the central nervous system both structurally and functionally. The effects of these variables on the cerebral cortex astroglia were assessed in two groups of post-weanning rats: A (N=4), sensory, motor and social stimulation for two months; B (N=4), isolation. Brains were processed for immunostaining of acid gliofibrilar protein (GFAP) (a-GFAP, Biogenex, 1:5000). Samples from the Frontal (Fr 1-3) and Cingulate region (Cg 1-3) (Zilles, 1985) were taken at three distances from the frontal pole (1, 3 and 5 mm). The GFAP-IR expression was measured in layers I to VI in the frontal region and I to IV in the cingulate using an automatized system of image analysis (Optimas Bioscan). Data were transformed trigonometrically and analyzed according to the Multiple Variance Analysis. The following results were obtained: a) in group A the GFAP-IR had a greater expression than in group B (Frontal $p<0.0001$, $F=828.07$; Cingulate $p<0.0001$, $F=686.98$); b) differences were statistically significant between both groups as regards to hemispheres (Frontal $p=0.02$, $F=5.855$; Cingulate $p=0.02$, $F=5.429$), segments (Frontal $p<0.0001$, $F=16.160$; Cingulate $p<0.0001$, $F=12.667$), and layers (Frontal $p=0.02$, $F=3.244$; Cingulate $p=0.013$, $F=3.60$); c) a greater GFAP-IR expression in layers I and VI in the Frontal region of group A. These results show that: a) the environmental variables applied altered in a differential manner cerebral cortex GFAP-IR astroglia. A greater GFAP-IR expression was observed in the experimentally enriched; and b) this variation is interhemispheric asymmetric and cytoarchitectonic (laminar) heterogeneous. Acknowledgements: Corpo Médica, Fundación Conectar, CEMIC, CONICET, Fundación Bunge & Born.

REGIONAL DIFFERENCES IN ASTROGLIAL MATURATION DURING BRAIN DEVELOPMENT IN EX-VIVO CONDITIONS.

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Morphological cell differentiation in response to cAMP was evaluated in rat astroglial subcultures from different brain regions at prenatal (E14, E16, E21) or postnatal (PN 5-6) ages. Purified astroglia was characterized by immunocytochemistry as GFAP +/-, vimentin +, S100 +, fibronectin +/-. Primary cultures of prenatal (E16) or postnatal (PN5-6) origin were seeded on poly-l-lysine and fed with DMEM-F12 supplemented with B27 nutrient mixture. Cells were evaluated 3 hours after 0.1 or 0.5 mM cAMP addition. E14 and E16 cerebral cortex (CC) and ventral mesencephalic (VM) astroglia responded in a dose dependent manner to cAMP addition. Reactive/total cell ratio (R/T) in 0.5 mM cAMP was: for CC and VM: 0.41 and 0.63 at E14 and 0.67 and 0.95 at E16, respectively ($p<0.001$ with respect to DMEM). Striatal (S) astroglia of the same ages showed R/T lower than 0.15 ($p<0.001$ respect to CC and VM). At E21 CC astroglia reached maximal R/T values (0.92) similar to PN astroglia. E21 S astroglia, though more reactive than the former ages (0.69, $p<0.001$ respect to E16) did not show R/T values of PN astroglia (0.86, $p<0.001$ referred to E21) from the same region. E16 S astroglia at various prenatal ages became responsive to cAMP when cocultured with PN primary cultures from the same three regions in cell contact conditions. VM and S primary cultures produced the highest R/T values (VM= 0.87, S= 0.83, $p<0.001$ and $p<0.01$, respectively, compared with CC (R/T= 0.31). This inductive effect was significantly reduced if neuronal elements were absent. Primary cultures from prenatal (E16) origin did not induce changes in R/T values in either of the two coculture conditions. S astroglia showed an immature feature related to its ability to respond with astrocitation to cAMP. This was also evidenced by a high fibronectin and low GFAP immunoreactivity during the same period. Age-related increase in cAMP reactivity correlated with an increase in GFAP immunolabelling (R= 0.814). This developmental lag in striatal maturation was overcome by cell contact with postnatal mixed cell populations from VM, S or CC. Aknowledgements: Fundación CONECTAR, CEMIC, CONICET, A. Von Humboldt Foundation, CORPOMÉDICA.

A NEW COGNITIVE ENHANCER KA-672.HCL IS UNCOMPETITIVE VOLTAGE-DEPENDENT NMDA RECEPTOR ANTAGONIST

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Here we demonstrate that the novel neuronal activator and antidementia agent KA-672.HCl (7-Methoxy-6 [-3-[-4-(2-methoxyphenyl)piperazin-1-yl] propoxy]- 3,4- dimethyl-2*H*-1 benzopyran-2-one hydrochloride) is a low affinity NMDA receptor-operated channel blocker which lacks the use-dependent mode of action. KA-672.HCl (KA-672) is a new substance demonstrating antidementia properties. It shows modulatory effects on several neurotransmitter systems known to be affected the patients with Alzheimer disease. In our study the action of KA-672 on the NMDA receptors was examined by applying patch-clamp techniques to the acutely isolated hippocampal neurons. KA-672 antagonizes NMDA responses in a voltage-dependent manner. At holding potential -90 mV the IC_{50} value for the blocking action of KA-672 is 20 ± 7μM. This action of KA-672 is independent on the concentration either of agonist or coagonist of NMDA receptor and does not interfere with ketamine-binding site. Evidently, KA-672.HCl is a weak NMDA receptor-operated channel blocker. This property may account for its pharmacological profile.

MEMORY RECONSOLIDATION FOLLOWING MEMORY RETRIEVAL IN PASSIVE AVOIDANCE TASK IN CHICKS: THE ROLE OF NMDA-RECEPTORS

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It is traditionally assumed that disruption of memory formation can occur only in a relatively short time interval after training. The aim of our experiments was to test whether a reminder (RM) treatment (presenting of environmental cue that was present during training procedure) can make the antagonist of glutamate NMDA-receptor MK-801 effective in eliciting amnesia at late times after training in chicks. Chicks were trained to avoid an aversive methylantranilate-coated red bead (d=3,2 cm) in a standard one-trial passive avoidance training task. A visual reminder stimulus (presenting of the dry red bead (d=3,2 cm) for 15 sec) was delivered at 2 or 24h after training. Chicks were injected with MK-801 (1) bilaterally into the IMHV area (6,25 nmol/hemisphere) 5 min before the RM, or (2) intraperitoneally (0,2 mg/kg) immediately after RM. Testing was conducted at 5 min, 0,5, 1, 3 and 24h after the REM. Administration of the MK-801 both intracerebrally and intraperitoneally when associated with RM produced a transient impairment in the task retention. The duration of the impairment decreased gradually with increase of the time interval between the original training and the RM. It is suggested that memory activated by the RM undergoes a reconsolidation process which requires a activation of NMDA-receptors. The gradual disappearance of vulnerability of memory to antagonist of NMDA-receptors suggests the existence of gradual memory consolidation process in the interval between 2 and 24h after training.

EFFECTS OF INTRAMUSCULAR INJECTIONS OF HYPERTONIC SALINE ON THE GAMA-MUSCLE SPINDLE SYSTEM

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Intramuscular injection of hypertonic saline (HS) is a common method to induce acute deep muscle pain in humans. We tested whether intramuscular injections of HS (5%) influence the activity of primary and secondary muscle spindle afferents (MSAs) from homonymous and heteronymous muscles. The experiments were performed on 6 cats anaesthetised with α-chloralose. Responses of 2-9 MSAs from gastrocnemius medialis and/or gastrocnemius lateralis muscles were recorded simultaneously, while HS was injected either to the receptor bearing muscle (homonymous responses) or to a close or a remote synergistic muscle (heteronymous responses). Out of 42 afferents tested (7 GM and 35 GS), 38 (90%) exhibited statistically significant responses to injections of HS either to homonymous and/or heteronymous muscles. The majority of the responses (63% to homonymous and 72% to heteronymous injections) were compatible with reflex action on static fusimotor neurones, whereas 23% and 19%, respectively, could be attributed to mixed static and dynamic fusimotor action. Injections of Tyrode solution did not induce any significant alterations in MSA responses. Changes in MSA activity related to HS injections were completely abolished after the nerves to corresponding muscles were cut, confirming the reflex nature of the effects. Intramuscular injections of HS induce reflex changes in MSAs activity from both homonymous as well as heteronymous muscles, most likely via fusimotor reflexes.

A NOVEL MECHANISM FOR THE GENERATION OF OSCILLATIONS IN THE INFERIOR OLIVE VIA SYMMETRY BREAKING

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Intracellular *in-vitro* measurements in inferior olive (IO) slices have revealed the existence of subthreshold synchronized oscillations in the membrane potential at frequencies 1-10Hz. Likewise, the action potentials of these cells were shown *in-vivo* to exhibit oscillatory activity at similar frequencies, which is correlated with rhythmic movement. Several considerations lead to the conclusion that a single IO cell is quiescent, and the appearance of synchronized oscillations result from the abundant electrical couplings between the IO cells. Electrical coupling is known to be capable of synchronizing neuronal activity. However, since these couplings tend to equalize the potentials of the coupled cells it is unclear how they can destabilize the quiescent state.

We present a novel mechanism for the generation of oscillations in an electrically coupled homogeneous neuronal network. Network oscillations in this model occur via a spontaneous breaking of the spatial symmetry which destabilizes the homogeneous state and leads to oscillations via a Hopf bifurcation in a non-homogeneous direction. A realization of this mechanism, a model based on currents measured in IO slices, is presented to explain the generation of the subthreshold oscillations in the IO. An analytical method for predicting the spatio-temporal structure of the oscillations is described. Using this method, it is shown that near the bifurcation point the frequency of oscillations is determined by the single cell properties, whereas the relative phases and amplitudes of the oscillations are dependent on the connectivity matrix of the network.

ENDOGENOUS PHARMACOLOGICAL STUDIES OF NEUROTROPHINO-TROPIN-FACTOR FOR FUTURE ALZHEIMER'S DISEASE THERAPY (INDIVIDUAL TREATMENT)

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Senile dementia of Alzheimer's type was associated with high level structural damages in the different parts of brain cells, especially in dendrites of cerebrocortex large pyramidal neurons.
Such brain pathology was accompanied with drastic neurological disorders which superseded by the phase of compensation. This phase is associated with accumulation in tissues of endogenous factors – some substances of peptide origin (oligopeptides, cytokines). This substances generated in response to injury are capable of non-specific therapeutic action (Cotman C et al., 1986). After modeling haemorrhagic stroke animal's brain was generated Neurotrophinotropin-factor SNC-TTP in tissue, which has proved to be capable of pronounced therapeutic action for the therapy not only the stroke but in experimental forms of infantile cerebral pulsy and multiple sclerosis. In the activation of defense and reparative functions in brain are highly potent.
From position of "endogenous pharmacology" (A.N.Makarenko et al., 1994) after first stage of senilien disorders, which modelling in the stress-induced animals, was received new-peptide substance Neurotrophinotropin-factor aDA (ADEMENT) by original method of purification. Brain-derived aDA was founded after modelling experimental brain pathology only by matriculation in the brain of animals-recipients specific for Alzheimer disease (proteins Alz50; A-63), together with cells and proteins of animal-recipient.
Neurotrophinotropin (ADEMENT) was rendered high effective protective action on the mental function and the memory of 64-year patient B., which previously was a donor of specific for Alzheimer's disease proteins. ADEMENT substantionally increase level of such Amino Acids as GLU, ASP, PRO, GLY and, especially, GABA, much more then in control and SNC-TTP.
Senile dementia treatment by Neurotrophinotropin aDA (ADEMENT) is start scientific basis formation of posibility realized an individual therapy of some organic brain diseases, long-time retardate of speed demential progress and fatal prognosis in patients with Alzheimer's disease.

EFFECTS OF INTERACTION BETWEEN GAMMAHYDROXY-BUTYRATE AND SCH 23390 ON AGONISTIC ENCOUNTERS IN MALE MICE

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Gammahydroxybutyric acid (GHB), a recreationally used drug popularly known as "liquid ecstasy", is a metabolite of GABA which also acts as neurotransmitter in the mammalian brain. There is experimental evidence suggesting the existence of a central interaction between GHB and D2 dopamine receptors in rodents (1). However, the possible interaction between GHB system and D1 receptors has not been explored. The aim of this study was to assess the effects of coadministration of GHB and SCH 23390 (a selective antagonist of D1 receptors), on social interactions between male mice. For this purpose, we compared the actions of GHB and SCH 23390, either alone or in combination, on agonistic encounters between mice, using an animal model of isolation-induced aggression. Animals were assigned to six different groups receiving: 1. GHB (80 mg/kg)+sal; 2. GHB (120 mg/kg)+sal; 3. SCH 23390 (0.03 mg/kg)+sal; 4. GHB (80 mg/kg)+SCH 23390; 5. GHB (120 mg/kg)+SCH 23390, and 6. Sal+sal. 10 min of diadic interactions were staged between a singly housed and an anosmic mouse in a neutral area. These encounters were videotaped and the accumulated time allocated by subjects to ten broad behavioral categories was estimated. Besides other behaviors, the aggressive (threat and attack) and motor behaviors were evaluated 30 min after injection of the drugs using an ethologically based analysis. Non-parametric Kruskal-Wallis and Mann-Whitney U-tests were used. Results indicated that mice treated with GHB+sal and GHB+SCH 23390 showed a significant decrease in offensive behaviors, without affecting immobility, in comparison with saline group ($p<0.05$). The antiaggressive action of coadministration of GHB and SCH 23390 was equal to the sum of the effects of each drug separately, evidencing a mere additive action of both drugs on aggression. In conclusion, these results suggest that GHB does not interacts with central dopamine D1 transmission. Further studies are needed to elucidate the interaction mechanisms between GHBergic and DA systems.

(1) Navarro JF et al (1998) Prog Neuropsychopharmacol Biol Psychiatry 22, 835-844.

ANXIOGENIC-LIKE ACTIVITY OF MDMA (ECSTASY) IN SOCIAL ENCOUNTERS BETWEEN MALE MICE IS NOT REVERTED BY DIAZEPAM ADMINISTRATION

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In a recent study, it has been found that MDMA (5-20 mg/kg), a synthetic amphetamine popularly known as "ecstasy", exhibits a behavioral profile characterized by a reduction of aggression without affecting immobility, accompanied by a decrease of social investigation and an increase of avoidance/flee and defense/submission behaviors. This ethopharmacological profile suggests an anxiogenic-like activity of this compound in mice (1). The aim of this study was to assess if this anxiogenic action of MDMA may be reverted by diazepam administration. For this purpose, we compared the effects of MDMA (1 , 8, 15 mg/kg, i.p) and diazepam (0.5 mg/kg, i.p), either alone or in combination, on agonistic encounters between mice, using an animal model of isolation-induced aggression. Animals were assigned to eight different groups receiving: 1. MDMA (1 mg/kg)+sal; 2. MDMA (8 mg/kg)+sal; 3. MDMA (15 mg/kg)+sal; 4. Diazepam (0.5 mg/kg)+sal; 5. MDMA (1 mg/kg)+diazepam; 6. MDMA (8 mg/kg)+diazepam; 7. MDMA (15 mg/kg)+diazepam, and 8. Sal+sal.10 min of diadic interactions were staged between a singly housed and an anosmic mouse in a neutral area. These encounters were videotaped and the accumulated time allocated by subjects to ten broad behavioral categories was estimated. Besides other behaviors, the aggressive (threat and attack), defensive, and motor behaviors (immobility) were evaluated 30 min after injection of the drugs using an ethologically based analysis. Results indicated that mice treated with MDMA (8 and 15 mg/kg) showed a significant increase of avoidance/flee, defense/submission and exploration from a distance as well as a decrease of social investigation and offensive behaviors, as compared with the saline group ($p<0.01$). Animals co-treated with MDMA and diazepam exhibited a very similar behavioral profile to that showed by mice treated with MDMA+sal. It is concluded that anxiogenic-like activity of MDMA in social encounters between male mice is not reverted by diazepam administration.

(1) Navarro JF and Maldonado E (1999) Prog Neuropsychopharmacol Biol Psychiatry 23 (in press).

PLASTICITY OF FREQUENCY-DEPENDENT INHIBITION IN THE RAT DENTATE GYRUS: POTENTIAL RELEVANCE TO LEARNING AND MEMORY.

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Most studies of hippocampal plasticity concentrate on modifications of principal cells. However, accumulating data suggests that inhibitory neurons in the hippocampus are rather plastic and can show several forms of activity-dependent modulation. Frequency-dependent inhibition (FDI) is a form of local circuit activity, involving the activation of inhibitory interneurons. This form of local circuit activity was found to be NMDA-dependent (Rosenblum et al., 1998).
In the present study we investigated first, the effects of theta bursts stimulation (TBS) on the level of FDI. In the second set of experiments we evaluated the effects of aging on FDI and FDI plasticity in relation to aging-associated impairments in spatial learning abilities.
TBS significantly reduced FDI, suggesting a form of plasticity of local circuit activity. In aged rats FDI levels were similar to those in young rats. However, TBS, which reduced FDI in young rats, was ineffective in the aged rats. Furthermore, this age-associated reduction of local circuit plasticity was correlated with an age-associated impairment in performance in a reversal spatial memory task. The results indicate that TBS induces a form of local circuit plasticity and suggest that this form of plasticity may be relevant to some aspects of spatial memory.

LATTICE ORGANIZATION OF CAVEOLIN-3 AT THE MYOFIBER SURFACE IN NORMAL AND DYSTROPHIC MUSCLES.

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Caveolin-3 is the major constituent of caveolae in skeletal, cardiac and smooth muscles. Caveolin-3 co-fractionates with members of the dystrophin-associated protein complex, and immunofluorescence detection of caveolin-3 has shown its localization in the sarcolemma of skeletal muscle fibers. However, it is not known whether caveolin-3 distribution in the sarcolemma is similar to that of other membrane associated proteins, such as dystrophin. Moreover, in the dystrophin-deficient mdx mouse, caveolin-3 is over-expressed, but it is not clear whether its subcellular distribution is altered or not, due to the lack of dystrophin. We have therefore studied the fine distribution of caveolin-3 and its possible co-localization with dystrophin by double immunofluorescence and confocal microscopy in skeletal muscle fibers of normal and mdx mice. We report that caveolin-3 is mostly associated with the sarcolemma in normal myofibers, where it is organized in a fine bidimensional lattice, composed by regular, transverse elements, similar to the costameres, and by marked longitudinal strands. The former are coincident with the distribution of dystrophin, while the latter seem to be more specific of caveolin-3. The bidimensional lattice observed by immunofluorescence is reminiscent of the ultrastructural distribution of caveolae. In mdx muscles, the sarcolemmal lattice is conserved; furthermore, a fine intermyofibrillar network is visible, particularly in regenerated fibers. These results show that caveolin-3 partially co-localizes with dystrophin in the sarcolemma, and keeps its typical distribution even in the absence of dystrophin. Finally, the reported over-expression of caveolin-3 in mdx muscle may be due at least in part to an additional localization in the T-tubules of regenerated fibers, mimicking a pattern described during normal muscle differentiation.

POSSIBLE MECHANISM OF SMP-69 PROTEIN PARTICIPATION IN MEMORY CONSOLIDATION THROUGH REGULATION OF ANTICONSOLIDATION PROTEIN SYNTHESIS

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The novel serotonin-modulating SMP-69 protein was identified and purified from rat brain (Mr69 kDa, pI 6,0). The anti-SMP-69 protein polyclonal antibodies, being injected intracerebrally, increased rat exploratory behavior in open field test. Controls were administered the rabbit non-immune γ-globulins. The antibodies administration to rats 24 h prior to learning session impaired memory processes in retention sessions 48h thereafter, while their administration 48h after the learning session and 24h pror to retension sessions didn't have any effect on memory processes. So, the antibodies impaired memory trace consolidation without having any effect on memory storage and retrieval. As this protein proposed to be the "early" genes product and as its antibodies had impaired the memory consolidation, the study of the antibodies effect on genome activity was carried out. The antibodies intracerebral administration 24h prior to rat decapitation brought to increase of both RNA and protein synthesis to 30%.

On this grounds the idea of increased "anticonsolidation" protein synthesis underlying the anticonsolidation effects of the SMP-69 protein antibodies was proposed.

The proposed "anticonsolidation" protein is considered to participate in regulation of incoming information recording in nerve cells in normal conditions.

PET STUDY OF MACAQUE MONKEYS DURING VISUAL GUIDED SACCADE TO RANDOMLY VS. REGULARLY PRESENTED CUES.

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To recognize a visual object, we often select a cue relevant to its recognition and exhibit saccadic eye movement in the direction of that cue. During this process, both the attentive process and the motor process are involved. In order to dissociate these processes, the regional cerebral blood flow (rCBF) was measured with 15O-labeled H2O and positron emission tomography (PET) in three rhesus monkeys. They were trained to make a saccadic eye movement toward a target presented on a computer monitor (saccade task). In the randomly presented cue condition (attentive condition), when the eyes reached to a target, the target was distinguished and a new target was presented. Thus the monkey made saccadic eye movement toward randomly presented one of eight targets repeatedly. In the regularly presented cue condition (less-attentive condition), cues were presented either left or right. In a control task (fixation task), the monkey was fixating a central target continuously during the scan. We found stronger activation in the frontal and parietal association corteces in random cue condition than in the regular cue condition. The results suggest the involvement of these areas in the attentive process.

PRESYNAPTIC PROTEINS GAP-43 AND BASP1 FORM MULTIMERS UNDER PHYSIOLOGICAL CONDITIONS

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Abundant in nerve terminals proteins GAP-43 (B-50, neuromodulin) and BASP1 (CAP-23, NAP-22) show a number of similar molecular and biochemical properties. Using size-exclusion chromatography and sucrose gradient ultracentrifugation, it was shown that GAP-43 and BASP1 possess hydrodynamic properties, characteristic to high molecular weight proteins. Our results show that both proteins form multimer complexes of several tens of molecules per particle under physiological conditions (pH, ionic strength). these complexes elute at the void volume of the gel-filtration columns with high exclusion limit (up to 1,000,000 Da). Study of this phenomenon under the conditions that diminish either electrostatic (high ionic strength or pH 11.5) or hydrophobic (0.02% Triton X-100) interaction has shown that both participate in multimerization of GAP-43 and BASP1. Contribution of hydrophobic constituent to BASP1 multimerization is greater than that to GAP-43 multimerization. Possibly, this is due to BASP1 myristoylation. Under the conditions of disruption of electrostatic or hydrophobic interactions, monomers of GAP-43 and BASP1 are released. Their Stokes radii are about 4.7 and 4.0 nm respectively. Taking into account low molecular masses of these proteins (of about 25 kDa), their molecules must be highly asymmetric (this was first demonstrated for GAP-43 by Masure et al. Biochemistry 1986, v. 25, 7553-7560; Benowitz et al. J. Neurochem. 1987, v. 48, 1640-1647; Chan et al. J. Neurosci. 1986, v. 6, 3618-3627). Very elongated shape of GAP-43 and BASP1 molecules is favourable for their multimerization. We suggest that multimerizationcan be important for local accumulation of these proteins'in the space between plasma membrane and cortical cytoskeleton, hereby influencing cell surface dynamics, axonal growth etc. (see Wiederkehr et al. Exp. Cell Res. 1998, v. 236, 103-116).
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OXIDATIVE DOWNMODULATION OF THE TRANSIENT K-CURRENT IA BY INTRACELLULAR ARACHIDONIC ACID IN RAT HIPPOCAMPAL NEURONS

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Arachidonic acid (AA) is a second messenger that is liberated by phospholipase-A2 in a calcium (Ca)-dependent way in neurons as well as in astrocytes. Because of its supposed membrane permeability it has been suggested as a transcellular messenger in synaptic plasticity and neurodegeneration. Using intracellular and extracellular application in whole cell path clamp configuration of cultured rat hippocampal neurons we studied modulation of voltage-dependent K-currents by intracellularly and extracellularly applied AA. We show that AA is extremely potent in selectively reducing IA when applied through the patch pipette (50 % blockade at 1 pM), whereas extracellular application required a 106 fold concentration. The non-metabolized AA analog ETYA as well as blockade of the cyclooxygenase pathway of AA metabolism by indomethacin (20 µM) mimicked the effect of AA while the lipoxygenase blocker NDGA (10 µM) and the P450 blocker proadifem (5 µM) had no effect on IA. The effect of AA was blocked by ascorbic acid (20 µM), indicating involvement of an oxidative mechanism, most likely of the channel protein. In order to clarify whether the site of oxidation is rather extracellularly or intracellularly, we applied the membrane impermeable cellular reducing agent glutathione (GSH). Intracellular application of GSH (2 mM) completely blocked the effect of AA while extracellular application was much less effective, indicating an intracellular site of oxidation. Application of hydrogen peroxide blocked the A-current only at an extremely high concentration (800 µM) supporting that oxidation by AA is highly specific and physologically relevant. We conclude that IA in mature neurons is highly sensitive to free radicals generated by AA inside cells. Because of this extreme sensitivity moderate extracellular AA concentrations are effective despite a rather poor transmembrane permeability. Depending on subcellular oxidative status and, hence, glutathion levels, AA is likely to effectively control excitability and thereby action potential waveform and release of neurotranmitters.

MULTISITE OPTICAL RECORDING OF EXCITABILITY IN THE ENTERIC NERVOUS SYSTEM.

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The aim of this study was to establish a multisite optical recording technique (MSORT) in the enteric nervous system (ENS) to investigate differential activation of enteric neurones and to study excitability spread within enteric circuits. The MSORT consisted of a 464 photodiode array to measure changes in transmembrane potentials (Vm) in guinea-pig and mouse ENS stained with the voltage sensitive dye Di-8-ANEPPS. Optical recordings of Vm changes in enteric neurones mirrored the Vm changes measured intracellularly in the same neurone. Optical recordings after electrical stimulation of interganglionic nerve strands revealed slow EPSPs and nicotinergic fast EPSPs in both myenteric as well as submucosal ganglia. Electrical stimulation of individual nerve strands connected to enteric ganglia revealed distinct activation patterns which would indicate selective activation of ganglion cells. At the same time excitability spread along nerve strands entering and leaving the ganglia could be detected. The optical mapping made it possible to record action potentials simultaneously in a large number of neurones with high spatio-temporal resolution that is unattainable by conventional techniques. This technique presents a powerful tool to study excitability spread within enteric circuits and to assess differential activation of enteric populations in response to a number of stimuli which modulate neuronal activity directly or through synaptic mechanisms.
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ACTIVATION OF CENTRAL PATTERN GENERATOR BY EPIDURAL SPINAL CORD STIMULATION IN CHRONICALLY SPINALIZED CATS

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At the first days after spinal cord transection (Th6 level), the low-frequency (3-5Hz) epidural spinal cord stimulation (SCS) of the L3-S1 segments evoked mono- or oligosynaptic responses, as well as late (150-200 ms latency) polysynaptic responses in the hindlimb muscles. At the beginning of 10-33 Hz SCS, the early responses were modulated in amplitude and then were transformed into the burst activity. SCS of L3-L4 segments evoked responses of thigh muscles, while the SCS of L5-S1 segments, in addition, involved responses of the leg and foot muscles. The SCS of 20-33 Hz resulted in appearance of locomotor-like activity. In one group of the cats, the SCS was performed every day, another group of cats was not trained. In the first group of animals 22-28 days after spinalization, only the SCS of L5-S1 segments evoked rhythmic movements of hindlimbs. Locomotor-like hindlimb activity arised under stimulation with 20-80 Hz.. Moreover, with increase in stimulation frequency this activity was more pronounced. In this case, the EMG activity was simultaneous in the flexor and extensor muscles, while the interlimb movements were reciprocal. After cessation of the SCS, the rhythmic polysynaptic muscle activity continues for 5-15 seconds. In contrary to the trained cats, in untrained ones the SCS of L5-L6 segments did not evoke the late polysynaptic responses and, consequently, could not evoke the rhythmic hindlimb activity. After cessation of the SCS, the burst rhythmic activity was not observed too. After unilateral transection of the L5 and L6 dorsal roots, the SCS of these segments resulted in decrease of the oligosynaptic and polysynaptic responses, and the burst activity in the hindlimb muscles. Transection of the dorsolateral funiculi also decreased both the oligosynaptic and polysynaptic responses, as well as the burst responses in the hindlimb muscles under SCS above the site of injury. We suppose, that local transection of dorsolateral funiculi disrupt majority but not all intersegmental connections. These data point to the important role of these structures in CPG activation and initiation of rhythmic muscle activity. It is suggested that SCS activates the afferents of dorsal roots and, as a consequence, the propriospinal interneurones. In chronically spinalized cats, when presynaptic inhibition is decreased and the afferent influences are increased, the SCS results in interaction of the oligosynaptic and polysynaptic systems that activates polysynaptic chains and provokes CPG activity. The regular training of these systems by SCS organizes the intraspinal interneuronal relations that improves the ability of the spinal cord to produce the locomotor activity. Supported by grants from Russian Foundation of Basic Researches 98-04-49097 and 97-04-49193.

ROLE OF THE D4 FAMILY PROTEINS IN DETERMINATION OF CELL FATE AND DEVELOPMENT OF THE NERVOUS SYSTEM

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Neuro-d4, the first member of the d4 family, was cloned as a neurospecific, developmentally regulated gene. More recently two other genes, requiem/ubi-d4 and cer-d4, that encode proteins closely related to neuro-d4 were identified. A hallmark of the family is the d4-domain, a double-paired finger motif that consists of two tandemly arranged PHD finger domains. A single Kr ppel-type zinc finger was found in the N-terminal part of the most d4 proteins, but because of differential splicing, some d4 proteins lack this finger along with a nuclear localisation signal and a stretch of negatively charged amino acids. It was suggested that d4 proteins comprise a family of nuclear factors, although some of these proteins could have cytoplasmic function(s). Requiem/ubi-d4 is involved in regulation of programmed cell death in the immune system because in the IL-3 dependent myeloid cell line, expression of this gene is necessary for apoptosis. Using microinjections of neuro-d4 expression plasmids into nuclei of cultured sensory neurons we demonstrated that overexpression of neuro-d4 rescues these neurons from the death after withdrawal of NGF. In the presence of growth factors increased level of neuro-d4 has a stimulating effect on neurite outgrowth. These results and the pattern of expression of neuro-d4 are consistent with the idea that this protein plays an important role in embryonic and postnatal development of the nervous systems. Supported by The Wellcome Trust

GABA is excitatory transmitter in the feeding neural system of a mollusc *Clione limacina.*

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GABA is known as the main inhibitory neurotransmitter in the CNS. However, it appears that GABA works mostly as an excitatory transmitter in the feeding neural circuitry of the mollusc *Clione limacina.* Exogenous GABA produced high-amplitude depolarization of the cerebral neurons that control prey capture reactions (Cr-A neurons). GABA-induced depolarizations easily reached spike thresholds and strongly activated the target cells. When seawater in the recording chamber was replaced with 2% Na^+ solution, responses to GABA completely disappeared suggesting that GABA-induced depolarizations were Na^+-dependent. Neuronal responses to 1 μM GABA were completely and reversibly blocked by 50 μM piperidine-4-sulfonic acid, indicating that it works as a GABA antagonist in this system.

Two pairs of excitatory interneurons, which play an important role in the feeding neural system, have been previously identified. It was demonstrated in this study that strong excitatory monosynaptic inputs produced by these interneurons to Cr-A neurons were GABAergic. GABA antagonist, 5-aminovaleric acid (10 μM), produced a reversible blocking effect on the synaptic transmission from interneurons to Cr-A neurons. Piperidine-4-sulfonic acid (50 μM) completely and reversibly blocked synaptic transmission from interneurons to Cr-A neurons. Nipecotic acid (50 μM), a GABA uptake inhibitor, significantly enhanced Cr-A neuron EPSPs increasing their amplitudes, but most dramatically their durations. In addition, double-labeling experiments demonstrated that both pairs of interneurons were GABA-immunoreactive. Thus, excitatory synaptic transmission from these key interneurons of the feeding neural system, which coordinate and activate a large population of prey capture Cr-A neurons, was demonstrated to be GABAergic.

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ROLE OF AFFERENT NERVE FIBRES OF SMALL INTESTINE IN COMMUNICATION BETWEEN NERVOUS AND IMMUNE SYSTEMS

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Experiments were performed on female Sprague-Dowley rats. One group of animals was sensitized with a single intraperitoneal injection of chicken egg albumin (EA) with complete Freund's adjuvant. The second group of unmanipulated animals was used as a control one.

12-20 days later experiments in presensitized animals were performed to investigate the effect of challenge of EA both on afferent impulse activity of mesenteric nerves of small intestine in rats and on morphometric characteristics of mast cells. The data obtained from sensitized animals were compared to those of the control group.

In acute electrophysiological experiments intraarterial or intraluminal challenge of EA enhanced the resting activity of mesenteric nerves of small intestine in animals previously sensitized. The effect does not depend on kind of injection chosen. The average increase in mesenteric nerve discharge rate was 10±1.6 %. Afferent nerve activity in control animals did not statistically change during EA challenge.

Morphometry under the light microscope was performed on plastic embedded, semithin, 0.5μm cross-sections from the upper part of the intestine stained with toluidine blue. Morphometry has shown that the mean number of granulated mast cells per square unit differed statistically significantly in two groups of rats: 11.28 ± 1.05 and 6.11 ± 1.11 in the control and experimental groups, respectively, which suggests activation of the degranulation process.

It is known that compound 48/80 caused a degranulation of mast cells. In our experiments intraluminal injection of compound 48/80 (20-30 mg) caused a prolonged significant increase of afferent impulse activity in unmanipulated rats. Antagonist of H_1 histamine receptors clemastine ($2 \cdot 10^{-3}$ M) reduced the effect of compound 48/80.

Results provide evidence that visceral afferent fibers play an important role in cross-communication between nervous and immune systems.

PRE- AND POSTSYNAPTIC CALCIUM IS INVOLVED IN LTP OF THE SYNAPSE BETWEEN CORTICAL L5 PYRAMIDAL NEURONS ORA OHANA AND BERT SAKMANN

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Synchronous activity of neurons within a neural network is one form of transferring and storing information. On the cellular level, it had been shown that coincident activity of synaptically connected neurons results in changes of the synaptic strength, such as LTP. In the synapse between L5 pyramidal neurons in the rat somatosensory cortex, LTP is induced by a coincidence protocol of incoming synaptic input and back-propagating action potentials. We have investigated the mechanisms underlying this LTP and concentrated on dissecting pre- and postsynaptic involvement of Ca2+ in induction and expression of LTP. Simultaneous pre- and postsynaptic recordings were made from L5 pyramidal neurons in rat somatosensory slices in the whole-cell patch clamp configuration. Induction of LTP is NMDA-R -dependent as it is blocked by 50 mM AP-5. BAPTA (4 mM) loaded into the postsynaptic neurons prior to induction blocked LTP. Whereas, loading of BAPTA after induction of LTP did not affect LTP magnitude, indicating that postsynaptic elevation of Ca2+ is required for the induction of LTP. We loaded presynaptic terminals with BAPTA or EGTA to test whether presynaptic [Ca2+]in or release probability underly the expression of LTP. The coincident stimuli for LTP-induction was applied, but caused a long-term depression (LTD) of the EPSP amplitude, rather than LTP. Our results suggest a complex involvement of pre- and postsynaptic mechanisms in the expression of LTP.

HYPOTHALAMIC PROJECTIONS TO THE AUDITORY THALAMUS IN THE RAT

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It has been shown that the medial nucleus of the medial geniculate body (MGm) and the surrounding perigeniculate (PG) nuclei (peripeduncular nucleus, nucleus subparafascicularis lateralis and posterior intralaminar nucleus) play a central role in giving the auditory information emotional significance. Additionally, it has been also shown that the hypothalamus also play a central role in performing fear responses. To study if hypothalamic projections reach the MGm/PG complex retrograde and anterograde tracers were injected in the MGm/PG complex and in tdifferent hypothalamic nuclei respectively. Cholera toxin B subunit injectionsin the MGm/PG complex resulted in retrograde labeling located in the ventromedial hypothalamic nucleus, nucleus arcuatus, medial tuberal area, dorsomedial hypothalamic nucleus, medial preoptic area, anteroventral preoptic area and lateral hypothalamic nucleus. Different patterns of retrograde hypothalamic labeling was obtained following CTB injections in the surrounding substantia nigra or the brachium of the inferior colliculus. Additional restricted biotinilated dextran amine (BDA) injections in the lateral hypothalamic area, ventromedial hypothalamic nucleus, and dorsomedial hypothalamic nucleus resulted in anterograde terminal-like labeling in the MGm/PG complex. Even larger BDA injections in the hypothalamus left the ventral nucleus of the medial geniculate body unlabeled and dense terminal-like labeling in the MGm/PG complex. These results lead to conclude that projections form hypothalamic nuclei and areas to the acoutic thalamus may control ascending auditory information progressing from the auditory brainstem to the lateral nucleus of the amygdala.

HOW DO MONKEYS RECALL ORDERED SEQUENCES OF STIMULI ?

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Previous studies have shown that monkeys are able to recall the serial order of a list of learned items. To discern what are the non-verbal strategies used in such a memory test, we trained two macaque monkeys on a novel delayed sequence recall task. We used 30 stimuli, divided into ten triplets, presented in a fixed temporal order. On each trial the monkeys viewed one triplet, i.e. 3 sequentially presented visual "sample" stimuli, followed by the "test" presentation, in which the same stimuli were shown simultaneously on the screen at random positions, together with a distractor stimulus (chosen randomly from the other stimuli in the set). The monkeys' task was to touch the three stimuli on the screen in the order of their prior presentation, without touching the distractor. Analysis of the errors made during the learning stage, showed that the monkeys erred more often by touching the distractor stimulus, when it belonged to the same ordinal number category as the correct stimulus. When the task was performed without seeing the initial sequence of sample stimuli, so that working memory could not be used, performance was slightly hampered. By using reshuffled stimuli *within categories* as sample stimuli, we showed that monkeys also make use of associations between members of each triplet to solve the problem. We conclude that monkeys' basic strategy for recalling ordinal number sequences was retrieval of ordinal categories from long term memory. Both use of working memory and recall of associations between triplet members were utilized as additional secondary strategies.

BASIC FIBROBLAST GROWTH FACTOR EXPRESSION ACCOMPANIES BY THE ISCHEMIC INJURIES FOLLOWING TRANSIENT FOCAL CEREBRAL ISCHEMIA, AND THE ROLE OF ELECTROACUPUNCTURE

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ABSTRACT: In this study, we investigated the expression changes of basic fibroblast growth factor (bFGF) like immunoreactivity (IR) and cerebral injuries in rat following transient middle cerebral artery occlusion(MCAO). In addition, the effect of electroacupuncture (EA) on rat cerebral injuries and expression of bFGF-like IR were also observed. METHODS: Animal models of 2h MCAO and 24h reperfusion were used. After reperfusing for 24 hours, rats were sacrificed and cut into brain sections (30μm and 50μm). The 30μm sections of each rat were stained by creyl violet and used for measuring the injuries (infarction and swelling). By using fluoro-immunohistochemical assay, one of the 50μm sections (bregma: –0.40mm, containing caudate putamen and frontoparietal cortex) was prepared for observing the bFGF-like IR expression and neuron loss in peri-infarct striatum as well as frontoparietal cortex. Electroacupuncture (EA, lasting 1h) applied in ischemia or reperfusion at points of "Bai Hui"and "Ren Zhong". The results indicate that gross neuronal damages include infarction, swelling and neuron loss occurred, accompanying by increased bFGF-like IR expression following MCAO. In striatum, the increase patterns of bFGF-like IR were different according the ischemic extent. bFGF-like IR was mainly located in astrocytes except some neurons also showed an upregulation of the IR in peri-infarct striatum. In frontoparietal cortex, strong induction of bFGF-like IR was mostly seen in neurons. Both the EA applied during ischemia and reperfusion could evidently alleviate cerebral lesion extent, notably upregulate the expression of bFGF-like IR in striatum and cortex, but there was no significant difference between the effects of EA applied during ischemia and reperfusion, except EA applied during reperfusion seem to be more effective in reducing the cerebral swelling. The results implied that, in striatum, astrocytes might play an important role in the protection of neuron via the expression of bFGF; whereas in cortex, neurons may exert an autoprotection through secreting bFGF themselves. One possible protective effect of EA lies in regulating the endogenous expression of bFGF.

ROLE OF DL-α-LIPOIC ACID TO PROTECT THE STRUCTURAL INTEGRITY OF NEURONS DURING EXPERIMENTAL TOXIC NEUROPATHIES

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The structural integrity of the neurons is the primary factor required for proper neurofunction. This study was aimed to establish the role of lipoic acid in reducing the myelin breakdown during toxic neuropathies. Mercury (0.3mg/kg b.wt/day,im) and acrylamide (10mg/kg b.wt/day,ip) were chosen to produce neurotoxic models along with prophylactic therapy of lipoic acid (10mg/kg b.wt/day,ip) for a period of 35 days. Glycolipids, phospholipids of the nervous tissues showed a depletion on mercury and acrylamide intoxication. But the total phospholipid content was differentially altered between the brain and nerve tissues on acrylamide exposure. Such differential susceptibility emphasize acrylamide to be inclined to PNS poisoning rather than CNS. The results are further confirmed by histopathological evaluation.Lipoic acid restored the myelin lipids towards normal levels by minimizing the availability of the toxins to cause myelin breakdown. So, the loss of structural integrity would only be a secondary effect on prolonged mercury exposure whereas, active demyelination resulted due to increased phosphoinositide turnover, incase of acrylamide.

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EFFECT OF HIGH ALTITUDE ON AVOIDANCE LEARNING DURING ENDURANCE PERFOMANCE IN RATS

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Stay at extreme altitude leads to several deleterious effects on normal physiological functioning. One significant but distressing complication is an impairment in learning and memory. The present work proposed to study this aspect in the laboratory. The study aimed to evaluate the effect of exposure to simulated altitude of 20,000 ft on avoidance learning during endurance performance. Male wistar rats (n=30) were trained for shock avoidance . The experimental group (n=15) was exposed to an altitude of 20,000 ft in a decompression chamber for 21 hrs daily for 21 days ; the age-matched control group (n=15) was maintained at sea-level. The recordings were obtained in the 1st, 2nd and 3rd week each pre-exposure, during exposure and post-exposure. The results showed significant differences in avoidance learning between the two groups. The number of stimuli avoided reduced by 34.2 % as compared to the pre-exposure values in the first week ($p<0.01$) and recovered to 58.6% lower than pre-exposure values in the third week of exposure ($p<0.05$). A complete recovery in avoidance learning was not attained even by third week post-exposure with the values remaining 29.1% lower than pre-exposure values. There was a reduction in endurance performance during HA exposure as reflected by a reduction in run speed (cm/s), run time (s) and distance travelled (cm). as compared to pre-exposure values. The control group did not register any significant changes in avoidance learning and endurance performance. High altitude equivalent to 20,000 ft appears to impair avoidance learning in rats.

EFFECTS OF SEROTONIN DEPLETION ON THE DEVELOPMENTAL PATTERN OF CORTICAL PEPTIDERGIC NEURONS

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From early development to adulthood, mammalian neocortex receives vast serotoninergic (5-HT) input. We have recently shown that 5-HT fibers selectively innervate somatostatin (SRIF)- and neuropeptide Y (NPY)- but not vasoactive intestinal polypeptide (VIP)-containing non-pyramidal neurons in the adult rat neocortex. Given the involvement of 5-HT in the cytoarchitectonic maturation and neurochemical differentiation of the cerebral cortex, the present study was designed to examine the putative role of 5-HT input in the postnatal development of the above peptidergic populations. The selective neurotoxin 5,7-dihydroxytryptamine was administered to newborn rats and the consequences of 5-HT depletion on the peptidergic neurons were evaluated immunocytochemically at the end of the 1st, 2nd, 3rd and 4th postnatal week (PW). The absence of 5-HT innervation was not seen to affect the overall density and morphology of peptidergic neurons at the end of the 1st PW. Similarly, during the 2nd PW, NPY and VIP populations remained unaffected, and only SRIF density was decreased. In the 3rd PW, cell-density of all peptidergic populations was significantly higher in 5-HT denervated cortices, as compared to the control values. However, at the end of the following week, cell-density was fully (SRIF and VIP) or largely (NPY) restored to control levels. These findings suggest an inhibitory 5-HT control, exerted relatively late in development, on the neurochemical differentiation of cortical peptidergic populations. It is remarkable however that peptidergic cells somehow compensate for serotonin depletion and normally develop following a pattern that appears genetically pre-set for each population.

THE INFLUENCE OF NMDA RECEPTOR INHIBITION ON NO-CONNECTED PPI METABOLISM IN OLFACTORY CORTEX SLICES DURING LTP

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The linkage of phosphoinositide signalling system with NO-mediated NMDA receptor activation was investigated in the rat olfactory cortex slices during long-term potentiation (LTP) by examining the effects of MK-801 – NMDA antagonist and sodium nitroprusside (SNP) – donor of nitric oxide (NO). LTP was induced by the high frequency stimulation of the lateral olfactory tract (3V, 100/s, 15 s). Labeling of the polyphosphoinositide (PPI) was carried out with radioactive phosphate. At the time examined (10 min after stimulation) the phosphate groups turnover and the content of the PPI were decreased about 25%. Treatment of the tetanized slices with SNP caused more strong inhibition of PPI metabolism (65% in comparison with control slices). Exposure to MK-801 together with SNP resulted in the recovery of the PPI metabolism in tetanized slices. It is concluded that the involvement of NO in PPI second messenger system regulation during LTP initiation is under the NMDA receptor control.

PHYLOGENESIS OF THE NERVOUS SYSTEM: I. PHYLUM CNIDARIA AND PHYLUM PLATYHELMINTHES

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Different theories have been proposed to explain the origin of the nervous tissue based on the studies realized in organisms as primitive as the sponges or the coelenterata. In the study of neurophylogenesis and synaptophylogenesis, our goals are: first, to know the moment during the evolutionary process of the animal when the first cellular structure, showing the characteristics of a neuronal cell, appeared and from there on to find the continuity of acquisition of neuronal elements which phylogenetically have contributed to the complexity of the nervous system; and second, to know the origin of interneuronal communication, that is, how synapses have originated. To obtain this information, we have analyzed by transmission electron microscopy the cellular composition of two phyla along the phylogenetic tree: cnidaria (coelenterata) and platyhelminthes, represented by the hydra and planaria, respectively. The results obtained in this study have shown that primitive animals, as the hydra, show a limited cellular diversity, however, we have identified in this phylum a cell type with certain characteristics of a primitive neuron. In the planaria, there already exists a neuronal organization, as the cephalic ganglia, which are in continuity with the surrounding tissues. These characteristics demonstrate that the nervous tissue of the planaria, among the bilateral metazoans, represents an important stage in the evolutionary process of the nervous system in which the internalization and the consequent cephalization of the neurons has occurred. Moreover, we have shown in the planaria the formation of the first true synapse with its neurotransmitter appparatus and the corresponding postsynaptic molecular stuctures. It is also important to indicate that in the cephalic ganglia of the planaria, glial elements were not seen.

REGULATION OF MOUSE SCHWANN CELLS K+ CURRENTS BY TYROSINE KINASES: A ROLE IN PROLIFERATION.

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Schwann cells are the myelin forming cells in the peripheral nervous system. They express a variety of voltage-dependent K+ channel a subunits, producing a transient (IA) and a slowly-inactivating delayed-rectifier (IK) K+ currents. The whole-cell configuration of the patch-clamp technique and immunoprecipitation experiments were used to investigate the effects of tyrosine kinases on the activity of voltage-dependent K+ currents in cultured mouse Schwann cells. Genistein, a broad-spectrum tyrosine kinase inhibitor, markedly reduced the amplitude of the voltage-dependent outward K+ currents and changes the gating of IA. Application of herbimycin A (2μM), abolished completely the IK component leaving the IA component intact. Unlike herbimycin A, genistein produced additional effects on the remaining transient K+ currents (IA) by profoundly affecting the gating properties. These changes consisted of slower activation kinetics, a positive shift in the voltage dependence of activation (by +30 mV), and an acceleration of channel deactivation. The action of genistein and herbimycin A on voltage-dependent outward K+ currents was accompanied by a decrease in tyrosine phosphorylation of the slowly-inactivating delayed-rectifier Kv1.5 and Kv2.1 and of the transient Kv1.4 K+ channel subunits. In addition, K+ current inhibition produced by either K+ channel blockers or by tyrosine kinase inhibitors, potently depressed cell proliferation. In conclusion, the present study shows that tyrosine phosphorylation markedly affects the amplitude of the delayed-rectifier IK and finely tunes the gating properties of the transient K+ current IA in Schwann cells. This modulation may be functionally relevant in the control of K+ channel activity during Schwann cell development and peripheral myelinogenesis.

CATALEPTIC ACTION OF ZOLPIDEM: PRELIMINARY PHARMACOLOGICAL ANALYSIS.

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It has been commonly suggested that GABA manipulation influences dopaminergic neurotransmition involved in stereotyped and cataleptic behaviors. For example it was found that GABA neurotransmition in the zona incerta/lateral hypothalamus is involved in the mediation of haloperidol-induced catalepsy (Wardas et al. 1988). Furthermore, the behavioral picture of catalepsy was observed after injection of muscimol (an agonist of GABA-A receptors) into this brain area. Recently, we have observed a cataleptic-like action of GABA-A/benzodiazepine (BZD) receptor agonist, zolpidem, a short-action non-benzodiazepine hypnotic drug belonging to the imidazopyridine derivatives. This compound binds selectively to the BZD -1 receptor subtype in the central nervous system. Catalepsy tests were evaluated in Wistar male rats according to Writh et al. (1958). Zolpidem produced short-lasting and dose-related (2.5-10 mg/kg) catalepsy with exaggerated muscle tone and intact righting reflexes. In contrast to zolpidem, midazolam and diazepam (full non-specific BZD receptor agonists) were able to produce only weak effect which occurred after high doses, and was accompanied with myorelaxation. Zolpidem-induced catalepsy was dose-dependently antagonised by pretreatment with a BZD receptor antagonist, flumazenil, an NMDA receptor antagonist dizocilpine, and dopamine D_2 receptor agonist quinpirole. Further, naltrexone (an antagonist of opioid receptors) failed to influence zolpidem-induced catalepsy. From these results it can be concluded that the picture and pharmacological characteristics of zolpidem catalepsy is closer to the neuroleptic-like catalepsy than the morphine-like catalepsy.

Wardas J., Ossowska K., Wolfarth S.: Brain Res. 1988. 462: 378-383
Writh W. R., Gossowald R.,Horein V., Risse K. H.: Arch. Int. Pharmacodyn. 1958, 115: 1 -31

WITHDRAWAL EFFECT OF THEOPHYLLINE ON BRAIN REGIONAL GAMMA-AMINOBUTYRIC ACID

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Theophylline (Th) has been found to inhibit the central GABAergic activity under nontolerant condition,whereas under its tolerant condition there was no such effect on central GABA.In the present investigation,our interest is to study the withdrawal effect of Th on the central GABAergic activity.Withdrawal of Th,following development of its [20 mg/kg body weight/day(p.o.) for 14 consecutive days] tolerance,reduced the locomotor activity (LA) with time.Maximum reduction (54%) in LA was observed at 48 h after its last administration and then gradually became normal with time.Withdrawal of Th enhanced the steady state level of GABA,the activity of its synthesizing enzyme GAD and [^{3}H]-GABA binding to its receptors and reduced the glutamic acid level,activity of GABA-T and ethanolamine-O-sulphate-induced GABA accumulation in cerebral cortex,hippocampus and cerebellum.Neither of these parameters in corpus striatum,hypothalamus and pons-medulla were changed excepting a reduction in steady state level of glutamic acid under this condition.These results,thus,suggest that (a) the withdrawal of Th following the development of its tolerance activates the GABAergic activity in cerebral cortex, hippocampus and cerebellum; whereas, in corpus striatum, hypothalamus and pons-medulla the GABAergic activity remains unaffected under the Th withdrawal condition and (b) activation of central GABAergic activity may reduce the LA under Th withdrawal condition.
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LONG-TERM EXPOSURE TO CHLORPROMAZINE POTENTIATES HIGHER ENVIRONMENTAL TEMPERATURE-INDUCED HYPERTHERMIA : ROLE OF HYPOTHALAMIC GABA

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Since higher environmental temperature (HET) affects body temperature (BT) through interregulation of central GABAergic,dopaminergic and cholinergic systems and chlorpromazine (CPZ),a well known anticholinergic drug, produces hypothermia at normal temperature, the present investigation have been carried out to study the involvement of hypothalamic GABAergic system under CPZ-induced change in BT at 28°C (normal) and 40°C (HET).Long-term treatment of CPZ (1mg/Kg,i.p.,for 30 consecutive days) to adult male albino rats (125-150 g b.wt.) at normal room temperature (28°C) produced significant hypothermia without changing the hypothalamic GABAergic activity (HGA) [measured by the steady state levels of GABA and glutamate,activities of GAD and GABA-T,EOS-induced GABA accumulation and [^{3}H]- GABA binding].Multiple exposures of rats to HET (40°C for 2 h/day ,for 30 consecutive days) increased BT of rats with a significant decrease in HGA but attenuated the increase in BT and decrease in HGA which was observed following single exposure to HET (40°C for 2 h).Multiple exposures of rat to HET as well as CPZ potentiated significantly the HET (following multiple exposures) - induced hyperthermia . These results,thus, suggest that (a) HGA is not associated with the reduction of BT following long-term treatment with CPZ, (b) long-term HET exposure to rats caused hyperthermia by decreasing the HGA and (c) potentiating effect of long-term CPZ treatment on long-term HET- induced hyperthermia may be possible by (i) reduction of HGA, (ii) heat dissipation and (iii) supersensitization of neurotransmitter receptor.
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EFFECT OF DIETARY PROTEIN ON RAT HYPOTHALAMIC GLUTAMATERGIC ACTIVITY AND IMMUNE RESPONSE DURING AGING

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Manipulation of dietary protein has recently been found to be an useful dictator in the age-associated changes of inhibitory neurotransmitter activity and immune response (IR) in mammals.In the present investigation, we studied the effect of dietary protein on rat hypothalamic glutamatergic activity (HGluA) (studying the levels of GABA, glutamate, glutamine; activities of GAD,GABA-T,glutamine synthetase,glutaminase and ^{14}C-glutamic acid binding) and IR (studying lymphocyte viability,its proliferating activity and cytotoxicity in blood and spleen) during aging.With the increase of age of rats from 3 to 9 months maintained with normal (20%) protein diet, the HGluA was apparently increased and IR was decreased which became significant with further increase of age upto 18 months. Short-term (for 7 consecutive days) intake of low (5%) protein diet (LPD) or high (40%) protein diet (HPD) failed to alter significantly the HGluA as well as IR irrespective of age (3 to 18 months). Long-term (for 30 consecutive days) consumption of LPD increased the HGluA and decreased the IR in 3 months old rats ; whereas, in 9,12 and 18 months old rats, the HGluA was decreased along with an immunopotentiation irrespective of age under similar condition of LPD consumption. Unlike LPD, HPD intake for long-term period reduced the HGluA and increased the IR in 3 months old rats. However,HPD under similar condition increased the HGluA along with an immunosuppression in 12 and 18 months old rats. Further,it was observed that the age-induced (3 to18 months) immunosuppression and increase in HGluA were reversed and potentiated with long-term intake of LPD and HPD respectively. The results, thus, suggest that long-term intake of protein rich or protein poor diet modulates the HGluA as well as the IR depending on the age.
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SOME FISH SPECIES CAN DETECT ULTRASOUND B BUT HOW?

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Some fish in the teleost order Clupeiformes can detect ultrasound to 180 kHz. This capability probably evolved to enable clupeids to detect a major predator, echolocating dolphins. While there are no studies that show how ultrasound is detected by these fish, we suggest that the highly derived utricle of the inner ear may serve this purpose. This utricle is unlike that found in any other vertebrate group and includes a closely allied air bubble that attaches to the swim bladder via a thin connecting tube
In this paper we present the results of several studies addressing this issue. Behavioral investigations show that best hearing occurs in two ranges, from 300 to 2,000 Hz and from 20,000 to 180,000 Hz. At the same time, morphological investigations of the ear of the American shad show that the structure of the sensory epithelium of one part of the utricle is highly specialized. This middle region of the utricular epithelium, unlike the other regions, is suspended above the air bubble of the bulla, suggesting that its involvement in hearing is different from that of other ear regions in this, and other, fishes. Results suggest that while the American shad can detect a wide range of ultrasound, there is probably no frequency selectivity within this bandwidth. (Work supported by NSF and NIH).

IMAGING OF ACTIVE VOLTAGE-DEPENDENT SODIUM CHANNELS

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We have tried to monitor the distribution of voltage-gated sodium channels, which are responsible for the generation and conduction of fast action potentials, in the slowly adapting stretch receptor neuron in the crayfish. The mechanical stretch of the abdominal muscle in the crayfish is encoded directly into the frequency of action potentials in this sensory neuron, a process which may be influenced by the sodium channel distribution. Isolated stretch receptor neurons were loaded with the sodium-sensitive dye sodium binding benzofuran isophthalate (SBFI) by iontophoretic injection. The changes in the fluorescence ratio at excitiation wave-lengths 340nm/380 nm, indicating the changes in intracellular sodium, were monitored during application of the alkaloid veratridine (150 (M) and during superfusion with sodium- and potassium-free saline. Veratridine, which opens voltage-gated sodium channels, evoked a rapid and large increase in the fluorescence ratio, indicating a prominent sodium influx through voltage-gated sodium channels into the cell. In different regions of the cell, including dendritic, somatic and axonal compartments, greatly different fluorescence changes were recorded. The largest fluorescence changes were observed in the proximal parts of the axon and dendrites, possibly indicating the highest density of sodium chanels in these regions. Removal of external sodium resulted in a decrease of SBFI fluorescence, indicating a decrease of intracellular sodium. In potassium-free saline, where the Na/K pump is inhibited, an increase in SBFI fluorescence ratio was observed, indicating a sodium increase in the cell. While the sodium changes following veratridine application were distinctly heterogenous in various cellular compartments, the changes evoked by sodium- and potassium-free saline were the same in soma, dendrites and axon. Our results show that the technique of sodium-sensitive fluorescence imaging might be a useful approach to obtain information about the distribution of sodium channels in the neuronal cell membrane. We now plan to record sodium changes during single action potentials with confocal laser scanning microscopy.

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RECOVERY OF MEMORY AFTER AMNESIA INDUCED BY PROTEIN SYNTHESIS INHIBITORS AND *NMDA*-RECEPTORS ANTAGONIST: STUDY OF A «REMINDER PHENOMENON» IN CHICKS.

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The consolidation theory interprets an experimentally induced amnesia as a disruption of processes of memory formation by amnesic agents. One implication of this theory is that retrograde amnesia should represent a permanent loss of memory. However, data are available that memory disrupted in rats or mice by protein synthesis inhibitors (PSI) can be recovered by a reminder treatment — i.e. presentation to the animal of one of the environmental cues that constituted a situation of training. The aim of the present study was to find out whether memory, disrupted in chicks by NMDA-receptors antagonists and PSI can be recovered by a reminder treatment. One-day old chicks were trained to avoid an aversive methylantranilate-coated bead in a standard one-trial passive avoidance learning task. Experimental amnesia was produced by bilateral intracerebral (into IMHV area) injections of 1) cycloheximide (CYC) [20 mkg], 2) anisomycin (ANI) [80 mkg] 3) MK-801 [10nmol] or by 4) i.p. injections of MK-801 [0.2 mg/kg]. A reminder was delivered 24h after training. We used a methylantranilate-coated bead of different size and color than the one used for initial training as a reminder treatment. Chicks that were intracranially injected with CYC, ANY, MK-801 and tested 24 or 48 hours later had a pronounced amnesia compared to saline-injected control chicks, whereas chicks that received intracranial injection of CYC, ANY, MK-801 and a reminder treatment showed good avoidance compared to chicks without a reminder. On the other hand, amnesia produced in chicks by i.p. injection of MK-801 was resistant to reminder procedure. Investigation of the dynamics of memory recovery has shown that it occurred between 5 and 7 h after reminder treatment. Injection of CYC, ANY, MK-801 into IMHV before the «reminder» prevented the recovery of memory. Thus our results suggest that protein synthesis and activation of glutamate NMDA-receptors in the area of IMHV during learning is not crucial for long-term memory formation and storage in this task since disrupted memory can be recovered by the reminder procedure. However these processes are necessary for subsequent retrieval of memory. On the other hand, activation of NMDA-receptors into other regions of chicks brain should be critical for long-term memory formation and storage as demonstrated by systemic injections of NMDA-receptor antagonist MK-801.

INFLUENCES OF HANDEDNESS AND GENDER ON THE GROOVED PEGBOARD TEST

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We studied performance on the Grooved Pegboard Test upon repeated trials and transfer of training between the hands in the first trial. We employed three trials for each hand and two different protocols for the order in which the hands started the test. For the three trials combined, women were faster than men. From the first to the second trial, there was an improvement in performance for both sexes. Within the first trial, sex differences reached significance and the protocol interacted with handedness. In this trial, only left-handed men were found to benefit from previous opposite-hand performance. It is speculated that a larger corpus callosum in left-handed men allows for the greater transfer of training between the hands.

BASOLATERAL AMYGDALA EFFECTS ON DENATATE GYRUS SYNAPTIC PLASTICITY: COMPARISON WITH THE EFFECTS OF STRESS

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Explicit memory is considered to be dependent upon the hippocampus, while the amygdala is considered as an emotional memory system.
We examined the effects of priming of the basolateral group of the amygdala (the BLA) on LTP in the dentate gyrus (DG). Compared to the control group, which received just high-frequency stimulation (HFS) to the perforant path (PP), priming the BLA resulted in an enhanced LTP in the DG 30, 90, 150 and 180 minutes post-HFS. However, exposure to a behavioral stress (putting the rat for 10 minutes on a platform in the center of a water-maze) inhibited LTP at 30 minutes post-HFS. From 90-minute post-HFS onward, LTP was the same as in controls. Since the amygdala is involved in emotional responses, we examined this apparent discrepancy between the effects of behavioral stress and of amygdala priming on hippocampal plasticity. Priming the BLA an hour prior to HFS (in order to equalize the interval between the manipulation and HFS) inhibited LTP in a way resembling the effects of the behavioral stress.
We propose a model in which both manipulations trigger two mechanisms: the first is activated immediately and decays quickly (a facilitating effect on synaptic plasticity), the second one takes longer to be activated, but has a long lasting influence (an inhibiting effect).

SIGNALLING PATHWAYS UNDERLYING LTP

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We are interested in understanding the signalling pathways involved in long term potentiation (LTP). Tetanic stimulation of the Schaffer collateral inputs into CA1 neurons in the hippocampal slice induced a rapid (1 min.) phosphorylation and activation of the tyrosine kinase, Src, as detected biochemically. Our data supports a model whereby activated Src then acted on NMDARs directly , or indirectly, to induce LTP. . Hence, the induction of LTP in CA1 by tetanic stimulation was prevented by blocking Src. Directly activating Src, in contrast, enhanced the postsynaptic EPSCs. Finally, Src-enhanced EPSCs and tetanic stimulation mutually occluded the induction of LTP. Therefore, we found that Src was necessary and sufficient for the induction of LTP. The mechanism of Src action on the NMDAR did not involve a displacement of Zn2+ inhibition of NMDAR function. Our recent experiments in cultured CA1 neurons show that Src augmented the open probability of the NMDAR channel; which could account for the increased amplitude of EPSCs during LTP. Src enhancement of NMDAREPSCs was Ca2+-independent. Once Ca2+ entered through the NMDAR channel, it is thought to activate CaMKII and other kinases (PKC) which phosphorylate ion channels (AMPAR), thereby increasing their EPSC amplitude. In order to study G-protein signalling we have created null mutations Patch-clamp analysis of CA1 neurons in hippocampal slices from mice lacking mGluR5 showed decreased enhancement of the excitatory postsynaptic current NMDAEPSC component, but normal AMPAEPSC. Since phorbol esters rescued LTP in mutants, and since inhibitors, such as PKC1, selectively blocked LTPNMDA but not LTPAMPA when injected into postsynaptic neurons, our data argues that mGluR5 is coupled through PKC to NMDAR channels. Our recent data in CA1 cultures shows that PKC effects on NMDAR currents are Src-dependent. Our results show that the mechanisms for generating LTPAMPA and LTPNMDA are distinct. These mGluR5-deficient mice also showed impaired learning performance and spatial memory in the Morris water maze and context dependent fear conditioning. Non-spatial learning and memory was normal. These experiments support the hypothesis that these glutamate receptors are involved in both LTP and learning and memory In addition they establish new pathways for the involvement of specific kinases, Src and PKC, in LTP.

DECREASED PRESYNAPTIC GLUTAMATE FUNCTION IN AN ANIMAL MODEL FOR ATTENTION-DEFICIT HYPERACTIVITY DISORDER

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The spontaneously hypertensive rat (SHR) is used as a model for Attention-Deficit Hyperactivity Disorder (ADHD), since it has behavioural characteristics (hyperactivity, impulsiveness and discrimination problems) similar to those of ADHD. The aim of this study was to investigate whether there was a disturbance in glutamatergic transmission in the cerebral cortex of SHR compared to their normotensive Wistar-Kyoto control rats (WKY). Synaptosomes were prepared from SHR and WKY cerebral cortices and depolarization- or glutamate- stimulated $^{45}Ca^{2+}$ uptake was determined. (Calcium uptake is directly coupled to neurotransmitter release and provides an indirect measure of neurotransmitter release). It was found that whereas K^+-stimulated $^{45}Ca^{2+}$ uptake was greater than or equal to that of WKY, glutamate-stimulated $^{45}Ca^{2+}$ uptake was depressed. Non-stimulated $^{45}Ca^{2+}$ uptake into SHR synaptosomes was significantly lower than uptake into WKY synaptosomes. This suggested that whereas depolarization-induced release of neurotransmitter appeared to be normal in SHR cerebral cortex, calcium uptake, and neurotransmitter release as a result of activation of presynaptic glutamate receptors might be impaired. The reason for this is not clear, it could be due to the presence of fewer neuron terminals or fewer stimulatory glutamate receptors located on presynaptic terminals in the brain cortex of SHR. This finding has important implications for the understanding of the mechanism underlying the behavioural disturbances of ADHD. The results suggest that SHR may have impaired glutamate-mediated positive feedback mechanisms that are required for learning and memory formation. If these results are confirmed, they could provide an important link between the behavioural disturbances of ADHD and learning impairment often seen in these children.

THE DISCOVERY OF LOCAL PART IN RATS STRIATE CORTEX SPECIALISED FOR REALISATION OF DIFFICULT SENSO-MOTOR FUNCTIONS

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The mainstream in the complex of contemporary investigations of integrative brain activity is study of physiological basis of the interaction between sensory and motor systems. In the bounds of this trend of research, the tasks of revealing and investigation of brain anatomical loci with strongly pronounced functional properties have a special actuality. During our investigations it was established. First, the ability for formation of skills that requested the difficult visual and motor behavior depended on small part of cerebral cortex in rats (the zone of functional specificity of complicated visual stimuli discrimination reflex). Secondly, this locus dispose on the board between Oc1 (field 17) and Oc 2.1 (field 18a) on the 5,3 mm caudal from bregma. It don't coinside with K.S.Lashley's field "c" and "b". Although this locus include the large part of field "b". Thirdly, after extirpation of this part of cerebral cortex the operated rats discriminated visual stimuli very well (it was showed in the experiments with electro-shock confirmation). The motor functions in operated rats kept too (it was showed in the experiments of brightness discrimination). But the ability for integration of visual and motor components of complicated visual stimuli discrimination reflex (jump to the visual stimuli) was lost irreversibility. So in spite of that the associative areas of cerebral cortex in rats expressed badly the strict functional accordance between extirpation of narrow part of rat striate cortex and the irreversible loss of correspondence functions was determined. This accordance is more typical for animals that take up the higher levels in the evolution (carnivora, primates). Therefore the discovered part of rat cerebral cortex that highly specialised for certain functions can used as handly and highly specific physiological model for investigation of changes in the morphological and functional state of neocortex under various influences on the learning and memory processes.

ZINC ENHANCES, BUT COMPLEX TRACE METAL AND VITAMIN SUPPLEMENTATION PREVENTS β-AMYLOID NEUROTOXICITY *IN VIVO*

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A multitude of clinical and experimental evidence indicate that trace metals (TM) play pivotal roles in the maintenance of physiological neural functions. Both the lack and pathologically enhanced accumulation of essential TM might result in severe neural dysfunctions. Recent neuropathological and *in vitro* experimental data pointed out that excess zinc (Zn) accumulation enhanced β-amyloid (Aβ) neurotoxicity which may be substantially involved in the progression of Alzheimer's disease. In contrast, other minerals (e.g. Mg, Se, Mn) or vitamins with free radical-scavenging potentials were shown to decrease the extent of neurotoxic insults. In the present study we set out to investigate the influence of several TM and their combinations with vitamin E and C on Aβ neurotoxicity *in vivo*.

$\beta A_{(1-42)}$ was unilaterally injected into the *nucleus basalis* of rats (0.2 nmol/1 μl). Pharmacological characteristics of TM supplementations with (1) **Zn**, (2) vitamin E+C, (3) vitamin E+C+Mg, (4) vitamin E+C+Mg+Se+Mn+Cu+**Zn** and (5) vitamin E+C+Mg+Se+Mn+Cu were tested. A standard vitamin E and C concentration (150 mg/kg) and feeding protocol (administered from 48 hrs pre- up to 48 hrs post-lesion) were used throughout the experiments. The degree of neurodegeneration was determined by testing the animals in consecutive series of behavioral tasks including elevated plus maze (d5, d7), passive avoidance learning (d10-12), small open-field (d14) and open-field (d14) paradigms. These were followed by determinations of acetylcholinesterase (AChE), choline-acetyltransferase (ChAT) and superoxide dismutase (SOD) activities in the parietal cortex by means of biochemical methods.

Whereas complex vitamin and TM supplementations markedly decreased the Aβ-induced behavioral dysfunctions and the loss of AChE, ChAT and SOD activities in the parietal cortex, Zn administration significantly enhanced Aβ neurotoxicity in the rat brain. Interestingly, co-administration of vitamin E+C and Mg, Se, Mn, Cu with Zn significantly ameliorated Zn-induced enhancement of Aβ neurotoxicity.

The present data prove the bioactivity and neuroprotective action of antioxidants and TM, such as Mg, Se, Mn, Cu, and the neurotoxicity-enhancing potential of Zn in a Aβ lesion paradigm. Underlying mechanisms may involve neuromodulatory action of TM on NMDA receptor function and changes in the regulation of intracellular Ca^{2+} homeostasis.

IMMUNE CHANGE IN PSYCHIATRIC DISORDERS: CATEGORICAL VS DIMENSIONAL EFFECTS

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Studies of immunity in patients with major depressive disorders (MD) have shown variable, at times apparently inconsistent, effects. A series of studies from our group (restricted to medically well, medication free subjects) suggests that immune differences are related to age and clinical characteristics such as coexisting anxiety. In adults, we found ($p<0.05$) decreased circulating lymphocyte subsets and mitogen induced lymphocyte proliferation in older MD patients but no change or elevated measures in younger depressed adults. Natural killer cell activity (NKCA), in contrast, appears to be decreased in depression throughout the adult age range. While we found that depressed prepubertal children showed mitogen and NKCA changes comparable to that of young adults, depressed adolescents showed decreased mitogen responses and elevated NKCA. The differing patterns in depressed adolescents may relate to co-occurring stress and anxiety as well as to developmental neuroendocrine factors. In other studies, we have found that comorbid anxiety disorders mitigated immune effects associated with MD while no changes were found in mitogen response or NKCA in currently asymptomatic (but unremitted) patients with panic disorder or in a well characterized medically healthy group of chronic alcoholics. Symptoms of anxiety in response to a major life stress (a family member's life threatening trauma), in contrast, were associated with increased mitogen response and depressive reactions were associated with decreased mitogen proliferation. The data together suggest that immune system changes in psychiatric disorders may be linked primarily to contingent and interacting factors such as stress, acute mood disturbances, and age rather than to persistent CNS states associated with the diagnostic entities per se.

EFFECTS OF HANDEDNESS AND GENDER ON MOTOR PERFORMANCE IN A COMPUTERIZED FINGER-TAPPING TASK.

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We studied gender and handedness effects on performance in a finger-tapping task. Two measures were assessed: speed of tapping and intertap variability. Raw data indicated that men performed more quickly than women on both the preferred and nonpreferred hand. Men performed more regularly only on the nondominant hand. When dispersion associated with sex differences was corrected, a significant handedness effect emerged. Right-handers showed significantly greater intermanual discrepancies than left-handers. It was concluded that adequate judgments on intermanual discrepancies, specially for the intertap variability measure, depend on normative studies using samples stratified according to handedness. The finding that left-handers do not show large intermanual discrepancy scores is interpreted considering that their larger corpus callosum might allow for greater interhemispheric communication.

PHYTOTHERAPY IN PERSONS WITH HIV INFECTION: CHANGES IN MARKERS OF DISEASE PROGRESSION TO AIDS.

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Background: HIV infected persons search for cure in Zimbabwe of a human immunodeficiency virus have made them to resort to traditional treatment. The study's aim was to examine herbs' effect in persons with HIV infection on changing in markers of disease progression to AIDS.
Methods and Material: This is a community-based open label uncontrolled study. Patients were recruited from two sources. One group was on phytotherapy (PT) plus conventional medical care (CMC), while the other group was on CMC only. To ensure compliance patients volunteered for one method of treatment but were done by Brief Psychiatric Rating Scale, the Montgomery-Asberg Depression Rating Scale, and WHO Quality of Life Instrument.
Results: The patients mean age (St) was 34.44 (7.4) years for patients on PT+CMC, whereas for patients on CMC was 36.4 (6.6) years. At three months' follow-ups, patients on CMC treatment (63.6%) group were 0.32 times more likely to suffer from psychiatric disorders than those on PT+CMC (95% CI=0.11-0.95, P=0.035). Also, quality of life assessment showed that patients scored higher in psychological status (P=0.009), and in Level of Independence (P<0.001).
Conclusion: Patients on CMC alone suffered more psychiatric disorders than those on PT+CMC. Male patients sought both traditional and conventional cares than the female patients.

THE RELATION BETWEEN THE CNS AND THE PHARYNGEAL NERVOUS SYSTEM IN A PLANARIAN

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Flatworms are regarded as the most ancient group possesing a CNS and a PNS. Planarians are free-living, predatory flatworms, with a high capacity of regeneration. In order to study the relationship between the CNS and the nervous system (NS) of the pharynx (Ph) in *Girardia tigrina,* the feeding behaviour was investigated. When *G. tigrina* feeds it (1) searches for prey, (2) it approaches the prey, (3) it throws out the pharynx (Ph) and (4) it eats. When the head ganglion is amputated the worm becomes unable to search for and approach the prey. If the worm by chance touches the prey, it throws out the Ph and eats. Thus the motoric activity of the Ph is independent of the head ganglion i.e. the NS system of the Ph functions autonomically. If the Ph is amputated the worm still searches for and approaches the prey, but is unable to eat. After some unsuccessful approaches the worm stops searching. The searching is resumed after the regeneration of Ph. The innervation of the head ganglion and the Ph was studied immunocytochemically, using anti-5-HT and anti-FaRP:s. In the head blastema the 5-HT-IR neuronal elements appear first and the FaRP-IR neuronal elements later. In the regenerating Ph the order is opposite, the FaRP-IR neuronal elements appear first and the 5-HT-IR elements later. The peptidergic nerves cling to the muscle fibres. Conclusions: (1) The head ganglion completely controls the feeding behaviour. (2) The autonomous NS of the Ph controls independently only the motor acitivity of Ph. (3) The function of the Ph is dependent on the development of the peptidergic NS.
The work was supported by INTAS 96-1365.

DAYTIME INCREASE OF PINEAL PEPTIDE SECRETION IN RATS WITH TUMORS OF LARGE INTESTINE OR IN OSMOTIC STRESS SUBJECTED

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In mammals the pineal gland (PG) is known to take part in circadian rhythms formation, together with suprachiasmatic nucleus of the basal hypothalamus. It's night-time increase of melatonin secretion and electrical activity is under the sympathetic control from the superior cervical ganglia. The day-time melatonin secretion level and electrical activity in the pineal gland is usually insignificant, so the PG is usually studied at night. It is important to say, that PG electrical activity reflects the intensity of protein and peptide secretion, but not melatonin. In McCance (1996) experiments N-acetyltransferase blocking didn't affect pinealocytes (PC) depolarization and at the same time the suppression of electrical activity with nifedipine didn't influence melatonin secretion. The aim of this investigation was to study the daytime pineal electrical activity in rats with induced large intestine tumors or in osmotic stress.

Wistar rats (n=35) were used extracellular microelectrode registration of PC spontaneous activity. We have demonstrated that colchicine microinjections through the microelectrode tip blocked exocytosis and stopped depolarization of PC. It confirms the link between PC spikes and exocytosis.

Some rats were injected with dimethylhydrazine 5 months before experiments, which 100% caused tumor progression in the large intestine. It lead to increase of day-time PC electrical activity up to the night-time level, due to increase of number of cells with high frequency of discharging (regular with frequency > 4 spikes/sec and pattern types of spikes). It points on the intensification of pineal peptide secretion, because of an accumulation of peptide containing vesicles in the body and in the processes of PC. Besides, daytime pineal activation in stress involves not all PC, but only largest of "light" cells. It have been revealed in light and electrone-microscopic study (Kovalenko et al., Russ. J. Physiol., 83(8): 87-93, 1997). In this conditions blood melatonin concentration in the day-time remained low (Anisimov V.N., et al, in press). The same results were obtained in 48-hour food and water deprived rats.

The discovered day-time activation of pineal electrical activity, which appears in stress (in deprived or in tumor bearing rats) is not of sympathetic origin, because in our study noradrenaline microinjections didn't influence PC spikes frequency, and α- and β-adrenoblockators (anapriline and phentolamine) didn't inhibit PC electrical activity. Furthermore, sympathetic influence usually stimulates melatonin production, but in our experiments it's blood concentration remained low.

CONTRIBUTION OF GABA AND 5-HT SYSTEMS TO RAT HABITUATION TO SPATIAL NOVELTY – A BEHAVIORAL AND AUTORADIOGRAPHICAL STUDY

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The following study was designed to evaluate the implicated role of GABA and 5-HT systems in adaptation of exploratory behavior over repeated exposures in the open field test (OFT) of neophobia. In the present experiment unhabituated animals were pretreated i.p. with various compounds acting at $GABA_A$/benzodiazepine (BDZ) receptor complex (diazepam, midazolam, zolpidem, muscimol, picrotoxin, bicuculline) as well as with $5HT_{1A}$ receptor agonist, buspirone, and subsequently subjected to test (after 30 min) and re-test (24 h later) in the OFT. During each 15-min. session ambulatory activity, exploratory parameters and thigmotaxis were scored. Changes in receptoral binding after buspirone pretreatment were assessed with a help of [^{3}H]muscimol autoradiography. All drugs, except bicuculline, in lower doses diminished the extent of, and in higher doses blocked the day-to-day decrease in ambulation and exploratory scores, although clear-cut differing in profile of action on the first testing day. Buspirone at the highest dose showed a lack of *ex tempore* effect and emerging distantly in time anxiolytic-like action, whereas e.g. BDZ failed to produce similar effect. Moreover the autoradiography revealed an increase of [^{3}H]muscimol binding in the frontal cortex after the buspirone pretreatment. In conclusion, the obtained data shedded some light on the manner in which 5-HT/GABA interaction in habituation to spatial novelty may be accomplished. The delayed action of buspirone, widely observed in clinical studies, seems be on account of time-dependent sensitization, shaking the assumption that the efficacy every time directly depends on pharmacokinetics, and simultaneously points at the underlying increase in GABA system activity after the buspirone injection.

THE UNITARY POSTSYNAPTIC MECHANISMS OF EXCITATORY AND INHIBITORY SYNAPTIC PLASTICITY IN THE NEOCORTEX, HIPPOCAMPUS, CEREBELLUM

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The unitary postsynaptic mechanism of excitatory and inhibitory synaptic plasticity for the neocortical, hippocampal and cerebellar neurons is proposed. It is postulated that properties of receptors on cerebellar cells are similar to those on neocortical or hippocampal cells. Therefore, in all these structures LTP/LTD of excitation is the result of AMPA receptors phosphorylation/dephosphorylation while LTP/LTD of inhibition is the result of GABAa receptors dephosphorylation/ phosphorylation. It is proposed that the participation of cGMP in synaptic plasticity of cerebellar granule cells, Purkinje cells and deep cerebellar nuclei cells, and the involvement of cAMP in synaptic plasticity of neocortical, hippocampal cells and cerebellar Golgi cells underlie the opposite calcium-dependent modification rules in these groups of neurons. This assumption is based on the data that cGMP level is down-regulated by calcium/calmodulin as distinct from positive correlation between the rise of calcium and cAMP level. To provide the fulfillment of Hebbian rule it is postulated that only synapses activated by the transmitter are modifiable. It is proposed that heterosynaptic effects occur if homosynaptic and heterosynaptic afferents activate both, a target cell and a "common" inhibitory neuron, which activates GABAb receptors on a target cell. Using the computational model of the posttetanic biochemical processes occurring in dendritic spines it was shown that stimulation frequency (calcium rise) necessary for LTP or LTD induction is relative but not absolute. Synaptic modification is the result of the shift in posttetanic calcium and cyclic nucleotide concentration in reference to the concentration produced by prior stimulation. Therefore, the dependence of the previous history is intrinsic property of synaptic plasticity. The following conditions are necessary and sufficient for synaptic modification: the coincidence of pre- and postsynaptic cells activity; the changes of pre- and/or postsynaptic cell activity during the time sufficient for the shift of the ratio between protein kinases and protein phosphatases. The results of suggested model are supported by known experimental data.

EFFECT OF HIGH ALTITUDE ON BRAINSTEM AUDITORY EVOKED RESPONSES

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Altitude above 3500 m have been invariably shown to cause symptoms of Acute Mountain Sickness and psychophysiological changes in an individual. The present study was taken to evaluate Brainstem Auditory Evoked Responses (BAER) on exposure to high altitude. Study was carried out on 25 normal healthy male volunteers in the age group of 20-30 years with no previous exposure to high altitude. The BAER recording from both left and right ear were carried out at sea-level (Silliguri) and at an altitude of 3500-m and 4,270 m and on return to sea-level using portable Compact - 4, Nicolet. After recording their base line data at sea-level the human volunteers were taken by road to an altitude of 3500 m for six days for initial acclimatization, and then transported to 4270 m where they stayed for ten days before reinduction to sea level. The results indicated a decrease in the interpeak latencies of wave III-V for right ear BAER at 3500 m as compared to corresponding sea level value and this decrease continued at 4270m with slight improvement, however the decrease was not significant statisticaly. The recording from left ear BAER did not show any change. The present finding indicate that induction of low landers to high altitude may not cause changes in the Brainstem Auditory evoked responses.

EFFECT OF ALTERED LIGHT DARK CYCLE AND TEMPERATURE ON CIRCADIAN RHYTHMICITY OF SUPERIOR CERVICAL GANGLIONECTOMIZED RATS

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The present study investigates the role of pineal and melatonin rhythm on the coupling of various circadian rhythms (CR) under combined influence of altered light dark cycle (LD) and cold temperature (C) in rats. To achieve this, normal, sham operated (S) and superior cervical ganglionectomized (SCGx) rats (n=6 in each) were exposed to 12:12 LD at 26±2oC and 23:1 LD at 8±2 oC (LL-Cold) for 15 days while continuous recording of body temperature (BT) and activity (ACT) was done telemetrically. On 16th day, four hourly blood sampling was done for melatonin estimation. All the CRs were analysed by Cosinor analysis. LL-C on control and S rats caused uncoupling along with disruption of BT and ACT rhythms while melatonin was undetectable. Whereas, LL-C of SCGx rats resulted in a highly significant melatonin CR (PR 96.47) with increased mesor (from 63.21±4.33 to 85.28±6.17 pg/ml)), amplitude and phase delay (from 0604h from 0149h) while other other rhythms remained uncoupled in comparison to 12:12 LD. Therefore, increased mesor with significant CR of melatonin might be due to activation of extrasympathetic stimulation (eg. NPYergic) of pineal in absence of sympathetic control or due to extrapineal release of melatonin in a feedback manner when pineal melatonin is absent. But the melatonin induced synchronization of other rhythms seem to be determined not by its circadian nature but upon its mean secretory level, amplitude and acrophase.

APOPTOSIS IN ALZHEIMER'S DISEASE. "IN VIVO" VISUALIZATION IN HUMANS USING RADIACTIVE ANTICASPASA.

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Radionucleidic Lab. Dept. Physiology. Fac. Medicine

We label an anticaspasa inhibitor compound with radionuclides (Tecnesium 99 metaestabel) and take scan image in 4 normal volunteers and four patient with Alzheimer's Disease. The normal volunteers don't have captation of the radiactive anticaspase ("negative image") v.s. the "positive image" that occurs in the pathologic brain in all the patients studied. Nobody of the patients or volunteers have reactions or complication during six months of "follow-up" after the use of the new radiopharmaco and method.

The roll and clinical implication of apoptosis in Alzheimer's Disease and the facilities of his "in vivo" visualization without damage, deserves more research in this exciting new camp of Neurology

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CYCLIC GMP MIMICS THE EFFECTS OF NOOTROPIC DRUG, VINPOCETINE, ON DIFFERENT TYPES OF POTASSIUM CHANNELS IN MOLLUSCAN NEURONS

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Vinpocetine (Cavinton) is a clinically empolyed nootropic drug which is known to be effective in a variety of dementias. To date, the beneficial effect of vinpocetine seems to be due to both cerebral vasodilation (Miyazaki, 1995) and direct action of the drug on the nervous cells. In nervous cells, vinpocetine was shown to block voltage-gated Na-channels (Molnar et al., 1995) and Ca- channels (Kaneko et al., 1990). The goal of present work was to study the influence of vinpocetine on different types of K-currents of neuronal membrane. The experiments were conducted in isolated neurones of land snail Helix. Using two microelectrodes voltage clamp method, five types of voltage-operated ionic currents were recorded: 1) Ca-current (I.Ca), 2) Ca-dependent K-current (I.K(Ca)), 3) delayed rectifier K-current (I.KD), 4) high threshold A-type of K- current (I.Ahth), 5) low threshold A-type of K-current (I.Alth). I.Ca was inhibited only by high concentration of vinpocetine (300-600 mkM). However, all four types of K-currents were sensitive to small and pharmaclogically relevant concentrations of the drug (1-100 mkM). The definite regularity for vinpocetine effects on K-currents was found: K-currents having weak inactivation (I.K(Ca) and I.KD) were depressed by the drug, and K-currents having strong inactivation (I.Ahth and I.Alth) were enhanced or remained unchanged. It is known from literature that vinpocetine is a strong inhibitor of phosphodiesterase of cyclic nucleotides (Beavo, 1992). The question was whether the effects of vinpocetine on K-currents were mediated by cyclic nucleotides, cAMP and cGMP. It was shown in our experiments that dibutyryl cAMP failed to mimic the effects of vinpocetine. At the same time, 0.5-1 mM dibutyryl cGMP mimics the effects of vinpocetine in the most of the cells tested. So, there is a statistically significant correlation (r=0.60, n=23) between the effects of vinpocetine and dbcGMP on K-currents. Physiological significance of observed changes in K-channels work may lie in the changing of the Ca-influx into the cell during an action potential. Because of the opposite action of vinpocetine on different types of K-currents, intracellular Ca-concentration may also be changed in opposite manner in different cells. This work was supported by a grant (No. 98-04-48904) from Russian Fund for Fundamental Investigation.

STUDY OF THE EFFECTS OF A SELECTIVE $GABA_b$ ANTAGONIST ON GABA-INDUCED CHANGES IN BIOELECTRICAL AND MECHANICAL ACTIVITY IN AUTONOMIC NEUROTRANSMISSION

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There are data that the GABA acts as a neurotransmitter in enteric plexus of autonomic nervous system and the $GABA_B$ receptors are involved in the tonic and phasic contractions of cat ileum preparation. The aim was to study the in vitro effects of GABA and a selective $GABA_B$ antagonist CGP55845A = [3-{{1-(S)-3,4-Dihydrophenyl(ethylamino)}-2-(S)-hydroxypropyl} phenylmethyl}-phosphinic acid hydrochloride on guinea pig ileum. Longitudinal smooth muscle preparation in modified Krebs with Carbogen aeration was used. The mechanical activity was registered by isometric tensotransducer Swema and recorded by Linseis. GABA showed bifasic contraction/relaxation reaction. It was found the well pronounced dose-response relationship of GABA effects. The maximum depolarization was 0.7 + 0.09mV. The selective $GABA_B$ antagonist CGP55845A caused the relaxation. On the bioelectrical activity a $GABA_B$ antagonist CGP55845A showed hyperpolarization and minimized the amplitude of the spontaneous bioelectrical activity. On GABA-induced changes in mechanical and bioelectrical activity CGP55845A reduced the relaxation phase or it is completely missing. The dose-dependent changes in the contractile phase were observed. It is conluded that GABA and its selective $GABA_B$ antagonist CGP55845A changed the mechanical and bioelectrical activity in enteric plexus of autonomic neurotransmission.

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ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF PREAUTONOMIC NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN).

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The PVN is a heterogeneous region containing magnocellular and parvocellular neurons. Neuroanatomical studies demonstrated that separate populations of neurons within the parvocellular division project to the median eminence (neuroendocrine neurons), the brain stem and spinal cord (preautonomic neurons). The main objective of this study was to characterize the electrophysiological and morphological properties of identified preautonomic PVN neurons. Extrahypothalamic projecting neurons in the PVN were retrogradely labelled by small injections of the fluorescent tracer DiI into the dorsal vagal complex in the brain stem. Whole-cell recordings were obtained from hypothalamic slices containing the PVN, where labelled neurons were visualized with fluorescence microscopy. Following the recordings, neurons were filled with biocytin, and then reconstructed in three dimensions. In general, as opposed to their neighboring magnocellular neurons, preautonomic neurons were characterized by the presence of a Ni2+-sensitive low threshold depolarization, and by a strong inward rectification elicited by hyperpolarizing pulses from depolarized membrane potentials. Furthermore, the great majority of preautonomic neurons were hyperpolarized when norepinephrine (10 μM) was added to the bath. Preautonomic neurons also differed from magnocellular neurons in terms of their morphology, displaying in general a more complex dendritic arborization. In summary, these experiments provide new information on the electrophysiological and morphological features of this discrete population of PVN neurons involved in the control of cardiovascular and autonomic functions.

CHANGES IN THE INFERIOR COLLICULUS EVOKED RESPONSES AND UNIT ACTIVITY IN RATAFTER FUNCTIONAL ABLATION OF THE AUDITORY CORTEX

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The morphology of descending fibers from the auditory cortex (AC) to the inferior colliculus (IC) is well documented, but the functional role of these fibers in the processing of auditory signals has not been studied in detail. We recorded click-evoked responses (IC-ER) and single and multiple unit activity from the IC and investigated the effects of functional ablation of the AC by the local intracortical application of a sodium channel blocker, tetrodotoxin (TTX). Evoked responses and single unit activity were recorded in rats slightly anesthetized with xylazine (0.03 ml/100g) with two metal electrodes (insulated nichrome wire, diameter 0.002 inches) implanted to different depths in the IC. The TTX was applied into the ipsilateral AC through three implanted canulas (to cover the whole extent of the AC) in a total dose of 30 ng. The function of the AC was checked by recording click-evoked middle latency responses (MLR) from an electrode (platinum-iridium ball electrode) implanted on the surface of the AC. Bipolar clicks or click pairs were presented from a loudspeaker in free field conditions. Sound stimulation and response acquisition were performed with a TDT system. The MLR disappeared almost totaly 5-10 minutes after TTX application, and this effect lasted for several hours. The full recovery of MLR was observed 1-3 days postinjection. Inactivation of the AC resulted in changes in the IC-ER amplitude and in a slight prolongation of the latencies of individual waves. In many experiments, mainly the later components of the IC-ER (occurring with a latency around 40 ms) were depressed during AC inactivation. The IC-ER amplitudes and latencies recovered within 2-3 days. The changes in IC-ER parameters were correlated with the electrode position within the IC. In addition, the response changes of the IC neurons during the test sessions were evaluated on the basis of peristimulus time histograms (PSTH). The results show that during the period of functional decortication of the AC, by which time the evoked responses from the AC are largely diminished, the unit activity may be enhanced, suppressed or in some animals essentially unchanged. The changes in the neuronal activity inversely corresponded to the functional recovery of the AC. The above observations have been, in a few cases, accompanied by threshold and/or CF shifts. A related on-going study seeks to correlate these observed changes in neuronal activity with the spatial location of the recording electrode so as to deduce possible implications for understanding the role of cortico-collicular pathways. The results document the complex interaction of descending cortico-tectal pathways with neuronal function in different parts of the IC.

PRE- AND/OR POST-SYNAPTIC LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTOR 1α (MGLUR1α) AND 2/3 (MGLUR2/3) IN THE RAT SPINAL CORD

Key Words: Pre- and post-synaptic localization; mGluR1α; mGluR2/3; spinal cord.

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By immunohistochemical and immunocytochemical study, it has been shown that in the lamina I, there existed weakly stained mGluR1α immunoreactive product, however, mGluR2/3 immunoreactivity was almost undetectable in this lamina and outer layer of the lamina II. In lamina II, strongly stained mGluR1α immunoreactive product was demonstrated, such a staining of mGluR2/3 was shown in the inner layer of lamina II and bordering part of lamina III. From lamina III to lamina X, weakly to moderately stained mGluR1α immunoreactive product was demonstrated. The similar staining was also shown from lamina III to lamina VI and in lamina X for mGluR2/3. However, few mGluR2/3 immunoreactivities were detected from lamina VII to lamina IX. Under electron microscope, mGluR1α immunoreactivity was shown in neuronal cell body and dendrites in lamina II of the dorsal horn. In the lateral and ventral horns, only dendrites of neurons were mGluR1α immunopositive. In lamina II of the dorsal horn, lateral and ventral horns, some mGluR2/3 immunopositive dendrites were demonstrated, however. In the ventral horn, mGluR2/3 immunopositive axon and axon terminals were shown. Some mGluR2/3 immunopositive astrocytes were also demonstrated in the three areas and their strongly stained processes wrapped neuronal cell bodies and synapses. It suggests that (1) in the dorsal horn, postsynaptic activation of mGluR1α and mGluR2/3 may be related to the spinal hyperexcitability under various peripheral stimulations; (2) in the lateral horn, postsynaptic mGluR1α and mGluR2/3 mediate the activity of the sympathetic preganglionic neurons; (3) in the ventral horn, both presynaptic group II and III mGluRs are involved in the regulaton of motoneuron activity; and dual action of mGluR agonists on neuronal excitability and synaptic transmission may be through activation of post- and pre-synaptic mGluR2/3 and (4) mGluR2/3 may be an important receptor for glutamate activation of intercellular glial signaling in the spinal cord.

ANXIOLYTIC EFFECTS OF KINDLING OF THE LATERAL SEPTUM IN RATS.

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An increase in anxiety or fearfulness typically occurs as a result of kindling of the amygdala, a region of the brain that may be described as anxiogenic. In contrast to kindling of the amygdala, we report here that kindling of the lateral septum has an anxiolytic effect in rats. Two groups of rats were implanted with stimulating electrodes in the right lateral septum. One group of animals received one second of lateral septal stimulation (0.25 ma, 100 Hz square waves with a pulse duration of 1 ms) three times a day for 20 consecutive days. The second group was placed in the stimulation chamber for the same amount of time but received no stimulation. Animals were rated for kindling on a five-point epilepsy scale devised by Pinel and Rovner (1978). Subsequently, animals were tested for anxiety in an elevated plus-maze. Kindled animals spent significantly more time on the open arms and had more entries into the open arms than the non-kindled animals In a subsequent test on the plus-maze, animals were tested for the effects of the benzodiazepine antagonist, flumazenil (10 mg/kg). Kindled rats administered flumazenil were essentially similar to the unkindled animals in time on the open arms and number of open-arm entries. The findings suggest that increasing the excitability of the lateral septum has a long term anxiolytic effect, consistent with an anxiolytic function of the lateral septum, and that the anxiolytic effect may involve the BZD-GABA-Cl^- receptor complex. Supported by NIMH grant 54674

DEVELOPMENT OF INTRINSIC RHYTMICITY OF THE RAT SUPRACHIASMATIC NUCLEUS

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The rat circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. On the bases of morphological and physiological characteristics, the SCN can be divided into the ventrolateral (VL) and dorsomedial (DM) part. The DM-SCN exhibits, besides other rhythms, the rhythm in the spontaneous c-Fos immunoreactivity in darkness, with maximum in the morning and through during the night (1). The VL-SCN exhibits the rhythm in c-Fos photoinduction; the gate for the photoinduction is open only during the subjective night. Both the DM- and VL-SCN rhythms depend on the photoperiod (2; in preparation).

The aim of the present study was to characterize development of intrinsic rhythmicity of the rat SCN under a long and a short photoperiod. Newborn rats were maintained in LD 16:8 or in LD 8:16 and rhythms in the spontaneous c-Fos immunoreactivity in the DM-SCN and in c-Fos photoinduction in the VL-SCN were followed on the 3rd and 10th postnatal day. The endogenous rhythm of c-Fos immunoreactivity was developed already in the DM-SCN of 10- as well as of only 3-day old rats, but was not yet photoperiod dependent. The gate for c-Fos photoinduction, though not yet photoperiod dependent, was present in the VL-SCN of only 10-day old rats; in 3-day old animals, light induced c-Fos at any day time. The data show a different development of both parts of the SCN and suggest a higher degree of synchronization among neurons of the newborn rat DM-SCN than among neurons of the VL-SCN.

1) Sumová et al., Brain Res., 801: 254-258, (1998).
2) Sumová et al., in preparation

EFFECT OF THE CHOLINESTERASE INHIBITOR GALANTHAMINE ON THE STUDYING ABILITY AND LOCOMOTIVE ACTIVITY IN PROLONGED ALCOHOL-INTAKE RAT MODEL OF ACETYLCHOLINE DEFICIT

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Chronic brain acetylcholine deficit is a typical feature of Alzheimer's disease and chronic alcohol abuse. In this and in previous works we have studied the effect of the acetylcholinesterase inhibitor galanthamine on such conditions. As a continuation of our previous investigations in which we have studied the spatial orientation of animals, subjected to prolonged alcohol intake we have examined the speed of learning and motor activity of the animals. Four groups of 6 male Wistar rats were studied. The first group (*ctrl*) and the fourth group (*ctrl+gal*) were controls and the rats received tap water as drinking fluid. In the second group (*alc*) and in the third group (*alc+gal*) we induced chronic acetylcholine deficit using a 16 week-long alcohol intake model (20 % v/v ethanol as a sole drinking fluid). After 12 days free of alcohol we began to administer galanthamine (2.5 mg/kg i.p) in *alc+gal* and *ctrl+gal*. One week later we performed the shuttle box test for evaluation the studying ability and the rota rod test for evaluation of locomotive activity. The results from the shuttle box test showed that *alc* had the lowest speed of learning compared to the controls and to *alc+gal* ($p<0.05$). As short term memory evaluation we examined the performance of the animals after 7 days rest and the results showed only significant impairment of the performance of *alc*. The only significant difference ($p<0.05$) on the rota rod test was between the results of *alc+gal* and *ctrl*. All these results suggest that galanthamine at that dosage improves the memory abilities in conditions of prolonged alcohol intake cognitive impairment.

CHANGES IN AUTONOMIC BALANCE: POWER SPECTRAL ANALYSIS OF HEART PERIOD VARIABILITY IN ASTHMATIC CHILDREN

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Cardiac interbeat interval sequences (heart periods (HP) or RR-intervals) show quasi-periodic fluctuations. This heart period variability (HPV) is largely determined by a balance between levels of activity in the cardiac sympathetic and parasympathetic nerves. Analysis of HPV in the frequency domain is a tool to assess autonomic balance. Short-term fluctuations in HPV are concentrated in three principal peaks of the power spectrum. Two of them were important for the present study: the vagally mediated high frequency (HF) peak (0.15-0.4 Hz), that reflects the respiratory sinus arrhythmia (RSA),and the mid frequency (MF) peak, that occurs around 0.1 Hz and is mediated jointly by the vagus and sympathetic nerves. Significantly higher spectral power was found in HF band in the asthmatic group as compared to the control group during rest ($p<0.023$). During postural change from supine to standing, the control group showed a substantial increase of MF component ($p<0.001$) reflecting a compensatory increase of beta-adrenergic activity. The asthmatic group failed to show this increase of MF component. This finding indicates an impaired baroreflex, a deranged sympathetic surge in response to orthostatic stress in asthmatic children. As these results show, an increased activity of the vagus nerve and a decreased sympathetic (a tentative beta-adrenergic) response to orthostatic load were found in asthma bronchiale.

THE MECHANISM OF MUCLE NO-SYNTHASE NEURONAL CONTROL

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The muscle fibers (MF) are known to contain two isoforms of NO-synthase (NOS): the neuronal and endothelial. The neuronal one is concentrated near postsynaptic membrane and disappears after denervation. It suggests that this enzyme is neuronally controlled. Using the electrophysiological and tissue culture methods it has been shown that the non-quantal (NQ) Ach released from motor nerve endings (NE) and probably Glu might be the neuronal signals controlling functioning of NOS in the MF end-plate area. In fact, the nanomolar Ach secreted from NE in a NQ manner activates the M1-muscarinic receptors in MF with the subsequent opening of Ca^{2+}-channels. Glu appears to be a co-factor of NQAch activating the Ca^{2+}-channel of the postsynaptic NMDA receptors. The M1-receptors mediated Ca^{2+}-influx through the voltage-dependent channels and Ca^{2+}-entry through the channel of NMDA receptors can activate the NOS in MF with the release of NO. The NO molecules seem also to act as retrograde signal in the neuromuscular junction stimulating the production of cGMP in the NE and controlling the amount of released NQAch as a negative feed-back mechanism. The early drop of membrane potential in denervated MF could be triggered by a decrease of NO synthesis caused by a deficiency of NQAch and Glu secretion. The denervation-like depolarization develops also in innervated MF from rats treated *in vivo* with NOS inhibitors.
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ADDRESSED INTERACTIONS IN THE NON-SPECIFIC SYSTEMS OF THE BRAIN AND DEVELOPMENT OF THE SLOW WAVE SLEEP IN CIRCADIAN CYCLE

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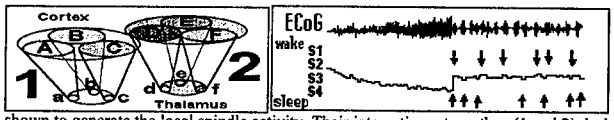
The conception of the non-specific systems of the brain has been formed since the works of Moruzzi, Magoun (1949) and Jasper (1963), showed that the structures of the non-specific thalamus are connected with correspondent cortical areas and form the united non-specific thalamo-cortical system. The system has been considered as a homogeneous with mainly diffuse relationships.

However, microelectrode studies upon rabbits and cats by matrices, placed both into the thalamus (NCM, CM-Pf) and the cortex (SMC, SSC) with simultaneous bioelectrical activity recordings make us drew another conclusion. So, the studies of: 1) spacial-temporal spindle activity organization (spontaneous and evoked by microstimulation); 2) spacial distribution of EPs thresholds, the dynamics of their recovery as a response to paired stimuli approximated by the linear regression; 3) the mathematical simulation of the interaction between identified, functionally united thalamic and cortical "blocks" at different sleep levels - revealed numerous mutually connected cortical and thalamic functional zones organized into thalamo-cortical units (e.g., A-a,B-b, C-c, D-d, E-e, F-f) which, in their turn, are united in thalamo-cortical blocks (e.g., 1 - [A-a, B-b, C-c], 2 - [D-d,E-e,F-f]). These blocks were



shown to generate the local spindle activity. Their interactions strengthen (1 and 2) during the slow wave sleep.The computer control of the blocks interaction (indicated by ECoG and hypnogram), produced by different regimes of thalamic microstimulation demonstrates new possibilities to control the slow wave sleep disorders. This approach could be applied mainly in studying sleep disorders, which are characterized by the prolongation of one slow wave sleep stage owing to another. So, one of the mechanisms of the slow wave sleep in circadian cycle appears to be the change of addressed interactions in the "non-specific" thalamo-cortical system of the brain.

ELECTROPHYSIOLOGICAL INVESTIGATION OF CABAc RECEPTORS IN THE TURTLE RETINA

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The aim of the present work was to elaborate appropriate methods for pharmacological isolation in the turtle retina of the recently described new type GABA receptors, so called GABAc receptors. The experiments were carried out on excised open eyecups of freshwater turtle *Emys orbicularis*. The electroretinogramm (ERG) was recorded before, during and affter application of several substances, thought to be specific GABAc receptors antagonists, and the amplitude and time characteristics of the ERG waves were measured.

Imidazole-4-acetic acid (I4AA), proven to be GABAc receptors antagonist in rat and fish retina, was applied alone and in combination with GABA. When applied alone, I4AA diminished the ERG *b*- and *d*- waves to 78,6 ±13,56 % and 79,9±10,32 % resp. Applied in combination with GABA, it failed to abolish its inhibitory action on the ERG. Therefore, a conclusion was made that I4AA does not act as GABAc antagonist in the turtle retina.

Picrotoxin (Pt), known to antagonize simultaneously GABAa and GABAc receptors, was applied alone and after preliminary full blocking of GABAa receptors with *Bicuculline (Bic)*. *Bic* increased the amplitudes of ERG waves. *Pt*, applied after *Bic* in saturating concentrations, led to an additional increase of the *b*- and *d*- wave amplitude by 139,7±4,6 % and 157±10,5 % resp. and delayed their time characteristics. A conclusion was made that these *Pt* effects were due to GABAc receptors blocking. Hence, *Picrotoxin*, applied after *Bicuculline,* acts as antagonist of GABAc receptors in the turtle retina.

The different time characteristics of the inhibitory processes, mediated by GABAa and GABAc receptors, were analysed also.

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MICROGLIA-ASTROCYTE INTERACTION IN ALZHEIMER DISEASE: SYNERGIC ACTIVATION BY -A MYLOID AND PROINFLAMMATORY MOLECULES.

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Activated microglia and astrocytes associate to mature amyloid plaques in Alzheimer Disease. We examined if cell activation induced by various amyloid β (A–β) peptides and pro-inflammatory molecules was modified when mixed cultures were used instead of pure microglia or astrocytes cultures. Using primary cells co-culture, we evaluated by morphological, immunocytochemical and biochemical methods several markers for cell activation and physiological functions, like phagocytosis, reduction capability, inducible nitric oxide synthase (iNOS) expression and apoptosis. We found that treatment with A–β peptides in both aggregated and monomeric forms activated glia although the magnitude of the activation differed depending on which marker was evaluated. For example aggregated peptides were better for inducing iNOS while soluble peptide produced more profound changes in the reduction activity of the cells. The same was true when comparing pure or mixed microglia and astrocyte cultures. Microglia exposed to A–β peptides was more activated when cultured alone than when cultured as a mixed population with astrocytes. The pro-inflammatory molecules showed a synergy with the A–β peptides that was specially conspicuous for astrocytes, which were poorly responsive to any of the compound when used alone. Our data suggest that the presence of a mixed cell population and the relative state of inflammatory co-activation of the glial cells plays a role in the reactive response of glial cells when exposed to A–β peptides.

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RIGHT HEMISHERIC CORTICAL ACTIVATION EVOKED BY BARORECEPTOR STIMULATION IN HUMANS

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The aim of this study was to determine the cortical representation of carotis sinus baroreceptors and especially its asymmetry in humans.On the basis of animal studies we hypothesized that baroreceptors project primarily to the insular cortex and that this projection is stronger in the right hemisphere. Some human psychophysiological studies also indicated that baroreceptor stimuli are preferentially processed in the right hemisphere, especially in the frontal lobe, and that baroreceptor stimulation has a more pronounced influence on right hemispheric functions.The assumption to be tested was that cortical baroreceptor representation is lateralized in favor of the right hemisphere.Experimental subjects were 8 right-handed males. A neck chamber and a pump were used to apply rhythmical suctions on the neck to stimulate carotis sinus baroreceptors. There were two control conditions: in one condition, there was no stimulation at all, and in the other, the suctions were applied on the upper part of the thorax. The effectivity of baroreceptor stimulation was assessed by power spectral density analysis of heart periods.For assessing activation of brain areas regional cerebral blood flow (3D positron emission tomography, [15O] butanol) was measured. The subjects were scanned 9 times, three 2 minutes' scans in each of the conditions. To improve localization NMR images were obtained for each subject.The results confirmed both of our assumptions. First, the cortical areas significantly activated by baroreceptor stimulation as compared to thorax stimulation and to rest were localized in the insular cortex and in the inferior frontal gyrus ($p < 0.01$). Second, in both locations this activation was much more marked in the right hemisphere than in the left.

This investigation provided the first direct evidence regarding the asymmetry of the cortical representation of autonomic sensory information. This finding might be relevant as regards the role of autonomic influences in the generation of emotions and the lateralization of emotions.

COGNITIVE DISORDERS IN APHASIA

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The study presents an analysis of cognitive functions in 62 adult aphasic patients. The following etiologies were represented in the sample: traumatic patients (35) and vascular patients (27). The main goal of this examination was to establish which cognitive functions are impaired in aphasics, as well as their influence on clinical picture and recovery in aphasia. In the examination the standardized neuropsychological tests were used: Trail making test, Rey-Osterrieth complex figure test, Auditory verbal learning test and Raven's progressive matrices. The results revealed memory disorders, visuoperceptual and attention deficits, and difficulties in problem solving abilities, while general intellectual ability were intact in most aphasics. It was concluded first, that the severity of cognitive disorders are in relation to severity and type of aphsia, and second that recovery of language abilities leads to improvement of cognitive functions, and vice versa.

NOVEL COGNITION ENHANCER GVS-111 FACILITATES THE FEAR CONDITIONING TO CONTEXTUAL BUT NOT CUED STIMULI IN RATS

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The influence of cognition enhancing dipeptide N-phenylacetyl-L-prolylglycine ethylester (GVS-111) (US Patent 5439930; Aug. 1995) on long-term memory was studied after massed and distributed training in cued and contextual fear conditioning in rats. In massed protocol three training trials were separated by 1 min intervals, in distributed - by 1 h. In each training trial animals received a footshock, paired with a 30 sec tone. GVS-111 (1 mg/kg i.p.) was injected 24 h after the first training. Animals were tested: to context, to cue with some contextual components and to cue only 72, 96 and 144 h after training accordingly. Control animals with massed training demonstrated lower freezing in all tests with contextual components than those with distributed one. GVS-111 increased freezing in the group with massed training but decreased it in case of distributed protocol in both tests. There were no significant differences between any of the groups in the cue test. These results demonstrate that (1) GVS-111 is effective being injected 24 h after training (2) it facilitates only the deficient learning caused by massive training (3) contextual, but not cued memory is influenced.

This research was supported by RFBR grant 98-04-48587.

SEX DIFFERENCES IN THE COGNITIVE EFFECTS AND INHIBITION OF BRAIN CHOLINESTERASE BY RIVASTIGMINE (EXELON) IN RATS.

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Two clinical reports suggest that women may be more sensitive than men to cholinesterase (ChE) inhibitors. A larger increase was produced by physostigmine in plasma ACTH, cortisol and ∃-endorphin in women than in men and in growth hormone releasing hormone by pyridostigmine. However, it is not known whether the drug effect was greater at the level of the enzyme or elsewhere in the cholinergic system. The present study compared inhibition by rivastigmine, a drug used in the treatment of Alzheimer's disease (AD), of ChE activity in brain, heart, skeletal muscle and plasma in male and female rats, and assessed its effects on the deficits in reference and working memory induced by scopolamine (0.5 mg/kg) in the Morris water maze. Rivastigmine (0.75 and 1.5 mg/kg) produced significantly greater ChE inhibition in the cortex, hippocampus and striatum, but not in the periphery, in females than in males. A similar sex difference was obtained with physostigmine (0.05mg/kg) on brain ChE. Rivastigmine (0.75 mg/kg) was also more effective in females than in males in antagonizing scopolamine-induced deficits in reference and working memory. However, there were no sex differences in ChE inhibition by either drug of enzymes prepared from the 3 brain regions *in vitro*. Ovariectomy had no effect on ChE inhibition by rivastigmine but orchidectomy abolished the sex differences. This suggests that testosterone reduces the amount of drug entering the brain or its interaction with the enzyme. It remains to be seen if women with AD are also more sensitive to the cognitive effects of this drug.

EFFECT OF THE ALBINO NEUROLOGICAL MUTANT AT THE TYROSINASE LOCUS ON VISUAL SYSTEM DEVELOPMENT IN AN ANIMAL MODEL OF ALBINISM: WESTENBERG-LONG-EVANS (WLE) SEGREGATING INBRED STRAIN ALBINIC RAT PUPS WITH PIGMENTED LITTERMATES.

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The albino neurological mutant, ***Tyr^{c}***, is a recessive allele at the tyrosinase locus, ***Tyr***. Albino genes affect the enzyme tyrosinase, which is necessary for the first metabolic step in the production of the pigment chemical melanin. A pair of albino genes at ***Tyr*** causes albinism, the lack pigmentation in the eyes, skin, hair and inner ear of mammalian species often used in research. In these species albinism is linked with visual system anomalies similar to those of albinic humans. These anomalies may be related to albinic-pigmented differences in developmental timing, e.g., of cells in the eye. A very early study suggested that a correlate of albinism in rats is premature eye-opening; however, the albinic and pigmented rats compared were not of the same inbred strain. Thus, the genetic difference at ***Tyr*** was confounded with genetic differences at other loci. I avoided this problem by using a design similar to that of a study on a mouse model of albinism with proper genetic controls; it compared albinic vs. pigmented mice of the same inbred strain. In that study littermates differed at ***Tyr*** but were identical at all other loci; the single-locus ***Tyr*** difference was not confounded with genetic differences at other loci. Eye-opening in the albinic mice was not premature; in some cases it was delayed. To extend the mouse findings I used a rat model of albinism, comparing albinic vs. pigmented Westenberg-Long-Evans (WLE) segregating inbred strain rats. Within each WLE litter albinic and pigmented pups were matched in developmental age, prenatal and postnatal environment, etc., and were roughly comparable in number. Littermates were genetically identical at virtually every locus except ***Tyr***, where albinic pups were homozygous with a pair of mutant ***Tyr^{c}*** genes (***Tyr^{c}*** / ***Tyr^{c}***) while pigmented pups were heterozygous, with one mutant ***Tyr^{c}*** gene and one normal, wild type ***+Tyr*** gene (***Tyr^{c}*** /***+Tyr***). In some WLE litters eye-opening of all of the albinic pups lagged that of all of their pigmented littermates. This did not occur as consistently as it did in an earlier study involving litters of hybrid rats with F344 mothers and WLE fathers (F3WLF1). This suggests that the effects of the albino mutation may vary depending on the genetic background of the strain. The albinic-pigmented difference might have been more striking had the pigmented pups been homozygous with a pair of wild type genes at ***Tyr*** (***+Tyr/+Tyr***). Future studies on the effects of mutant albino genes must incorporate appropriate genetic controls.

[***Tyr*** is the former albino locus, ***c*** in mouse and ***C*** in rat; ***Tyr^{c}*** is the former ***c***.]

EFFECT OF HIGH DOSES OF CORTICOSTERONE ON PASSIVE AVOIDANCE BEHAVIOR IN RATS.

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We investigated the effect of a subchronic (3 days) or chronic (22 days) treatment with corticosterone on a passive avoidance test in rats. Sprague Dawley adult rats were subcutaneously implanted with two pellets of 100 mg of either corticosterone or cholesterol (as a control). Serum corticosterone levels (μg/dl) rised up to 83.8 ± 18.1 in CORT group and were of 5.3± 1.9 in CONTROL group on day 3 ($p<0.01$) and persisted elevated (CORT 10.8 ± 1.6; CONTROL 4.8 ± 0.9) until day 22. Passive avoidance behavior was studied in a one-trial learning step-through test (footshock:0.5 mA, 2s). Subchronic treatment with corticosterone increased training latency but we found no differences between test and training session latencies.On the contrary chronically treated animals showed no differences on training session latencies but the CORT group showed a significantly lower test latency than CONTROL group ($p<0.05$). We conclude that a chronic exposure to high corticosterone serum levels produces memory impairment in a passive avoidance behavior. High levels of corticosterone in a subchronic schedule induces a different profile, as behavior on the acquisition session was affected.

This work was supported by grants from CONICET (Argentina) and Roche Lab. (Argentina).

ROLE OF ENDOGENOUS NITRIC OXIDE IN FUNCTION OF NEURO-MUSCULAR SYNAPSE.

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The influence of endogeneous nitric oxide (NO) on function of neuro-muscular synapce was investigated using method of extracellular recording of end plate potentials (EPP) and miniature end plate potentials (MEPP). Experiments were performed on frog Cutaneous pectoris muscle. L-arginine which is substratum for NO syntesis in concentration 0.1Mm/l decreased the amplitude of EPPs to 65,3% The NO-sintase ingibitor nitro-L-arginine in the concentration 0.01Mm/l, to 205%.Addition of hemoglobin to the superfusion solution did not change evoked transmitter release.It was concluded that endogeneous NO decreased the transmitter release from nerve endings.It was suggested that endogenous NO is produced in the nerve ending.

MÜLLER CELLS EXPRESS $GABA_B$ RECEPTORS IN THE BULLFROG RETINA

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γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the vertebrate retina. Although GABA receptors in the retina have been extensively characterized, the localization of $GABA_b$ receptors in the retina is little known. In the present study, we used a specific antiserum to localize $GABA_b$ receptors in the Bullfrog retina immunocytochemically. At the light microscopic level, immunolabelling was detected in both the external and inner limiting membranes, as well as in the inner and outer plexiform layers (IPL and OPL). Müller cells in the inner nuclear layer, identified by the co-localization of immunoreactivity to glial fibrillary acidic protein (GFAP), express $GABA_b$ receptors-like immunoreactivity (IR), and radially oriented primary processes of these cells in the IPL and vitreal end feet are also labeled. We further observed radial processes and soma of Müller cells with $GABA_b$ receptors-like IR at the ultrastructural level. All these results suggest that $GABA_b$ receptors are expressed in Müller cells of the Bullfrog retina and these receptors may be involved in modulation of synaptic transmission in the retina. This study provides the first evidence that glial elements in the vertebrate retina express $GABA_b$ receptor. (Supported by a grant from National Natural Science Foundation of China, 39800041).

INVOLVEMENT IN RESPIRATORY RHYTHMOGENESIS AND NEUROCHEMICAL PROPERTIES OF PREBOTZINGER COMPLEX IN ADULT RATS

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Pre-Botzinger complex (PBC) is considered to be essential for respiratory rhythmogenesis in neonatal rat preparations in vitro. The present study examined the functional importance in rhythmic respiration and the neurochemical properties of the neurons of the PBC in adult rat in vivo. A microinjection of excitatory amino acid, L-glutamate (L-Glu), into the PBC shortened the duration of expiration. An injection of excitatory neurotoxin, kainic acid, initially shortened the duration of expiration, lengthened the duration of inspiration and increased the respiratory frequency, and then terminated the respiration. An injection of inhibitory amino acid, glycine (Gly) or g-amino butyric acid (GABA), shortened the duration of inspiration. The discharge patterns and responses to iontophoretic application of L-Glu, Gly and GABA and their antagonists of spontaneous discharge neurons in PBC were analyzed with the multibarrel microelectrode techniques. All types of respiratory neurons, including pre-inspiratory ones, were recorded in the PBC region. The neurons could be excited by L-Glu and inhibited by Gly and GABA. These effects could be reversed by AP-5, strychnine and bicuculline, respectively. The neurons with positive immunohistochemical reaction of Leu-enkephalin, neuropeptide Y, substance P and serotonin were observed in the PBC region. These results indicate that the PBC plays an important role in promoting the phase transition from expiration to inspiration, that Glu, Gly and GABA might be involved in the synaptic transmission of signals in the PBC, and that Leu-enkephalin, neuropeptide Y, substance P and serotonin could act as transmitters or modulators of the PBC neurons, in adult rats.

DEVELOPMENT OF GLYCINE- AND GABA-MEDIATED INHIBITORY SYNAPTIC CURRENTS IN THE RAT SPINAL CORD.

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Glycine and GABA are the major inhibitory neurotransmitters in the mammalian spinal cord, but little is known about their relative roles in mediating inhibitory synaptic transmission in newly formed neural networks in the developing rat spinal cord. In this study, properties of miniature inhibitory postsynaptic currents (mIPSCs) were examined in spinal motoneurons of embryonic (E17-18) and postnatal (P1-3) rats. Three mIPSC components were distinguished based on their decay time constant(s): (1) fast-decaying, bicuculline-resistant, glycine receptor (glycineR)-mediated mIPSCs, (2) slow-decaying, strychnine-resistant, $GABA_A$ receptor ($GABA_AR$)-mediated mIPSCs, and (3) dual-component, glycineR- and $GABA_AR$-mediated mIPSCs with bi-exponential decay time. At E17-18, more than 50% of mIPSCs consisted of $GABA_AR$-mediated currents, while similar, small proportions of dual-component and glycineR-mediated currents comprised the rest of mIPSC population. In contrast, glycineR-mediated mIPSCs constituted more than 50% of the currents after birth, and the fraction of dual-component mIPSCs increased. Our data suggest that early in spinal network formation, GABAergic synapses dominate spontaneous inhibitory synaptic transmission, while glycine contribution is predominant after birth, resulting primarily from an increase in either the number of glycinergic synapses and/or the probability of glycine release. The postnatal increase in mIPSC unitary current is attributed to an increase in glycine receptor density and/or increase in glycine quantal content. The larger fraction of dual-component mIPSCs implies that both the quantal corelease of glycine and GABA and the colocalization of glycine and $GABA_A$ receptors at postsynaptic sites increase after birth. This work was supported by NIH grant NS23808.

THE RELATIONSHIP BETWEEN REPETITION PRIMING EFFECTS AND SKILL LEARNING IN AN ENUMERATION TASK

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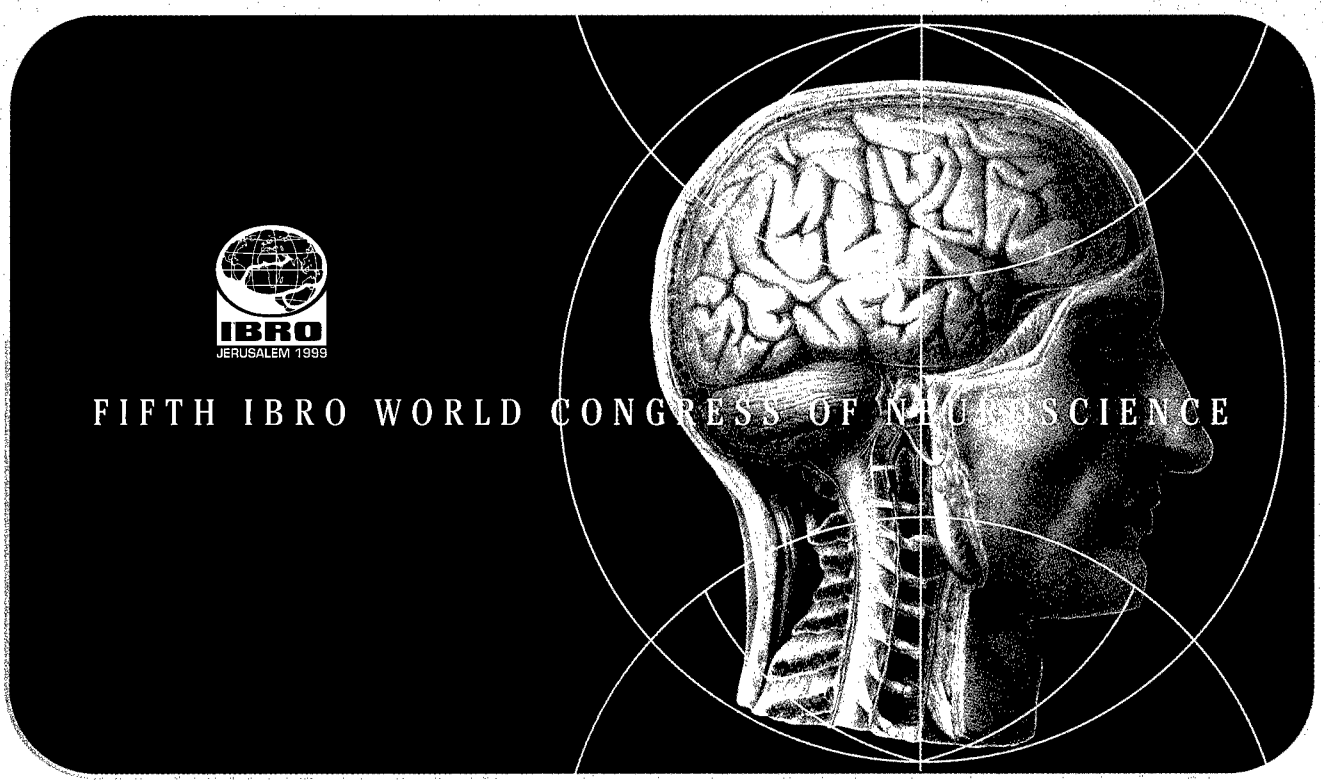
Repetition priming and skill learning are suggested to represent different forms of implicit memory. Little is known, however, of how priming effects are related to later stages of item repetition and skill learning. Recently it was suggested that skilled performance is characterized by a distinct time-course: initial within-session gains are followed by delayed, between-session gains in performance (consolidation) provided sufficient practice was given (Karni & Sagi, Nature 1993).

To investigate the relationship between priming and the consolidation phase of skill learning we designed a paradigm of spaced item repetition where Ss had to decide if letter strings (non-words of 3-6 letters) were odd or even. 16 items were presented in pairs of blocks: in the first block in a sequenced order (primer) and in the following block in a random order (test). Average RTs on each block as well as the gain in RT (delta-RT) between primer and test were computed. In the first session 14 Ss trained on either 3 or 10 pairs of blocks and were retested on the next day.

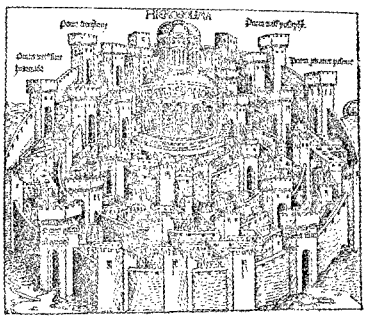
Priming (delta-RT) showed a distinctive time course with significant effects only within the first 4 pairs of blocks. After the 6th pair, delta-RT became zero. Mean RTs on test blocks showed no further improvement after the 4th pair. By the next day, while delta-RTs remained zero, significant reductions in RTs were found indicating an inter-session improvement. Similarly, between-session but no within-session gains were found when subjects were re-tested in a third session. On the other hand, Ss who performed only 3 paired block repetitions showed no inter-session gains by the next day but showed within-session gains which asymptoted after 3 pairs of blocks. Again, continued training in the second session resulted in inter-session improvement by the third session.

Both groups showed only small transfer effects to increasing numbers of letters and priming effects reappeared. However, the training dependent gains showed good transfer to changes in letter size, font, case and color suggesting that the learning relates to an increasing ability to estimate string length rather than to the discrimination of specific stimulus features.

We propose that the zeroing of delta-RT (i.e., the disappearance of priming) reflects a system switch in task and stimulus processing which is a necessary step to induce slow learning processes.



ADDITIONAL ABSTRACTS



DUAL FORMS OF EXCITOTOXIC CELL DEATH TRIGGERED BY AMPA AND KAINATE RECEPTORS IN OLIGODENDROGLIAL CULTURES

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Recent studies indicate that oligodendrocytes are vulnerable to excitotoxic insults mediated by glutamate receptors. The present study was performed to characterize the type of glutamate receptors triggering cell death in cultures of oligodendrocytes derived from the perinatal optic nerve. Acute (15 min) activation of either AMPA or kainate receptors was toxic to oligodendrocytes as measured 24 h after drug application. In contrast, exposure to agonists of the NMDA and metabotropic glutamate receptors had no effect on cell viability. Interestingly, dose-response curves showed that cell death triggered by activation of kainate receptors had two components. The high affinity component displayed a bell-shaped curve which peaks at around 3 μM kainate while the low affinity component had an EC50 for kainate of 488 μM. Toxicity was reduced in cultures exposed to AMPA and kainate receptor agonists in Ca2+ -free medium or when Na+ was substituted for choline+. In addition, cells underwent apoptosis or necrosis depending on whether agonists were applied at low or high concentrations respectively. Thus, cell death triggered by 1 μM kainate, in the presence of the AMPA receptor antagonist GYKI53655, or by 10 μM AMPA, was nearly abolished in cultures treated with ZVAD-FMK, an inhibitor of the CED3/ICE-like family proteases, indicating that it was apoptotic. In contrast, toxicity levels were not altered after incubation with higher agonist concentrations in the aforementioned experimental conditions. These results indicate that activation of AMPA and/or kainate receptors can be toxic to oligodendrocytes and that the contribution of apoptosis and necrosis to cell death depends on the intensity of the excitotoxic signal.

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KA1 RECEPTOR SUBUNIT IMMUNOREACTIVITY IN NEURONS AND GLIA USING A NOVEL ANTI-PEPTIDE ANTIBODY.

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Functional kainate receptors can be formed by various combinations of subunits with low (GluR5, GluR6 and GluR7) or high affinity for kainate (KA1 and KA2). The precise contribution of each subunit to native receptors as well as their distribution within the central nervous system (CNS) is still unclear. Here, we describe the presence of KA1 immunostaining in both neurons and glial cells of the CNS, using a newly developed antiserum to an extended carboxy terminus epitope of the KA1 subunit. In Western Blot, this antiserum specifically recognized a unique molecular species of ~105 kDa in rat, bovine, mouse and human brain protein homogenates. Immunohistochemistry of post-mortem human neocortex revealed intense staining in the soma and apical dendrites of a subset of pyramidal neurons. In the rat CNS, immunoreactivity was observed in all fields of the hippocampus and was particularly abundant in the CA3 area. Within this subfield, immunostaining was found to be present in dendritic spines postsynaptic to commisural-associational fibers, but only very rarely in those contacted by mossy fiber terminals. In the cerebral cortex, stained neurons were both pyramidal and non-pyramidal in morphology and a subpopulation of gamma-amino-butyric acidergic cells were KA1 immunoreactive. In the cerebellum, Purkinje cell somata and their dendrites as well as Bergmann glial processes were most notably labeled. In addition to neurons, macroglia were also stained by the KA1 antiserum. Thus, optic nerve oligodendrocytes both in vitro and in situ and cultured astrocytes were intensely labeled. Taken together, these results indicate that the KA1 subunit is widely distributed throughout the CNS. This newly developed antiserum, which also recognizes the human KA1 antigen in situ, may help to clarify the properties of KA1-containing kainate receptors within the normal and pathological brain.

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DYNAMICS OF VISUAL SHORT-TERM MEMORY

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Low-contrast visual stimuli were shown to produce a memory trace, enhancing following target detection for as long as 16 seconds (Tanaka and Sagi, 1998; PNAS 95, 12729-33). Here we show that the memory trace depends on dynamic interactions between low-level stimulus properties and a higher-level gating process. Contrast detection thresholds were measured (2AFC) for a foveal Gabor Signal (GS; sigma=lambda=0.15 deg.) using a temporal-cueing method. The target was preceded either by (1) a peripheral temporal cue consisting of two peripheral crosses (control condition), or (2) a temporal cue and a foveal-GS prime (prime condition). Different prime orientations (0, 15, 45, 60, and 90 deg.) and different prime contrast were tested. Time between cue and target (SOA) was set between 0 to 1800~ms, with cues and targets presented for 90~ms. Experimental blocks of trials were either of fixed SOA or of mixed (temporal uncertainty) SOAs. Results indicate that temporal cueing without GS prime (control) reduced GS detection~thresholds by 40% (SE=5%, n=5 observers) at SOA of 360-540~ms relative to thresholds at SOA=0. Larger SOAs yielded baseline (SOA=0) performance. The presence of a low-contrast GS prime induced long-lasting (1800ms) enhancement. Detection thresholds were reduced by 39% (SE=6%) relative to baseline with prime orientations identical (0 deg.) to the target. Tilted primes (15-60deg.) yielded long-lasting suppression, with threshold increases of 28% (SE=7%) relative to baseline. Orthogonal primes (90 deg.) yielded no effect. Both enhancement and suppression were maximal with prime contrast near-threshold, and disappeared with high-contrast primes. Temporal uncertainty abolished both the temporal-cueing effect and the long-lasting GS facilitation and suppression. These results suggest a two-step process in which attention affects transition between perception and memory: a non-selective process that gates competition between overlapping representations in low-level vision.

SPATIAL POTASSIUM BUFFERING IN THE HIPPOCAMPUS OF ANIMALS AND HUMANS SUFFERING FROM MESIAL TEMPORAL LOBE EPILEPSY

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Pharmacologically resistant mesial temporal lobe epilepsy (mTLE) is associated with hippocampal sclerosis characterized by loss of neurones and gliosis. The objective of the present study is to investigate whether astrocytes in such tissue are less able to take up and redistribute potassium ions released during high neuronal activity. Therefore, we measured changes of stimulus induced rises of $[K^+]_o$ before and after blocking glial inwardly rectifying and leak potassium currents with 2 mM $BaCl_2$ added to ACSF in hippocampal slices from amygdala-kindled rats, chronic epileptic rats (pilocarpine model) and from surgically removed hippocampal specimens of patients with mTLE.
In area CA1 of slices from control rats barium significantly augmented rises of $[K^+]_o$ induced by repetitive antidromic stimulation, also in presence of 2-APV and NBQX. Similar results were observed in the CA1 from kindled rats as well as in less sclerotic CA1 and DG regions from human tissue (increases by 160% as well as by 100% and 120%, respectively). In contrast, the barium-effect was missing in highly sclerotic CA1 regions from chronic epileptic rats and human hippocampal specimens. This indicates an impairment of glial barium-sensitive potassium buffering in sclerotic hippocampal regions from animals and humans with mTLE but not in gliotic regions from kindled rats and less altered human tissue. It is concluded that reduction of the glial potassium buffering capacity is restricted to sclerotic regions and may be induced in astrocytes sensing death or absence of neurones.
(Supported by the SFB 507)

WHO SEES WHAT: DIFFERENTIAL RESPONSE OF PRIMATE V1 AND V2 TO REAL AND ILLUSORY CONTOURS.

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In the primate visual cortex, approximately a third of neurons in the V2 have been reported to respond preferentially to higher order contours, such as illusory contours, a response not found in V1 neurons (Peterhans and von der Heydt, 1989). To investigate the relative roles of V1 and V2 in lower vs higher order feature perception, optically imaged real contour orientation maps of areas V1 and V2 of anesthetized monkeys were compared with illusory contour orientation maps. Illusory contour imaging revealed clustered responses in V2 similar in size to real orientation domains in V2. Strongest V2 responses to illusory (vertical) stimuli often co-localized with responses to matching real (vertical) stimuli. In contrast, V1 responses to illusory contour stimuli were least in columns matching the illusory contour orientation, suggesting a possible suppressive interaction. Electrophysiological recordings from targeted illusory domains revealed that some V2 neurons were tuned to matching real and illusory orientations, while almost all V1 neurons were tuned to the orientation of the real line stimuli. These data thus suggest that V2 'sees' higher order orientation percept, while V1 'sees' the orientations of the lower order elements. Moreover, in conjunction with illusory (vertical) activation in V2 is suppression of V1 real (vertical) regions, suggesting that activation of perceptually salient features is paralleled by inhibition of potentially conflicting information. Support: NIH-EY11744, Whitehall, Sloan, Brown-Coxe Foundations.

THE VISUAL CORTICAL HIERARCHY AND PERCEPTUAL LEARNING

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We have been studying the roles of attention in learning and in skilled performance and recently proposed a theoretical framework for two basic learning phenomena, based on physiological characteristics of the visual cortical hierarchy. These phenomena are learning transfer (the useful transfer of learning under one set of conditions to performance under conditions) and the easy-to-hard case learning cascade (the need for subjects to train on easy conditions before hard ones). According to this framework, early training affects high cortical levels and the appropriate low-level mechanisms can only be recruited later by guidance from high level regions. The key role attributed to the attentional search mechanism is the allocation of the appropriate neural population for learning under the specific training conditions. We have now tested a number of predictions of this model regarding procedures to facilitate and guide visual search by cueing with visual stimuli. Related to the reverse hierarchy theory, we also found that the attention needed for learning can not be divided even between two simple visual tasks. Subjects generally learn first one and then the other task. Furthermore, following training with both tasks making one of them more difficult affects performance of both tasks. These findings indicate that attention and the allocation of limited resources remain relevant even following considerable training, and perceptual tasks do not become automatic in this sense.

THE CELLULAR BASES OF LOCOMOTION IN A LOWER VERTEBRATE - MECHANISMS OF MODULATION

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Goal-directed locomotion can be initiated via visual and olfactory stimuli in the lamprey. Afferents from the olfactory bulb and the optic nerve activate neurons in the ventral thalamus which in turn monosynaptically activate reticulospinal neurons which turn on the spinal pattern generating circuitry resulting in coordinated swimming within a broad frequency range. The pattern generating circuitry consists of glutamatergic (NMDA, AMPA/kainate, mGluR) excitatory ipsilateral interneurons and glycinergic crossed inhibitory interneurons. Several ionic mechanisms, including calcium dependent potassium channels of different types, play an important role in determining the output pattern. A number of different modulators target different presynaptic or somadendritic ion channels which alter the activity of single types of neurons and thereby the network motor pattern. Monoamine and peptidergic modulators produce specific changes of the motor pattern during a constant excitatory drive on the locomotor network. Tachykinins induce long-lasting changes (>24 h) with a need for protein synthesis (Parker et al 1998). Stretch receptors that sense the undulatory movements act on the spinal circuit to adapt the motor pattern to external events.

NEURAL PROCESSING OF BINOCULAR DISPARITY AND SURFACE DEPTHS IN MONKEY INFERIOR TEMPORAL CORTEX.

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When binocular disparity is given to a part of 2-dimensional (2-D) shape, we perceive a 3-D structure consisting of multiple surfaces at different depths and orientations. While such 2-D retinal images may be interpreted in many ways, the visual system reliably calculates the most probable 3-D structure from sparse local disparity cues. A neural representation of the perceived 3-D structure is, therefore, created somewhere in the brain, after the detection of binocular disparity.

Neurons in the ventral visual pathway leading to the inferior temporal cortex (IT) have been known to respond to surface characteristics of objects such as color, 2-D shape, and texture. On the other hand, neurons responding to binocular disparity has been found along the magnocellular-dominated stream in earlier cortices and then along the dorsal pathway leading to the parietal cortex. In order to perceive 3-D surfaces, however, the brain must know the depth as well as other attributes of a surface such as its shape. We have recently found that the IT in the macaque monkey contains many neurons (more than 50% of the neurons tested) sensitive to both disparity and shape. We have further demonstrated that the activity of some IT neurons encode information on the perceived relative depths of surfaces rather than the local disparity cues of the stimulus. We suggest that the IT integrates shape and disparity information, and the ventral visual pathway leading to the IT is involved in reconstructing 3-D structures from local disparity cues.

INVOLVEMENT OF DIFFERENT OPIOID RECEPTORS AND CREB IN OPIOID-INDUCED DEPENDENCE AND REWARDING EFFECTS

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The neurobiological mechanisms involved in the development and expression of opiate dependence have been recently investigated by using mice with a genetic disruption of genes related to the opioid responses. The pharmacological effects induced by morphine were first evaluated in mice lacking the two major transcriptionally active isoforms of cAMP-responsive element-binding protein (CREBaD) gene. No change in morphine-induced antinociception was observed in these mice after acute administration. After repeated morphine treatment, CREBaD mutant mice develop tolerance to morphine antinociception, but to a lesser extent than wild-type mice. These animals did not show any modification on the rewarding properties induced by morphine in the place preference paradigm. The expression of the somatic symptoms of naloxone-precipitated morphine withdrawal was strongly attenuated in CREBaD deficient mice. However, the immediate early genes c-fos and c-jun were still induced in the locus coeruleus and the amygdala following precipitation of morphine withdrawal in these mutant mice. Some behavioral responses produced by the repeated exposure to cocaine were also investigated in these animals. The sensitization to cocaine locomotor responses induced by its repeated administration was evaluated by using a non-context specific procedure and cocaine rewarding effects were measured by using the place preference paradigm. Both cocaine behavioral responses remained unaffected in CREBaD deficient mice. Morphine responses were then evaluated in mutant mice lacking opioid receptor genes. Mu deficient mice did not show any antinociceptive or rewarding response after morphine administration. Besides, chronic morphine did not develop any behavioral or biochemical manifestation of dependence in these mice. However, antinociceptive and rewarding effects of morphine were not modified in kappa deficient mice, whereas the development of morphine-dependence was only slightly attenuated in these animals. These results reveal a crucial role for the mu opioid receptors in the different components of opiate dependence whereas the transcription factor CREB seems to be selectively implicated in the somatic expression of opiate abstinence.

CODING OF INTENTIONS AND SPACE IN THE POSTERIOR PARIETAL CORTEX

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The posterior parietal cortex is situated between sensory cortical areas, which encode spatial information, and motor cortical areas, which code movements. Recent studies have shown that neural activity in this area is not restricted to purely sensory or motor functions, but rather reflect intermediate stages in the sensory-motor transformation process. I will describe experiments from our laboratory which demonstrate activity related to the early planning of actions. Moreover, there appears to be an anatomical organization with respect to movement selection, with the lateral intraparietal area (LIP) specialized for saccadic eye movements, and the parietal reach region (PRR) specialized for limb movements.

We have recently examined the spatial reference frames in which these nascent plans are coded. Area LIP codes saccade targets in eye coordinates for visual stimuli, and a majority of LIP neurons also code auditory targets in eye coordinates. Such an encoding is consistent with the specification of the targets in motor error coordinates, i.e. the direction and amplitude of an eye movement that would foveate the target. We have recently examined the spatial reference frame in PRR. Surprisingly, these cells do not generally code planned reaches in limb coordinates, i.e. they do not specify the direction and amplitude the limb would need to move to acquire potential reach targets. Rather, PRR neurons code visual targets in eye coordinates. Coding of arm movements in an eye-centered reference frame may be advantageous because obstacles that affect planning as well as errors in reaching are registered in this reference frame. Also, eye movements are planned in eye coordinates, and the use of similar coordinates for reaching may facilitate hand-eye coordination.

The above observations have led to the idea that early movement plans, independent of the type of movement or sensory modality of the target, might be coded in an eye reference frame. To test this hypothesis further, we examined the reference frame of reach plans to auditory targets. A priori, there is no reason to believe that reach plans to auditory targets should be in an eye-centered reference frame; the head-centered representation of a sound source can, in principle, be converted directly into a limb-centered reference frame that is useful for arm movements, without going through an eye-centered representation. However, we found that a significant number of PRR neurons encode intended reaches to auditory stimuli in an eye-centered reference frame.

Finally, we have found that cells in LIP and PRR are gain modulated by eye position signals, and the PRR cells are also modulated by limb position. These influences may reflect an early stage in the transformation of these signals into the non-retinal coordinate frames which are found in later stages of motor programming.

CAN FUNCTIONAL BRAIN IMAGING BE USED TO "SEE" PAIN?

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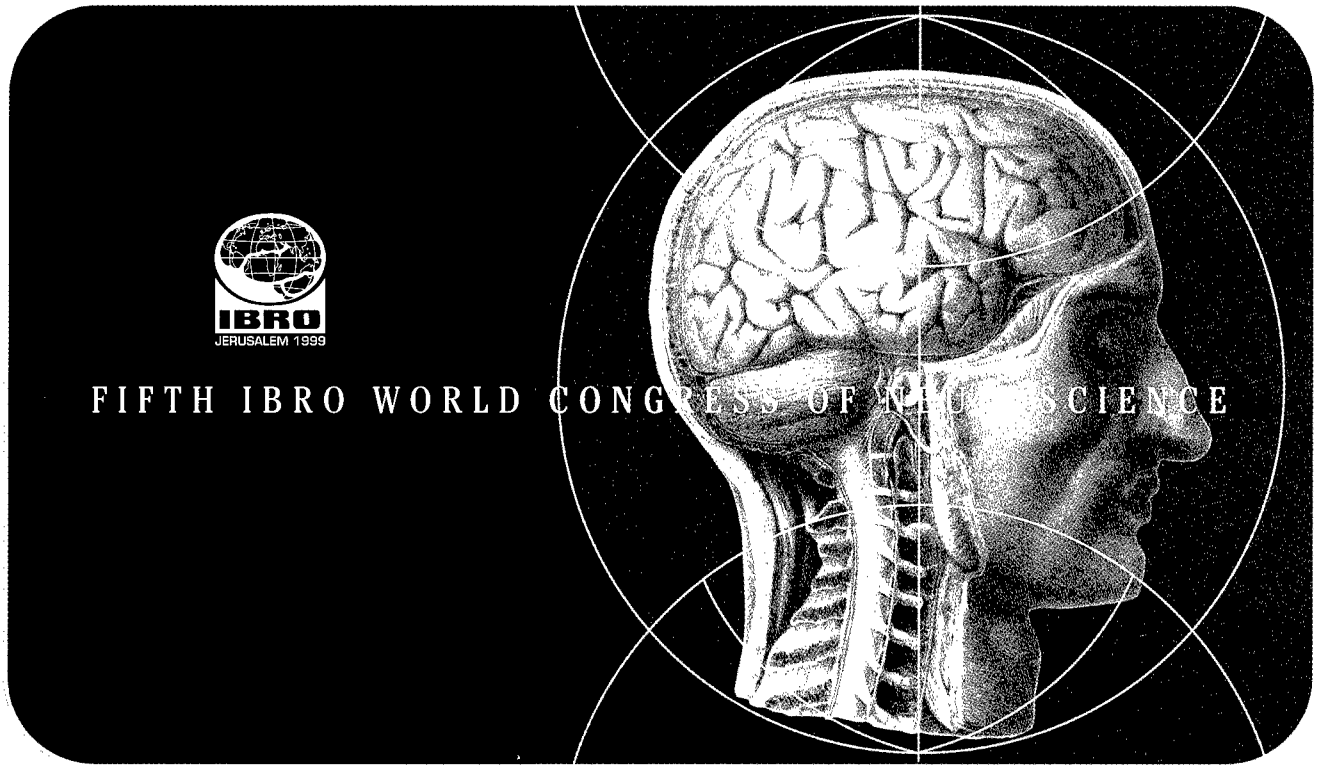
Technological advances in functional brain imaging (e.g., PET, fMRI) over the last decade have allowed for the identification of brain regions that show changes in blood flow or oxygenation, so called "activations", associated with the performance of particular tasks or application of stimuli to awake humans. These techniques have been used to study a variety of cognitive, motor and sensory processes, including pain. Many studies have now identified distinct cortical regions of increased blood flow and oxygenation during application of painful stimuli in awake humans. The most consistently identified regions of pain-related activation include the anterior insula, anterior cingulate cortex and somatosensory cortex. However, the question remains whether these results pertain to pain perception or some other process which is co-activated by nociceptive stimuli. Data will be presented from a variety of laboratories to address this issue, including those studies which manipulated stimulus intensity or affect and from studies that included motor or attention task controls. In particular, the correlation between the stimulus-evoked pain perception and regions of activation will be discussed. Finally, data from imaging studies of chronic, ongoing pain will be compared to those from acute pain studies.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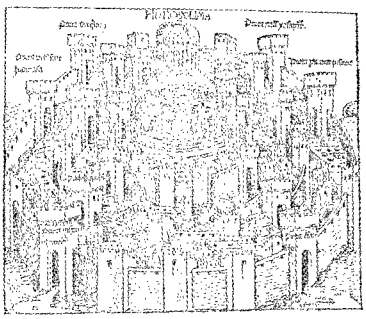

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