PLENARY LECTURES

Synaptic transmission, cellular specificity and cortical reorganization in the lesioned visual system

Eysel, Ulf
Department of Neurophysiology, Ruhr-University, Bochum, Germany
eysel@rub.de

Lesions in the visual system lead to significant reorganization of single cell properties and of topographic maps in the visual cortex. This is observed in the adult brain after cortical lesions as well as after retinal lesions. Reprogramming of functional connectivity of the visual cortex follows a characteristic sequence of mechanisms including upregulation of excitatory and downregulation of inhibitory synaptic transmission, local hyperactivity and facilitation of long term potentiation (LTP) in the region that undergoes functional reorganization. Visual cortical neurons are newly connected to previously subthreshold inputs by increasing synaptic weights. Reorganized cells take over additional or even new functions. The cortical retinotopic map is reprogrammed. We investigate the lesion-induced dynamics in the cat visual cortex *in vivo* and we determine the underlying mechanisms in the rat visual cortex *in vitro*. Changes of the functional representation of visual space *in vivo* (enlarged receptive fields, shifts in retinotopy) indicate changes of synaptic connectivity. The parallel *in vitro* studies of synaptic plasticity and immunohistochemical and molecular approaches revealed underlying mechanisms that are reminiscent of patterns of plasticity that are present in early postnatal live. Following lesions in the visual system, like in early postnatal life, the potential for synaptic remodeling is increased by modifications of the glutamate receptors (AMPA and NMDA), a shift of balance between excitation and inhibition towards increased excitability, and a resulting increase of synaptic plasticity (LTP).
Adult visual cortical plasticity appears optimized for localized cortical reprogramming to compensate for functional loss and to allow for use-dependent reorganisation, that can be useful for post-lesion rehabilitation of function in the visual system.

At the intersection of neuropharmacology and cell biology: Constitutive activation drives compartment selective endocytosis and axonal targeting of several axonal GPCRs such as CB1 cannabinoid and 5HT1B serotonin receptors

Lenkei Zsolt
Laboratory of Neurobiology of Jean Rossie, ESPCI, Paris, France
zsolt.lenkei@espci.fr

Axonal GPCRs (G-protein coupled receptors) critically regulate neurotransmission and are important targets of therapeutic and abused drugs. Several axonal GPCRs, such as CB1 cannabinoid and 5-HT1B receptors display significant constitutive activity (i.e. spontaneous activation in the absence of ligand) *in vitro*. In transfected cell lines this constitutive activity results in a permanent cycle of endocytosis and recycling. In neurons, however, this cycle is restricted to the somatodendritic compartment. Thus, GPCRs are continuously removed by endocytosis from the somatodendritic plasma membrane while in axons these receptors accumulate on surface. Blocking constitutive activity or somatodendritic endocytosis results in sequestration of recycled GPCRs on the somatodendritic plasma membrane. Long-term inhibition of endocytosis results in impaired axonal polarization of surface-bound GPCRs. Indeed, kinetic analysis shows that the majority of newly synthesized GPCRs arrive first to the somatodendritic plasma membrane, from where they are rapidly removed by constitutive

endocytosis before being delivered to axons. In axons, a significant but surmountable natural blockade of endocytosis leads to sequestration of GPCRs on the plasma membrane, predominantly in an extrasynaptic localization. Thus, constitutive activity driven somatodendritic endocytosis is required for proper axonal targeting, representing a novel, conformation-dependent targeting mechanism for axonal GPCRs. This model of axonal GPCR function could provide a novel pharmacodynamical framework for understanding the development of tolerance as well as the chronic effects of inverse agonist drugs.

Left-right asymmetry of hippocampal synapses

Ryuichi Shigemoto
National Institute for Physiological Sciences, Okazaki, Japan
shigemot@nips.ac.jp

Left-right asymmetry in higher-order neural function is a fundamental concept of neuroscience. However, the molecular basis of brain asymmetry remains unclear. We discovered that synaptic distribution of N-methyl-D-aspartate (NMDA) receptor NR2B subunits in the adult mouse hippocampus is asymmetrical between the left and right and between the apical and basal dendrites of single neurons. These asymmetrical allocations of NR2B subunits differentiate the properties of NMDA receptors and synaptic plasticity between the left and right hippocampus. Next, we analyzed the hippocampal circuitry of the inversus viscerum (iv) mouse, which has a randomized laterality of internal organs. The iv mouse exhibits bilateral right-sidedness in the distribution of NR2B, irrespective of the laterality of visceral organs. This right isomerism of the hippocampus is the first evidence that a distinct mechanism downstream of the iv mutation generates brain asymmetry. Finally, to investigate functional difference between left and right hippocampus, we analyzed spatial learning ability of wild-type mice with transected ventral hippocampal commissure and corpus callosum. To test spatial learning ability of left or right hippocampus, left or right eye of these mice was enucleated, respectively, so that visual cues are mainly processed within one side of the hemisphere (90% of visual stimuli to the eye are transferred into the opposite side of the cortex in mouse). During 15 days training in Barnes' maze, those two groups of mice did not show significant difference in acquiring spatial learning. However, in the probe test phase after training, right eye deprived mice stayed at correct platform for significantly longer time than left eye deprived group. This result suggests that right hippocampus performs better in spatial memory retention than left hippocampus, and demonstrates the existence of functional left-right asymmetry of the mouse hippocampus.

Time and space in cooperative neuronal circuits: the chronocircuitry of the hippocampus

Somogyi Peter
MRC Anatomical Neuropharmacology Unit, University of Oxford, Oxford, UK
peter.somogyi@pharm.ox.ac.uk

Nervous systems evolved to enable organisms to move and interact with the external and internal environments. Interactions take place on various time scales reflected in the temporal pattern of neuronal activity. Adaptation to the required temporal parameters dictated by the environment drives evolution of the nervous system and is reflected in the properties of molecules, synapses and cells that we observe and measure. How could we define cell types in a biologically meaningful way? Capturing the temporal differentiation in the activity of

cortical neurons during behaviourally relevant activity gives the essential metric for defining cell types and their contribution to the organism.
In the cortical network, accurately timed and located GABA release co-operates with the information-carrying glutamatergic inputs to govern the spike timing of pyramidal cells responsible for representations (refs 1,2). Space and time in the network forms an indivisible unity of evolutionary design. The spatial and temporal blueprint is most easily accessible in the CA1 area of the hippocampus where a relatively uniform population of pyramidal cells and their inputs follow an instantly recognisable laminated pattern and act within behaviour contingent network oscillations. The robust and reproducible network oscillations provide a temporal reference frame to monitor the spike timing of both the GABAergic neurons and pyramidal cells. Labelling and molecular dissection of cells recorded *in vivo* helps to explain the spatio-temporal design. Based on input output differences (when known) and molecular differentiation, currently we recognise 3 types of pyramidal cells and at least 21 types of GABAergic neuron in the CA1 area of the hippocampus (refs 2-8)
Why are so many independent sources of GABA needed for shaping the activity of pyramidal cells? A specialisation of GABA release times to different pyramidal cell surface domains, as reflected in the distinct action potential patterns of interneurons relative to network oscillations, is one explanation for the need for independent sources of GABA (refs 2-8). But the same domain may also receive GABA at different times from distinct presynaptic cells during one type of network activity. Activity dependent changes in GABA release are governed by presynaptic receptors in a synapse specific manner. In addition, distinct GABAergic cell types change their firing patterns differentially in different brain states leading to oscillatory network activity with distinct frequencies. Cell type specific and temporally modulated timing of interneuron firing, together with the spatial restriction of GABA release sites to certain cellular domains, regulate and promote the formation of cell assemblies and representations in the hippocampus. Translating these general principles to explicit explanations of specific neuronal events remains a major challenge, because the synaptic wiring and plasticity of the inputs to interneurons remain largely unknown even in this most simple of cortical areas.

Retinotectal connections - revisited

Székely György
Department of Anatomy, Histology and Embryology, University of Debrecen, Debrecen, Hungary
Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
szekely@chondron.anat.dote.hu

Current views about the establishment of ordered retinotectal connexions are reviewed on the basis of previous and recent works. A number of experiments, including the ipsilateral (indirect) projection of the visual field on the optic tectum, the retinotectal projections of eyes composed of identical halves of eye primordials (expansion), projections from normal eyes upon half tecta (compression), the development of the retinal topology in retinotectal projections (synapse slidings), indicate that the visual response cannot be regarded as series of simple reflexes in which the interneuronal connexions are specifically wired with the aid of specific guiding molecules. Histological studies on the optic tectum reveal at least a 1:10 convergence of retinal fibres upon the cortex-like structure of the optic tectum. The intimate and reciprocal intrerconnexions of the diencephalic, mesencephalic and rhombencephalic (isthmic nucleus) optic centres provide such active interconnexions which point towards self-organizing mechanisms in the establishment of the ordered retinotopic connexions in the centres. The process of regeneration of the cut optic nerve in frogs and lizards , as well as

theoretical considerations, support the proposition of a self-organizing mechanism in ordering the interneuronal connexions. Epigenetic effects may be invoked in the establishment of order in neural structures.

SYMPOSIUM LECTURES

Adenylyl cyclases: sites of signal integration in nerve cells

Antoni Ferenc
Department of Molecular Pharmacology, EGIS Pharmaceutical Plc, Budapest, Hungary
ferenc.antoni@egis.hu

Adenylyl cyclases (ACs) synthesize the intracellular messenger cyclic adenosine 3'5'monophosphate (cAMP). Vertebrate genomes have 10 genes of AC, 9 of these are membrane-bound whilst there is a single soluble enzyme. All membrane-bound ACs are expressed in the brain and the presence of several AC paralogues in individual nerve cells appears to be the norm. There are marked differences in the regulatory properties of ACs and three main categories of paralogue-specific control are apparent: heterotrimeric G proteins, Ca^{2+} and protein phosphorylation. These factors all control the enzymatic acitivity of ACs. In turn, the factors controlling ACs are subject to multiple regulatory processes themselves. Thus, ACs sample a large number of cellular signalling events and translate these into cAMP synthesis. Knowledge of the regulation and the physiological relevance of AC paralogues expressed in the brain is still sketchy. Ca^{2+}/calmodulin activated ACs appear to play a role in synaptic plasticity, anxiety and early development. Moreover, AC1 appears to confer sensitivity to glutamate mediated toxicity to cortical neurons. The complexity of AC phosphorylation is formidable: AC9, which is expressed by all nerve cells, is phosphorylated on at least 11 different sites when expressed in human embryonic kidney cells. The phosphorylations are found in the paralogue-specific segments indicating the importance of the paralogue-specific control mechanisms. Protein kinases that phosphorylate AC9-specific sites include cdk5/p35 and protein kinase A. One of these sites, Ser374, appears to be inhibitory to cAMP synthesis when in the phosphorylated state, and may constitute a feedback pathway to tune cAMP accumulation. Yet another facet of AC9 in the brain appears to be proteolytic cleavage. Studies with antibodies directed against the N- and C-terminal segments of the enzyme have shown different patterns of C-terminally cleaved enzyme in brain regions. The function of the C-terminus appears to be the inhibition of the coupling of AC9 to activated Gsα. If the paralogue specific C terminal portion is cleaved, the enzyme is much more reactive to stimulation by Gsα. The findings raise the intriguing possibility that proteolytic conversion of AC9 may have a major impact on cAMP responses induced by various neurotransmitters in individual nerve cells.

Dopaminergic neurotransmission and behavior

Beaulieu, *Jean-Martin*
Department of Anatomie et Physiologie, Unité de Neurobiologie Cellulaire Centre de Recherche, Cell Biology, Université Laval Robert-Giffard, Beauport, Canada
Martin.Beaulieu@crulrg.ulaval.ca

Béta-arrestin 2, which has a role in G-protein-coupled receptor (GPCR) desensitization. Here we will present an overview on how Akt/GSK-3 mediated signaling contributes to the regulation of behavior by dopamine and how this pathway can contribute to the action of psychiatric drugs such as lithium and antipsychotics.

Chronic changes in the electrical activity of individual neurons

Burrone, Juan; Humphreys, Lawrence; Leiwe, Marcus; Grubb, Matthew S.
MRC Centre for Developmental Neurobiology, Kings College London, London, UK
juan.burrone@kcl.ac.uk

Chronic changes in neuronal activity result in homeostatic forms of synaptic plasticity. Here, we use genetically-encoded modulators of neuronal activity to selectively manipulate activity patterns in individual hippocampal neurons in culture. We then assess the effects of these manipulations on the anatomical and functional development of glutamatergic and GABAergic synapses.

We find that silencing the entire neuronal network for 2 days, by blocking Na^+-dependent action potentials with TTX or by blocking glutamatergic synaptic transmission with CNQX/APV, results in an increase in the levels of presynaptic vesicle proteins, such as VAMP2 and vGlut, measured with immunofluorescence. Silencing an individual neuron by means of a specific sodium channel shRNA, also produces an increase in vGlut levels. This increase occurs specifically on boutons forming direct contacts onto the silent neuron. On the other hand, vGlut levels on the axonal output of the silent neuron did not change. Our studies reveal that both global and single cell silencing have the effect of increasing presynaptic strength, suggesting that this form of plasticity is cell autonomous and, more specifically, that postsynaptic activity is a key variable mediating this form of presynaptic plasticity. We are currently performing electrophysiological recordings to establish how synaptic function changes after chronic silencing. As well as silencing single cells, we are also controlling the activity of individual neurons through the use of the light-activated channel channelrhodopsin-2 (ChR2). Using a custom made cell culture plate containing LED lights on each culture well, we have grown neurons under various light-stimulated conditions to electrically stimulate the ChR2-expressing cells. Initial immunofluorescence data show that ChR2-mediated firing can counteract homeostatic plasticity mechanisms induced by chronic CNQX/APV blockade. Electrophysiological recordings are currently underway to investigate the functional synaptic consequences of patterned ChR2-controlled stimulation.

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Unraveling functional selectivity of horizontal spread in cat visual cortex using VSDI

Chavane, Frederick
CNRS, Université de la Méditerranéé, Marseille, France
Frederic.Chavane@incm.cnrs-mrs.fr

While cortical neurons within a vertical column share common functional references, long-range horizontal axons connect distant neurons with possibly different receptive field properties. Previous anatomical and extracellular cross-correlations studies have suggested that these horizontal axons preferentially connect neurons with similar orientation preference. We used optical imaging of voltage sensitive dyes and intracellular recordings to characterize the tuning of the post-synaptic integration of horizontal spread in response to small oriented stimuli in the primary visual cortex of the anaesthetized cat. At the population level, using VSDI imaging, we found that orientation selective activity was mostly confined to a feedforward cortical imprint of the local stimulus. The iso-orientation bias of remote cortical territories activated via long-range horizontal connectivity diminished progressively with

distance. Iso-orientation preference and tuning selectivity of post synaptic responses decreased exponentially with a cortical space constant of about the diameter of a hypercolumn. Intracellular recordings were used to directly measure synaptic responses evoked by local oriented stimuli, in single cells. We found that area 17 cells receive a diversity of subthreshold synaptic input from horizontal connections, either tuned with variable orientation preferences or untuned. This integrated study shows that the lateral influence of local stimuli is more diverse than initially proposed. In contrast, by using complementary center and surround stimuli we found both at the population and single cell levels that associative cortical mechanisms are required to propagate iso-orientation preference beyond the hypercolumn scale. We propose that the cooperative recruitment of recurrent columnar circuits allows the expression and propagation of a functional orientation preference.

Mechanisms underlying the formation of vestibular circuitry

Glover C. Joel
Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
joel.glover@medisin.uio.no

Using voltage-sensitive and calcium-sensitive dye recording, the development of functional circuitry in central auditory and vestibular pathways has been charted in the chicken embryo. Appropriately specific functional connections, detected as optical responses in central nuclei following stimulation of peripheral afferents, form in a characteristic sequence starting at around 7 days of embryonic development. Pharmacological manipulation allows identification of the functional sign of the connections, and in the vestibular system demonstrates that excitatory and inhibitory connections form contemporaneously in the appropriate patterns for reflex activation of motoneuron targets. The specificity of these premotor connections develops in the presence of chronic N-methyl-D-aspartate (NMDA) receptor blockade and spontaneous synchronous activity, suggesting that activity is not a critical factor in establishing the proper synaptic connectivity in the vestibular pathways. The development of functional connections revealed by optical recording will be related to anatomical and genetic studies of axon path-finding and the neuroepithelial origin of nuclear groups.

Imaging early development of orientation selectivity in ferret visual cortex at single cell resolution

Hübener, Mark, Leong J.S., Leinweber M., Mrsic-Flogel T.D., Bonhoeffer T.
Department of Cellular and Systems Neurobiology, Max-Planck Institute of Neurobiology, Munich, Germany
mark@neuro.mpg.de

In the visual cortex of higher mammals, preferred stimulus orientation is mapped across the cortical surface in a smooth fashion, except for pinwheel-like discontinuities, around which all orientation preferences occur. With intrinsic signal imaging, orientation maps in ferret visual cortex were first detected around the time of eye-opening, and once apparent were found to remain stable over the following weeks. However, intrinsic signal imaging relies on the concerted activity of neuronal populations and cannot resolve the activity of individual neurons. In fact, electrical recordings have revealed orientation selective single units about ten

days before the earliest orientation maps have been reported, but whether these neurons are organized into a map remains unknown.
We are using two-photon calcium imaging to map orientation preference at cellular resolution in the developing ferret visual cortex. Around postnatal day 19, neurons exhibit large, spontaneous calcium transients, but visual stimulation evokes only unreliable responses, if any. Within days, and as early as ten days before eye opening, we find a high proportion of visually responsive neurons. Surprisingly, all such neurons at this early time point respond almost exclusively to horizontal stimuli and are distributed uniformly across the cortical surface. Electrical recordings confirm this unusual regime of functional organization. Subsequently, around the time of eye opening, all orientation preferences are present. While most neurons exhibit broad orientation tuning, they are clustered spatially according to preferred orientation, and we never observe a mixing of cells preferring different orientations. Adult-like maps emerge as orientation tuning sharpens over the following days.
Our results suggest the following sequence of events in the development of orientation preference. Initially, all visually responsive neurons strongly prefer horizontal stimuli. This early bias could be caused by neuronal activity, or reflect activity-independent mechanisms such as anisotropic connectivity during early axon ingrowth and/or the remodeling of dendritic arbors. Orientation tuning then broadens for all cells as the horizontal bias is lost. Most neurons attain a new preferred orientation, indicating that a fast and dramatic change in orientation preference is a key feature of early orientation map development. Since neurons' new preferred orientations develop in a clustered fashion, we conclude that the development of a neuron's orientation preference is intimately linked to the formation of the orientation map.
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Regulation of angiotensin and cannabinoid receptors
Hunyady László
Department of Physiology, Semmelveis University, Budapest, Hungary
hunyady@puskin.sote.hu

Angiotensin II (Ang II) exerts its biological effects via AT_1 and AT_2 angiotensin receptors (AT_1Rs and AT_2Rs). Signaling of AT_1Rs is regulated by ß-arrestins, which bind to activated AT_1Rs, uncouple them form G proteins, and initiate their internalization via clathrin-coated pits and cause G protein independent MAP kinase activation. AT_1Rs internalize via ß-arrestin-dependent and independent mechanisms, whereas AT_2Rs do not internalize. In transiently transfected HEK 293 and CHO cells, Ang II induced rapid association of AT_1Rs with green fluorescent protein- (GFP)-labeled ß-arrestin2 at the plasma membrane and in endosomal vesicles was observed with confocal microscopy. However, homogenous cytosolic distribution of GFP-ß-arrestin2 was not affected by Ang II treatment in cells expressing AT_2Rs. Bioluminescence resonance energy transfer (BRET) was employed to verify that direct molecular interaction occurs between stimulated AT_1Rs and ß-arrestin2. In agreement with the confocal data, no BRET interaction was detected between AT_2Rs and ß-arrestin2. The cannabinoid CB1 receptor (CB1R) couples mainly to the $G_{i/o}$ family of G proteins. CB1R displays basal activity and internalizes both constitutively and after agonist stimulation. In CHO cells stimulation of CB1R with WIN55,212-2 lead to translocation of β-arrestin2 to the cell membrane, but its presence was not observed in internalized intracellular vesicles, indicating that CB1R belongs to the class A group of receptors, which do not form stable complexes with β-arrestin molecules after receptor internalization. In CHO cells co-expressing CB1R and AT_1Rs, Ang II-induced G_o protein activation, which was blocked both

with AM251, a CB1R antagonist, and tetrahydrolipstatin (THL), an inhibitor of DAG lipases. In cells separately transfected with the receptors, Ang II-induced, AT_1R-mediated paracrine transactivation of CB1 receptors was also detected.
These data demonstrate that agonist-induced stimulation of AT_1Rs and CB1Rs leads to association of ß-arrestins with the receptors with different stability, whereas Ang II does not induce association of ß-arrestins with AT_2Rs. They also suggest that AT_1 receptor activation leads to activation of CB1 receptors with autocrine and paracrine mechanisms.
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Topography of single cortical cells correlates with visual feature representations
Karube, Fuyuki
Department of Anatomy, Histology and Embryology, University Debrecen, Debrecen, Hungary
fuyuki@chondron.anat.dote.hu

In the cat visual cortex, there is a map-like representation of multiple visual features such as visual field position (retinotopy) and preferred orientation of feature elements. It has been assumed that the neuronal connectivity scheme underlying these maps can be ascribed in accordance to the "like connects to like" concept. So far, this concept has gained support from the iso-orientation specific connectivity of layer III lateral connections. However, little is known about the validity of this concept with regard to lateral connections of other layers.
Here, we studied the connectivity of layer IV and layer VI spiny cells to functional maps using *in vivo* intrinsic signal optical imaging (orientation map), electrophysiological recording (retinotopic map) and 3D-reconstruction of labeled spiny cells in the primary visual cortex of the cat (A18). Orientation maps were characterized by largely latero-medially elongated iso-orientation domains (slab), which ran almost parallel to iso-elevation lines. Direct comparison between these maps and layer IV spiny cells (N=18) showed that for 6 cells distal projections terminated preferentially at iso-orientation sites, whereas for 7 cells, targeted cross-orientation sites with respect to the orientation preference of the parent soma. Importantly, the axonal fields of iso-orientation preferring cells were elongated latero-medially parallel to the representation of iso-elevation. On the other hand, cross-orientation preferring cells provided connections, which were elongated antero-posteriorly parallel to the representation of iso-azimuth. Layer VI cells (N=17) also had distal projections, however, showed only a weak bias to iso-orientations.
Taken together, the present and previously published data on the functional specificity of single cells suggest that the three main cortical tiers of A18 (layers II/III, IV, V/VI) employ rather different horizontal connectivity schemes, each of which represent a distinct stage for processing visual information.
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Modulation of dendritic spikes in the prefrontal cortex
Lendvai Balázs
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
lendvai@koki.hu

While excitation of subcellular ion dynamics occurs after activation of the receptors, inhibition may also develop during spontaneous activity by the activation of

hyperpolarization-activated cation currents. Backpropagating action potentials are considered as determinant players of synaptic plasticity. This form of back-reporting in the dendrites is likely involved in the memory mechanisms that store the previous activities of the neuron. To explore the modulatory actions of nonsynaptic receptor agonists on the backpropagating action potential (bAP)-evoked Ca^{2+} transients in apical dendrites and spines we performed two-photon imaging of neocortical layer 5 pyramidal neurons. Perfusion of drugs induced site-dependent modulation of the bAP-evoked Ca^{2+} transients. Dendritic-spikes were seen during the perfusion of drugs. This complex modulatory effect of alpha-adrenergic drugs on dendritic integration should expand the computational power of pyramidal dendrites and may help understand the modulatory possibilities of cognitive function.

Unraveling cortical development and plasticity using intrinsic signal optical imaging

Löwel, Sigrid
Institute Allgemeine Zoologie und Tierphysiologie, Friedrich-Schiller Universitat, Jena, Germany
siegrid.loewel@uni-jena.de

Visualizing brain activity by optical recording of "intrinsic signals" (as opposed e.g. to optical imaging with voltage sensitive dyes) has been a fascinating technique in neuroscience for more than two decades. It has been known for over 50 years that such intrinsic signals are associated with metabolic or electrical activity in many tissues, including the brain. Nevertheless, these small signals were first exploited for the *in vivo* mapping of cortical activity about 20 years ago. Since intrinsic imaging offers very high spatial resolution (in fact the highest resolution of any *in vivo* imaging technique until 2-photon *in vivo* Ca-imaging has been introduced 2 years ago) the technique is ideally suited to map sensory areas of the cerebral cortex.
For researchers interested in long-term or learning-induced changes of cortical maps, repeated optical imaging of the same region of cortex can provide a "movie" of the observed map changes. I will present an example from the adult cat visual cortex, in which intracortical microstimulation of defined functional domains, a method to temporally synchronize neuronal discharges, induced long-range and long-term changes of orientation column map layout which might underlie perceptual learning phenomena.
Likewise, in research on experience-dependent changes in neuronal circuitry, optical imaging of intrinsic signals combined with tracer injections yields detailed information about the functional selectivity of labelled nerve cell connections. Using these techniques, we were able to demonstrate that i) the layout of the long-range horizontal circuitry in cats with strabismic amblyopia is significantly different from control animals and from non-amblyopic squinters and that ii) network modifications are consistent with the perceptual deficits of strabismic amblyopes.
Finally, we could recently establish optical imaging of intrinsic signals as a fast and reliable screening tool for visual cortical plasticity in mice. As has been shown already a decade ago, ocular dominance plasticity induced by monocular eyelid suture is present also in mice and follows similar rules as in higher mammals, thus establishing the mouse as a valid model system to study ocular dominance plasticity. Using optical imaging of intrinsic signals, experience-dependent changes in cortical activity patterns can be visualized in less than 30 minutes. This technique has the potential to greatly accelerate the studies using transgenic mice to investigate the molecular and cellular mechanisms underlying cortical plasticity.

Plasticity of dendritic spikes in hippocampal CA1 pyramidal cells

Makara Judit; Losonczy Attila; Magee C. Jeffrey
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, USA
jmakara@koki.hu

Local dendritic spikes, evoked by synchronously activated synaptic inputs in cortical pyramidal cells, represent a nonlinear form of integration that enables the cell to decode spatio-temporally correlated input patterns. We study the properties of dendritic spikes in radial oblique and basal dendrites of CA1 pyramidal cells evoked by synchronous two-photon uncaging of glutamate at multiple spines on a dendritic segment. Propagation of the fast Na+ spike along the dendrite (measured with two-photon Ca^{2+} imaging) was remarkably variable among individual branches, resulting in different strength at the soma (amplitude and rate of rise [dV/dt]) and different precision of action potential output. Spikes propagated weakly in most of the dendrites (weak branches; dV/dt < 2 V/s, ~80 %), however, in a group of branches spikes propagated much more effectively (strong branches; dV/dt > 2 V/s, ~20 %). Spike strength was related to the distance from the soma and to the order of the branch within the dendritic family. Spikes in lower order non-terminal dendrites were usually strong, whereas higher order terminal branches were predominantly weak, although in a subset of terminal dendrites spike propagation was strong enough to recruit the strong spike of the non-terminal dendrite ("branch coupling").
We found that spike propagation and branch coupling are not static features, as pairing repetitive local spikes with either backpropagating action potentials (bAPs) or with application of the cholinergic agonist carbachol markedly enhanced dV/dt ("branch strength potentiation") and increased the probability of branch coupling in an NMDA receptor dependent fashion. This potentiation could be mimicked by the A-type K^+ current blocker Ba^{2+}, the specificity of which was confirmed by the lack of its effect in Kv4.2 subunit knockout mice. Furthermore, branch strength potentiation could not be induced by either bAP- or carbachol-pairing in Kv4.2 knockout mice, indicating that regulation of A-type K^+ currents play a critical role in this form of plasticity.
We propose that branch strength potentiation represents a form of information storage that is distinct from that produced by changes in synaptic efficacy. This novel form of plasticity may be particularly effective in supporting temporally structured neuronal activity, such as that found during hippocampal sharp-waves.

Morphological background underlying the gaze and posture control

Matesz Klára
Department of Anatomy, Histology and Embryology, Medical and Health Science Center, University of Debrecen and HAS-UD Neuroscience Research Group H-4012 Debrecen, Hungary
matesz@chondron.anat.dote.hu

Governing of gaze fixation, maintenance of equilibrium and orientation in tree-dimensional space depend on different sensory impulses arising from the retina, labyrinthine organs and sensory receptors of skin, muscles and joints. During body displacement the very fine and continuous tuning of gaze fixation and the rapid control of limb movements requires a very fast modification of the motor activity to obtain the proper muscle contraction. Despite of its functional importance, the morphological background of gaze and posture control is not yet fully understood. By using different neuronal labeling techniques we have characterized the neuronal elements of the vestibulo-ocular and vestibulo-spinal circuitry in frog and rat. We

have found a significant overlap of vestibular and proprioceptive terminals in parts of the CNS related to motor coordination suggesting the convergence of sensory modalities involved in the control of eye movements and sense of balance. In the visuo-motor system we have found that the dendritic bundles of the oculomotor nucleus extend into the territory of trochlear motoneurons and vice versa. Electron microscopic studies revealed long, direct membrane appositions without any morphological specialization between the motoneurons of oculomotor and trochlear nuclei providing the morphological substrate of ephaptic interactions. Activation of ipsilateral motoneurons may spread very rapidly by the way of electrically coupled dendritic bundles to their contralateral counterpart and may contribute to the synchronized contraction of bilateral muscles of the eyeball. Besides the conventional chemical impulse transmission from vestibular and proprioceptive afferent fibers, we have demonstrated their dye-coupled connections, indicative of gap junctions at light microscopical level, in the spinal cord, brainstem and the cerebellum. Gap junctional couplings may synchronize and amplify the signals leading to a very rapid modification of motor activity in the vestibulo-cerebello-spinal circuit. The convergence of inputs from vestibular and proprioceptive receptors may provide further amplification of signals. Combination of chemical and electrical impulse transmission may be the possible morphological correlate for the sequential activation of motoneurons.
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D2 dopamine receptor signaling and tonic inhibitory regulation of prolactin secretion

Nagy M. György
Department of Human Morphology, HAS-Semmelweis University, Budapest, Hungary
nagy-gm@ana2.sote.hu

Secretion of prolactin (PRL) by the pituitary gland is under a persistent (tonic) inhibitory control exerted by the hypophyseotrophic dopamine (DA). However, the mechanisms involved in this inhibition remain to be defined.
We have previously observed that mammotropes obtained from lactating rats become partially resistant to DA inhibition following a brief suckling period compared to non-suckled mothers. This, so-called “desensitization” (and a parallel appearance of “tolerance”) to DA is mediated through by a significant reduction of protein phosphatase 2A (PP2A) activity in the pituitary gland. Besides the known Gi/o-cAMP-PKA pathway, stimulation of D2-receptor (D2-R) leads to the activation of the p44/42 extracellular-regulated kinase (ERK1/2) in the pituitary gland. Recently, a new signal-transduction pathway has been described in relation with the striatal D2-R that is a □-arrestin dependent (at the same time G-protein independent) mechanism. In this signaling □-arrestin is coupled with PP2A that dephosphorylates, therefore, inactivates protein kinase B (Akt). Detailed characterization of the changes in phosphorylation of ERK1/2 and Akt following physiological (suckling) and/or pharmacological (inhibitor of DA biosynthesis and/or D2-R antagonist) manipulations of the hypophyseotrophic DAergic system clearly suggest that inactivation (dephosphorylation) of Akt is coupled with the tonic inhibitory influence of DA on mammotropes, which may help to explain the observed dissociation between “tolerance” and “dependence” during the persistent tone of hypophyseotrophic DA signaling existing on pituitary mammotropes. This work was supported by the Hungarian National Found (OTKA K-68170) and the Hungarian Ministry of Health (ETT T-177/2006) to NGM.

Diverse cell surface distribution of voltage-gated ion channels

Nusser Zoltán
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
nusser@koki.hu

Most nerve cells receive inputs from thousands of synapses distributed on an extensive dendritic tree. The way synaptic inputs affect the output generation of a cell does not only depend on the properties of the synapses, but also on the distribution of a large number of voltage-gated ion channels on the somato-dendritic surface of the cell. Here I will summarize recent experimental evidences for complex cell surface distribution of transient A-type K^+ channels. The Kv4.3 subunit of the A-type channels has an uneven distribution on the surface of cerebellar stellate cells. There is a high density of channels in unique membrane specializations between GABAergic interneurons and climbing fibers. Our ultrastructural and molecular neuroanatomical experiments demonstrated that these specializations are distinct from known chemical and electrical synapses. We investigated whether such potassium channel-rich membrane specializations are unique for this subunit and for this cell type or are widespread in the CNS. Both the Kv4.2 and Kv4.3 subunits show characteristic, clustered distribution in olfactory bulb neurons. The Kv4.2 subunit is enriched in membrane specializations between olfactory bulb granule and mitral cells. Such specializations contain high density of channels in both membranes, symmetrically. The Kv4.2 subunit in external tufted cells and the Kv4.3 subunit in deep short-axon cells and in some periglomerular cells are present in such potassium channel-rich specializations asymmetrically; only one side of the opposing membranes contains the channels. Our results reveal a highly non-homogeneous distribution of the Kv4.2 and Kv4.3 A-type K^+ channels subunits on the surface of a variety of neurons and predict their novel roles in intercellular communication.

Dendritic integration in hippocampal interneurons
Rózsa Balázs; Katona Gergely; Kaszás Attila; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
rozsabal@koki.huű

We developed a new, real-time, 3 dimensional (3D) two-photon microscope technique ("Roller Coaster Scanning") in order to image the rapid physiological activity with 1 kHz time resolution in the dendrites and spines of neurons.
Although interneurons play a major role in the central nervous system, little is known about how their dendrites integrate inputs. Freehand 3D lines were placed following the curvature of single long dendritic segments to monitor the calcium signals elicited by focal electric stimulation. Using our novel rapid two-photon 3D linescan techniques, which enabled the measurement of long dendritic segments (typically > 30 µm, sometimes 50 µm), we detected more forms of regenerative activity (dendritic spikes) in the dendrites of interneurons. We used multisite focal extracellular stimulation to deliver synaptic input to single dendritic branches of interneurons while eliciting dendritic spikes with the second electrode. We found a novel type of synaptic plasticity: the dendritic spike changed the gain of synaptic inputs depending on the time difference between the synaptic inputs and the dendritic spike. Our results suggest that similarly to pyramidal neurons dendritic spikes exist in the short dendrites of interneurons and these spikes may contribute to synaptic plasticity.

Dendritic integration of few quanta produces high spike precision in a spontaneously active cerebellar interneuron
Silver, Angus; Roby T. Kanichay

Department of Physiology, University College London, London, UK
a.silver@ucl.ac.uk

Precise representation of the timing of sensory stimuli is essential for rapid motor coordination, a core function of the cerebellum. Many of the neurons in motor systems, particularly in the cerebellum, are spontaneously active and several of these participate in feed-forward inhibitory loops, that are thought to preserve temporal signalling as sensory signals flow through networks. But, relatively little is known about how temporal processing is accomplished in the presence of intrinsic activity in these feed-forward interneurons.
We have investigated the mechanisms underlying temporal signalling in the input layer of the rat cerebellar cortex. We establish that the previously uncharacterized mossy fibre (MF) - Golgi cell (GoC) - granule cell (GrC) pathway acts a functional feed-forward inhibitory circuit. A small number of MFs, each releasing 1-2 quanta onto GoC dendrites, can reset spontaneous GoC firing with high temporal precision. Precision was maintained over a range of stimulation frequencies, but GoC firing rate was only weakly modulated due to dominant pacemaker currents underlying the intrinsic activity. Feed-forward excitation of GoCs induced temporally precise inhibition in GrCs, reducing their time window for synaptic integration. Our results suggest that the synaptic properties of the feed-forward input and the conductances underlying spontaneous GoC activity are tuned to signal the precise timing of the onset of sensory stimuli.
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Interactions between vestibular and locomotor signals for gaze control

John Simmers[1]; Denis Combes[1]; Didier Le Ray[1]; Francois M. Lambert[2]; Hans Straka[2]
[1]University of Bordeaux, Laboratory of Mouvement Adaptation Cognition, Bordeaux, France
[2]Laboratoire de Neurobiologie des Réseaux Sensorimoteurs, Centre National de la Recherche Scientifique, Unité Mixte de Recherche, Université Paris Descartes, Paris, France
j.simmers@lnr.u-bordeaux.fr

Self-motion derived perturbation of visual information processing requires compensatory eye and head-adjustments that are driven by movement-encoding vestibular, optokinetic and proprioceptive signals. We are investigating the novel possibility that rhythmic locomotor commands produced by central pattern-generating circuitry in the spinal cord also accesses the brainstem extraocular motor nuclei and contributes to gaze stabilization during active body movements. We use isolated head-spinal cord preparations of larval *Xenopus laevis* (stage 55) in which strong lateral head excursions occur during undulatory swimming in the intact animal. During spontaneous episodes of fictive swimming *in vitro* (without movement-related sensory feedback), extracellularly-recorded activity in bilateral extraocular motor nerves was found to be phase-coupled to bilaterally-alternating spinal ventral root bursts that drive swimming in the intact animal. The strongest coupling between locomotor and extraocular motor output occurred in the lateral and medial rectus, was only weak in the superior rectus and inferior oblique, and was absent in the superior oblique and inferior rectus nerves. The locomotory timed activity in each lateral rectus nerve was in-phase with bursts in both the contralateral medial rectus and contralateral spinal ventral roots, consistent with the production of conjugate eye movements that would counteract oppositely-directed horizontal head displacements during undulatory swimming. Moreover, the spatio-temporal specificity of the locomotor-extraocular motor coupling seen *in vitro* corresponded closely to the *in vivo* activation of the different eye muscles by horizontal semicircular canal input during

sinusoidal rotation about a vertical axis. Our results therefore suggest that in *Xenopus*, copies of centrally-generated locomotor commands are used to "predict" the visual consequences of body movements and are integrated with indirect, and therefore slower, sensory signals to produce gaze stabilization.

Co-adapted membrane and network properties determine differential signal processing in central vestibular neurons

Hans, Straka; Pfanzelt, Sandra; Beraneck, Mathieu
Laboratoire de Neurobiologie des Réseaux Sensorimoteurs, Centre National de la Recherche Scientifique, Unité Mixte de Recherche, Université Paris Descartes, Paris, France
hans.straka@univ-paris5.fr

Central vestibular neurons play a key role in the sensory-motor transformation of head movement-related signals into commands for gaze and postural stabilization. Frog second-order vestibular neurons (2°VN) offer an excellent possibility to study *in vitro* in an isolated whole brain the respective roles of intrinsic membrane and emerging network properties in the processing of labyrinthine nerve afferent signals. Frog 2°VN subdivide into phasic and tonic neurons on the basis of distinct differences in intrinsic membrane properties. Tonic 2°VN are characterized by a continuous discharge during depolarization and largely linear membrane properties that assign to these neurons classical low-pass filter properties. In contrast, phasic 2°VN are characterized by a powerful spike discharge adaptation and highly non-linear membrane properties. The marked impedance resonance at ~40 Hz in these neurons results from the activation of voltage-dependent potassium conductances and assigns to these neurons band-pass filter properties. Artificial depolarization or synaptic activation causes an increase of the impedance in tonic as opposed to a decrease in phasic 2°VN. These complementary properties render tonic 2°VN particularly suitable for signal integration and phasic 2°VN for signal detection. The role of vestibular networks for the signal processing was revealed by applying sinusoidally modulated trains of single electrical pulses to individual vestibular nerves to mimic natural inputs. Compound responses of tonic 2°VN exhibited a fairly linear behavior with a sinusoidal waveform and a small phase lead. In contrast, compound responses of phasic 2°VN exhibited a rather non-linear behavior with a large phase lead of up to 50°. This phase lead is caused by a delayed recruitment of inhibitory feed-forward side-loops and complies with the intrinsic phasic response dynamics of this neuronal subpopulation. The inhibition is mediated by glycine- as well as $GABA_A$-receptors that are activated by local inhibitory vestibular interneurons and cerebellar pathways. The different circuits, in which tonic and phasic 2° VN are embedded, thus comply with the different intrinsic signal processing modes of the two neuronal types and suggest a coadaptation of intrinsic membrane properties and emerging network properties.

POSTER PRESENTATIONS

Behavioural effects of acute changes of plasma corticosterone levels in cannabinoid receptor CB1 knockout and wild-type mice

Aliczki Manó; Mikics Éva; Mergl Zsuzsa; Zelena Dóra; Haller József
Department of Behavioural Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
aliczki_mano@yahoo.com

Recent studies showed that glucocorticoids – besides their genomic effects – can affect behaviour through non-genomic mechanisms. In rats, acute changes in plasma corticosterone levels induced context-dependent behavioural changes: while the glucocorticoid-synthesis inhibitor metyrapone significantly decreased territorial aggression in social challenges and risk-assessment behaviour in non-social challenges, acute corticosterone treatment reversed these behavioural effects via a non-genomic mechanism. The exact molecular mechanisms are poorly known, however, recent studies suggest that the endocannabinoid system may play a crucial role in mediating the rapid effects of glucocorticoids. Therefore, the aim of the present study was to investigate rapid behavioural effects of glucocorticoids in cannabinoid receptor CB1 knockout and wild-type mice, that showed different behaviour and HPA-axis activity. We studied the effects of three different dose of metyrapone on plasma corticosterone levels and locomotion in CD1 mice, then the effects on plasma corticosterone levels and aggressive behaviour in the resident-intruder test in CB1 knockout and wild-type mice. In the first experiment, metyrapone significantly decreased plasma corticosterone levels in 25 minutes in three different doses, but had no effects on locomotion. In the second experiment metyrapone significantly decreased plasma corticosterone levels in both genotypes, but had no effect on territorial aggression. We found that CB1 KO mice were significantly less aggressive than WT's, which is in contradiction to a previous study, where CB1 KO animals showed greater aggression than others. These preliminary data suggests that metyrapone is effective in mice, but has no acute effects on aggression. It is likely that the difference between the aggression of the CB1 KO and WT animals is not caused by acute changes in glucocorticoid background. Further studies are needed to investigate the effects of acute glucocorticoid treatment on other behavioural variables in mice.

Double- and triple-microdrive positioning headstage-assemblies for neuronal recording in freely moving mice

Averkin Robert G.[1,2]*; Korshunov Victor A.*[3]
[1]Department of Physiology, University of Szeged, Hungary
[2]Bay Zoltan Foundation for Applied Research, Szeged, Hungary
[3]Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
averkinrobert@yahoo.com

We present new lightweight and miniature double-microdrive and triple-microdrive headstage-assemblies for recording neuronal activity in freely moving mice with sharpened high impedance electrodes. The devices can be used for neuronal recording from different brain structures. The advantage of this construction involves the possibility to change the aiming angles of the electrode penetrations by rotation of the driver holders (up to 20 degree). Each microdrive is fixed in the base that has it's own axis of rotation. Thus, the double microdrive construction provides independent rotation of the microdrives in two parallel plane around one axis and suitable for targeting the homogeneous structures in both hemispheres or in two different structures in the same hemisphere. The triple-microdrive assembly provides independent rotation (up to 20 degree) of the microdrives around three parallel axes and thus make possible to direct the electrodes to one structure or three different structures in the brain. Both assemblies were successfully tested in freely moving mice. Single unit recordings were made in different cortical areas and hippocampus. In some cases the single extracellular signal was stable up to 16 hours in all recorded channels. Both constructions are lightweight (thus the weight of one microdrive less than 45 mg in weight, and the whole assembly with 3 microdrives is less 225 mg); this provides light load even for mice.

Mapping of the parathyroide hormone 2 receptor in human diencephalon and brainstem

Bagó G. Attila[1]; Palkovits Miklós[2]; Ted B. Usdin[3]; Seress László[4]; Dobolyi Árpád [2]
[1]National Institute of Neurosurgery, Budapest, Hungary
[2]Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[3]Section on Fundamental Neuroscience, National Institute of Mental Health, Bethesda, Maryland, USA
[4]Central Electron Microscopic Laboratory, University of Pécs, Pécs, Hungary
bagoatt@hotmail.com

The parathyroid hormone 2 receptor (PTH2R) was identified on the basis of its sequence homology to other polypeptide-recognizing G protein coupled receptors. Pharmacological and anatomical evidence suggested that tuberoinfundibular peptide of 39 residues (TIP39) and not parathyroid hormone or parathyroid hormone-related peptide is the endogenous ligand of the PTH2R. Initial functional studies suggest that the TIP39-PTH2R neuromodulator system is involved in viscerosensory functions of the central nervous system. As a first step towards potential clinical applications of the TIP39-PTH2R neuromodulator system, here we describe the presence and distribution of the PTH2R in human diencephalon and brainstem. Hypothalamus and medulla oblongata were dissected to isolate mRNA. Subsequent RT-PCR experiments and sequencing of PCR products demonstrated the expression of PTH2R mRNA in the human brain. The distribution of PTH2R was determined with fluorescent amplification immunochemistry using specific anti-PTH2R primary antibody on free floating brain sections. No immunolabeled cell bodies were detected. However, a high density of PTH2R-immunoreactive fibers were found in many brain regions including dense fiber networks in the nucleus of the solitary tract, the spinal trigeminal nucleus, the dorsal reticular nucleus, and the dorsal horn of the spinal cord. These findings are compared to rodent data with which they are highly consistent. PTH2R-immunoreactive cell bodies were often unvisible in regions of mice brain that expressed PTH2R mRNA, and also in transgenic mice that express beta-galactosidase driven by the PTH2R promoter suggesting that PTH2R is promptly transported from cell bodies towards the fibers. The distribution of PTH2R-immunoreactive fibers in the human brain is also similar to that reported in mice and rats suggesting a similar role of the PTH2R in human as in rodents. This finding will have important implications when experimental data obtained on the function of the TIP39-PTH2R neuromodulator system in rodents are to be utilized in human.
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Age-related variations of alpha-MSH-induced anorexia

Balaskó Márta; Cséplő Péter, Soós Szilvia; Pétervári Erika; Székely Miklós
Department of Pathophysiology and Gerontology, University of Pécs, Pécs, Hungary
marta.balasko@aok.pte.hu

In otherwise healthy humans as well as in rodents, the long-term regulation of body weight and energy balance encompasses two problems: one is called the age-related obesity, which is followed by the other one, described as anorexia of aging. Alpha-MSH derives from pro-

opiomelanocortin, which is produced mainly in the ventrolateral part of the hypothalamus. It is regarded the most important endogenous agonist of the melanocortin receptors, particularly those of MC3R and MC4R. Activation of these receptors has been shown to suppress food intake and to decrease body weight, while endogenous/exogenous antagonists are known to act in the opposite way. The question arises whether changes in the activity or effectiveness of alpha-MSH may have any role in such age-related changes of food intake and body weight regulations. The consumption of powdered chow was measured in a Feedscale system (Columbus) in male Wistar rats aging 3-4, 6, 12 or 24 months, following injection of vehicle or 5 µg alpha-MSH in a volume of 5 µl intracerebroventricularly (ICV). The rats were normally maintained in a room with lights on between 6 a.m. and 6 p.m. and they were provided with powdered chow and water ad libitum. For the injection, an ICV guide cannula was surgically implanted at least 1 week prior to the tests. Some rats received the injection at 6 p.m. (right before the onset of the circadian activity and spontaneous feeding), while others were injected at 9 a.m. following a 24-h period of food deprivation. As compared with the controls, in 3 month-old rats alpha-MSH significantly suppressed the spontaneous food intake for about 4 hours and the rats did not make up for the deficit within a day. In rats treated with alpha-MSH the post-deprivation food intake was also smaller than in controls. However, the severity of suppression was different in various age-groups: by the first hour of re-feeding the consumption was reduced to about 41, 61, 65 and 16% in 3-4, 6, 12 and 24 months-old rats, respectively. In the second hour these values were 42, 48, 70 and 16%, while in the third hour they were 39, 58, 58 and 29%, respectively. In conclusion, in rats of 6 or 12 months the alpha-MSH-induced anorexia was less pronounced than in the youngest and oldest groups. This may suggest that melanocortins have some role in the age-related changes in regulation of food intake and body weight.

Bilateral protection of ischemia-induced alterations in the hippocampus by unilateral preconditioning

Balázsa Tamás[1]; Nyitrai Gabriella[2]; Kardos Julianna[2]; Palkovits Miklós[1]
[1]Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
balazs@ana.sote.hu

Ischemic preconditioning (IPC) represents an important phenomenon of adaptation of the central nervous system to sublethal short-term ischemia, which results in an increased tolerance to the lethal ischemia. Previous studies have demonstrated that bilateral ischemic preconditioning decrease ischemia/reperfusion-associated *c-fos* expression in the brain. The purpose of this study was to determine whether transient unilateral preconditioning is capable of inducing bilateral ischemic tolerance. Fos immunostaining was applied to serial sections of the forebrain of adult, male Sprague Dawley rats subjected to 10 min global ischemia with or without 4 min of sublethal unilateral ischemic preconditioning. The number and density (cell number/area measured) of Fos-immunopositive (Fos+) neurons were determined in all layers of the hippocampal CA1, CA2, CA3 areas (stratum oriens, st. pyramidale, st. radiatum, st. lucidum, st. lacunosum moleculare) and the dentate gyrus (molecular layer, granular layer, polymorph layer) of both ischemic and preconditioned rats, on both sides individually. Global ischemia without IPC induced massive *c-fos* activation in hippocampal neurons in all investigated layers and areas. Based on the quantification results, we found that unilateral preconditioning reduces *c-fos* expression in the hippocampal areas and dental gyrus layers

significantly both ipsi- and contralateral to the site of the occlusions. There were no statistically significant differences in the number of the Fos+ principal cells or of the Fos+ interneurons between the ipsilateral and contralateral sides. In one of our pilot studies we found that intrahippocampal transection did not alter the effect of unilateral IPC on the *c-fos* expression. These data suggest that the induction of the ischemic tolerance does not require neuronal interconnections between the two sides of the hippocampus. Recent studies have suggested that pCREB may be involved in acquisition of ischemic tolerance, which occurs after sublethal ischemic stress. We found that 10 min global ischemia deceased pCREB expression. In contrast, we found elevated pCREB expression in those sections which underwent unilateral IPC prior to bilateral ischemic insult. The pCREB expression markedly elevated both ipsi- and contralaterally in the dental gyrus granular layer and all the pyramidal layers. In coclusion, *unilateral* ischemic preconditioning can elicit a neuroprotective state in both sides of the hippocampus, indicating the possible role of humoral agents in this mechanism.
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Subunit switching of NMDAR in early development of mouse barrel cortex
Banerjee Abhishek ; Antonio Rodríguez-Moreno; Ole Paulsen
Department of Physiology, Anatomy and Genetics, University of Oxford, UK
abhi.synap@gmail.com

Synapse formation and refinement of cortical connections and topographic maps during development rely on experience dependent processes. Synaptic N-methyl-D-aspartate (NMDA) receptors play an important role in regulation of neuronal communication, synaptic function and plasticity working as a coincidence detector. Changes in the subunit composition of NMDAR have been suggested as a mechanism of developmental plasticity in the cortex where NR2 subunit is critical in determining biophysical and pharmacological properties relating physiological functions. Spike time dependent long term potentiation and long-term depression is also coordinated with the development of NMDA subtype specific current in early postnatal life. Using the whole-cell patch clamp technique, we recorded EPSCs from an identified cell group of layer 2/3 pyramidal neurons evoked by stimulation of layer 4, and looked for involvement of different subtype of NMDA receptors, namely NR2A and NR2B. NVP-AAM077 (100 uM), a NR2A preferring competitive antagonist blocked 12.88 ± 3.41 % current in P 6-8 C57/BL-6 mice (n = 9) indicating the presence of mostly NR2B enriched synapses in Layer 2/3 cells in early life. With the development and maturation of circuitry, NR2A expression increases as NVP takes away 36.44 ± 8.03 % of NMDAR mediated current in P 11-15 mice (n = 10). This study correlates well with the published result in other cortical areas with the early expression of NR2B followed by an increased expression of NR2A (Monyer et.al, 1997). On the other hand, a selective blocker of NR2B subunit of NMDAR, Ro 25-6981 (0.5 uM) blocks the excitatory transmission between Layer 4 and Layer 2/3 by 21.88 ± 7.4 % in P 11-15 mice (n = 4) and a greater reduction, 45 ± 3.4 % in transmission, is seen in younger (P 6-8) animals (n = 2). This increased expression of NR2A component of NMDAR subunit in the second postnatal week in developing mouse barrel cortex decreases the decay time constant of NMDAR mediated current and regulates the Ca++ influx which might be crucial for the long term plasticity processes. Initial results show the induction of Spike timing dependent LTP in second postnatal week in Layer 2/3 pyramidal neurons which can be blocked when NR2A component of the NMDAR is blocked with 100nM NVP-AAM077.

Traumatic experience facilitates expression of morphine-induced place preference

Barsay Boglárka; Barsvari Beáta; Haller József
Department of Behavioural Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
barsay@koki.hu

Large number of people suffers Post-Traumatic Stress Disorder (PTSD) recognized at the time of Vietnam War. Since that its causing traumas have been widened with terrorist attacks, accidents, sexual assault, social or familiar problems, etc. In an earlier study we have shown that employing electric foot-shock is a good laboratory model of inducing traumatic stress. However, many people with PTSD have gone through some medical treatment receiving morphine, which is still the most widely used anaesthetic drug in serious emergencies. Thus, in the present study we investigated the effect of PTSD on morphine induced addictive behaviour in rats. First, electric shock (3 mA) was employed and then, 24 days later, conditioned place preference (CPP) was applied as experimental model to assess the development of drug-seeking behaviour to subcutaneous morphine challenge (10 mg/kg). After conditioning phase, the drug-seeking behaviour was monitored in the test phase lasted for minimum 2 weeks (4 or more times). Morphine treated animals spent more time in the drug-associated compartment than in the saline administered one. This effect calmed down and ceased at the end of the first week. In contrast, in the group of shock-experienced, morphine treated rats, this effect lasted for 2 weeks. These results indicate that morphine-seeking behaviour was significantly prolonged after traumatic stress compared to the controls. Moreover, the behaviour of foot-shocked animals (receiving no morphine) showed high variability in place preference, possibly due to the traumatic experience itself. Taken together, we showed here the first time that going through once a trauma – causing PTSD – has a high impact on development of drug addiction.

Hyperpolarization-activated current (Ih) and alpha2-adrenergic receptors modulate dendritic spike initiation

Barth A. Miklós; Vizi E. Szilveszter; Zelles Tibor; B. LendvaiBalázs
Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
barth@koki.hu

Recent data highlighted the integrative role of the backpropagating action potentials in neurons. These dendritic signals carry information to the active synapses about the recent activities of the cell in order to detect coincidence with current inputs. In our experiments the role of hyperpolarization-activated current (Ih) was investigated on the backpropagating action potentials and dendritic spike generation in layer 5 pyramidal neurons of the prefrontal cortex. Two-photon laser scanning microscopy was used to detect the calcium transients and dendritic spikes in the apical dendrite evoked by backpropagating action potential trains with different frequencies. Low frequency trains of backpropagating action potentials were filtered out at a gating region. High-frequency trains avoided filtering and initiated all-or-none-type Ca2+ transients corresponding to dendritic spikes. The block of hyperpolarization-activated currents (Ih) by ZD7288 could shift the frequency threshold and decreased the number of action potentials required to initiate spikes. The alpha2-adrenergic agonist clonidine could also shift the frequency coding of spikes to lower frequencies. Our data revealed that receptor activation can influence dendritic spike generation by modulation of Ih.

Projection of retinal afferents to the oculomotor neurons in the frog

Bácskai Timea [1]; Veress Gabor [1]; Matesz Klara [1,2]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center
[2]Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
timi@chondron.anat.dote.hu

The accessory optic system (AOS) is involved in the optokinetic nystagmus and stabilization of retinal image in mammalian and non-mammalian species. The AOS of the frog includes the nucleus of the basal optic root (NBO) and the nucleus lentiformis mesencephali. The AOS influences the activity of the eye moving cranial nerve nuclei by polysynaptic pathways via the pretectal areas as well as the nucleus of the medial longitudinal fascicle. In our earlier cobalt labeling studies we have found that the ventrolateral dendritic array of the nucleus of the oculomotor nerve extends into the area of the NBO suggesting the presence of the monosynaptic connection between the retinal afferents and the oculomotor neurons. In order to examine this possibility we have labeled simultaneously the optic and oculomotor nerve in the frog. On the anesthetized animals, crystals of fluorescein binding dextran amine were applied to the cut end of the oculomotor nerve while the tetramethylrhodamine dextran amine was put on the cut end of the contralateral optic nerve. After 4-5 survival days cross sections of the mesencephalon were made and the specimens were examined in fluorescent microscope. Dendrites of oculomotor motoneurons intermingled with the retinal afferent fibers and terminals within the area of NBO. By using of confocal laser scanning microscope we have found close appositions between the optic nerve terminals and the oculomotor dendrites. The distance between the neighboring profiles suggested close membrane appositions without intercalating glial or neuronal elements. Our results revealed that monosynaptic connection exists between the optic nerve and the oculomotor motoneurons providing the morphological background of the very fast eye movement during body displacement. This work was supported by OTKA K 67641 and MTA-TKI 242.

Diencephalic and brainstem projections of the nucleus accumbens in the domestic chick

Bálint Eszter; Csillag András
Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
pupalibera@yahoo.com

The connectivity of the core (AcC) and shell (AcS) areas of nucleus accumbens (Ac), a part of the avian basal ganglia, was studied using the anterograde tracer biotinylated dextran amine (BDA, 10 kDa). Pressure injections were placed mainly in the AcS, while some injection sites partially covered the core subregion and the mediodorsal region of the medial striatum. The projection patterns of these areas were largely similar, with minor alterations. Most of the efferents remained ipsilateral, however, a few contralateral fibers were apparent primarily in the brainstem. Within the diencephalon, terminating axons were found in the limbic dorsal thalamus, as well as in the nucleus paramedianus internus, habenular complex, mammillary nuclei and in the lateral and paraventricular hypothalamic areas. A massive projection from Ac was detected in the midbrain central gray and also in the catecholaminergic areas including the dopaminergic nuclei substantia nigra and ventral tegmental area, and the noradrenergic locus coeruleus and subcoeruleus nuclei. In the lower brainstem, efferents from AcC, but not AcS, were apparent in the hypoglossal nucleus, the nucleus intercalatus and

nucleus tractus solitarii, reflecting a minor difference between the projection patterns. Retrogradely labelled somata were seen bilaterally in the nucleus tegmentalis ventralis and ipsilaterally in the substantia nigra and the habenular complex. According to our findings, the efferent descending projections of nucleus accumbens in the domestic chick are highly similar to those described in mammals. The projections are consistent with the presumed roles of nucleus accumbens in decision-making, reinforcement learning of locomotor patterns of behavioural relevance (e.g. feeding), and arousal. Grant sponsors: ETT 119/2006, Semmelweis University School of Doctoral Studies

Neuro-ophthalmological changes in migraine

Benedek Krisztina[1]; Janáky Márta[2]; Tuboly Gábor[3]; Braunitzer Gábor[3]; Benedek György[3]; Tajti János[1]; Vécsei László[1]
[1]Department of Neurology, [2]Ophthalmology and [3]Physiology, Faculty of Medicine, University of Szeged, Hungary
benedek@phys.szote.u-szeged.hu

Recent cross-sectional imaging data pointed out that migraine with aura may be progressive. Evoked potential studies of migraine describe decreasing P100 amplitude of VEPs (visual evoked potentials) in migraine with aura as a function of disease duration. Amplitude and latency changes of visual evoked potentials (VEPs) were demonstrated along the time course of the disease, as well as during a single attack. Observations on the of amplitude and latency changes of VEP and PERG recordings in long term disease duration provided controversial results. In our cross-sectional study we applied various electrophysiological methods in order to investigate potentially progressive neuro-ophtalmological changes. Twenty-two adult patients with episodes of migraine with visual aura (diagnosed according to the IHS criteria of the Federation of Neurology), as well as 15 age-matched controls were involved in the study. In the case of migraineurs duration of the illness ranged between 2 months and 33 years. Subjects underwent ERG, Pattern ERG and Pattern VEP measurements. Having performed statistical analysis we found that the amplitude of the oscillatory potentials were increased in patients with short illness history, while longer histories showed a tendency with progressively decreased amplitudes. PERG amplitudes showed a similar decreasing pattern. Pattern ERG peaks and the N135 component of VEP showed increasing latency values. Our results, thus, seem to support the notion on the progressive nature of visual impairment in migraine.

Molecular basis of stress sensitivity

Bilkei-Gorzó András; Astrid Becker; Lihua Wang-Eckhardt; Otto Martin; Baermann Ágnes; Andreas Zimmer
University of Bonn, Bonn, Germany
abiklei@uni-bonn.de

Stress sensitivity varies highly between individuals, and elevated contributes to the pathogenesis of stress related diseases like anxiety disorders or depression. The molecular mechanism underlying these individual differences are not well understood. In this study we have used two genetic mouse models with a deletion of the enkephalin encoding gene Penk1 and the substance P/neurokinin A encoding gene Tac1. These mice are highly stress sensitive (Penk1-/-) or insensitive (Tac1-/-) respectively. To identify brain areas differently activated by stressors in these mouse strains, we evaluated c-Fos activity pattern in limbic structures

after exposure to a zero-maze test. We found a significant difference in the cFos expression upon stress exposition in the amygdaloid nuclei. We next established gene-expression profiles from this brain area in animals kept in stress-free conditions and in animals two hours after the zero-maze stress. Genes showing a significant and more than twofold expression changes were selected and evaluated. We identified two major systems showing opposing changes in the two knockout strains. These may contribute to their opposing anxiety-related phenotype. The expression of transcripts related to the excitatory glutamatergic system was higher in Penk1-/-, while the inhibitory GABA system lower in the Tac1-/- mice as in the wild types. These changes could contribute to the different stress reactivity of the strains. Also, the level of tyrosine kinases and phosphatases changed in different directions in these knockout mouse strains. In agreement with the expression studies, the phosphorylation level of proteins in the amygdala differed after stress in stress sensitive and resistant mice. We suppose that ration of inhibitory and excitatory signalling and differences in protein phosphorylation in the amygdala could be responsible for the differences in stress reactivity.

Phosphorylation/activation of tyrosine hydroxylase (TH) at the median eminence/arcuate nucleus (ME/ARC) region is strictly related to the rate of pituitary prolactin (PRL) secretion

Bodnár Ibolya; Daniel Hechtl; Oláh Márk; Nagy M. György
Neuromorphological and Neuroendocrine Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
bodnar@ana2.sote.hu

Hypothalamic dopamine (DA), the physiological regulator of pituitary PRL secretion, is synthesized in the neuroendocrine DAergic (NEDA) neurons that projects to the median eminence (ME) and the neurointermediate lobe of the pituitary gland. The rate-limiting step of DA biosynthesis is catalyzed by tyrosine hydroxylase (TH) that produces L-3,4-dihydroxy-phenylalanine (L-DOPA) from tyrosine. The activity of TH can be modulated by two mechanisms: 1., medium-, to long-term regulation by changing gene expression and 2., short-term regulation by changing the enzyme activity. The phosphorylation of TH at different Ser residues (Ser31 and Ser40) is critical for the activation of this enzyme. It must be noted that the dephosphorylated TH is inactive. Phosphorylation of TH at Ser40 in situ is the most important as far as the increases of the enzyme activity is concerned. The aim of our present study was to investigate whether two main physiological stimuli, suckling stimulus and separation of the mother from their pups are able to influence the phosphorylation of TH in the arcuate nucleus-ME region of lactating dams. Immunohistochemistry, using antibodies raised against to native as well as phosphorylated TH (pTH) were used to compare their distributions in the area of the hypothalamic NEDA system and in the pituitary of lactating rats without separation (continuously suckling) or following 4 as well as 24 h separation and compaired with the same region of ovariectomized (OVX) females. In OVX females intensely stained TH and pTH-immunoreactive cells at the midlevel of the arcuate nucleus and fairly rich network of inmunoreactive terminals at the external zone of the ME could be detected. In non-separated (continuously suckled) dams no pTH-immunoreactive perikarya or terminals can be found in the same regions. In contrast, after 4 hours separation pTH-immunoreactivity can be visualized in the external zone of ME. Moreover, in dams separated for 24 hours numerous pTH immunopositive cells were seen in the arcuate nucleus. Our findings are the first neuromorphological observations indicating that without suckling stimulus significant changes can occur in the pTH immunoreactivity of the arcuate nucleus and the ME. This work

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Anatomical comparison of 3D ultrastructure of rat and monkey nigro-thalamic terminals

Bodor Ágnes Lívia[1]; Giber Kristóf[1]; Rovó Zita[1]; Ulbert István[2]; Acsády László[1]
[1]Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
acsady@koki.hu

Giant inhibitory terminals with multiple active zones (AZ), the counterparts of excitatory "detonator" or "driver" terminals have not been described in the forebrain. Using 3D reconstructions of electron microscopic images, here we quantitatively characterize a GABAergic pathway, which establishes synaptic contacts exclusively via multiple AZs. Anterogradely labeled axon terminals from the rat substantia nigra pars reticulata (SNR) formed 3-26 symmetrical synapses preferentially on large diameter dendrites and somata of relay cells in the ventromedial nucleus of the thalamus. All synapses of a given terminal converged on a single postsynaptic element. The majority of the AZs was localized close to the edge of the bouton surface facing the postsynaptic membranes and was not separated by astrocytic processes. The central part of the synapse bearing surface was occupied by a network of puncta adhaerentia (PA). All terminals were ensheathed by glial processes. Nigrothalamic terminals in the macaque monkey showed the same ultrastructural features both in qualitative and quantitative terms (average bouton volume, 2.3 µm3 vs. 3.0 µm3; number of AZs per bouton, 10.2 vs. 8.5; nearest neighbor synaptic distances, 190 nm vs. 226 nm in the rat and monkey respectively). We propose that the structural features of nigrothalamic terminals underlie the efficacy of this pathway. The arrangement of AZs permits intersynaptic spillover of GABA among the multiple release sites of a single bouton. The possible consequences of such spillover are larger charge transfer, lower trial to trial postsynaptic response variability and a less pronounced short-term depression during high frequency activity.

Adaptation of glutamatergic transmission following repeated 4-aminopyridine treatment. A hippocampal slice study

Borbély Sándor[1]; Dobó Endre[2]; Czégé Dávid[1]; Mihály András[2]; Világi Ildikó[1]
[1]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
[2]Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary
bosa@ludens.elte.hu

Convulsants usually cause serious insults in brain, including neuronal loss, or axon sprouting, leading to hiperexcitability or developing of secondary seizures. The goal of our present study was to investigate the effect of repeated 4-AP administration on neuronal excitability.
Experiments were carried out on adult male rats treated with 4.5 mg/kg 4-AP (4-AP treated group) or physiological saline (control group) on 12 consecutive days. In electrophysiological investigations the changes of evoked population spike responses following single or paired-pulse stimulations were analyzed in hippocampal CA1 region. Eliciting of long-term potentiation (LTP) and inhibition of glutamate receptors (APV and GYKI 52466 application) were compared in control and 4-AP treated group. Alterations in non-NMDA receptors

activity were also tested by quantitative Co^{2+} uptake determination. Structure and localization of glutamate receptor subtypes was analyzed in 10µm horizontal slices by histoblot method.
The amplitude of population spike was not affected by APV and was enhanced after tetanization in the same degree in both treatment groups. GYKI 52466 exerted stronger inhibition in control group compared to 4-AP treated group. Glutamate receptor antagonists had no effect on short-term facilitation; however, following LTP induction short-term depression developed at 100 ms and 50 ms intervals, which was more pronounced in 4-AP treated slices. Study on Co^{2+} uptake revealed weak rise of non-NMDA receptor conductivity in CA1 and CA3 area caused by 4-AP administration. Histoblot staining showed reduction of GluR2 subtype and increase of GluR1flop and NR2A receptor subtypes in CA1 area. In CA3 region, however, rising number of GluR1, GluR1flop and NR2A subtypes was observed.
Our data indicate that 4-aminopyridine may induce tolerance against subsequent convulsive actions. Repeated exposure of convulsant (4-aminopyridine) affects mainly AMPA receptor activation in hippocampus, supposing an important receptor subunit composition rearrangement.

Embryonic development of a neuropeptidergic system in the earthworm Eisenia fetida

Boros Ákos; Molnár László; Pollák Edit; Herbert Zsófia
Department of General Zoology, University of Pécs, Pécs, Hungary
borosakos@gmail.com

Capa peptides are the most abundant neuropeptides of the insect neurosecretory and neurohaemal system and they are occurring in certain interneurones, as well. Members of the Capa peptide family (periviscerokinins and pyrokinins) were primarily found in insect species investigated. Recently we have identified six new Capa peptides in the central nervous system of the adult specimens of the earthworm E. fetida. This study focuses on the investigation of the development of CAPA peptide expressing structures during the earthworm embryogenesis. The first Capa peptide expressing neurons were detected in the brain and subesophageal ganglion at E1 embryonic 20%). In the ventral nerve cord (VNC) labelled cells with≈developmental stage (ipsilateral projections were found at E2 developmental stage. During embryogenesis additional Capa peptide-expressing neurons with contralateral projections appeared in the central nervous system till the last E5 developmental stage (hatching). Pattern of labelled somata and fibres were similar to those found in adult specimens, however, the cell number was significantly lower in E5 embryos than in adult worms suggesting that the development of this peptidergic system does not terminate at hatching. Capa peptide expressing cells, identified as primary sensory cells, occurred in the ectoderm from E2 developmental stage. The number of labelled primary sensory cells increased till hatching when their pattern became similar to those found in adult worms. Quantitative analysis of Capa peptides expression during embryogenesis showed steady increase of the peptide level in embryos that supports our immunohistochemical results.

Responses to a chromatic and cone-isolating contrast in the primary visual cortex of dichromatic marmosets revealed by optical imaging

Buzás Péter[1,2]; Helen Lockrey[2]; Brett A. Szmajda[2]; Paul R. Martin[2]
[1]Institute of Physiology, University of Pécs, Pécs, Hungary
[2]National Vision Research Institute and Department of Optometric and Vision Sciences, University of Melbourne, Australia
peter.buzas@aok.pte.hu

Signals from short-wavelength-sensitive (S-) cones of the primate retina travel primarily in the koniocellular layers of the lateral geniculate nucleus. It has been, however, controversial whether they remain spatially segregated at the level of the primary visual cortex (V1), where detailed mapping has been hampered by the sparseness of S-cone driven neurones. Male marmosets provide an ideal system to study S-cone signals, since they only possess a single cone type sensitive to medium-long wavelength (ML) light whereas their S-cone pathways appear identical to those of trichromatic individuals. Here, we investigated the dependence of the intrinsic optical signal on luminance contrast and colour in V1 of anaesthetised dichromatic marmosets (n=3). Stimuli were drifting gratings (0.4 cycles/deg spatial frequency, 4 Hz temporal frequency), modulated along achromatic, ML- or S-cone isolating chromatic axes. Optical imaging of the V1 foveal representation was performed and the baseline-normalised change of the negative oximetry signal (\"initial dip\") was used as response measure. The response peak appeared 6-7 s after stimulus onset in all stimulus conditions. Contrast response to achromatic gratings showed Naka-Rushton type characteristics with a half-saturation contrast around 32% on average. Both ML- and S-cone isolating responses were significantly above the blank response but revealed no consistent spatial pattern. The ML-cone isolating responses were 5.4±0.77 times greater than S-isolating ones at equal cone contrast. These relative signal amplitudes are slightly greater than predicted from the low (~5%) proportion of S-cones in the retina.

Reciprocal modulation of G-protein signaling between cannabinoid CB1 and GABAB receptors in rat brain hippocampus

Cinar Resat[1]; Katona István[2]; Mackie Ken[3]; Freund F. Tamás[2]; Szűcs Mária[1]
[1]Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
[2]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[3]Department Psychology and Brain Sciences, Indiana University, Bloomington, USA
resat@brc.hu

Cannabinoid CB1 and GABAB receptors have been shown to display several similar pharmacological effects and co-localization in certain brain regions. Previous studies have reported a functional link between the two systems. As a first step to investigate the underlying molecular mechanism, here we show cross-inhibition of G-protein signaling between GABAB and CB1 receptors in rat hippocampal membranes. The CB1 agonists R-Win55,212-2 and CP55,940 displayed high potency and efficacy in stimulating Guanosine-5'-O-(3-[35S]thio)triphosphate, [35S]GTPγS binding. Their effects were completely blocked by the CB1 antagonists AM251 suggesting that the signaling was via CB1 receptors. The GABAB agonist baclofen and SKF97541 also elevated [35S]GTPγS binding by about 60%, with potency values in the micromolar range. Phaclofen behaved as a low potency antagonist with an an ED50 ≈ 1 mM . However, an ultra-low dose (10 nM) of phaclofen slightly but significantly lowered the maximal stimulation of [35S]GTPγS binding compared to that obtained with the CB1 agonist Win55,212-2 alone. Combination of the specific CB1 antagonist AM251 (1 nM) with the GABAB receptor agonist SKF97541 also uncovered a new functional entity that displayed a ligand binding profile distinct from that of the individual receptors. These results imply that GABAB and cannabinoid CB1 receptor interactions reciprocally inhibit G-protein signaling and profoundly change the pharmacology of the individual receptors. Supported by NKTH DNT 08/2004 and OTKA TS 049817 research grants.

Repeated mild seizures reduce non-NMDA receptors activity in rat somatosensory cortex

Czégé Dávid[1]; Dobó Endre[2]; Borbély Sándor[1]; Mihály András[2]; Világi Ildikó[1]
[1]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
[2]Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary
felagund@tolkien.hu

Kainate or pilocarpine provoke serious seizure and cause permanent discharges following application in one dose. Convulsant effect of 4-aminopyridine (4-AP) is, however, temporary, status epilepticus never develops following the treatment. In the present series of our experiments the alterations were investigated which developed in somatosensory cortex of rats as a consequence of repeated mild seizures following 4-AP application. Changes in basic excitability, synaptic plasticity and glutamaterg receptor sensitivity were investigated in electrophysiological and hystochemical investigations.
The permanent 12-day-long 4-AP pretreatment resulted in a decrease in basic excitability, amplitude of the early component of evoked field potentials become significantly lesser, the late component, however, showed mild increase. Following repetitive stimulation smaller evoked response increase was detected. AMPA type glutamate receptor antagonist GYKI 52466 effectively decreased the amplitude of early component in control slices, but it had slighter effect in 4-AP pretreated slices. In the case of late component the effect of NMDA antagonist APV was more enhanced in the 4-AP treated slices, while it was moderate in controls. Co^{2+}-uptake experiments showed a decreased AMPA receptor activation in slices from pretreated rats comparing to controls. Results of histoblot investigations show that the number of GluR1flop AMPA receptor subtype decreased considerably, while level of NR2A and KA2 receptors increased, respectively.
In summary we can conclude that basic excitability of slices from 4-AP pretreated animals decreased versus control slices. Our data suggest that this reduced activity presumably caused by the shrinkage of the active AMPA receptor fraction or by changes of their excitability. The small increase in NMDA receptor activation might underlay in the enhanced late component after the 4-AP treatment. The overall hypoexcitability is presumably due to the AMPA-receptor decrease, which also regulates NMDA receptor activity through the appropriate current generation for ion-channel opening. The unexpected reduction in the late component after the repetitive stimuli suggests that there are also other potent uninvestigated factors that have influence in the regulation of this process.

Neurochemical characteristics of basalocortical projecting neurons located in the basal forebrain, and their relationships to glutamatergic innervation

Csáki Ágnes[1]; Csaba Zsolt[1]; Halász Béla[2]; Kiss József[2]
[1]Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary
[2]Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
csaki@ana2.sote.hu

Neurons located in basal forebrain cholinergic (BFC) regions may contain cell types different in transmitter-content, and peptide-containing neurons which are known to project to various cortical areas. We examined the neurochemical nature of neurons in the internal capsule, projecting to the entorhinal cortex (EG). Retrograde tracing of parent cells, immunostained

for choline-acetyltransferase (ChAT), parvalbumin (PV) or calretinin (CR) was investigated after microinjections of wheatgerm-agglutinin (WGA)- conjugated with colloidal gold, fluoro-labelled 0.04 micrometer microbeads, or fluorogold as retrograde tracers into the entorhinal cortex. The contributions of glutamatergic axon terminals containing vesicular glutamate transporters type1 or 2 (VGluT1; VGluT2) in their axon terminals in the innervation of BFC-neurons projecting to the entorhinal cortex have been investigated. For this study, light-, and electron microscopic (LM; EM) immunocytochemistry by fluorescence or silverintensified immunogold labelling were used. – LM-WGA-gold labelling revealed large number of silver-intensified gold-labelled neurons in the internal capsule. The majority of the labelled cells were observed in these regions, containing a continuous collection of cholinergic projection neurons referred to as cholinergic space. WGA-gold labelling was similar to those contained retrogradely fluoroscence labelled neurons. Combinations of retrograde labelling of the parent cells projecting to EC with immunocytochemistry for ChAT, PV and CR the neurochemical characters of basalo-cortical projecting neurons were also revealed. The majority of retrogradely labelled neurons (350 double-labelled out of 664 single WGAgold positive) was also immunoreactive for ChAT; less of labelled cells (269 out 562 WGAgold labelled) contained also CR and only few neurons (10 out of 228 retrogradely labelled) were PV-immunoreactive. EM investigations revealed: occurrence of VGluT1 and VGluT2-labelled axon terminals in the caudal area of the internal capsule; VGluT2-positive boutons were seen to establish asymmetric type of synaptic contacts with ChAT-positive dendrites.- Due to our EM findings it may be supposed that glutamatergic inputs on basalocortical projecting neurons can modulate the effects of this afferentation in the entorhinal cortex. Support: OTKA T-049455 (J.K.), ETT 020/2006, Hung. Acad. Sci.

Special features of sleep slow oscillation in humans

Csercsa Richárd[1]; Magony Andor[1]; Fabó Dániel[1]; Entz László[2]; Wittner Luca[1]; Erőss Loránd[2]; Ulbert István[1]
[1]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
[2]National Institute of Neurosurgery, Budapest, Hungary
csercsa@cogpsyphy.hu

Slow oscillation (SO) detected in deep stages of sleep plays an important role in memory consolidation and other homeostatic brain functions. SO in vitro, under anesthesia, and in natural slow wave sleep (SWS) in animals is composed of rhythmically recurring phases of hyperpolarization with cell firing secession called down-state, and depolarization with high firing rates called up-state. In the human scalp EEG, down-state appears as a surface negative and the up-state as a surface positive potential with superimposed spindle and higher frequency oscillations. However, we have no information about the cellular and trans-membrane events occurring in different layers of the human cortex during SWS. Here we provide the first direct experimental evidences that supragranular layers play a leading role in SO generation, moreover cell firing during up-state is relatively sparse, unstructured and near probabilistic. Patients with drug resistant epilepsy were implanted with laminar multielectrodes in the frontal cortical region. Current source density (CSD), multiunit activity (MUA), single unit activity (SUA) and time frequency analysis (TFR) were performed on the signal recorded during overnight sleep. We found that supragranular oscillatory power, cell firing and estimated trans-membrane currents were larger than the same measures in infragranular layers. Supragranular cells fired more often earlier after the onset of the up-state than infragranular cells. These observations contrast the idea that SO is generated in the infragranular layers. Firing rates were about 1/10th of what was previously observed

emphasizing excitability differences between human and animal cortical neurons. Compared to lower level mammals, primate and human brain size and cognitive capacity is enormously enlarged reflected in increased metabolism and corticalization, which is compatible with the observed major supragranular activation. The increased human glia-neuron ratio may enhance glial buffering and thus implicate relatively lower cellular excitability. In addition, sparse representation of long term memory traces in humans may be better supported by a sparse firing strategy during memory consolidation periods of SWS.

Reaction kinetic models of rapid (g-protein-dependent) and slow (beta-arrestin dependent) transmission

Csercsik Dávid[1]*; Hangos M. Katalin*[1]*; Nagy M. György*[2]
[1]Process Control Research Group, Systems and Control Laboratory, Hungarian Academy of Sciences
[2]Department of Human Morphology, Semmelweis University, Budapest, Hungary
csercsik@scl.sztaki.hu

A diverse array of external stimuli, including neurotransmitters, hormones, phospholipids, photons, odorants, taste ligands and mitogens, bind to specific G-protein coupled receptors (GPCRs) on the cell surface, which subsequently interact with their respective G proteins to induce downstream intracellular signaling. Following GPCR activation, The ligand-bind receptor can be phosphorylated by GPCR kinases (GRKs). Thereafter Beta-arrestins bind to the receptors to uncouple them from G-proteins (desensitization) leading to an attenuation of G-protein-dependent GPCR signaling, and the formation of an endocytic protein signaling complex. In our present study the qualitative dynamic behavior of reaction kinetics of G-protein-dependent as well as the Beta-arrestin-dependent signaling is examined via mathematical modeling based on the theory of reaction kinetic networks. A simplified basic G-protein signaling structure is defined, which is extended to be able to take into account the Beta-arrestin-dependent or slow transmission as well as the RGS mediated and ERK/phosphatase mediated feedback regulation. The resulting model gives rise to an acceptable qualitative approximation of the G-protein-dependent and independent ERK activation dynamics. This work was supported by the Hungarian National Fund (OTKA K-68170 and 102 266) and Ministry of Health (ETT T-448/2006) to NGM.

Potential signaling role of aspartate in striatal synaptic circuits of the domestic chick

Csillag András; Ádám S. Ágota; Zachar Gábor; Bálint Eszter
Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
csillag@ana.sote.hu

Subpallial structures are known to be highly conserved across the different vertebrate species. They are instrumental in the neural processing relevant to adaptive learning, decision making, motivation and behavioural strategies. The medial striatum (MSt), formerly known as the lobus parolfactorius (LPO) has now been parcellated into subregions, also including part of the nucleus accumbens (Ac). Similar to mammals, the avian Ac and MSt receive input from the pallium, the dopaminergic centres of midbrain (substantia nigra, ventral tegmental area), and the arcopallium/amygdala. Furthermore, a critically timed and balanced coincidence between the glutamatergic and dopaminergic synaptic activities in the ventral/medial striatum, including the Ac, is required for memory to be formed for a given pairing of stimulus and

hedonic quality or behavioural salience. The underlying mechanism involves the activation of NMDA and D1 dopaminergic receptors, as well as the phosphorylation of DARPP-32. Using ultrastructural electron microscopy of chick specimens double-labelled against glutamate and DARPP-32 we observed direct synaptic connections between glutamate and aspartate immunoreactive axon terminals and DARPP-32 labelled dendrites in the MSt. In our studies we described the excitatory amino acid L-aspartate (Asp) to partially colocalise with L-glutamate in a number of neurons of the amygdala and other arcopallial regions of chicks. A considerable percentage of such neurons, however, was found to contain only Asp. Asp-immunoreactive axon terminals with or without colocalisation of glutamate were found to form asymmetrical (putative excitatory) synapses with dendritic profiles of the MSt and nucleus accumbens. We also verified the amygdalar origin of such axon terminals by pathway tracing (BDA) combined with postembedding immunoelectron microscopy. It can be surmised that Asp may play the role of a signalling molecule in the avian MSt/accumbens, potentially associated with the aversive response. The presence of Asp in the intermediate medial mesopallium (former IMHV) - arcopallium/amygdala – MSt loop, implicated in passive avoidance learning of domestic chicks (Csillag, 1999), may represent a novel mechanism of neural modulation associated with early adaptive learning and motivation. Grant sponsors: ETT 119/2006; Semmelweis University School of Doctoral Studies.

Oncomodulin-immunopositive axons in parvalbumin knock-out animals: axonal regeneration in the adult central nervous system

Csillik Bertalan[1]; Mihály András[1]; Losonczi Erika[1]; Knyihár-Csillik Elizabeth[1]; Schwaller Beata[2]
[1]Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary
[2]Unit of Anatomy, Department of Medicine, University of Fribourg, Fribourg, Switzerland
csillik@anatomi.szote.u-szeged.hu

Coexistence of GABA and the calcium binding proteins in recently discovered giantic calyciform presynaptic terminals of the reticular thalamic nucleus (Csillik et al, 2002, 2005) raises the question whether or not, GABA and calcium binding proteins are not only structurally but also genetically related to eachother (Csillik et al, 2006). In young adult male homozygous parvalbumin-knockout (PV -/-) mice (Schwaller et al, 1999) we found that GABA immunoreaction of giantic calyciform presynaptic terminals persisted while PV disappeared completely, not only from neuronal parikarya but also from calyciform presynaptic terminals. At the same time, numerous, partly varicous PV immunoreactive axons appeared in the diencephalon, mainly in the lamina medullaris externa surrounding the thalamus.These axons were found to contain oncomodulin or beta-PV, a protein which was known until now to be present in normal mammals only in the Corti organ of the inner ear (Yang et al, 2004) . Newly generated oncomodulin-immunopositive fibres are closely related to oncomodulin-immunoreactive cells scattered in the thalamus. Accordingly, our studies seem to support a recent theory about the origin of oncomodulin from macrophages (Yin et al, 2006), and, nevertheless, prove the involvement of oncomodulin in promoting axonal outgrowth in the diencephalon of parvalbumin-depleted animals. The therapeutical possibility to improve axonal regeneration in the central nervous system by application of oncomodulin presents an unsurpassed challenge for neuropharmacologists and clinical neurologists alike. References Csillik B, Pálfi A, Gulya K, Mihály A, Knyihár-Csillik E: Somato-dendritic synapses in the nucleus reticularis thalami of the rat. Acta Biol Hung 53: 33-41. 2002. Csillik B, Pálfi A, Gulya K, Samsam M, Mihály A, Vécsei L, Knyihár-Csillik E: Parvalbumin immunopositive large presynaptic complexes in the reticular nucleus of the rat. Acta Physiol.

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Pharmacological characterization of LPS-induced Il-1β release in murine brain-an in vivo study

Csölle Cecilia; Sperlágh Beáta
Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Hungary Academy of Sciences, Budapest, Hungary
csolle@koki.hu

1. Interleukin 1β (IL-1β) is a key regulator of inflammation and the immune response. Extracellular ATP and its analogue can trigger the release of IL-1β through its binding to P2 purinergic receptors, especially the $P2X_7$ receptor ($P2X_7R$), which expressed predominantly by microglial cells, the main source of proinflammatory cytokine in the brain. We investigated cytokine response of hippocampus to bacterial lipopolysaccharide (LPS) in vivo was altered in rat and in $P2X_7R^{-/-}$ mice compared to wild-type (WT) mice. We have also pharmacologically characterized the P2 receptor involved in LPS-induced release of IL-1β.
2. Intraperitoneal injection of LPS (*Escherichia coli*) caused release of IL-1β in rat hippocampus in a concentration-dependent (300-500 μg kg^{-1}) and time-dependent manner (2-6 h). Both of endogenous ATP- and LPS (300 μg kg^{-1})-induced response was attenuated by PPADS (25 mg kg^{-1}) and by the selective $P2X_7$ receptor antagonists BBG (100 mg kg^{-1}) and oxidised ATP (oATP; 100μM kg^{-1} at 1ml kg^{-1}) injected intraperitoneally before the induction of infammation.
3. LPS (250 μg kg^{-1}) significantly increased IL-1β in the hippocampus of WT mice 6 h after treatment. This increase in concentrations of IL-1β were almost totally reduced by BBG (100 mg kg^{-1}) and oATP (300μM kg^{-1} at 1ml kg^{-1}). Level of IL-1β was significantly less in the hippocampus of $P2X_7R^{-/-}$ than in the hippocampus of WT mice in response to systemic endotoxin. LPS-induced response was also strongly inhibited by oATP (300μM kg^{-1} at 1ml kg^{-1}) and by BBG (100 mg kg^{-1}) in the absence of $P2X_7$ receptor.
4. In conclusion our data confirm the involvement of purinergic signalling, especially the $P2X_7R$ in LPS-induced increases in the concentraion of Il-1β in murine hippocampus.

Differences in neurochemical features and autonomic innervation of brown and white adipose tissue

Darida Miklós[1]; Dénes Ádám[1]; Windisch Katalin[2]; Vizi E. Szilveszter[2]; Kovács J. Krisztina[1]
[1]Laboratory of Molecular Neuroendocrinology

[2]Laboratory of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
darida@koki.hu

The physiological role of brown (BAT) and white adipose tissue (WAT) in metabolism is different. WAT serves as an energy storage and releases fuels as necessary, while BAT dissipates energy in the form of heat. Several studies have focused on the local innervation and the central neural control of these tissues. However, the common and distinct neuronal circuits involved in the central regulation of BAT and WAT as well as existence of parasympathetic innervation in WAT are not clear. To address these questions, two isogenic pseudorabies virus (PRV) derivatives expressing different reporter genes were used for retrograde transneuronal viral tracing. BDL (expressing beta galactosidase) was injected into the epididymal WAT, BDG (expressing GFP) was inoculated into the interscapular BAT. In general, the majority of the infected brain areas were overlapping in the case of the two virus strains but only a small population of neurons were infected with both viruses. Somatotopic appearance of BDG and BDL was observed in several brain sites, within the same nuclei. In the dorsal motor nucleus of the vagus (DMV), only retrograde BDL infection from the epididymal WAT was present, which was also confirmed by monosynaptic cholera toxin labeling. This suggest that parasympathetic nerve fibers innervate the WAT. To test this hypothesis we studied the uptake of H3-labelled norepinephrine (NE) and acetylcholine (ACh) in BAT and WAT in vitro, and their release evoked by electric field stimulation. Both types of adipose tissues were found to take up both transmitters, altough ACh uptake was 10 times, NE uptake was 5 times higher in BAT than in WAT. In contrast, ACh release after stimulation was 10 times higher in WAT than in BAT, and NE realease from WAT was approximately 65% that of BAT, compared to their basic uptake. Both local and intraperitoneal administration of the noradrenergic cytotoxin 6-hydroxi-dopamine (6OH-DA) profoundly reduced NE uptake and abolished NE release, but retrograde viral infection from WAT was prevented only when 6OH-DA was injected locally. Our results indicate that (1) the parasympathetic nervous system contributes to the innervation of WAT and (2) raise the possibility that fat cells might be directly involved in the peripheral regulation of NE in adipose tissues.

Effect of labyrinthectomy on the molecular composition of perineuronal net in the vestibular nuclei of the rat

Deak Adam[1]; Bacskai Timea[1]; Matesz Klara[1,2]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center
[2]Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
adam@chondron.anat.dote.hu

Earlier studies demonstrated that labyrinthectomy results in postural deficits and disturbances in the eye movements of mammalian species. The symptoms are restored spontaneously during the process of vestibular compensation. The molecular mechanism in the background of vestibular compensation is not yet fully understood. Recent studies have shown that macromolecules of the extracellular matrix (ECM) have an important role in the neural plasticity having either a permissive or non-permissive role in the restoration of function. A characteristic place of occurrence of ECM is the so-called perineuronal net (PN) around the perikaryon, proximal dendrites and axon hillock. The PN plays an important protective role in the maintenance of normal function of neurons and in the stabilization of synaptic

connections. In our previous study we have demonstrated that the expression pattern of hyaluronan (HA) is changing in the vestibular nuclei of the rat during the vestibular compensation. The present work was undertaken to examine the expression of chondroitinsulfate proteoglycans (CSPG) in the PN of the vestibular nuclei. Experiments were performed on adult rats. On anesthetized animals the labyrinth was approached via the external auditory meatus and the vestibular sense organs were destroyed mechanically. The animals were sacrificed at different times and the excised brainstem was immersed into Sainte-Marie's fixative. The paraffin embedded specimens were cut in transverse plane and biotinylated Wisteria floribunda was applied to detect the CSPG. During the 1st, 3rd postoperative days the intensity of WFA reaction decreased in the PN around the neurons of vestibular nuclei bilaterally with more pronounced decrease on the operated side. The perineuronal net was not distinguished from the surrounding neuropil indicating the desorganization of its molecular structure. By the time of 7th day the intensity of WFA reaction increased at both sides and the morphology of PN restored indicating the stabilization of newly established synaptic contacts. Our results demonstrated that the expression pattern of CSPG in the PN of the vestibular nuclei of the rat shows similar changes to that of the HA during the vestibular compensation. The dynamic of changes shows close correlation with the restoration of vestibular function. This work was supported by OTKA K 67641 and MTA-TKI 242.

Type 1 cannabinoid receptor (CB1) is present in axons innervating hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus (PVN)

Deli Levente[1], Wittmann Gábor[1]; Lechan Ronald M.[2]; Watanabe Masahiko[3]; Liposits Zsolt[1]; Fekete Csaba[1,2]

[1]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

[2]Tupper Research Institute and Department of Medicine, Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, New England Medical Center, Boston, USA

[3]Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan

turekne.marta@koki.hu

Hypophysiotropic thyrotropin-releasing hormone (TRH) - synthesizing neurons of the hypothalamic paraventricular nucleus (PVN) have a critical role in the regulation of the energy homeostasis through control of the hypothalamic-pituitary-thyroid axis. Recently, endocannabinoids have been shown to exert inhibitory effects on TRH neurons via the type 1 cannabinoid receptor (CB1) (Di et al., J Neurosci 2003). To understand the anatomical basis for this regulatory mechanism, we determined whether CB1 is contained in axons innervating hypophysiotropic TRH neurons using a recently developed antiserum against the C-terminal portion of mouse CB1 (Fukudome et al., Eur J Neurosci, 2004 Kawamura et al., J Neurosci 2006). CB1-immunoreactive (IR) axons innervated the majority of hypothalamic nuclei, including the PVN, where a dense network of CB1-IR axons was observed with punctuate appearance (Wittmann et al., J Comp Neurol 2007). Ultrastructurally, CB1-immunoreactivity was observed in the preterminal portion of axons establishing both symmetric and asymmetric synaptic specializations with parvocellular neurons and with the perikarya and dendrites of all TRH neurons in the PVN.

These data demonstrate that CB1 is abundantly present in axons that are in synaptic association with hypophysiotropic TRH neurons, indicating an important role for endocannabinoids in the regulation of the hypothalamic-pituitary-thyroid axis. The presence

of both symmetric and asymmetric type CB1 synapses on TRH neurons in the PVN suggests that endocannabinoids may have both excitatory and inhibitory effects on these neurons.
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CX3CR1 (fractalkine receptor) deficiency results in reduced brain damage and neuronal apoptosis in a mouse model of focal cerebral ischemia

Dénes Ádám[1]; Halász József[2]; Ferenczi Szilamér[1]; Kovács J. Krisztina[1]
[1]Laboratory of Molecular Neuroendocrinology, Institute of Experimetnal Medicine, Budapest, Hungary
[2]Laboratory of Behavioural and Stress Studies, Institute of Experimetnal Medicine, Budapest, Hungary
kovacs@koki.hu

CX3CR1 is the unique known receptor of fractalkine, which has been implicated in several inflammatory processes such as recruitment, adhesion and vascular transmigration of leukocytes. In the brain, preserved function of CX3CR1 was shown to be important for sustaining normal microglial activity, while absence of the receptor results in cell-autonomous microglial neurotoxicity *in vivo*. Transient occlusion of the middle cerebral artery (tMCAo) is considered to be proper experimental model of human stroke, in which, role of microglial cells (both acutely and late after reperfusion) is not well understood. Since microglia is the only resident cell type in the brain, which constitutively express CX3CR1, we performed 60 minutes MCAo followed by different reperfusion intervals to examine microglial inflammatory function in stroke. Surprisingly, we observed significant reduction of ischemic brain damage in CX3CR1 deficient (CX3CR1 -/-) animals compared to CX3CR1 +/- and wild type mice after 72 hours of reperfusion. The rostro-caudal extension of the damage in the ipsilateral cerebral cortex was much smaller in K.O. mice, while the thalamus as well as the hippocampus were usually not affected, unlike that seen in mice expressing CX3CR1. Blood brain barrier damage identified by IgG staining showed similar results. General recovery of CX3CR1-/- animals was much better after tMCAo, which was associated with significantly less sensory-motor disfunction as identified by sticky tape test. Annexin V immunostaining revealed more apoptotic cells in mice expressing CX3CR1, resulting in increased cell loss in the cortex, thalamus and hippocampus. Similarly to our previous findings, larger cortical infarcts in heterozygous and wild type mice leaded to reduced microglial cell numbers in the ischemic cortex. In contrast, no difference in the number of IL-1 beta expressing microglia or in plasma IL-1 beta concentration was observed among mice with different CX3CR1 genotypes. These findings indicate that acute microglial neurotoxicity itself does not result in increase of brain damage after tMCAo, furthermore, absence of CX3CR1-fractalkine signaling might cause reduced capacity of peripheral inflammatory cells to contribute to the exacerbation of ischemic damage after stroke.

Immunolabelling of vimentin in the hypothalamo-neurohypophysal system of one humped camel Camelus dromedarius

Djazouli Alim Fatma Zohra[1]; Lebaili Nemcha[2]; Mahy Nicole[3]
[1]Department of Biology Faculty of Agro-veterinary and Biological Science University Saad Dahleb, Blida, Algeria
[2]Laboratory of Animal Physiology, ecole normal Kouba, Algeria

[3]Facultat de Medicina Unitat de bioquimica universitat de Barcelona, Spain
zahralim@yahoo.com

Our work is interested to study the structural relation dynamic of astrocytes glial cells with Magnocellular neuron of dromadary hypothalamo-neurohypophysal system (HNS) by immuno-peroxydase labelling of vimentin a cytoskeletal intermediate filament which is expressed primordially in radial glial cells of developing brain; conferring to them a high degree of flexibility. The results obtained reveal a positive immunoreactivity of an astrocytic filamentous structures in the different levels of the HNS comparing to its adjacent areas. In the supraoptic and paraventricular hypothalamic nuclei; these prolongement are arranged between MCN presenting an abundance nerosececretory material in their cytoplasm. In the neural lobe these astrocytic immunopositive structure are concentrated outside the islet containing neurosecretary endings of MCN witch are known to engulf them in unstimulated case. This funding allowed us to conclude that the HNS of dromedary is a very dynamic system and that vimentin would take part in the great plasticity of its astrocytes, by preserving their immature character they may best change their anatomical shape to support MCN and to enhance the liberation of neuropeptides into blood stream maintaining consequently water balance.

Distribution of mRNAs encoding transforming growth factor beta 1, 2 and 3 in the rat brain

Dobolyi Árpád
Department of Anatomy, Histology, Embryology, Semmelweis University and Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Science, Budapest, Hungary
dobolyi@ana.sote.hu

Transforming growth factor beta (TGFbeta) 1, 2 and 3 form a small group of related proteins that are involved in the regulation of proliferation, differentiation and survival of various cell types. In the central nervous system, TGFbetas were recently demonstrated to be neuroprotective following ischemic insult, increase survival of midbrain dopaminergic neurons and motoneurons, regulate neurogenesis, neuronal differentiation and plasticity in the hippocampus, and directly influence autonomic and neuroendocrine functions. TGFbetas are activated in the extracellular space *via* latent TGFbeta binding proteins, with which they are co-released in an inactive complex. We recently reported distinct distributional patterns of the 4 types of latent TGFbeta binding proteins in the rat brain. Based on these data, we hypothesized different patterns for expression of TGFbetas, as well. Probes were produced for all 3 types of TGFbetas and radioactive *in situ* hybridization histochemistry was performed on serial rat brain sections followed by emulsion autoradiography. We demonstrated significant expression of TGFbeta 1 in layer IV of the cerebral cortex, hippocampus, medial preoptic area, paraventricular hypothalamic and central amygdaloid nuclei, brainstem motoneurons, area postrema and cerebellum. In contrast, TGFbeta 2 was expressed in deep layers of cerebral cortex, anterior olfactory nucleus, dentate gyrus, midline thalamic nuclei, supraoptic nucleus, mamillary body, brainstem auditory nuclei, areas of monoaminergic neurons, nucleus of the solitary tract, area postrema, and the choroid plexus while TGFbeta 3 mRNA was found in the cerebral cortex, CA2 region of the hippocampus, dentate gyrus, midline and reticular thalamic nuclei, supramamillary and lateral amygdaloid nuclei, periaquenductal gray, superior colliculus, tegmental nuclei, brainstem auditory nuclei, motoneurons, and inferior olive. The different distributional patterns of the expression of the subtypes of TGFbetas suggest that

they are involved in the regulation of different neurons. Our results also suggest that TGFbeta subtypes bind different latent TGFbeta binding proteins enabling them to be activated by different stimuli.
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Comparison of auditory information processing in sleep and anesthesia

Dombovári Balázs; Grand László; Wittner Lucia; Karmos György; Ulbert István
[1]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
dombovari@cogpsyphy.hu

The auditory information stream invades the acoustic system continuously, with virtually no opportunity of intentional attenuation. As the vigilance state changes towards quiescence and sleep, one of the most important modality in alerting is audition. Sleep maintenance and steady environmental monitoring is an antagonistic task. The acoustic system has to protect sleep, while it also has to activate arousal mechanisms on demand. Our aim was to investigate the nature of cortical information processing and compare it in natural non-REM sleep and ketamine anesthesia. Cats were chronically implanted with laminar electrodes into the auditory cortex in addition to epidural, hippocampal, EOG, EMG electrodes and bone conductor to deliver auditory stimuli. To map acoustic responses, we used single click stimulus paradigm. Slow oscillation (SO) in ketamine anesthesia is described by two alternating states: depolarized up-states and hyperpolarized down-states. Up-states were characterized by surface positivity, inward currents in the middle cortical layers, increased cell firing and increased oscillatory power. Down-states were typified by surface negativity, outward currents in the middle cortical layers, decreased cell firing and decreased oscillatory power. Single click stimuli in the up-state often evoked a down-state after an initial transient response and vice versa under ketamine anesthesia. In the cat auditory cortex, during natural non-REM sleep, although the sleep cycle seemed to be intact, we failed to observe the typical signs of SO, despite of months of sleep training. Single click stimuli delivered during natural non-REM sleep, evoked a surface negative field potential component similar to down-state. This component was also characterized by decreased cell firing and oscillatory power. The main difference between the sound evoked hyperpolarization in natural non-REM sleep and ketamine anesthesia was their duration, being 100ms and 200ms respectively. Our results show that in natural sleep, auditory cortex may not be involved in SO generation to the extent that other brain regions are, however, ketamine anesthesia can bring the acoustic cortex into a slow oscillatory state. Observed in both natural sleep and anesthesia, evoked down-state seems to inhibit acoustic responses via the profound hyperpolarization of cortical neurons for a brief period of time.

Neurochemical changes in the spinal dorsal horn in neuropathy following limb-lengthening in rabbits

Domos Gyula[1]; Pap Károly[1]; Szőke György[1]; Lukácsi Erika[2]; Kozsurek Márk[2]; Puskár Zita[2]
[1]Department of Orthopaedics
[2]Szentagothai Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
domosgy@gmail.com

Limb-lengthening is a widely used surgical procedure to correct bone deformities of the extremities. Nerve injury is one of the most serious complications associated with this type of operation resulting in neuropathy with pain, sensory loss or motor weakness. Despite of the fact that limb-lengthening is a common practice, changes in the nervous system underlying the symptoms are still unknown. We showed earlier that following limb lengthening, huge vacuoles appeared in large dorsal root ganglion (DRG) cells on the ipsilateral side of the operation. In addition, the number of substance P- (SP) immunoreactive cells in the DRGs decreased (by 10%), while the total number of DRG neurons was unchanged. In this study, we examined the neurochemical changes in the spinal dorsal horn associated with limb-lengthening in rabbits. Four groups of rabbits (5 rabbits/group) were used. In group A, B and C (sham operated) tibial osteotomy, external fixateur application and 7-day bone compression were carried out. After the callus formation in group A and B, 1 mm distraction was applied once a day for 20 days to achieve 120% of the original length. Untreated animals formed the group D. All animals were sacrificed after the lengthening had been terminated except those in group B that reached full recovery before. Using immunohistochemistry and confocal image analysis, SP-, calcitonin gene-related peptide- (CGRP) immunoreactivity and Bandeireae simplicifolia lectin (IB4) binding were measured in the dorsal horn of the L5 segments in the four groups. SP and CGRP immunoreactivity and IB4 binding decreased in the ipsilateral spinal dorsal horn in areas corresponding to the termination zone of the sciatic nerve in all groups compared to the control one. The decrease was the greatest in group A, but was also seen in group B. A minor decrease was present in the sham group as well. Our results suggest that both the surgical procedure and the distraction process cause neurochemical changes in the spinal cord, which last longer than the lengthening procedure itself. The deteriorated peptide expression and IB4 binding imply that both the peptidergic and non-peptidergic C primary afferents are affected and could contribute to the development of different pain symptoms in patients during and after limb-lengthening.

Effects of electrical stimulation on cortical activity in different vigilance states in humans

Entz László[1]; Fabó Dániel[1]; Erőss Loránd[2]; Halász Péter[3]; Wittner Lucia[1]; Karmos György[1]; Ulbert István[1]

[1]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary

[2]National Institute of Neurosurgery, Budapest, Hungary

[3]National Institute of Psychiatry and Neurology, Budapest, Hungary

entz@gmx.net

Cortical electrical stimulation (CES) in epilepsy diagnosis is an essential tool for localizing eloquent areas but in some cases, it can also aid seizure focus identification. Besides diagnosis, electrical stimulation has the potential to suppress paroxysmal activity in epileptic patients. Rhythmic electrical stimulation was found to enhance slow oscillation in non-REM sleep and improve declarative memory performance in normal subjects. In addition, recent transcranial magnetic stimulation (TMS) studies brought direct evidences, that magnetic stimulation triggers slow oscillation (SO) in deep stages of sleep. Our aim was to characterize CES evoked responses during different states of vigilance: under anesthesia, non-REM sleep and wakefulness. Patients with drug resistant epilepsy (n=4) were implanted with subdural grid and strip electrodes and intracortical laminar multielectrodes. Brief single current pulses were injected into adjacent grid contacts (10mA, 0.1ms, 0.5Hz stimulation rate) and responses were averaged both from the grids and the laminar electrodes. In most of the cases, we have found that current pulses awake an initial biphasic activation, which is followed by a wide

spread, long lasting surface negative potential and a rebound positivity. The surface negative followed by positive deflection sequence highly resembled to the SO detected in deepest stages of non-REM sleep. The negativity was accompanied by a wide spread reduction of oscillatory power in the 30-200Hz range and decreased cell firing, distinctive feature of the down-state, while the positivity was characterized by oscillatory power increase and enhanced cell firing, suggestive of the up-state of the SO. We were able to trigger the above sequence in all patients regardless of their vigilance state. Usually the down-state was the first, which emerged after the stimulation, while the up-state followed it. In one anesthetized patient we found that if the electrical stimulation was given in the up-state, it evoked a down-state, while if the stimulus was given in the down-state, it evoked an up-state. Here we have shown that CES initiates a wide spread hyperpolarization followed by a depolarization sequence, which is quite similar to a single wave of cortical slow oscillation. The existence of evoked state transitions implicates that CES may be a useful tool in modulating cortical excitability. This neuromodulatory effect can further be utilized in procedures aiming to suppress epileptic activity.

GABAergic deep short-axon cells of the rat main olfactory bulb form a novel extra-bulbar projection

Eyre Mark D.; Antal Miklós; Nusser Zoltán
Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
eyre@koki.hu

Mitral and tufted (M/T) cells are glutamatergic principal cells of the main olfactory bulb (MOB) that receive direct olfactory sensory inputs and are thought to provide the sole output of the MOB. Here, we investigated whether neurons distinct from M/T cells also project outside the MOB. Retrogradely-transported fluorescent latex beads were injected into the olfactory tubercle and the piriform cortex. Acute, in vitro slices were prepared from the MOB of these animals 3-14 days after tracer injection. Whole-cell patch-clamp recordings were made from neurons containing fluorescent beads located in the infra-mitral layers (granule cell layer and internal plexiform layer), and their physiological properties were investigated. Biocytin-filled cells were visualized after the recordings for light microscopic analysis and 3D reconstruction. Injection of retrogradely-transported beads in the olfactory tubercle or the piriform cortex did not only label M/T cells, but resulted in a large number of labeled cells in the infra-mitral layers. These neurons were distinct from granule, mitral and tufted cells and possessed electrical and morphological properties characteristic of deep short-axon cells (dSACs). These large GABAergic neurons, with dendrites restricted to the infra-mitral layers, do not receive direct sensory input. For eight cells, considerable axonal arbors were also recovered that sparsely innervated the infra-mitral layers of the MOB, allowing their identification as GCL-dSACs. Previous experiments in our laboratory have characterized GCL-dSACs as spontaneously active GABAergic neurons that exclusively innervate the somata and proximal dendrites of granule cells in the infra-mitral layers via GABAergic synapses and GABA-A receptors. Our results demonstrate that a subpopulation of dSACs constitute a previously unknown GABAergic projection from the MOB to downstream olfactory targets, which may provide a readout of MOB network activity instead of directly relaying sensory inputs.

Properties of in vivo interictal spike generation in the human subiculum

Fabó Dániel[1]; Maglóczky Zsófia[2]; Wittner Lucia[1]; Erőss Lóránd[3]; Halász Péter[3]; Freund F. Tamás[2]; Ulbert István[1]
[1]Institute of Psychology and [2]Institute of Experimental Medicine Hungarian Academy of Sciences, Budapest, Hungary
[3]National Institute of Neurosurgery, Budapest, Hungary
fabo.daniel@gmail.com

Subiculum is located in a central position within the limbic network. It controls the output of the hippocampus but also shares its inputs arising in the temporal cortical areas. The subiculum is also considered as a key structure in the generation and maintenance of epileptiform activity in temporal lobe epilepsy both in humans and in animal models. In order to investigate if and how the subiculum is involved in the generation of epileptic discharges in vivo, subicular laminar field potential gradient (FPG) and multiple unit activity (MUA), and lateral temporal electrocoricogramm were meantime recorded under anesthesia in drug resistant epilepsy patients undergoing temporal lobectomy. We analyzed the current source density (CSD) distribution, and the spectral content of spontaneous subicular interictal activity. Two types of interictal spikes were distinguished in the subiculum. The more frequently occurring spike started with an initial excitatory current (CSD sink) in the pyramidal cell layer associated with increased MUA in the same location, followed by later inhibitory currents (CSD source) and decreased MUA. In the other spike type, the initial excitation was confined to the apical dendritic region and it was associated with a less prominent MUA increase. Interictal spikes were highly synchronized at spatially distinct locations of the subiculum. Laminar data showed that the peak of the initial excitation occurred within 0-4ms at subicular sites separated by 6mm at the anterior-posterior axis, in addition, initial spike peak amplitudes were highly correlated in most recordings. A subset of subicular and temporal lobe spikes were also highly synchronous, in one case the subicular spikes reliably preceded the temporal lobe discharges. Based on our results we suggest that multiple spike generator mechanisms exist in the human epileptic subiculum reflecting complex network interplay between medial and lateral temporal structures during interictal epileptic activity. We hypothesize that both intrinsic and extrinsically triggered activity may contribute to the observed widespread intra-subicular synchrony that supports the hypothesis that subiculum may also play an active role in the distribution of epileptiform activity to other brain regions. Limited data suggest that subiculum might even play a pacemaker role in the generation of paroxysmal discharges.

Effects of ghrelin on neurons of the hypothalamic paraventricular nucleus requires retrograde endocannabinoid signaling

Farkas Imre; Fekete Csaba; Liposits Zsolt
Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
turekne.marta@koki.hu

Ghrelin is an orexigenic hormone produced in the mucosa of stomach. Ghrelin markedly increases appetite when injected intracerebroventricularly or into the hypothalamic paraventricular nucleus (PVN). However, administration of type 1 cannabinoid receptor (CB1) antagonist completely blocks the orexigenic effect of ghrelin injected into the PVN (Tucci et al., Br. J. Pharmacol. 2004/143/520-523). Parvocellular neurons of the PVN have been shown to express ghrelin receptor and are densely innervated by CB1 immunoreactive axons. Endocannabinoids, similarly to ghrelin, enhance food intake suggesting that ghrelin

may exert its orexigenic effect via activation of the endocannabinoid system in the PVN. To investigate the mechanism of the ghrelin-endocannabinoid interaction in the PVN, the effect of ghrelin on the miniature excitatory postsynaptic currents (mEPSCs) and modification of this effect by cannabinoid inhibitors were studied by whole cell clamp electrophysiology in the parvocellular neurosecretory neurons of the PVN in acute brain slices of 30 day-old male mice. The action potentials and inhibitory PSCs were blocked with TTX and picrotoxin. Effect of ghrelin on the mEPSCs was determined with or without pre-administration of a CB1 antagonist (AM251; 1 μM) or that of the intracellularly applied calcium chelator BAPTA (10 mM in the electrode solution), that blocked endocannabinoid synthesis in the postsynaptic cell. Ghrelin (100 nM) decreased frequency (60.6±6.95% of the control) and amplitude (86.46±2.86% of the control) of the mEPSCs significantly ($p<0.05$), thus inhibited the excitatory inputs. This effect was attenuated by administration of AM251 (amplitude: 98±2.09%; frequency: 96.4±9.91% of the AM251-control). The effect also disappeared in the presence of BAPTA (amplitude: 94.92±2.91%; frequency: 101.6±12.64% of the BAPTA control) demonstrating that the inhibited endocannabinoid synthesis in the postsynaptic cell blocked the effect of ghrelin. These results reveal that the ghrelin sensitive neurons in the PVN regulate their own synaptic input by endocannabinoid release and a retrograde endocannabinoid signaling pathway is necessary for the observed inhibitory effect of ghrelin.
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Transcriptional changes of the NPY hnRNA during hypoglycaemia

Ferenczi Szilamér; Kovács J. Krisztina
Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
ferenczi@koki.hu

Neuropeptide Y (NPY), synthesized in the arcuate nucleus of the hypothalamus, is an evolutionary conserved potent orexigenic neuropeptide. NPY plays a crucial role in the switch of food intake and regulation of the energy balance in vertebrates. The NPY neurons project to other hypothalamic nuclei, such as paraventricular nucleus (PVN), and it is reported that NPY contents in the PVN, but not NPY mRNA levels in the arcuate nucleus, changed rapidly after food consumption or food restriction. Many signals reflecting energy balance in the periphery are integrated at the NPY neurons, insulin, by influencing blood glucose level, has been implicated as one of the key regulators for NPY neurons. To assess rapid transcriptional changes in NPY gene in the arcuate nucleus we have designed intron-specific cRNA probes for in situ hybridization histochemistry. These probes hybridize to primary transcript, the heteroneuclear (hn)RNA of a given gene. Following overnight fast NPY hnRNA level has been doubled in the arcuate neurons compared to the fed controls. Insulin-induced hypoglycaemia resulted in a marked and rapid increase in NPY hnRNA that peaked at 1 hour and declined thereafter, by 2-4 hours after insulin injection. Throughout the time course examined, cells in the medial basal hypothalamus that exhibited NPY hnRNA signal remained overwhelmingly localized to the arcuate nucleus. This study highlights that monitoring of hnRNA is a precise indicator of the gene transcription and indicates rapid changes of NPY expression in response to metabolic and stress challenges.

Biochemical and immunhistochemical characterization of the 5-HTergic system in the buccal mass of the developing and adult pond snail, Lymnaea stagnalis

Filla Adrienn; Balog Gábor*; Hiripi László; Elekes Károly*
**equally contributing authors*
Department of Experimental Zoology, Balaton Limnological Research Institute, Hungarian Academy of Sciences, Tihany, Hungary
fillaa@citromail.hu

5-HTergic neurons play an important role in the regulation of feeding behavior of gastropods. Although the cellular physiology, neurochemistry and chemical-neuroanatomy of the 5-HTergic system in the snail CNS have been described in details, considerably less is known about the innervation of the buccal musculature, which is responsible for the execution of radula protraction. In this study, we investigated in the buccal mass of juvenile and adult Lymnaea the 5-HT level, 5-HT uptake-release system and 5-HT receptor binding characteristics, as well as the innervation by 5-HT-immunoreactive (IR) elements. 5-HT content of the buccal mass increased gradually during postembryogenesis (3,6 pmol/mg, P1; 6 pmol/mg, P6), then it was 5-HT uptake system was]3H[doubled (12.4 pmol/mg) in adults. A one component Mμdetected in juveniles, increased by 100% in adult muscle, showing 13,65 affinity to the transporter (Km) and 12,7 pmol/mg/20min transporter density 5-HT release from the isolated adult buccal mass,]3H[(Vmax). 100 mM K+ evoked 5-HT binding experiments a]3H[which decreased by 100% in Ca2+ free medium. In non-linear binding with 4,5 nM Kd and 2,4 fmol/mg Bmax values were obtained, indicating the presence of a single receptor type in the buccal mass. 5-HTergic agonists enhanced adenylate cyclase activity (cAMP level) in the isolated buccal M), whereμmass in a concentration-dependent way (0,1 to 100 5-carboxyamidotryptamine proved to be the most effective, followed by 5-HT, 5-methoxytryptamine and 8-OH-dipropylaminotetralin. 5-HTergic antagonists (100 M methiothepin, methysergide, BOL, metergolin and clozapine) significantly inhibited the 5-HT stimulated cAMP level. Immunocytochemistry revealed a gradual maturation of 5-HT-IR innervation forming a network of varicose fibers in the buccal mass during postembryogenesis, and developing parallel with the maturation of the musculature. A full innervation pattern was reached in late juveniles and adults. Our findings indicate that in Lymnaea i) 5-HT is a transmitter/modulator candidate at neuromuscular level in the feeding system (buccal mass); ii) 5-HT effect is mediated by a 5-HT7-like receptor; iii) 5-HT concentration and uptake-release activity, and 5-HT-IR innervation develop parallel in the buccal mass, correlating also with the maturation of the feeding behavior. Supported by OTKA grants #46580 and #T49090.

Modelling of firing mechanisms and underlying active processes in a hippocampal CA1 pyramidal cell

Florián-Szabó Mátyás[1]; Freund F. Tamás[2]; Káli Szabolcs[2]
[1]Péter Pázmány Catholic University, Budapest, Hungary
[2]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
florian@koki.hu

Cortical pyramidal neurons are able to produce multiple distinct firing patterns according to their inputs and internal state. The internal state of a neuron is dependent on many parameters, which cannot be measured in parallel. In order to gain deeper understanding of the active mechanisms responsible for the wide variety of firing patterns observed in in vivo and in vitro experiments, we constructed a computational model of a single CA1 pyramidal cell. We used a detailed morphological reconstruction and physiological data from previous recordings, and implemented it in the GENESIS simulation environment. We found that, when using active

conductances based on patch clamp measurements from published articles, the resulting model\'s firing characteristics were inconsistent with existing current clamp data. In particular, slow spiking could not be elicited by a near-threshold current injection, and a large amplitude afterhyperpolarization was observed. These differences can be due to variations in measuring conditions and methods in different experiments. Therefore we tried to change the parameters to fit firing patterns observed in in vitro recordings. We did this by altering the smallest possible number of parameters, giving preference to those which had a significant amount of experimental uncertainty. We used various methods such as automated as well as manual parameter searches and phase-space diagrams to find the best fitting parameters. We found that relatively small changes in the mid-point and slope of the activation and inactivation curves of a small number of conductances (namely, transient Na, the A-type and delayed rectifier K channels) resulted in a simulation with only minor deviations from measured firing behavior. Our model helps to understand the interaction of active mechanisms in the generation of neuronal output patterns and can be used in hippocampal network simulations.

Application of the Dynaflow patch clamp system in studying ion channel pharmacology

Fodor László; Tarnawa István
Gedeon Richter Pharmaceuticals Plc., Budapest, Hungary
l.fodor@richter.hu

Patch clamp technique is still the golden standard for studying interaction of drug molecules and ion channel receptors. Fast and reliable drug application is crucial for such interaction studies. The Dynaflow system uses cells lifted up from the bottom of the recording chamber and moves them in front of the openings of parallel drug ejection channels by a computer controlled system. Here we compare the Dynaflow system with conventional manifold-based drug application in whole-cell patch clamp experiments. Dofetilide and six Richter compounds inhibited human recombinant Kv11.1 voltage gated potassium channels (hERG) in HEK cells. There were no significant differences between drug potencies wheter determined by the Dyanflow or the conventional patch clamp method. Analysis of current traces indicated that the fluid exchange was much faster when the Dynaflow system was used, and drug application was also more reliable, resulting in an increased throughput of drug testing. The Dynaflow system can be equipped with a temperature control system. Experiments done at 37 C degrees were also performed. Comparison of the physiology and pharmacology of different subtypes of recombinant sodium channels did not show significant differences when conventional and the Dyanflow systems were used. Besides testing voltage gated sodium and potassium channels; the advanced drug application system of Dynaflow proved to be suitable for studying ligand gated ion channels, like NMDA, P2X, TRPV, and glycine receptors. In addition to HEK cells expressing recombinant ion channels; we also run experiments with one-day-old dorsal root ganglion cells. In conclusion the Dynaflow system produced pharmacological results comparable to that of conventional patch system, and proved to be superior concerning reliability and speed of drug application.

Changes in metabolic and stress parameters during morphine withdrawal

Földes Anna[1]; Markus Veronika[1]; Cristina Nunez[2]; Fatima Martin[2]; Milanes M. Victoria[2]; Kovács J. Krisztina[1]
[1]Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary

[2]Department of Pharmacology, University of Murcia, Murcia, Spain
foldes@koki.hu

Morphine use and abuse is accompanied by complex neuroendocrine, autonomic and behavioral alterations. Chronic exposure to opioids results in the development of tolerance and dependence. Naloxone precipitated morphine withdrawal produces complex behavioural, autonomic and endocrine changes, including stress-like activation of the HPA axis. Morphine abuse is often accompanied by alteration in food intake, energy metabolism and thermoregulation, however, the neurobiological basis of drug-induced autonomic changes are not fully characterized. Morphine dependence was achieved by implantation of increasing amounts of subcutaneous morphine pellets that was precipitated by a single injection of naloxone on day 7. Plasma corticosterone levels were 10-15x higher in morphine dependent, naloxone precipitated rats than in the controls. Significant induction of c-Fos, CRH and AVP expression were detected in the hypophyseotropic neurons of the PVH. In addition, autonomic related, projection neurons of the PVH also showed significant activation that might be due to increased sympathetic activity. Hypothalamic CRH is not only a neuropeptide that initiates the neuroendocrine stress response, but through its autonomic projections has a significant impact on the energy homeostasis, inhibit food intake and stimulates energy expenditure by activating sympathetic outflow. To characterize metabolic changes during chronic morphine treatment and withdrawal, we followed food and water intake, weight gain and caloric efficiency in rats that were morphine dependent. The body weight of the animals was dramatically decreased during naloxone-induced morphine withdrawal due to the breakdown of white adipose tissue. In morphine tolerant rat and during naloxone precipitated morphine withdrawal blood samples were obtained to measure glucose, leptin, insulin, adiponectin levels. Morphin-dependent rats did not display significant changes in blood glucose, adiponectin levels, however their insulin levels were slightly decreased and plasma leptin was significantly lower than in controls. Naloxon precipitated morphine withdrawal resulted significant increase in glucose levels and a slight decrease in insulin levels. Our results may help to understand the relationship between drug abuse, withdrawal, stress and metabolism.

Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats

Füzesi Tamás; Wittmann Gábor; Liposits Zsolt; Fekete Csaba
Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
turekne.marta@koki.hu

Hypophysiotropic thyrotropin-releasing hormone (TRH)- synthesizing neurons, the central regulators of the hypothalamic-pituitary-thyroid axis, are located in the paraventricular nucleus of the hypothalamus (PVN). Cold exposure is well known to stimulate the hypophysiotropic TRH neurons through activation of ascending brainstem pathways. The hypophysiotropic TRH neurons are heavily innervated by catecholaminergic nerve fibers that contain the dopamine-beta-hydroxylase (DBH) enzyme that generates noradrenaline. The DBH-containing axons in the PVN originate from six different cell groups of the brainstem, three of which also express the phenylethanolamine-N-methyltransferase (PNMT) enzyme that further converts noradrenaline to adrenaline. Both DBH and PNMT-containing axons densely innervate TRH neurons in the PVN, but, it was unknown whether in addition to the adrenergic neurons, the noradrenergic cell groups that lack PNMT also contribute to the innervation of TRH neurons. Therefore, we aimed to compare the contribution of adrenergic

and noradrenergic axons to the innervation of TRH neurons in the PVN. Thus, triple-labeling immunofluorescence was performed on rat hypothalamic sections to examine the association of proTRH neurons with DBH- and PNMT-containing fibers in the PVN. Using confocal microscopy, we counted the number of boutons containing both PNMT and DBH (adrenergic) and the number of boutons containing only DBH but not PNMT (noradrenergic) on the surface of proTRH neurons. We observed a very rich catecholaminergic innervation of TRH neurons in the PVN. Both noradrenergic and adrenergic fibers were found in close contact with nearly all TRH neurons (more than 98% in both cases). On the surface of TRH cell bodies and proximal dendrites, 12.5 adrenergic boutons and 7.1 noradrenergic boutons were observed on average. Thus, of all catecholaminergic axon-varicosities apposed to the surface of TRH neurons, 64% contained PNMT while the remaining 36% were immunopositive for only DBH. We conclude that both adrenergic and noradrenergic axons densely innervate hypophysiotropic TRH neurons, and there are about twice as much adrenergic varicosity in the proximity of TRH neurons than noradrenergic boutons. Further clarification is required to identify which adrenergic and/or noradrenergic cell groups participate in the cold-induced activation of TRH neurons.

Composition of the perineuronal net of the motoneurons in the frog

Gaál Botond[1,2]; Rácz Éva[1]; Mészár Zoltán[1]; Módis László[1]; Matesz Klára[1,2]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center
[2]Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
botond@chondron.anat.dote.hu

The perineuronal net (PN) is a condensation of extracellular matrix macromolecules (ECM) around the perikaryon, proximal dendrites and the axon hillock. It is generally accepted that in mammalian species the major components of the PN are the chondroitin-sulfate proteoglycans (CSPG) that bind to a variety of molecules such as the hyaluronan (HA) and tenascin-R. The stabilization of these molecular interactions is provided by the link proteins. In addition, the cell surface receptors and/or ionic channels of the neuronal membrane also contribute to the stabilization by anchoring the constituents of the PN. The molecular heterogeneity of PN was supposed to be related with the different functions of neurons and with their different vulnerability for the intrinsic or extrinsic noxious substances. Since the motoneurons of the brainstem show different response for the axotomy, we suppose that it is associated with different molecular organization of their PN. This study was undertaken to examine the chemical components of the PN around the somatomotor neurons of the frog. On the anesthetized animals the brainstem and spinal cord was excised and immersed into Sainte-Marie's fixative. Cross sections of the brainstem and spinal cord were made from the paraffin embedded specimens. The HA reaction was positive throughout the motoneurons of the brainstem and spinal cord showing the strongest intensity around the eye moving and spinal cord motoneurons. The Wisteria floribunda reaction, the general marker of the CSPG, was negative similarly to the results of the immunohistochemical reaction for the individual PG molecules, i.g. versican and neurocan. Our result suggests that the structure of the PN in the frog brainstem and spinal cord motoneurons is less organized than that of the PN in the vestibular nuclear complex of the frog or in mammalian species. We suppose that in the motoneuronal PN of the frog the function of the CSPG is provided by the phylogenetically ancient heparán-sulfate proteoglycans. This work was supported by OTKA K 67641 and MTA-TKI 242.

Electrophysiological investigation of antiepileptogenic and anticonvulsant effects of 16463 on the 4-aminopyridine epilepsy model in vivo

Gajda Zita; Martók Anikó; Tuka Bernadett; Kéri György; Szente Magdolna
Department of Comparative Physiology, University of Szeged, Szeged, Hungary
zitagajda@hotmail.com

In this study, the possible antiepileptogenic and/or anticonvulsant effects of 16463 molecule that supposedly acts on gap junction channels were tested on the 4-aminopyridine semi-chronic epilepsy model on anaesthetized rats. 16463 was applied intravenously either before the induction (pretreatment)-, or after 25-30 spontaneously repeated seizures (treatment). ECoG and EKG were recorded and analysed. Pretreatment increased the latency of the first seizure significantly in comparison to control data collected from a separate group of animals. The number of seizures decreased significantly below the control in the first 20 min of the active epileptic focus and stayed there even at the 140th min in comparison to the time-matched control. The average duration of seizures was slightly above the control through the experiments. The summated ictal activity (seizure numbers times seizure durations) decreased significantly in comparison to control in the first 20 min, and it was still significantly below the time-matched control around the 100th min, then gradually retained to control around the 120th min. The average amplitudes of seizure discharges decreased in comparison to control, the reduction was significant around the 60th min. Treatment turned out to be more efficient in comparison to pretreatment. Control measurements were taken from the same animals before the application of the drug. Anticonvulsant effects of 16463 manifested rather quickly and reached its peak around the 100th min after the application, then declined around the 240th min. The seizure number decreased significantly in comparison to control and reached the lowest value around the 240th min. The duration of seizures stayed slightly below the control until 160th min, and then increased gradually up to 200% of control around the 240th min. As a result, the summated ictal activity decreased significantly in comparison to control and was still slightly below it around the 240th min. The average amplitudes of seizure discharges decreased slightly through the experiments. The 16463 did not affect considerably either the heart frequency, or the configuration of EKG. Based on these observations, 16463 might be a promising candidate for the development of a new antiepileptogenic and anticonvulsant drug. This study was supported by DNT.

Effects of the selective 5-HT7 receptor antagonist SB-258719 on sleep wake cycle after partial serotonergic denervation

Garay Tamás[1]; Molnár Eszter[1]; Kátai Zita[1]; Kitka Tamás[1]; Lévay György[2]; Bagdy György[1,3]
[1]Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary
[2]EGIS Pharmaceuticals Plc., Budapest, Hungary
[3]Group of Neuropsychopharmacology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
garayt@gmail.com

Effects of the selective 5-HT7 receptor antagonist SB-258719 on sleep wake cycle after partial serotonergic denervation T. Garay1, E. Molnár1, Z. Kátai1, T. Kitka1, G. Levay2, G. Bagdy1,3 1Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, 2EGIS Pharmaceuticals Plc., 3HAS-SU Group of Neuropsychopharmacology garayt@gmail.com Since its discovery, the serotonin-7 (5-HT7)

receptor has been implicated in the regulation of circadian rhythms and sleep. MDMA, the active ingredient of the recreational drug ecstasy causes a long-term damage of serotonergic axons and terminals in rodents and non-human primates and very likely, also in human ecstasy users. Our aim was to investigate the function of this receptor in the regulation of the sleep-wake cycle using the 5-HT7 receptor-selective antagonists SB-258719. Furthermore, the possible alteration of the 5-HT7 receptor function after chronic partial damage caused by MDMA was studied. Three months after a pretreatment with a single dose of MDMA (15 mg kg-1 i.p.), the antagonist SB-258719 (20mg kg-1 i.p.) was administered to male Dark Agouti rats at light onset (beginning of passive phase). EEG, EMG, and motor activity were recorded during the subsequent 24 h. The following sleep stages were determined: active wake (AW), passive wake (PW), slow wave sleep 1 and 2 (S1, S2) and REM sleep (REM). Circadian rhythms of the above vigilance stages were analyzed by cosinor analysis. This latter means setting of a sinusoid curve on the measured values and determining amplitude, acrophase (time of the maximum over the 24 h cyle) and mesor (axis of the curve). SB-258719 significantly decreased mesor of REM sleep and slightly increased mesor of PW. In animals treated with MDMA three months earlier, significant reduction of serotonergic axon densities were found in several terminal areas, and sleep effects of SB-258719 were attenuated. In conclusion, our data provide evidence for the role of 5-HT7 receptors in vigilance cycles. Furthermore, partial lesion of the serotonergic system interferes with this function. Acknowledgement This work was supported by the 6th Framework Program of the EU, LSHM-CT-2004-503474

Plastic changes in Edinger-Westphal-Urocortin 1 neurons upon different stessors

Gaszner Balázs[2]; Nicole Derks[2]; Csernus Valér[1]; Eric W. Roubos[2]; Kozicz Tamás[2]
[1]Department of Anatomy, Faculty of Medicine, University of Pécs, Pécs, Hungary
[2]Department of Cellular Animal Physiology, Radboud University Nijmegen, The Netherlands
pecas_hu@yahoo.de

The crucial role of the hypothalamus-pituitary-adrenal (HPA) axis in the control of adaptation to stressors is well known, but in recent years new members of the corticotropin-releasing factor (CRF) peptide family have been identified that are involved in stress responses as well. In our research we focus on the role of urocortin 1 (Ucn1), expressed predominantly in the non-preganglionic Edinger-Westphal nucleus (npEW). Ucn1 stimulates ACTH, binds with high affinity to both known CRF receptors, and reveals strong co-expression in the npEW with brain-derived neurotrophic factor (BDNF), a growth factor implicated in the pathogenesis of stress-related mood disorders, such as anxiety and depression. The purpose of the present study was to assess the effects of different stress paradigms known to cause mood disorders in a gender-specific way, on the dynamics of the npEW-Ucn1 system. Firstly, using immunocytochemical triple labeling for Ucn1, BDNF and c-Fos, and morphometry, we observed a substantial increase (by ca. 100%; $p<0.001$) in the Ucn1-specific signal density (SSD) following acute restraint stress, in both male and female rats; in contrast, chronic, variable stress did not alter the Ucn1 SSD compared to controls. Similarly, using radioactive in situ hybridization, we found increases in Ucn1 mRNA contents (by ca. 25%; $p<0.05$) after both acute and chronic stress, in both sexes. Secondly, using immunocytochemistry, we demonstrated that BDNF protein contents (SSD) increased upon both acute and chronic stress, in females (by ca. 20%; $P<0.05$), but not in males. Total BDNF mRNA expression increased in both sexes upon stress revealed by quantitative RT-PCR. In particular, the up-regulation occurred in promoter-specific manner: we found increased amount of exon 1, 3, 4 and 6 transcripts. The assessment of c-Fos immunoreactive cell numbers in the npEW showed a 25-

times increase following acute and chronic stress ($p<0.001$) in males. Females showed 7-times ($p<0.05$) elevation in c-Fos response to acute stress but no significant response upon chronic variable stress. The evaluation of corticosterone (CORT) levels revealed that both acute and chronic stressors were effective, and caused ca. 400% and 300% rises in males, respectively ($p<0.001$); females, however, responded to both stress paradigms with an only 80% increase ($p<0.05$) in CORT level compared to controls. We conclude that the dynamics of the npEW-Ucn1 system are influenced by both acute and chronic stress paradigms, in a largely gender-specific manner, which supports our hypothesis that this system plays an important role in the pathogenesis of stress-related mood disorders.

The herpes simplex virus entry receptor (nectin-1) in cerbrocortical development

Gellért Levente[1]; Prandovszky Emese[1]; Toldi József[2]; Janka Zoltán[1]; Horváth Szatmár[1]
[1]Department of Psychiatry, Faculty of Medicine and [2]Department of Comparative Physiology, Faculty of Sciences, University of Szeged, Szeged, Hungary
huxley@citromail.hu

Neonatal herpes simplex encephalitis is characterized by widespread cortical necrosis and is typically associated with a more severe and devastating process than in the adult brain. Similarly to humans, neonatal mice are much more sensitive to HSV infection than adult mice. To elucidate the potential role of the major neural entry receptor of HSV, nectin-1, in age-dependent susceptibility of cortical neurons to viral encephalitis, we examined the spatiotemporal changes in the distribution of the major neural entry receptor for HSV (nectin-1) in the developing human and mouse cerebral cortex. We have identified N1-positive neuronal and other cell populations in the neocortex and allocortex of the immature brain. Interestingly, the undifferentiated cells in the ventricular zone and neurons in the outermost areas of the fetal neocortex were N1 negative. Importantly, we detected strong N1 expression in a subset of cells that regulate migration of neurons, including CR neurons and radial glial cells. These data raise the possibility that the susceptibility of immature neurons to HSV may be related to an increased expression of neuronal surface molecules acting as viral receptors.

GFAP in the visual pathway of the rat

Gerics Balázs; Szalay Ferenc; Hajós Ferenc
Department of Anatomy and Histology, Faculty of Veterinary Sciences, Szent István University, Budapest, Hungary
Gerics.balazs@aotk.szie.hu

The astroglial marker, glial fibrillary acidic protein (GFAP) was investigated by immunohistochemistry in various brain areas in order to see its fluctuations in various functional states. Different neuronal states were either experimentally induced or studied under physiological conditions. To produce experimental alterations the visual system was chosen as a model. Upon lesioning of the lateral geniculate body with the stereotaxic injection of ibotenic acid an increase in GFAP immunoreactivity could be induced in layers III and IV of the ipsilateral visual cortex where geniculo-cortical fibres terminate. Electron microscopy has revealed a synchronous degeneration of synaptic terminals and the hypertrophy of perisynaptic astrocyte processes. To study changes in the intact animal the effect of illumination was observed. In the lateral geniculate body the dorsal subnucleus was found immunonegative when studied at day and positive at night.

It was concluded that changes in GFAP immunoreactivity can indicate synaptic events whithin a circumscribed area of the brain.

Expression of cadherin-13 an anusual member of the cadherin family during mammalian CNS development

Géczi Kornél; Mayer Mátyás; Bercsényi Kinga; Szabó Gábor; Lele Zsolt
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
lelezs@koki.hu

Cadherin 13 is a unique member of the cadherin superfamily. It has a normal 5 extracellular cadherin-binding domain structure, but it lacks transmembrane and intracellular domains and it is linked to the cell membrane extracellularly via a GPI-anchor.[1] Earlier Cdh13 has been implicated in vascular development [2] and in adipogenesis as a coreceptor of adiponectin [3]. It is upregulated in tumor vasculature and its hypermethylation and deletion is closely related with increased malignancy of various tumors[4]. In vitro data demonstrated its potential as a negative guidance cue in spinal motor axon growth [5] and its role in synapse development [6] but in vivo data regarding its function in neural development and function is still lacking. Here we give a detailed characterization of its spatio-temporal pattern of expression using single and double in situ hybridization as well as immunhistochemistry. We are also in the process of cloning constructs for electroporation and microinjection which would help us in elucidating its function. 1. B Ranscht, MT Dours-Zimmermann: T-cadherin, a novel cadherin cell adhesion molecule in the nervous system lacks the conserved cytoplasmic region. Neuron 1991, 7:391-402. 2. D Ivanov, M Philippova, V Tkachuk, P Erne, T Resink: Cell adhesion molecule T-cadherin regulates vascular cell adhesion, phenotype and motility. Experimental cell research 2004, 293:207-18. 3. C Hug, J Wang, NS Ahmad, JS Bogan, TS Tsao, HF Lodish: T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proceedings of the National Academy of Sciences of the United States of America 2004, 101:10308-13. 4. S Toyooka, KO Toyooka, K Harada, K Miyajima, P Makarla, UG Sathyanarayana, J Yin, F Sato, N Shivapurkar, SJ Meltzer, et al: Aberrant methylation of the CDH13 (H-cadherin) promoter region in colorectal cancers and adenomas. Cancer research 2002, 62:3382-6. 5. BJ Fredette, J Miller, B Ranscht: Inhibition of motor axon growth by T-cadherin substrata. Development (Cambridge, England) 1996, 122:3163-71. 6. S Paradis, DB Harrar, Y Lin, AC Koon, JL Hauser, EC Griffith, L Zhu, LF Brass, C Chen, ME Greenberg: An RNAi-based approach identifies molecules required for glutamatergic and GABAergic synapse development. Neuron 2007, 53:217-32.

Patterning of intra- and internuclear inhibitory interneurons in the oculomotor nucleus of neonatal mouse

Halasi Gábor; Joel C. Glover
Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
gaborg@medisin.uio.no

The motoneurons of the extraocular motor nuclei represent the final site of covergence of premotor signals related to the control of eye movements. Premotor afferents to the abducens, trochlear and oculomotor nuclei comprise both excitatory and inhibitory synaptic connections. The afferents that give inhibitory, dominantly GABAergic inputs on oculomotor motoneurons mainly arise from the vestibular nuclei (SVN), the rostral interstitial nucleus of the MLF

(RIMLF), the supraoculomotor area (SOA).
Several populations of internuclear neurons with diverse projection targets, such as spinal cord, the cerebellum, the abducens nucleus have been identified within and around the oculomotor nucleus. The presence of oculomotor internuclear neurons in lower vertebrates as lampreys and in mammals has been reported but their connections and role in ocular coordination are poorly understood. The best investigated of there interneurons are the oculomotor internuclear neurons (OMN-INTs) lying within the oculomotor nucleus and in the supraoculomotor area projecting bilaterally to the abducens nucleus. OMN-INTs in primates are predominantly excitatory, but a smaller percentage (20% in cats and 30% in lampreys) is GABAergic. GABAergic internuclear neurons without direct connection to the abducens nucleus within and around the oculomotor nucleus has been described, but the functional role of these GABAergic OMN-INTs is not clear.
The aim of the present study was to investigate the presence and localization of glutamate decarboxylase-67 (GAD67) positive neurons, within and in the surroundings of the oculomotor nucleus in newborn GAD67 transgenic mouse, in order to elucidate whether GABA is involved in the modulation of the ocular motor system.
In this study we found that there are GAD67-GFP positive GABAergic interneurons in the oculomotor nucleus and in the supraoculomotor region of newborn mouse. The RIMLF, which is important in eye-movements control, also contains GABAerg neurons in newborn mouse. The GABAergic interneurons are situated in the position where they have been described in other species in adults and their rostral dominance of the distribution in the oculomotor nucleus correlates with earlier findings in cats. GABAergic intra- and internuclear interneurons can be distinguished by morphology from the motoneurons of the oculomotor nucleus.

Density changes in some neural (CART) and glial (GFAP) markers induced by food deprivation in the lateral septum of male rats

Halasy Katalin[1]; Janzsó Gergely[1]; Lendvai Zsuzsanna[1]; Szőke Balázs[1]; Ábrahám Hajnalka[2]; Kozicz Tamás[3]
[1]Department of Anatomy and Histology, Faculty of Veterinary Sciences, Szent István University, Budapest, Hungary
[2]Central Electron Microscopic Laboratory, Faculty of Medicine, University of Pécs, Hungary
[3]Department of Cellular Animal Physiology, Radboud University, Nijmegen, The Netherlands
halasy.katalin@aotk.szie.hu

Changes in the immunocytochemically detectable amount of cocaine- and amphetamine-regulated trascript (CART) and the astroglial marker glial fibrillary acidic protein (GFAP) were studied after one week of partial (40%) food deprivation in the lateral septum (LS) of male rats (n=12) with combined pre-embedding immunocytochemistry and computer-aided densitometry. Rats were perfused with 4 % buffered paraformaldehyde, 60 micrometer serial free-floating sections from the brain were incubated, then the sections were photographed and representative areas from the digitalized images were measured by three independent persons. Data were pooled and the Kaleidagraph program(Synergy Software) was used for statistical analysis. After one week of fasting the amount of the strongly anorexigenic CART peptide was significantly reduced along the entire rostrocaudal axis of the LS, as measured in microphotos of serial sections. At the same time the density of the astroglial marker GFAP showed a significant increase as a result of fasting, indicating that not only neural, but glial elements also heavily react to changes in environmental factors. These results are in accord with previous studies reporting density changes of other neuropeptides (neuropeptide Y,

galanin, opioid peptides) in the LS as a consequence of fasting. Since the lateral septum contains considerable amount of several orexigenic and anorexigenic peptides sensitively reacting to changes in the amount of food available, we can assume that this brain area may be involved indirectly in the regulation of food intake, via its extensive reciproc connections with the hypothalamus. Acknowledgements: the monoclonal mouse anti-CART antibody was kindly provided by J.T. Clausen (Novo Nordisk A/S, Bagdverd, Denmark). This work was supported by the Hungarian National Research Fund (OTKA, T 43170).

Semi-automatic burst detection in thalamic relay cells

Hangya Balázs[1]; Slezia Andrea[1]; Bokor Hajnalka[1]; Ulbert István[2]; Varga Viktor[1]; Acsady László[1]; Freund F. Tamás[1]
[1]Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences
[2]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
hangyab@koki.hu

Detection of putative low threshold burst from long spike train recordings of thalamic relay neurons provides a challenging in vivo analysis problem. Our aim was to develop a method that is able to identify visibly distinguishable putative low threshold bursts and provides reliable extrapolation to those cases where visual identification is ambiguous. We developed a cluster analysis-based semi-automatic burst detection method. Interspike intervals (ISI) were clustered using Ward's amalgamation rule and Euclidean metric, assuming that the ISI cluster containing the shortest interval corresponds to the intraburst intervals. Clustering was performed iteratively, with varying the number of clusters from 2 to the theoretical maximum. Four parameters were displayed in the function of cluster number: (i) average intraburst frequency, (ii) minimum of preburst silent period, (iii) difference between preburst silence and maximal intraburst ISI (termed here as "ISI gap"), (iv) minimal intraburst frequency. Instead of determining fixed limiting values for the above burst parameters, implausibly low values (ca. 120 Hz for minimal and average intraburst frequency, ca. 40 ms for preburst silent period and ISI gap) were used as excluding parameters. Largest non-excluded step in the minimal intraburst frequency function was determined, and the clustering corresponding to the cluster number after the step was chosen. Bursts were post-hoc verified by visual examination, and in a few cases, the chosen step was modified to a neighboring step in the minimal intraburst frequency function. Applying the above burst detection method we were able to separate putative low threshold bursts in spike trains of thalamic relay neurons and to monitor drug-induced changes of burst parameters.

The Wulst-VTA loop in the avian brain

Hanics János; Bálint Eszter; Székely Andrea D.
Department of Anatomy, Faculty of Medicine, Semmelweis University, Budapest, Hungary
hanicsjanos@freemail.hu

The ventral tegmental area (VTA) a midbrain monoaminergic cell group, is implicated in the generation of addictive behaviours possessing reciprocal connections with the medial prefrontal cortex (mPFC). The VTA is thought to be responsible for the processing of the hedonistic component in certain forms of learning and memory, while representing an essential link within the brain reward cycle. The mPFC plays an essential role in the regulation of emotional context and certain aspects of learning and memory. The major

afferents of the mammalian VTA stem from the limbic forebrain, including the ventral mPFC. In birds, the rostral Wulst area, together with the posterolateral telencephalon is thought to be part of a corresponding pallial structure which, similar to that found in mammals, may be reciprocally innervated by the VTA. In the present study, the rostral Wulst of one week old domestic chicks was injected with the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHAL, or biotinylated dextranamine – BDA) to verify the presence of Wulst efferents within VTA. Alternatively, in further animals, the Wulst was injected with horseradish peroxydase (HRP) to label VTA neurons projecting upon the rostral component of the putative 'avian prefrontal cortex'. The rostral Wulst gives rise to a massive fiber tract entering the septomesencephalic tract then the ventral hypothalamus to terminate presumably on the dopaminergic cells in the ipsilateral VTA. Similarily, HRP-filled granula were detected in some large perikarya bilaterally, but primarily in the ipsilateral VTA. The distribution of backfilled neurons and wulst efferents exhibited a substantial spatial overlap, thus supporting the presence of a VTA-Wulst neuronal circuit. This work was supported by a Bolyai fellowship to A.D. Székely

Neuronal and glial localization of the cannabinoid-1 receptor in the superficial dorsal horn of the rodent spinal cord

Hegyi Zoltán; Kis Gréta; Antal Miklós
Department of Anatomy, Histology and Embryology, Medical and Health Science Center, University of Debrecen, Hungary
hegyiz@chondron.anat.dote.hu

A long line of experimental evidence indicates that endogenous cannabinoid mechanisms play important roles in the modulation of pain processing in various areas of the nervous system including the superficial spinal dorsal horn. Although it is extensively documented that the cannabinoid-1 (CB1) receptor is strongly expressed in the superficial spinal dorsal horn, its cellular distribution is poorly defined that renders the interpretation of the effect of cannabinoids on pain processing spinal neural circuits very dubious. Thus, we investigated the cellular distribution of CB1 receptors in laminae I-II of the rodent spinal dorsal horn with immunocytochemical methods at the level of the light and electron microscopes. Presynaptic axonal varicosities showed a strong immunoreactivity for CB1 receptor, but we have not observed any CB1 receptor expression on the cell membrane of postsynaptic dendrites and somata. Investigating the co-localization of CB1 receptor with calcitonin gene related peptide, isolectin B4-binding, vesicular glutamate transporter 2 and glutamate decarboxilase 65/67, markers for peptidergic and non-petidergic primary afferents, and axon terminals of excitatory and inhibitory spinal neurons, respectively, we found that various subpopulations of axon terminals of both nociceptive primary afferents and excitatory as well as inhibitory neurons expressed CB1 receptors in the superficial spinal dorsal horn. In addition to neurons, CB1 immunoreactivity was observed also in glial cells. Near half of the astrocytic (GFAP-immunoreactive) and 80% of microglial (CD11b-immunoreactive) profiles showed immunoreactivity for CB1. The findings suggest that the activity dependent release of endogenous cannabinoids in acute pain may activate a complex signaling mechanism in pain processing neural circuits in the superficial spinal dorsal horn in which neurons and glial cells may equally contribute. This work was supported by: OTKA 46121, ETT 079/2006, MTA-TKI 242.

In vitro studies on the formation of the astroglial phenotype

Herberth Balázs; Varga Balázs; Berzsenyi Sára; Hádinger Nóra; Madarász Emília
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
madarasz@koki.hu

The "neurons first – glia second" principle is a common feature of neural development, both in vivo and in vitro. The majority of glial cells arise at or after the end of the main period of nerve cell generation. The mechanisms behind this timing are far from clear. Although several factors known to induce astrocyte differentiation (CNTF, LIF, BMPs) are present in the developing brain, their presence is not sufficient to promote an early formation of astrocytes. Thus, some inhibitory factors can be postulated, which do not allow the precocious generation of glia. In this study, a suspected glia-inhibitory effect of all-trans retinoic acid (RA) was analyzed in the course of in vitro astroglia formation. For studying the astroglial cell fate commitment, one-cell derived embryonic neural NE-4C stem cells and P19 embryonic carcinoma cells were used as in vitro model systems. Both cell lines give rise to neurons and later to astrocytes if induced by all-trans retinoic acid. The results indicated that RA reduced dramatically the formation of GFAP-positive cells in both cell lines, while did not influence the neuron formation or the persistence of non-committed stem-like cells. The effect was not due to a selective death of glial cells, either. The data suggest that RA exerts a direct effect on neural progenitors and inhibits their glial cell fate decision. In accordance with the in vitro results, we could show the presence of RA in the developing brain, in those regions where active neurogenesis takes place and which will produce astrocyte in later phases of neural tissue-genesis.

The neuronal background of dopamine-serotonin interaction in a simple nervous system

Hernádi László; Hiripi László; Vehovszky Ágnes
Balaton Limnological Research Institute, Tihany, Hungary
hernadi@tres.biki.hu

In mammalian brain several lines of evidences indicate that central serotonergic and dopaminergic systems play an important role in regulating normal and abnormal behaviors and their disfunctions are involved in the pathophysiology of neuropsychiatric disorders. In simple nervous systems as in gastropods the electrophysiologial and behavioral studies demonstrated that serotonergic system is involved in both the arousal and the modulation of the activity of central pattern generators (CPG) including the feeding CPG, whereas dopaminergic system is involved in the activation of CPGs with command properties. In this study we investigated the possible interactions of the 5HTergic and DAergic system in the snail Helix pomatia. We have described the presence of characteristic DAergic and 5HTergic cell groups in the CNS with identified neurons among them. Both 5HT and DAergic elements are present also in the buccal and cerebral ganglia containing feeding CPG. Double immunostaining revealed that both central DAergic neuron and peripheral DAergic sensory neurons receive 5HTergic fibers, whereas identified central 5HTergic neurons receive DAergic fibers only at their distal axon segments which fibers show sensory axon-like appearance. Intracellular recording from identified 5HTergic neurons shows that extracellularly applied DA inhibits the firing activity in a concentration dependent manner. Additionally these 5HTergic neurons receive excitatory 5HTergic inputs from central 5HTergic neurons indicating that the elements of 5HTergic system are interconnected and their activity is modulated by both DA and 5HT. When 5HT precursor the 5HTP was injected into the haemocoel, which increases both the 5HT level and the activity of 5HTergic neurons, it increased not only the 5HT but also the DA levels in both the CNS and peripheral organs

suggesting that it increased the activity of the DAergic system. Our present observations suggest that the 5HTergic and DAergic system interact in both the central and peripheral nervous system and additionally they may mutually modulate the activity of different CPG in the animals. Acknowledgement: This work was supported by Hungarian OTKA grants T 46580 and T63451.

The application of the TAqMan Low Density Array (TLDA) method for the investigation of gene expression in pain processing areas of the nervous system

Holló Krisztina[1]; Bakk Erzsébet[1]; Hegedűs Krisztina[1]; Papp Ildikó[1]; Nagy László[2]; Antal Miklós[1]

[1]Department of Anatomy, Histology and Embryology, [2]Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Hungary

kriszti@chondron.anat.dote.hu

Neural microcircuits of the superficial spinal dorsal horn represent the first relay station of the nociceptive sensory system. After local information processing, the nociceptive impulses are conveyed to cells of origin of ascending sensory pathways that transmit the impulses to higher brain centres, where these signals generate complex motivational-affective behaviours including acute pain. Peripheral tissue or nerve injuries and inflammations evoke long-lasting changes in nociceptive-processing microcircuits. This activity-evoked plasticity results in the development of central sensitization that is thought to critically contribute to conditions of chronic pain and hyperalgesia. Our research is focused on two major aims: 1) We would like to acquire an exhaustive understanding of fundamental aspects of the molecular organization of pain processing microcircuits. We intend to study the molecular properties of neurons at different parts of the pain processing apparatus of the central nervous system. 2) In addition to studying the nociceptive mechanisms under normal conditions, we also intend to elucidate how information processing in nociceptive neural networks may change in inflammatory andneuropathic pain states. Experimental models of inflammatory and neuropathic pain are used to study the induced changes in functional morphology at the molecular level. To investigate the molecular changes at the level of mRNA expression we are using the TLDA method. To find molecules which are possible key players in pain processing we have designed a panel of 45 molecules including various receptors, ion channels, chemokines and cytokines on a TaqMan microfluid card. We are investigating the changes in the expression of our customized collection of molecules in the dorsal root ganglion, spinal cord and anterior cingulate cortex. Here we present our first results obtained with this method. This work was supported by: OTKA 46121, ETT 079/2006 and MTA-TKI 242.

Behavioral and electrophysiological effects after subacute exposure of rats to low doses of methyl mercury

Hornyik Tibor; Horváth Edina; Oszlánczi Gábor; Lukács Anita; Vezér Tünde

Department of Public Health, Faculty of Medicine, University of Szeged, Szeged, Hungary

hornyik@puhe.szote.u-szeged-hu

Methyl mercury (MEM), a well-known environmental neurotoxicant, is found primarily in sea food as a result or biotransformation of inorganic mercury emitted in human activities. MEM can cause various neurological disorders in humans, including paraesthesia, psychomotor and memory problems. MEM can also severely disturb neural and mental development. In this work, young adult male Wistar rats were treated with MEM dissolved in sunflower oil, 5

times a week for 5 weeks. The doses were equivalent to 0.5 and 0.05 mg Hg/kg b.w. Before and during the treatment weeks, the rats had maze learning test sessions and rotarod tests. At the end of the treatment period, the rats open field (OF) behaviour, and rotarod performance, was investigated; and the acoustic startle response (ASR) test was done. Following that, the rats were prepared for electrophysiology. Electrocorticogram (ECoG) and cortical evoked potentials (EP), as well as tail nerve action potentials and electroneuromyogram (ENMG) of the sciatic nerve were recorded in urethane anesthesia. There was no significant difference in the maze performance, though the treated rats finished the sessions faster and with more errors. The rotarod performance showed a slight decline in thee treated rats, in which the pre-pulse inhibition of the ASR response was also somewhat decreased. In the OF, significant increase of local motility and immobility of the MEM-treated rats was observed. The band spectrum of the ECoG showed no clear trends. Significant changes were seen, however, in the latency and duration of the somatosensory, and the duration of the visual and auditory EP. The relative refractory period of the tail nerve, and the frequency-dependent amplitude decrease of the ENMG, was increased in the high dose group. These data further emphasize the neurotoxic risk of MEM present in some foodstuffs.

The antinociceptive effects of intrathecal injection of N-arachidonoyl-dopamine (NADA) are mediated by cannabinoid receptors

Horvath Gyöngyi; Farkas Ibolya; Tuboly Gábor; Benedek György
Department of Physiology, Faculty of Medicine, University of Szeged, Szeged, Hungary
horvath@phys.szote.u-szeged.hu

N-arachidonoyl-dopamine (NADA) is an endogenous ligand for TRPV1 and cannabinoid-1 (CB1) receptors. Both receptors are expressed on primary afferent nociceptors, therefore their activation can influence the pain sensitivity. Only one study suggested that NADA influences nociception at spinal level. The goal of this study was to characterize the antinociceptive potency of NADA and to determine the role of TRPV1 and CB1 receptors in its effect. Methods: After obtaining institutional ethical approval, intrathecal catheters were implanted into male Wistar rats. The nociceptive threshold was assessed by using paw withdrawal test. Thermal hypersensitivity was produced by injection of carrageenan (2mg/100µl) intraplantarly in one of the hindpaws. The pain threshold was obtained before carrageenan injection, 3 h after carrageenan administration and then in every 10 min interval for 90 min. NADA (1, 3 and 10 µg), AM 251 (10 µg; CB1 receptor antagonist), AMG 9810 (0.3 µg, TRPV1 antagonist) and their combinations were given. Analysis of variance (ANOVA) of the data for repeated measures was used for overall effects, followed by the Fisher LSD test. A probability level of 0.05 was considered significant. Results: NADA did not influence the pain sensitivity at normal side, but it produced dose-dependent antinociception at inflamed paws. The highest dose relieved the hyperalgesia. The highest dose of NADA caused temporary excitation during its injection. Neither AM 251 nor AMG 9810 influenced significantly the pain threshold at the normal and inflamed sides. AM 251 significantly decreased the antihyperalgesic potency of NADA, while AMG 9810 produced only a slight antagonism. In conclusion, intrathecally administered endogenous cannabinoid and TRPV1 receptor agonist NADA might be beneficial in inflammatory pain. The antinociceptive effect of NADA was mediated mainly by CB1 receptor. This work was supported by the National Research and Development Office, Hungary (OMFB-0066/2005/DNT) and by a Hungarian Research Grant (OTKA, K60278).

Demonstration of estrogen receptor-beta in human gonadotropin-releasing hormone neurons

Hrabovszky Erik[1]; Kalló Imre[1]; Keller Éva[2]; Merchenthaler István[3]; Liposits Zsolt[1]
[1]Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Forensic Medicine, Semmelweis University, Budapest, Hungary
[3]Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, USA
turekne.marta@koki.hu

The gonadotropin-releasing hormone (GnRH) neurosecretory system represents the final common hypothalamic pathway in the neuroendocrine control of reproduction. Changing levels of the ovarian sex steroid hormone 17β-estradiol (E_2) tightly regulate the activity of GnRH cells via feedback actions. Recently, our group has localized the second isoform of estrogen receptors (ER-β) within GnRH neurons of the rat brain, indicating that GnRH cells are capable of directly sensing circulating estrogens. To address the issue of whether GnRH neurons of the human hypothalamus also contain ER-β, we have carried out dual-label immunocytochemical studies on autopsy samples. Research protocols to obtain and handle tissues were reviewed and approved by the Regional Committee of Science and Research Ethics (TUKEB 49/1999). Combined technical efforts that minimized *post-mortem* interval (<24 h), optimized fixation conditions (use of a mixture of 2% paraformaldehyde and 4% acrolein) and sensitized the immunocytochemical detection (application of silver-intensified nickel-diaminobenzidine chromogen) allowed the visualization of nuclear ER-β immunoreactivity in 10.8-28.0 % of GnRH neurons in the preoptic/hypothalamic area of male human individuals. The demonstration of ER-β in human GnRH cells, which lack the classical ER-α receptor isoform, indicate that estrogens may exert direct actions upon GnRH cells selectively through ER-β. In the light of the differing ligand binding characteristics of ER-β from those of ER-α, this discovery offers a potential novel approach to influence estrogen feed-back mechanisms to GnRH neurons through the recently available ER-β-selective ligands.
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Decreased expression of connexin-43 after generalized seizures in the neonatal brain

Hugyecz Marietta[1]; Zimmermann Aliz[1]; Berczi Sándor[2]; Mátyás Adrienne[2]; Bari Ferenc[1]
[1]Department of Physiology and [2]Department of Anatomy, Faculty of Medicine, University of Szeged, Hungary
hugyeczmarietta@gmail.com

Generalized seizures are followed frequently by decreased cortical electrical activity, unconsciousness, impaired cognition and memory due to disturbed neuronal signaling. Astrocytes are connected to neurons via gap junctions and modulate neurotransmission in the brain. We tested if generalized seizures alter the expression of connexin 43 (CX43), the main gap junction protein expressed by astroglia. Anesthetized and ventilated newborn pigs were used (n=22). Seizures were induced with the $GABA_A$-receptor antagonist bicuculline (3 mg/kg iv) after muscle relaxation with pipecuronium bromide (0.5 mg/kg iv). Brains were removed 8 hours after 1) seizure induction, 2) sham operation or 3) immediately after anesthesia (untreated group). CX-43 expression was measured by Western blot analysis and immunohistochemistry. CX-43 levels as detected by Western blot analysis markedly decreased in the frontal cortex and hippocampus, but not in the temporal cortex after seizures

compared to sham operated and untreated pigs. Immunohistochemistry revealed that CX43 expression significantly decreased after seizures compared to sham in the stratum (str) moleculare of the cerebral cortex and in the str moleculare and oriens of the hippocampal CA1 region. Conversely, seizures did not change CX-43 expression in the upper cellular layers of the cerebral cortex, in the str lacunosum of the CA1 or in the str moleculare internum of the dentate gyrus. We conclude that generalized seizures decrease the levels of CX-43 in the cerebral cortex and hippocampus, which potentially contributes to abnormal postictal neurotransmission.
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Direct connection between the vestibular afferents and abducens motoneurons in the frog, Rana esculenta

Hunyadi Andrea[1]; Rácz Éva[1]; Veress Gábor[1]; Székely György[1,2]; Mihály Erzsébet[1]; Matesz Klára[1,2]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center
[2]Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
hunyadiandrea@freemail.hu

During body displacement the gaze fixation needs very fast and precise movements of the eye with the assistance of vestibulo-ocular reflex (VOR). The afferent fibers of the VOR originate in the labyrinthine organs and establish synaptic contact with the second order vestibular neurons (VN) of the vestibular nuclear complex of the brainstem. The VN send their axons either directly to the motoneurons of eye moving cranial nerves or by way of the interneurons located in the motor nucleus of the abducens nerve indicating polysynaptic pathway in the VOR. In our earlier cobalt labeling studies on the frog we have found that the dorsal dendritic arrays of the abducens motoneurons invade into the territory of the termination area of the vestibular afferent fibers suggesting the possibility of the monosynaptic connection between the afferent and efferent elements of the VOR in the horizontal direction. To examine this possibility, double labeling with different fluorescens tracers was performed in anesthetized animals. Crystals of fluorescein binding dextran amine were applied to the cut end of the vestibulocochlear nerve while the abducens nerve was labeled with tetramethylrhodamine dextran amine. After 4-5 survival days cross sections of the brainstem were made and the specimens were examined. Dendrites of abducens motoneurons intermingled with the vestibular afferent fibers and terminals. By using of confocal laser scanning microscope we have found close apposition between the vestibular terminals and the abducens dendrites. The distance between the neighboring profiles suggested close membrane appositions without intercalating glial or neuronal elements. Our results suggest a monosynaptic component of VOR providing the morphological background of a very fast compensatory eye movement during head displacement. This work was supported by OTKA K 67641 and MTA-TKI 242.

Changes in nitric oxide synthase (NOS), cyclooxygenase-2 (COX-2), superoxide dismutase (SOD) protein levels and cerebral autoregulatory capacity in the early and late phase of chronic cerebral hypoperfusion in rats

Institóris Ádám[1]; Mracskó Éva[1]; Hugyecz Marietta[1]; Berczi Sándor[2]; Farkas Eszter[2]; Bari Ferenc[1]
[1]Department of Physiology and [2]Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary

insti@phys.szote.u-szeged.hu

Chronic cerebral hypoperfusion is a condititon which contributes to cognitive trajectory in dementia. Its experimental model is the permanent, bilateral common carotid artery occlusion (2VO) in rats. Following 2VO a progressive restoration of cerebral perfusion occurs, which probably, in part is due to the induction of NOS and COX-2 enzymes, meanwhile their products might maintain a permanent oxidative stress. We purposed to detect the enzyme expressions immediately after 2VO induction, and at 6 and 12 month following 2VO. We also tested the autoregulatory capacity at the late phase (9 months) of hypoperfusion.
Male Wistar rats were imposed to 2VO (n=23) or sham operation (SHAM) (n=25). Tissue samples of the hippocampus and frontal cortex of both, 2VO and SHAM animals were taken 1 day (n=7), 3 days (n=10), 6 months (n=9) and 12 months (n=11) after the operation. Samples were used to determine the expression of endothelial NOS (eNOS), inducible NOS (iNOS), neuronal NOS (nNOS), COX-2 and manganase SOD (MnSOD) with Western blot analysis. In the remainder rats, 9 months after surgery, laser doppler flowmetry was applied to measure the autoregulation dynamics of 2VO and SHAM animals in the frontal cortex and in the hippocampus during hypertension (MABP≥ 170 mmHg) induced by the intravenous infusion of adrenalin (1-6 μg/min) or during hypotension (MABP≤ 40mmHg) created by desanguination.
Hypoperfusion induced a significant decrease in the level of iNOS and nNOS 1 and 3 days after 2VO. COX-2 level showed elevation at day 1, while MnSOD increased at day 3. 6 and 12 months after 2VO presented a slight elevation in nNOS level, while the other markers showed no change, compared to the controls. Autoregulation was found to be intact after 9 months of hypoperfusion in the 2VO rats.
We concluded that the early changes in enzyme expression returns to normal level, and maintains autoregulation in the cortex and in the hippocampus, while the elevation of nNOS might be a consequence of aging.
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Melanin-concentrating hormone neurons: why they express alpha-dystrobrevin?

Jancsik Veronika[1]; Várkonyi Emese[1]; Tárnok Krisztián[2]; Hazai Diana[1]
[1]Department of Anatomy and Histology, Faculty of Veterinary Sciences, Szent István University, Budapest, Hungary
[2]Department of Physiology and Neurobiology, Faculty of Science, Eötvös Loránd University, Budapest, Hungary
Jancsik.Veronika@aotk.szie.hu

Alpha-dystrobrevins (alpha-DBs) are key components of the plasma membrane-spanning dystrophin-associated protein complex, which participates in cell-cell and cell-extracellular matrix interactions, as well as in signal transduction from the cell surface towards its interior. In the adult brain alpha-DBs were thought to be highly specific for the glial cells, while other members of the dystrobrevin protein family, beta-dystrobrevins were localized in neurons. Previously we have identified a subset of neurons in the hypothalamus expressing the short alpha-DB isoform(s) alpha-DB-2 and/or alpha-DB-4 (alpha-DB-2/4). Immunoreactive neurons form clusters with bilateral symmetry, localized predominantly in the lateral hypothalamic area, with extensions into the zona incerta and the dorso-medial and ventro-medial hypothalamic regions. These neurons all express melanin concentrating hormone (MCH) as well (Hazai et al., IBRO Meeting, Budapest, 2006 and Jancsik et al., manuscipt in preparation). alpha-DBs are localized in neuronal perikarya and as puncta among the

perikarya corresponding to synaptic sites. For more accurate sub-cellular localisation of alpha-DB-2/4 immunoreactivity, detailed electron-microscopic analysis was carried out. This demonstated that alpha-DB-2/4 immunpositivity was confined exclusively to spine synapses. To get further data on kinesin as a potential intracellular binding partner of alpha-DB-2/4, double-label immunofluorescence studies were carried out. Kinesin immunoreactivity was found in all alpha-DB-2/4 immunopositive cells. Several alpha-DB-2/4 immunonegative neurons, on the other hand, were found to contain kinesin. Confocal laser scanning microscopy studies confirmed the above finding and showed that the immunoreactivity of the two proteins overlaps on the subcellular level. We suggest that the exclusive co-localization of alpha-DB-2/4 with MCH reflects a specific functional requirement. -DB-2/4, MCH and kinesin on the sub-cellular level Co-localization of alpha suggests that this association could play a role in the regulation of the intracellular targeting of MCH into specific subcellular compartments and/or membrane sites.

Enhancement of potassium chloride cotransporter type 2 expression and its relation to recurrent seizures and cell loss in pilocarpine-induced epilepsy

Karlocai Rita[1]; Tóth Kinga[1]; Varga Viktor[1]; Juhász Gábor[2]; Freund F. Tamás[1]; Maglóczky Zsófia[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Eötvös Lóránd University, Institute of Biology, Laboratory of Proteomics, Budapest, Hungary
karlocai@koki.hu

KCC2 is a membrane protein responsible for the regulation of cell volume and intracellular Cl- and K+ concentrations, and its reduced activity leads to a depolarizing effect of GABA. We studied the expression pattern of KCC2 in pilocarpine-induced epilepsy using CD1 mice. Based on behavioral signs of status epilepticus, mice were classified as “strongly” and “weakly” epileptic using the Racine-scale. In the strong group the seizures were frequent with intense motor symptoms (Racine 4-5), whereas in the weak group there were only a few mild seizures (Racine 1-3). 1-3 months after the injection we recorded EEG to investigate the presence of recurrent seizures. We recorded recurrent seizures in the strong group frequently and the hippocampi of mice showed sclerosis in most cases. In the weak group only some of the animals produced recurrent seizures and none of them were sclerotic, although interictal spikes were present in the EEG and scattered cell loss was found in the hippocampus (mainly in the CA3 region). In control animals some principal cell dendrites and spines were faintly, certain interneuron dendrites were strongly KCC2-immunoreactive. Only a few cell bodies were slightly stained. The distribution of KCC2-immunogold reaction product in pyramidal cells’ soma was inhomogeneous; some immunopositive clusters could be seen in the membrane. In interneurons the distribution of KCC2-immunoreactive clusters was more homogeneous. In epileptic animals the number of KCC2-positive interneurons has increased both in sclerotic and non-sclerotic animals. Enhanced density of KCC2 immunstaining was found also in principal cells. In the electron microscope, we found more numerous immunopositive profiles in the epileptic hippocampi than in the controls. Interneuronal cell bodies and dendrites showed stronger immunopositivity than the surviving principal cells. Surviving CA1 pyramids had slightly increased KCC2 expression, whereas KCC2-immunopositivity of CA3 pyramidal cells of epileptic animals were very similar to the controls. The enhancement of KCC2-expression in this model may represent a compensatory change of the tissue, since it may prevent excitotoxic damage (swelling) via increased efficacy of volume regulation. However, if the chloride (and potassium) transport is reversed

due to the high extracellular potassium concentration during seizures, GABA may become depolarizing and will aggravate seizures. Supported by the Szentagothai Knowledge Center.

Dendritic integration in hippocampal interneurons

Kaszás Attila; Katona Gergely; Rózsa J. Balázs; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
kaszas@koki.hu

Although interneurons play a major role in the central nervous system, little is known about how their dendrites integrate inputs. We used multisite focal extracellular stimulation to deliver different spatiotemporal input patterns to single dendritic branches of interneurons of stratum radiatum while simultaneously recording the evoked excitatory postsynaptic potentials and local calcium signals. We used a novel rapid two-photon linescan technique in order to measure long dendritic segments (typically > 30 μm). Freehand lines were placed following the curvature of single long dendritic segments to monitor the calcium signals elicited by focal electric stimulation (elicited by two electrodes, located at different relative distances). The scanning time being in the range of typically < 6 ms allowed us to analyze the spatiotemporal properties of calcium compartments. Appropriately timed and sized input patterns produced a supralinear summation of local calcium signals, although excitatory postsynaptic potentials, recorded somatically, summed sublinearly. We found that the spatiotemporal distribution of the supralinearity of summation depends on the distance between the neighboring activated compartments. Our results suggest that similarly to pyramidal neurons the aspiny dendrites of interneurons sum inputs nonlinearly, although the arithmetic of interneurons seems to be different.

Reconstructing the mechanism of hippocampal theta oscillations through a network model based on field potential and single unit recordings

Káli Szabolcs; Freund F. Tamás
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
kali@koki.hu

Theta frequency oscillations in the local field potential can be recorded in the hippocampus during specific behavioral states including exploration and REM sleep, and are thought to make an essential contribution to hippocampal computations. The spatio-temporal structure of these field oscillations, in the form of current source density (CSD) maps, has been identified. The firing patterns of various classes of hippocampal neurons in relation to the theta oscillation have also been determined. However, the nature of the mechanisms which give rise to the observed activity patterns, and to theta rhythmicity itself, has been controversial. In particular, it has been difficult to reconcile the data on the relative phases of current sinks and sources in the CSD map with the known preferred firing phases of neurons within and outside the hippocampus. Our aim was to analyze, using our existing computer model of the CA1 network, the link between the field potential and single cell firing during theta, as a function of the relative strength and phase of external inputs to area CA1. Our large-scale network simulations contained simplified models of CA1 pyramidal neurons, basket cells, and O-LM interneurons, and included external inputs from area CA3, entorhinal cortex, and the medial septum. We found that rhythmic neuronal activity could be induced by oscillatory input through various afferent connections, and could also be generated autonomously within area CA1. However, both the spatio-temporal distribution of sinks and sources in the CSD map

and the preferred firing phases of different neuronal classes depended critically on the mode of theta generation. By using the combined constraints of field potential and single unit data, we were able to determine the combination of strengths and phases of external inputs which gave rise to oscillatory activity in our model network that was generally consistent with the available data from in vivo recordings in both intact and lesioned animals.

Sleep effects of an inverse agonist selective for α5 subunit-containing GABA-A receptors

Kátai Zita[1]; Garay Tamás[1]; Kitka Tamás[1]; Lévay György[2]; Bagdy György[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary [2]EGIS Pharmaceuticals Plc., Budapest, Hungary
kataizita@gmail.com

Recently 19 GABAA receptor subunits have been indentified. Several physiological functions of GABAA receptors are mediated by benzodiazepines. The benzodiazepine (BZ) binding site is located at the interface of either α1, α2, α3 or α5 and γ2 subunits. The α5-containing GABAA receptors are located mainly in the hippocampus, so they are thought to play role in learning and memory, but the actual functions of GABAA receptors containing α5 subunit are poorly defined. BZ site agonists (eg. diazepam) increase the GABA-induced chloride influx, and have anxiolytic, sedative, hypnotic and antiepileptic effects. The BZ site antagonists (eg. flumazenil) have no effects on the efficacy of GABA. They are used to impede the effects of benzodiazepines. The nonselective BZ site inverse agonists (eg. the β-carboline DMCM) decrease the chloride influx. They show an anxiogenic-like profile and increase vigilance. The effects of benzodiazepines on time spent in different sleep stages include effects on rapide eye movement (REM) sleep, non-REM sleep and intermediate stage of sleep. For example, chlordiazepoxide markedly decreases intermediate stage of sleep even at relatively low anxiolytic doses (Kantor et al., 2005). The chronic administration of flumazenil has been proved to increase the time spent in REM sleep. To study the sleep-vigilance effects of the α5 subunit the inverse agonists selective for this subunit 3-(5-Methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3,4-a]phthalazine (we will mark this drug as α5IA) was administered to freely moving male Sprague-Dawley rats and polysomnographic recordings (EEG, EMG, motor activity) were performed for 2 h after treatment. The compound α5IA was administered (1, 3 and 5 mg/kg i.p.) at light onset. Active and passive wake (AW, PW), light and deep slow wave sleep (SWS-1, SWS-2) and REM sleep were evaluated. Our results show that the most robust effect of α5IA was a marked decrease in time spent in REM sleep in the second hour studied. In conclusion, based on our results, we propose that the endogenous activation of GABAA receptor α5 subunit is important in the sleep regulation. Acknowledgement This work was supported by EGIS Pharmaceutical Plc and EU, Framework 6, LSHM-CT-2004-503474. Kantor et al.: (2005) The Journal of Pharmacology and Experimental Therapeutics 315: 921–930

Interaction between the effects of nAChR activation and bAP on Ca2+ transients in dendrites of hippocampal interneurons

Katona Gergely; Rózsa Balázs; Kaszás Attila; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
katonag@koki.hu

In our combined patch clamp and two-photon laser scanning microscope experiments, we provided evidence for the first time that under conditions in which synaptic inputs were

blocked, the stimulation of alpha7-nAChRs localized nonsynaptically on the dendrites of interneurons in the stratum radiatum of the CA1 hippocampal region via rapid drug application or electrical stimulation elicited large Ca2+ transients. The amplitude of the transients showed a distance-dependent increase as a consequence of the decrease in dendrite diameter. The timing of activation of these receptors by cholinergic agonists (acetylcholine and choline) determined whether facilitation or depression of backpropagating action potential (bAP)-evoked Ca2+ transients occurred. Eliciting bAP at the onset (0.1 – 0.35 s) of the rapid cholinergic application induced facilitation while inducing bAP later (1 - 3 s) resulted in depression of bAP-evoked Ca2+ transients. Somatic current injection was able to mimick the effect of ACh in the facilitation. Our findings might explain the involvement of cholinergic innervation, whose axon terminals do not make synaptic contacts 93% of the time, in the modulation of dendritic excitability. Acetylcholine released from these boutons may diffuse far away from release sites and activate alpha7-nAChRs located extrasynaptically. This process may establish functional interactions between cholinergic innervation and neuronal circuitry in the hippocampus.

Molecular changes accompanying morphine withdrawal in rat brain

Kékesi Orsolya; Birkás Erika; Cinar Resat; Szűcs Mária
Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
kekorsi@yahoo.com

The highly potent opioid analgesic morphine is known to induce tolerance, dependence and reward upon continuous administration. Addiction to opioids is a major public health concern. A more thorough understanding of the molecular mechanisms of morphine addiction can lead to better treatment options in the future. Morphine acts via μ-opioid receptors that belong to the G-protein coupled receptor superfamily. In the present work, female Wistar rats were injected with increasing doses of morphine for 5.5 days (total dose 370 mg/kg, s.c.) to evoke analgesic tolerance/dependence as published (Fabian et al. (2002) JPET 302 (2): 774-780). Control animals received saline. We divided the rats into two groups afterwards. One group was used the study the effect of spontaneous withdrawal for 4 days. Rats were sacrificed and their brains removed on day 9. The other group received naltrexone (1 mg/kg, i.p.) two hours after the last morphine injection to precipitate withdrawal on the morning of the day 6. Fifteen minutes later the brains were removed. Synaptic plasma membrane (SPM) and microsomal (MI) fractions, that represent the cell surface respectively intracellular receptors, were isolated by sucrose density gradient centrifugation of the forebrain homogenates. Homologous displacement experiments were performed with the well-known μ-agonist ligand, DAGO ([D-Ala2, N-Me-Phe4,Gly5-ol]-enkephalin), and the affinity constant, KD and receptor density, Bmax values were determined. There were no significant changes either in the numbers of the receptors nor in their affinity in the SPM, while the Bmax increased from 413 ± 41 to 520 ± 63 fmol x mg protein-1 in the MI of 5-day morphine-treated rats. In contrast, the number of the surface μ-opioid receptors was up-regulated by 37 and 66% upon spontaneous and morphine-precipitated withdrawal, respectively. Supported by NKTH DNT 08/2004 and OTKA TS 049817 research grants.

Marked changes in the immunoreactivity of vesicular glutamate transporter type 1 and type 2 neuronal elements in the brain of ovariectomized and estrogen receptor knock-out mice

Kiss József[1]*; Bodnár Ibolya*[1]*; Kalló Imre*[2]*; Liposits Zsolt*[2]*; Halász Béla*[1]
[1]Department of Human Morphology and Developmental Biology, Neuromorpholgical and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
kissjo@ana2.sote.hu

Previously we have demonstrated that there are vesicular glutamate transporter type 2 (presumed glutamatergic) neurons in the . The aim of the presentαrat brain which contain also estrogen receptor investigations was to reveal whether ovariectomy or the lack of estrogen receptors affects the immunoreactivity for vesicular glutamate transporter type 1 (VGluT1) and/or VGluT2 in the brain. Immunocytochemistry was used at the light microscopy level to detect VGluT1 and VGluT2 immunoreactive neuronal elements in the brain of wild type (intact (n= 2), ovariectomized (n=3)) and estrogen -KO (n=2). Compared to intactβ-KO (n=3)) and αreceptor (ER) knock-out mice (-KO mice the density of VGLUT1 immunoreactive fibers wasαcontrols, in the ER significantly reduced in the cortex and the hippocampus, but it was markedly increased in the medial habenula. Numerous VGluT1 immunopositive cells were seen -KO miceβin the basolateral nucleus of the amygdala. In contrast, in the ER appreciable changes were not seen in the VGluT1 immunoreactivity. In wild-type OVX mice similar changes were observed in the dentate gyrus and in the medial habenula, in which the VGluT1 immunoreactivity appeared to be enhanced. Reduced number of VGluT2 immunopositive perikarya and a decreased density of immunoreactive fibers were found in the brain of both ERKO as well as OVX mice. The changes occurred primarily in the cortex and the hippocampus, and were most -KO mice, in which the VGluT2 immunoreactivity disappearedαpronounced in the ER from the pyramidal cells of the 5th cortical layer. The only change we observed in the hypothalamus of both ERKO mice was a decrease in the density of the immunoreactive fibers in the ventromedial nucleus. In contrast to intact controls, VGluT2 immunopositive neurons were not evident in the internal capsule of ERKO and OVX mice. Our findings are the first neuromorphological observations indicating that the lack of estrogen influence in the brain results in significant changes in the glutamatergic system manifesting in altered VGluT1 and VGluT2 immunoreactivities. Support: NKFP 1A002-04/2004 (B.H.), OTKA T049455 (J.K.), ETT 020/2006, Hungarian Academy of Sciences

Organisation of the sensory system of the earthworm body wall: a DiI tracing study
Kiszler Gábor; Várhalmi Eszter; Pollák Edit; Molnár László
Department of General Zoology, University of Pécs, Pécs, Hungary
kiszlerg@freemail.hu

This study focuses on the anatomical organisation of the peripheral and central sensory structures of the earthworm Lumbricus terrestris applying a fluorescent carbocyanine dye (DiI), as a neuronal tracer. When a large DiI crystal was directly applied to a dissected ventral nerve cord ganglion, isolated from the adjacent ganglia, high number of the labelled sensory cells and several free nerve endings were seen in the epithelium of the traced segment. Most of the labelled cells concentrated in sense organs located in setae rows and their central processes enter into the 2nd and 3rd segmental nerves while central processes of primary sensory cells situated in anterior part of the segment enter into the 1st segmental nerves. However, labelled cells, showing identical pattern that was found in the traced segment, were

detected in adjacent segments both anteriorly and posteriorly, proving that a segmental ganglion receives sensory inputs from several (up to 13) segments. Direct application of DiI crystal to the body wall epithelium showed that bifurcating central processes of the primary sensory cells can be detected in four neighbouring ganglia. Our result prove that the overlapping sensory units of Lumbricus terrestris build up from several sensory structures that are located in neighbouring segments.

Localization of synaptic vesicle protein-2 in rat and human hypophysis

Kocsis S. Zsuzsa[1]; Deli Levente[1]; Kalló Imre[1]; Horváth C. Mónika [2]; Keller Éva[2]; Liposits Zsolt[1]; Hrabovszky Erik[1]
[1]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Forensic Medicine, Semmelweis University, Budapest, Hungary
turekne.marta@koki.hu

Synaptic vesicle protein-2 is a glycoprotein integrated into the membrane of synaptic vesicles. In addition to occur in neurons, SV2 has been detected in various endocrine cell types. In this study, we investigated the immunocytochemical distribution of SV2 in the rat and human hypophyses. The posterior lobe of the rat exhibited SV2 immunoreactivity in magnocellular neuroendocrine terminals. The distribution of SV2 was identical with that of glutamate containing synaptic vesicles which also contained type-2 vesicular glutamate transporter (VGLUT2). In the intermediate lobe of the rat, most of the epithelial cells were also SV2 immunoreactive and they intermingled with SV2 immunoreactive axons. In the anterior lobe, a subset of relatively large epithelial cells exhibited heavy SV2 immunoreactivity, whereas some labeling was noticeable in the majority of anterior lobe cells. Dual-immunofluorescent studies identified the heavily labeled cells as gonadotropes, thyrotropes and corticotropes. In addition, in the anterior lobe we found a high degree of overlap in the distribution of SV2 and the glutamatergic cell marker VGLUT2. These observations raise the possibility that SV2 and VGLUT2 are co-contained in the same organelles, potentially synaptic-like microvesicles, within gonadotropes and thyrotropes. Future studies with electron microscopy will need to clear the subcellular localization of SV2 and VGLUT2 in synaptic-like microvesicles and/or secretory granules. In addition, the subtype of SV2 in the anterior lobe will be determined and the transcriptional regulation of its mRNA expression, in response to endocrine manipulations of the gonadal and thyroid axes, will be studied. This work was supported by grants from the National Science Foundation of Hungary (OTKA T46574 and K69127) and by NKFP (1A/002/2004).

Package information leaflets as possible determinants of choice of curatives

Komsa Ildikó; Köteles Ferenc; Bárdos György
Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
komsa.ildi@gmail.com

There are many factors that can influence patients\' choices from OTC-medicines of the same effect. Such factors are previous experiences, family traditions, perceptual characteristics of pills, ads, number of side effects, etc. In this study, a rarely considered factor: description of positive effects in the package information leaflets was examined. The appropriate parts of the package leaflets of 15 frequently used, freely accessible (OTC), NSAID drugs were rated by 202 participants (37% male, 63% female; mean age 20,1 years, SD = 2,90). In the ratings, two

independent aspects were considered: (a) how understandable and usable the descriptions of effects were; and (b) how attractive the drugs seemed based on the descriptions (what would be the chance of choosing them). Significant differences among the 15 drugs were found regarding both investigated aspects: there were several very positively and very negatively rated descriptions, respectively. As the two aspects intercorrelated very strongly (r=0.938**), the obvious conclusion could be drawn that understandability and content of practical information were the most important factors of choice. The most attractive descriptions were longer than average and listed more concrete illnesses and symptoms for which the curative was recommended to be used. Patient Information Leaflets (PILs) can make benefits (giving information) and harms (more side-effects) as well. Drug manufacturers are gradually recognizing the importance of the patients\' participation in designing better and more usable PILs. It is hoped that over the next years, patients will be provided with better designed and understandable PILs in Hungary as well.

Projections from the vestibular nuclei to the hypothalamic paraventricular nucleus: morphological evidence for the existence of a vestibular stress pathway in the rat brain

Kovacs Zsolt I.; Markia Balázs; Palkovits Miklós
Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
palkovits@ana.sote.hu

Although, it has been reported by several laboratories that vestibular stress activates the hypothalamo-pituitary-adrenocortical axis (HPA), the existence of neuronal connections between vestibular and hypothalamic paraventricular neurons have not been demonstrated yet. By the use of a virus-based retrograde trans-synaptic tracing technique in the rat, here we demonstrate vestibular projections to the paraventricular nucleus (PVN). Pseudorabies virus (Bartha strain, type BDL62) was injected into the PVN, and the progression of the infection along synaptically connected neurons was followed in the pons and the medulla, 3 and 4 days post-inoculation. Virus-infected neurons were revealed mainly in the medial vestibular nucleus. Labeled cells were scattered in the spinal, and very rarely in the superior nuclei, but none of them in the lateral vestibular nucleus. Injections of cholera toxin B subunit, a monosynaptic retrograde tracer into the PVN failed to label any cells in the vestibular nuclei. These results provide anatomical evidence for the existence of a vestibulo-paraventricular polysynaptic pathway and support the view that the HPA axis is modulated by vestibular stress.

Facilitation of spike-wave discharge activity by intraperitoneally and intraventricularly injected lipopolysaccharides in wag/rij rats

Kovács Zsolt; Kékesi A. Katalin; Bártfai Tamás; Juhász Gábor
Department of Zoology, Berzsenyi Dániel College, Szombathely, Hungary
zskovacs@bdf.hu

In normal rats the proinflammatory cytokines, which are induced by bacterial lipopolysaccharides (LPS), are able to control thalamo-cortical excitability by exerting strong effects on physiological and pathological synchronization like that in epileptic discharges. To investigate whether proinflammatory cytokines or LPS could modulate absence seizures, we used a genetically epileptic WAG/Rij rat strain, which is spontaneously generating high voltage spike-wave discharges (SWD). WAG/Rij rats responded with an increase of the

number of SWDs to LPS injection (from 10 to 1000 µg/kg; i.p., and 3 µg/rat i.c.v.). The LPS induced increase in SWDs was not directly correlated with the elevation of the core body temperature. Indomethacine (40 mg/kg, i. p.), the prostaglandin synthesis inhibitor, efficiently blocked LPS induced enhancement of SWD genesis suggesting that the SWD facilitating effect of LPS involves induction of cyclooxygenase 2 and subsequent synthesis and actions of prostaglandin E2 (PGE2).

Non-selective innervation of lamina I projection neurons by cocaine- and amphetamine-regulated transcript (CART) peptide-containing fibers in the rat spinal cord

Kozsurek Márk[1]; Lukácsi Erika[1]; Fekete Csaba[2]; Puskár Zita[1]
[1]Szentagothai Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary
[2]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
mark@kozsurek.hu

The role of CART(55-102) peptide in the spinal nociception has been suggested by behavioural studies. We have demonstrated that the majority of CART-immunoreactive fibres, forming dense plexus in the superficial laminae, originates from peptidergic nociceptive primary afferents containing both calcitonin gene-related peptide and substance P, and many of them terminate on supraspinally projecting neurons. In this study, we used immunocytochemistry combined with retrograde tract tracing method to determine the subclasses of the peptidergic fibres which contain CART and to quantify the contact density of the CART terminals on different populations of the lamina I projection neurons. It was shown that galanin (GAL) has pro- or antinociceptive effect in different pain states. We found that 80.6% of peptidergic primary afferents contain GAL in lamina I and 51.5% of them were also CART-positive. CART peptide was present in 48.5% of peptidergic primary afferents and 85.5% of them was also immunoreactive for GAL. All randomly selected projection neurons received contacts from CART-ergic axons. Quantitative analysis showed that 52.3% and 66.9% of the peptidergic contacts on substance P receptor- (NK1) immunoreactive and non-NK1-positive projection neurons were CART- immunoreactive, respectively. The contact density per unit surface area on NK1-positive and non-NK1-positive projection cells (201.5 and 222.2 boutons/104 µm2, respectively) and among the different morphological types of the NK1-immunoreactive projection neurons (187.3, 232.7 and 183.1 boutons/104 µm2 in the fusiform, pyramidal and multipolar types, respectively) were not significantly different. The coexistence of CART peptide with GAL in nociceptive primary afferents in lamina I further supports the possible role of CART peptide in different pain states. The fact that neither the composition nor the contact density of the CART-containing peptidergic fibres differed significantly on the different neurochemical and morphological types of projection cells suggests a non-selective innervation of the projection neurons from CART-ergic primary afferents.

Associations between the expected effectivity and the perceptual characteristics of medicines

Köteles Ferenc; Bárdos György
Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest
fecuska@gmail.com

As it is well-known from previous research, there are strong associations in people\'s mind between the look-and-feel of curatives and their expected effects. However, there hasn't been any data on to what extent these expectations could influence drug choice. In the present study, this question was addressed. Groups of 5-5 pictures of pills of different colour and/or shape were assigned to three commonly appreciated drug effects (analgesic-antipyretic, sedative-hypnotic and spasmolytic). The 181 participants (37% male, 63% female; mean age 20,1 years, SD = 2,90) were asked to rate in a five-grade scale that how possible was they would choose the different pills from the respective group in case of emergency. Significant differences among the pills within each group were found: the participants had clearly and strongly preferred certain tablets with given perceptual characteristics. The possible influence of the look-and-feel of curatives could modulate their effectivity in many ways: if the perceptual characteristics matched the patients\' expectations, this might increase non-specific effects, i.e. those that are not related to the drug itself; in addition, patients\' compliance to the prescribed therapy might also be improved. It is unfortunate that pharmaceutical industry neglects this aspect that could significantly enhance the effectivity of their products.

Inverse CSD methods for better localization of neural activity

Kőrössy Csaba[1]; Borbely Sándor[2]; Világi Ildikó[2]; Somogyvári Zoltán[1]; Érdi Péter[1,3]
[1]Department of Biophysics, Research Institute for Particle and Nuclear Physics, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
[3]Center for Complex System Studies, Kalamazzo, Michigan, USA
csabakorossy@yahoo.com

The traditional one dimensional current source density (CSD) analysis method is a widely used tool for spatial electrical potential patterns, recorded by multiple micro electrode arrays. In spite of the fact that the traditional CSD method is based on strong physical principles, such as Maxwell\'s first equation and Ohm\'s law, the application for a one dimensional data series includes assumptions, which are never proved and often obviously violated. It is rarely noticed, that neglecting the remaining two dimensions in the CSD calculation is equivalent with the assumption of the laminar homogeneity of the sources. Since the assumption of homogeneity fails the results of the calculation can be innacureate. A solution for this problem is the application of the inverse CSD (iCSD) methods. The iCSD method is based on the inverse solution of the discretized Maxwell-equation. The iCSD method calculates the spatial potential pattern of each activity disc in each lamina (the lead field) and calculates the source distribution from the measured spatial potential pattern via matrix inversion. Our in vitro slice preparation, and the combination of the intrinsic optical imaging with the multiple micro electrode array recordings provide optimal opportunity for the application of the iCSD method. The optical imaging of the slice provides information about the extent of the active area in each lamina, from which the lead fields can be determined. Based on the lead field matrix, the inverse solution can be calculated and applied to measured spatial potential patterns. This new method results much more accurate laminar distribution of the current source density than any other method before. Application of this new method to the evoked seizure like events or epileptic spikes improves our knowledge not only about the effect of different convulsants but about the cortical micro circuits also.

GABA signalling during mouse lens development: components and functionality

Kwakowsky Andrea[1]; Marija Schwirtlich[1]; Zova Katarova[1]; Yuchio Yanagawa[2]; Szabó Gábor[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi, Japan
csabakorossy@yahoo.com

The vertebrate lens because of its simplicity provides an ideal model for studying complex signaling pathways operating during gamma-aminobutyric that embryonic development. Previously, we have reported acid (GABA) and glutamic acid decarboxylase (GAD), the enzyme catalyzing GABA synthesis, are expressed in the mouse lens from early embryonic stages in a pattern suggesting a role in the lens fiber elongation, possibly by modulating the focal adhesion at the fiber tips. Here we report the expression patterns of different GABAA and GABAB receptor subunits, GABA transporters (vesicular VGAT and membrane GAT) in the developing mouse lens and in primary epithelial lens cultures (LEC) by semi-quantitative RT-PCR and immunohistochemistry. Furthermore we show that these components form a fully functional signaling pathway as GABA or specific GABA-R agonists evoked GABA receptor activation and transient increase of [Ca2+]I in primary LEC. To evaluate the role of GAD and GABA during lens development in vivo, we studied transgenic lenses overexpressing GAD67 under regulation of the alphaA-crystallin promoter and lenses of knock-out mice lacking both GAD forms. Morphological analysis of E12.5 embryonic lenses (stage lens vesicle) revealed precautious epithelial cell elongation in lenses overexpressing GAD67 and delayed elongation in GAD-/- mice. Adult lenses overexpressing GAD67 also displayed deformed fiber tips and lens sutures. Elevated GAD/GABA levels in the lens caused additional ocular defects like multilayered lens epithelium, cataract, lens-retina fusions, retinal foldings and ectopic retina. Taken together, our results show that GAD and GABA are involved in the cytoskeletal reorganisation during fiber cell differentiation by modulating the [Ca2+]I levels. Thus, the lens affords a unique model for studying the molecular mechanisms underlying the unconventional (non-synaptic) GABA signaling operating mostly during embryonic development, but also in the adult CNS and periphery.

Enhancement of passive avoidance learning by Neurotensin injected into the rat central nucleus of amygdale

László Kristóf; Tóth Krisztián; Bárdosi Réka; Molnár Ádám; Kertes Erika; Oláh-Várady Katalin; Lénárd László
Institute of Physiology and Neurophysyology Research Group, Hungarian Academy of Sciences, University of Pécs, Pécs, Hungary
kristof.laszlo@aok.pte.hu

Tridecapeptide Neurotensin (NT) heterogeneously distributed throughout the brain and in the periphery. In the central nervous system NT acts as a neurotransmitter and neuromodulator. The NT has been shown to be closely releated to central dopaminergic regulation. Via its receptors in the ventral mesencephalon NT stimulates dopamine cell firing and axonal dopamine release in limbic terminal fields. The central nucleus of amygdala (ACE), part of the limbic system, plays an important role in learning, memory, regulation of anxiety and emotional behaviour. It has been demonstrated that the amygdaloid body is rich in NT immunoreactive elements and NT-1 receptors. The aim of our study was to examine in the ACE the possible effects of NT on passive avoidance learning (PAV) in two-compartment passive avoidance paradigm. In the habituation trial male wistar rats were placed into the

lightened chamber and had free access to all parts of the apparatus for 180 s. In the conditioning trial animals were placed again into the large chamber. After entering the dark compartment the door was closed and rats were shocked with 0.4 mA and subsequently were microinjected bilaterally with 100 ng NT or 250 ng NT or 500 ng NT (Sigma: N 3010, dissolved in sterile saline, injected in volume of 0.4 µl) or 40 ng NT-1 receptor antagonist SR 48692 (Sanofi-Synthelabo) alone, or NT-1 receptor antagonist 15 min before 100 ng NT treatment or vehicle solution into the ACE. The test trials conducted 24 h and 7 days after conditioning. All of the doses of NT significantly increased the latency time. Prior treatment with the non-peptid NT-1 receptor antagonist (SR 48692), equimolar to NT treatment (0.18 nM and 0.18 nM, respectively) blocked the effects of NT. Antagonist in itself did not influence the passive avoidance learning. Our results show that in the rat ACE NT facilitates passive avoidance learning, via NT-1 receptors. Supported by ETT 317/2006, RET-0008/2005 MEDIPOLIS, NKTH-OTKA K68431 and by the HAS.

Mapping the biophysical properties of 28 sodium channel inhibitors

Lenkey Nóra; Mike Árpád; Karoly Robert; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
lenkeyn@koki.hu

All clinically used sodium channel inhibitors (SCIs) cause voltage- and use-dependent inhibition. However, biophysical properties of inhibition can differ widely even between use-dependent SCIs which seem to act similarly. Recently it has been proposed that, regarding the therapeutical potential of the drugs, the mechanism of inhibition can be more important than potency or isoform-selectivity (e.g. Ilyin et al., 2006). In this study we attempted to find out, if biophysical properties of the inhibition correlate to the therapeutic profile. We tested 28 use-dependent SCIs of different chemical structure and therapeutic indication including local anesthetics, anticonvulsants, antiarrhytmics, antidepressants, and antipsychotics. We examined the effects of SCIs on hNav1.2 channels expressed in HEK 293 cells, using an automated patch clamp apparatus (QPatch HT system, Sophion Bioscience, Denmark). We tested the development of inactivation in parallel with association, the recovery from inactivation in parallel with dissociation, and the preference toward fast- vs slow-inactivated states (using the protocol described in Lenkey et al, 2006). We found that properties of the inhibition correlate with therapeutic profile much more than with chemical structure, which suggests that biophysical property-sensitive screening of SCI drugs could be a useful strategy in drug development.

Cell type-dependent molecular composition of the axon initial segment

Lorincz Andrea; Nusser Zoltán
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
lorincz@koki.hu

The axon initial segment (AIS) is a functionally specialized region of the axon, which is believed to be critical for action potential generation and controling the output of the cell. The exact site of the initiation and the shape of the action potential are determined by the density and the distribution of voltage-gated sodium (Nav) and potassium (Kv) channels within the AIS. Here we used immunofluorescence reactions to determine the subcellular locations of Nav1.6, Kv1.2 and Kv7.3 subunits in the AIS of different cell types in the main olfactory bulb (MOB), neocortex, hippocampus and cerebellum. In mitral and tufted cells of

the MOB, immunolabeling for the Nav1.6 and Kv1.2 subunits devoids the proximal part and increases gradually towards the distal microdomain of the AIS, whereas in mitral cells the Kv7.3 subunit shows a slight decrease in density along the AIS towards distal locations. Hippocampal pyramidal cell AISs contain the Kv7.3 and Nav1.6 subunits at high densities, but the Kv1.2 subunit remains undetectable in this location. However, in a subpopulation of interneurons the AISs are strongly immunopositive for the Kv1.2 and Nav1.6 subunits, but negative for the Kv7.3 subunit. In neocortical pyramidal cells, the Nav1.6 subunit is distributed along the whole length of the AIS, whereas the Kv1.2 channels are mostly localized to the distal segment, where a reduction in the Kv7.3 subunit immunolabeling is observed. Out of these three subunits, only the Nav1.6 subunit is expressed in cerebellar Purkinje cell AISs at a detectable level. This precise cell type-specific molecular organization of the AIS is likely to contribute to the diversity of action potential shapes and neuronal firing properties observed in the CNS.

CB1 cannabinoid receptor and related molecular elements are down-regulated in the hippocampus of epileptic patients

Ludányi Anió.[1]*; Erőss Lóránd*[2]*; Czirják Sándor*[2]*; Vajda János*[2]*; Halász Péter*[3]*; Watanabe, Masahiko*[4]*; Palkovits Miklós.*[5]*; Maglóczky Zsófia*[1]*;Freund F. Tamás*[1]*; Katona István*[1]

[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]National Institute of Neurosurgery, Budapest, Hungary
[3]National Institute of Psychiatry and Neurology, Epilepsy Center, Budapest, Hungary
[4]Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan
[5]Department of Anatomy, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary
ludanyi@koki.hu

The endocannabinoid system is a recently recognized synaptic signaling system. Compelling evidence show that its perturbation prones animals to development of epileptic seizures indicating that endocannabinoids play an intrinsic protective role in controlling neuronal excitability. To elucidate whether potential long-term reorganization of endocannabinoid signaling occurs in chronic epileptic patients, we carried out comparative expression profiling along with quantitative electron microscopic analysis of epileptic hippocampal samples surgically removed from patients with intractable temporal lobe epilepsy and control postmortem hippocampal samples from subjects with no signs of neurological disorders. Quantitative PCR measurements revealed that mRNA level of the CB1 cannabinoid receptor was down regulated to one-third of its control value in epileptic hippocampus. Likewise, the expression level of the cannabinoid receptor interacting protein 1a was decreased, whereas that of the 1b isoform remained unaltered. Diacylglycerol lipase-alpha, the enzyme responsible for synthesis of 2-arachidonoylglycerol (2-AG) was also reduced by ~60%, while its related beta isoform remained unchanged. The expression of synthetic and degrading enzymes of anandamide, N-arachidonoyl-phosphatidylethanolamine phospholipase D and fatty acid amide hydrolase, respectively and 2-AG's degrading enzyme monoacylglycerol lipase did not show any differences. Parallel to the decline in CB1 mRNA, the density of CB1-immunolabeling was also decreased in the epileptic hippocampus, most robustly in the inner molecular layer of the dentate gyrus, where quantitative electron microscopic analysis uncovered reduction from 72% to 20% in the ratio of CB1-positive glutamatergic axon terminals. These findings reveal that endogenous neuroprotective signaling mediated by endocannabinoids may be impaired in epileptic human hippocampus and imply that down-regulation of CB1 receptors and related molecular components of the endocannabinoid system

at the glutamatergic axon terminals may be conducive to deleterious effects of increased network excitability.

Ionotropic neurotransmitter receptors: Activation and allosteric modulation

Maksay Gábor[1]; Fodor László[2]
[1]Department of Molecular Pharmacology, Institute of Biomolecular Chemistry, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Pharmacology,Gedeon Richter Plc, Budapest, Hungary
maksay@chemres.hu

Introduction: GABA and glycine are the main inhibitory neurotransmitters. GABA-A and glycine receptors (GlyR) form pentameric chloride channels and belong to the „Cys-loop" receptor superfamily with 5-HT3-type serotonin and nicotinic receptors. Homology-modelling has helped us to study the binding interactions of antagonists versus agonists in the binding interface of 5-HT3A receptors leading to ligand translocations, closure of the binding cavity and ionophore activation. Allosteric modulation of the ionotropic receptors enables the pharmacological fine-tuning of the corresponding neurotransmission. Methods: Radioligand ([3H]EBOB, [3H]strychnine) binding to native and recombinant receptors and whole-cell patch clamp electrophysiology in cultured rat cerebellar granule and cortical cells. Results: We describe a 17ß-derivative of the neurosteroid allopregnanolone to antagonize the potentiating effects of allopregnanolone selectively on a cerebellar (α6ßδ) population of GABA-A receptors with nanomolar potency. We also describe some tropeines (tropine esters) as bidirectional allosteric modulators of GlyRs, particularly a point mutant R271L of α1 GlyRs leading to hyperekplexia, an inherited neurological disorder. Nor-O-zatosetron is the highest affinity ligand of GlyRs reported so far. Conclusions: These allosteric modulators have unprecedentally high (nanomolar) potencies and serve as promising leads for the subunit-selective modulation of these ionotropic receptors.

The perception of cyclopean stimuli are independent from luminance-contrast

Markó Katalin[1]; Kiss Huba[1]; Mikó-Baráth Eszter[1]; Kovács Ilona[2]; Török Béla[3]; Jandó Gábor[1]
[1]Institute of Physiology, Faculty of Medicine, University of Pécs, Pécs, Hungary
[2]Department of Cognitive Science, University of Technology and Economics, Budapest, Hungary
[3]Department of Ophthalmology, Kantonsspital St. Gallen, Switzerland
kati_marko22@yahoo.com

Measuring visual evoked potential (VEP) response to dynamic random dot correlogram (DRDC) is a reliable method to detect binocular function even in non-verbal humans. Dynamic random dot stereograms (DRDS) can be used for a quick psychophysical assessment of stereopsis. Both DRDC-VEPs and depth perception are sensitive to the stimulus parameters. The objective of our present experiment was to study the effect of Michaelson contrast on the DRDC-VEP and depth perception. Sixteen young adult individuals (between 18-35 years of age) were tested. Binocular DRDC-VEPs and monocular yellow-black color checkerboard pattern reversal VEPs (PR-VEP) were used. Stimuli were presented on red-green channels of a computer monitor and viewed through red-green goggles for dichoptic viewing. Electrical scalp responses were recorded from Oz-Fz position. The Fourier component of the stimulus fundamental frequency in EEG was analyzed by T2circ statistics.

In the psychophysical tests the orientation of Snellen E dynamic random dot stereogram had to be determined by the subjects. Decreasing luminance caused an increase in the P100 latency and a decrease in amplitude in PR-VEPs, whereas neither the amplitude nor the phase of DRDC-VEPs showed correlation with the Michaelson contrast. About the same amplitude DRDC-VEPs could be recorded at 88%, 11% and in the 60% of the subjects even at 5.5% contrast when the PR-VEP became undetectable. This difference in dynamics can be due to the fact, that stereoscopic information and probably DRDC is processed by the magnocellular, while the high spatial frequency transient checkerboard stimuli are associated with the parvocellular system. The results of the psychophysical tests well correlated with our electrophysiological data. Supported by OTKA NF60806 to IK.

Oxaloacetate is efficient in recovering ischemia-induced impairment of long-term potentiation in rat hippocampal CA1 region

Marosi Máté; Fuzik János; Nagy Dávid; Rákos Gabriella; Kis Zsolt; Farkas Tamás; Toldi József
Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary
marosi.mate@gmail.com

Several acute brain pathological conditions are characterized by the presence of elevated glutamate concentrations. It is known from the literature that decreasing blood glutamate levels increases the driving force for an enhanced brain-to-blood efflux of glutamate, which removal could prevent the glutamate excitotoxicity seen in stroke conditions that causes long-lasting neurological deficits. The present study aimed the evaluating the effects of oxaloacetate - as a glutamate scavenger - on the impaired LTP in 2VO (2 vessel occlusion) ischemic model in rat. Transient (30 min) incomplete forebrain ischemia was carried out 72 hours before LTP induction. The transient brain hypoperfusion resulted in impaired LTP in the hippocampal CA1 region without damaging the basal synaptic transmission between the Schaffer collaterals and pyramidal neurons. Histochemical analysis (Fluoro-Jade B staining and NeuN-immunohistochemistry) revealed no injured cells in the CA1 region. Oxaloacetate administration immediately after the transient hypoperfusion prevented completely the impairment of LTP. Our result suggest that glutamate scavenging by oxaloacetate may contribute to diminishing of LTP impairment induced by ischemia.

Suppression of pseudorabies virus infection by the fluorescent tracer Fast Blue

Márton Gábor[1]; Szabó András[1]; Boldogköi Zsolt[2]; Nógrádi Antal[1]
[1]Laboratory of Neuromorphology, Department of Ophthalmology, University of Szeged, Szeged, Hungary
[2]Department of Medical Biology, University of Szeged, Szeged, Hungary
margavino@freemail.hu

The pseudorabies virus (Prv) inoculated into peripherial tissue infects neurons and spreads through presynaptically linked neurons (retrograde transsynaptic transmission) resulted in the lytic infection of chains of neurons. The fluorescent tracer Fast Blue (FB) is widely used in experimental neuroanatomical studies. The intriguing question raised, whether FB has an effect on the infection of the Prv upon double use of these tracers. In this study we inoculated Bartha strain of Prv into hindlimb muscles of SD rats and after 24 or 48 hours survival Fast Blue crystals were applied to the cut sciatic nerve of the animals and the spreading of the

virus was investigated. The virus infection was also studied in neuroblastoma cell culture in the presence of FB. In control rats the virus appeared in the spinal cord motoneurons 50 hours after the virus inoculation and spread into the linked interneurons by 80-96 hours. In animals where the sciatic nerve was labelled with FB 24 hours after the PrV injection the motoneurons did not become infected, but after 96 hours survival many interneurons were infected. In rats where FB was applied 48 hours after the PrV injection the virus appeared in both motoneurones and interneurones. The FB effect on the viability of neuroblastoma cells was studied by Trypan Blue dye exclusion test after 24 hours incubation with different concentrations of FB. At the highest concentration (0,8g/100ml culture medium) the rate of the viable cells in the FB incubated culture did not significantly differ (paired t-test) from that of untreated culture. At this concentration virus could not be detected in the treated cells after 19h hours of virus incubation. Our results show that FB inhibits the Prv infection in vitro in a dose dependent manner and the Fast Blue delivered to the primarily infected rat neurons within 24 hours after PrV injection prevents the PrV infection of the lumbar motoneurons whereas the virus is able to spread to the linked neurones inducing productive infection.

Topically applied endogenous ligands (endomorphin-1 and kynurenic acid) exhibit analgesic effects for joint pain

Mecs László[1]; Tuboly Gábor[2]; Benedek György[2]; Horvath Gyöngyi[2]
[1]Department of Orthopaedics and [2]Department of Physiology, Faculty of Medicine, University of Szeged, Szeged, Hungary
benedek@phys.szote.u-szeged.hu

Opioid effects are mediated by central and peripheral opioid receptors. The glutamate receptors are also demonstrated peripherally, and the local administration of the NMDA receptor antagonist ketamine produces antinociception. Here we examine the antinociceptive potential of the endogenous opioid peptide, endomorphin-1, and the endogenous NMDA receptor antagonist kynurenic acid and their interaction at peripheral level Methods: After obtaining institutional ethical approval, mechanical hypersensitivity was produced by injection of carrageenan (300 µg/20µl) into the tibiotarsal joint of the right hind leg. The mechanical pain threshold was assessed by von Frey filaments (0.064-110 g). After baseline paw withdrawal measurements (pre-carrageenan baseline value at -180 min) carrageenan was injected. The paw withdrawal latencies were obtained again 3 h after carrageenan injection (post-carrageenan baseline values at 0 min). Endomorphin-1 (30, 100 and 200 µg) and kynurenic acid (30, 100, 200 and 400 µg) and their combinations (20 µl) were given into the inflamed joint. After the injections mechanical hypersensivity was determined for 75 min after the drug adminstrations. Analysis of variance (ANOVA) of the data for repeated measures was used for overall effects, followed by the Fisher LSD test. A probability level of 0.05 was considered significant. Results: Both kynurenic acid and endomorphin-1 produced dose-dependent antihyperalgesia, however, none of them revealed the hyperalgesia. Endomorphin-1 had higher potency but shorter duration, compared to kynurenic acid. The coadministration of them caused an additive interaction. Neither endomorphin-1 nor kynurenic acid influenced tha pain threshold contralaterally. In conclusion, peripherally administered endogenous opioid agonist and NMDA receptor antagonist ligands might be beneficial in inflammatory pain. Since both drugs hardly cross the blood-brain barrier, their local administration does not cause central side effects. This work was supported by the National Research and Development Office, Hungary (OMFB-0066/2005/DNT) and by a Hungarian Research Grant (OTKA, K60278).

Contribution of enhanced adrenal gland sensitivity in dissociated ACTH and corticosterone release of vasopressin-deficient Brattleboro rat pups

Mergl Zsuzsa[1]; Subodh Kumar Jain[2]; Vass Ágnes[1]; Barna István[1]; Zelena Dóra[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Zoology and Biotechnology, Dk. H.S. Gour University, Sagar, India
zelena@koki.hu

Stressful early life events have special importance in the development permanently affecting the lifetime vulnerability to diseases. For interpretation of the long-term consequences it is important to understand the regulation of the hormonal responses in the neonatal period. As the role of vasopressin (AVP) in hypothalamo-pituitary-adrenal axis (HPA) regulation as well as in affective disorders seems to be important, we addressed the question if the congenital lack of AVP will modify the stress reactivity of the rat pups. AVP producing (di/+) and deficient (di/di) Brattleboro rat pups were used. Genotyping was performed postmortem by measuring pituitary AVP levels. In 10-day-old pups of both genotypes the ip Hypnorm® injection induced a remarkable corticosterone (B) elevation with measurable ACTH increase in the di/+ but just with small rise in the di/di pups. The dissociation of ACTH and B response might be the consequence of the enhanced sensitivity of the adrenal cortex to ACTH. We confirmed this theory in vitro adding 10-13, 10-12 and 10-11M ACTH to the incubation medium. However it is unlikely, that the 25-40% sensitivity elevation could fully explain the similar effectiveness of a 75-85 % lower ACTH elevation. Moreover the in vivo sensitivity of the 10-day-old pups was similar. During perinatal period AVP seems to be the dominant secretagogue of the ACTH release. Alternative (non-ACTH) stimuli of glucocorticoid secretion might come in front in neonates.

Experimental testing of the modulated- and guarded receptor hypotheses for the mechanism of use-dependent sodium channel inhibition

Mike Árpád; Károly Róbert; Lenkey Nóra; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungary Academy of Sciences Budapest, Hungary
mike@koki.hu

Common properties of use-dependent and voltage-dependent sodium channel inhibitor drugs can be explained by two major hypotheses: the modulated receptor hypothesis (MRH) and the guarded receptor hypothesis (GRH). We have developed a method for assessing which of these hypotheses better describes inhibitory effect of individual drugs. The method is based on monitoring the association of drugs to channels during prolonged trains of depolarizations (see accompanying poster). Different hyperpolarized / depolarized duration ratios were used; in one protocol channels were in resting conformation most of the time during the train, while in another protocol the ratio of inactivated channels was maximized. Increased inhibition was regarded as a sign of the MRH being relevant, while increased rate of association was considered to indicate the correctness of the GRH. Six fast inactivated state preferring drugs were investigated regarding their mechanism of effect, the anticonvulsant Carbamazepine, lamotrigine and phenytoin, the antiarrhythmics lidocaine and procainimide, and the spasmolytic tolperisone. Lidocaine and lamotrigine showed characteristics suggestive of the GRH, while in the case of phenytoin, and especially procainimide we found a mechanism closer to the MRH.

Disturbances in social communication and the emergence of violent aggression after early social deprivation in rats

Mikics Éva; Tóth Máté; Halász József; Barsy Boglárka; Haller József
Department of Behavioural Neurobiology, Institute of Experimental Medicine, Budapest, Hungary
mikics@koki.hu

Children raised in aversive social environments are at risk to develop serious psychopathologies in adulthood, including antisocial behaviours and an inclination towards violence. Similar phenomena were noticed in various animal species as well: socially deprived animals showed an increase in aggressive behaviour in a number of studies. However, qualitative changes in social communication and aggressive behaviour have not yet been investigated in socially deprived animals. Here were show that social isolation from weaning not only increases the level of aggressiveness, but results in abnormal attack patterns and deficits in social communication in male rats. In socially deprived rats, the share of attacks aimed at vulnerable body parts of opponents (head, throat and belly) dramatically increased and the attack/threat ratio was shifted towards attacks, suggesting a decrease in intention signaling. Moreover, the results of the Multiple Regression analysis show that the communication deficit (i.e. low intention signaling) and the abnormal features of aggressive behaviour (i.e. changes in attack targeting) were closely interrelated. Defensive behaviours were also increased by socially deprivation, although deprived rats delivered more attacks than controls. These data suggest that the social deprivation-induced abnormal forms of aggression model the aggression-related problems resulting from early social neglect in humans, and studies on its brain mechanisms may increase our understanding of the brain mechanisms underlying psychopathologies resulting from early social problems.

Reorganization of synaptic inputs in the hypothalamic paraventricular nucleus after chronic variable stress

Miklós H. Ildikó; Kovács J. Krisztina
Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
miklos@koki.hu

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis as a pathogenetic factor has been implicated in various stress-related affective disorders such as depression. The activity of HPA axis is governed by corticotropin-releasing hormone (CRH) and co-secretagogues synthesized by neurosecretory neurons in the dorsomedial parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN). According to the recently developed network hypothesis for depression, the disease is associated with changes in neuronal connectivity rather than changes in chemical/neurotransmitter balance. Morphological alterations have been revealed in various brain areas affected by depression, however the neuronal network in the dorsomedial parvocellular subdivision of the PVH has not examined yet. Our aim was to compare synaptic innervation of the stress-related CRH neurons of the PVH in control animals and after three weeks of chronic variable stress (CVS). We used combined pre-embedding CRH immunocytochemistry with postembedding GABA immunogold staining and calculated the number of GABAergic inhibitory- and non-GABAergic, excitatory synapses in the dorsomedial parvocellular part of the PVH using the quantitative disector. Our results indicate significantly enhanced synaptic neurotransmission in the PVH: the number of all synapses in the dorsomedial parvocellular subdivision increased by 96.1% after CVS. The

number of the GABAergic synapses increased by 93.0%, the number of all unlabeled synapses was also increased by 101.1%, however the ratio of the GABAergic and non-GABAergic synapses was not altered. Regarding the stress-related CRH neurons, the number of the GABAergic synapses on CRH-positive profiles increased by 63.8%, the number of the non-labeled synapses almost doubled, but the ratio of the GABAergic and non-labeled synapses were not changed. We analyzed the subcellular distribution of the GABAergic and non-labeled synapses terminated on CRH neurons and found that the ratio of the "input integrator" dendritic GABAergic inhibition was increased while the "output regulator" somatic GABAergic inhibition was decreased. The present result is the first ultrastructural evidence of synaptic reorganization on the stress-integrating neurosecretory neurons in the PVH in rats after chronic variable stress that clearly supports the network hypothesis of depression.

Serotonergic regulation of feed-forward networks activated by single pyramidal cells in the human cerebral cortex

Molnár Gábor; Oláh Szabolcs; Komlósi Gergely; Füle Miklós; Varga Csaba; Barzó Pál; Tamás Gábor
Cortical Microcircuits Research Group, Hungarian Academy of Sciences and University of Szeged, Szeged, Hungary
malnarg@bio.u-szeged.hu

Serotonin is a neurotransmitter suggested to influence cognitive functions and modulated by the most widely prescribed CNS drugs. However, the site of serotonin action in cortical circuits remains undefined. We performed simultaneous recordings from pairs, triplets and quadruplets of neurons and analyzed their interconnections in slices of human association cortices. Single action potentials in identified human pyramidal cells frequently triggered reliable and stereotyped series of multiple postsynaptic potentials in simultaneously recorded pyramidal cells and interneurons. Such temporally correlated series of postsynaptic events were composed of both excitatory and inhibitory responses and lasted up to ~35 ms. Single spike triggered long lasting network events could be effectively modulated pharmacologically. Serotonin and serotonin receptor 2 subtype agonist a-methyl-serotonin was found to reversibly eliminate pyramidal cell activated di- and polysynaptic inhibition, and axo-axonic cell triggered polysynaptic but not disynaptic events. This effect was due to the downregulation of glutamate release probability through 5HT2 receptors located presumably on presynaptic terminals of pyramidal cells. Thus, recruitment of proposed building blocks of higher order computation is feasible with single pyramidal axons which can be effectively modulated suggesting a microcircuit-specific site of action for serotoninergics of common therapeutic use.

Gamma-hydroxybutyrate elicits Ca2+ ion transients in the nucleus accumbens by activating baclofen-insensitive gamma-hydroxybutyrate receptors

Molnár Tünde[1]; Kardos Julianna[1]; Simon-Trompler Edit[2]; Rheinallt H. Parri[3]; Vincenzo Crunelli[4]; Palkovits Miklós[5]; Emri Zsuzsa[1]
[1] Institute of Biomolecular Chemistry, Department of Neurochemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
[2]Institute of Biomolecular Chemistry, Group for Radiosyntheses, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
[3]School of Life and Health Sciences, Aston University, Birmingham, UK

[4]School of Bioscinences, Cardiff University, Cardiff, UK
[5]Neuromorphological and Neuroendocrine Research Laboratory, Semmelweis University and Hungarian Academy of Sciences, Hungary
tmolnar@chemres.hu

Gamma-hydroxybutyrate (GHB) has diverse neuropharmacological and neurobiological properties, including activation of both GABAB receptors and putative GHB receptors, although conclusive evidence on the existence of GHB receptor-mediated responses to GHB has proved difficult due to the poor specificity of available pharmacological agents. GHB action on Ca2+ ion signalling was studied in rat (10-24 days old) nucleus accumbens (NA) slices incubated with the Ca2+ ion indicator dye Fluo-4AM and TTX (1µM). Responding cells were injected with Alexa-green, and identified as astrocytes by their morphology and S100b immunoreactivity. The number of astrocytes showing increases in [Ca2+]i was measured under different conditions, including the addition of antagonists for GABAB (CGP-54626, 10µM), glutamate (CNQX, 10µM; APV, 20µM; MPEP, 30µM) and putative GHB (NCS-382, 300µM) receptors. GHB, but not baclofen, induced transient [Ca2+]i elevation in astrocytes (2mM GHB: 3.7±0.6; 20µM baclofen: 0.4±0.3). The number of astrocytes responding to GHB application was unaffected by CGP 54626 (2.5±0.8) and NCS-382 (3.7±0.4) but was reduced by CNQX (1.7±0.6), MPEP (0.7±0.3) or APV (2.1±0.7). The GHB-induced [Ca2+]i elevations were abolished in a medium with no Ca2+ added (0.5±0.2) or after application of the SERCA inhibitor cyclopiazonic acid (10mM; 0.4±0.4), but persisted in slices incubated with bafilomycin (4mM; 2.9±0.4). Affinity screening of GHB, NCS-382, baclofen, CGP-55845 and the common metabolite of GHB and GABA, succinate were performed in [3H]GHB displacement measurements. Competition and saturation experiments showed only one GHB binding site (KD=0.9µM and BMAX=14 pmoles/mg protein) in rat rostral telencephalon membranes. GHB, succinate and NCS-382 inhibited [3H]GHB binding (KI,GHB=3µM, KI,SUC=172µM and KI,NCS=1µM), but baclofen and CGP-55845 (1nM-1mM) did not. A similar binding profile was found in human NA membranes. These data show a clear effect of GHB on astrocytes of the NA suggesting a role in the rewarding properties of this drug. This action of GHB appears not to involve GABAB receptors. Financial support of grants Wellcome Trust (68690 and 71436), MediChem2 (1/A/005/2004 NKFP) and Transporter Explorer (GVOP 3.1.1-2004-05-00) are acknowledged.

Changes caused by estrogen in the cytoskeleton and antioxidant system

Mórotz Gábor[1]; Szegő M. Éva[2]
[1]Eötvös Loránd University, Faculty of Science, Institute of Biology, Laboratory of Proteomics, Budapest, Hungary
[2]University of Szeged, Department of Medical Chemistry, Szeged, Hungary
morotz.gabor@gmail.com

Estrogen (E2) is one of the most important female sex hormons affecting many places the function of the nervous system. It induces protein expression in two ways; via activating the intracellular signaling pathways or through its receptors binding to DNA. Besides its well-known effects on transcription factors, our knowledge about estrogen-induced protein family is insufficient. Our group (Laboratory of Proteomics of ELU) proved that E2 can achieve „preventive" protective effects on the nervous system through modfying many intracellular processes. The aim of this research was to verify the results of the fluorescent two dimensional gelelelectrophoresis (DIGE) method, by using western-blot (WB) technique in order to measure protein changes caused by estrogen in the brain. Moreover we have

ivestigated the changes of the number of immunopositive cells in several brain areas. Three proteins were examined namely: calpain1, coronin1b and SOD2. Adult female C57/Bl6 mice were ovarectomised and injected with 17β-estradiol or ethil-oleat vehicle, and brains were removed 24 hours later for immunhistochemistry or for WB. DIGE results were confirmed by the western-blot analysis of the calpain1 and SOD2 proteins: levels of these two proteins were increased by estrogen in the cytoplasmic fraction. The expression of coronin1b was decreased but there was no significant change observed. At the same time there were no changes detected in the number of the immunopositive cell number within the examined brain areas. Summarising our results we can conclude that we verified our results from the proteomics experiment with a conventional method which confirms the reliability of this procedure in researches in neurobiology. Here we proved that estrogen changes the expression, among other things, of some antioxidant (SOD2), cytoskeletal (coronin1b) and cytoskeleton remodelling related (calpain1) proteins but it does not influences the number of cells which produce these proteins. Based on our results we provided further evidences of estrogen-induced neuroprotection.

Differential expression patterns of K+/Cl- co-transporter (KCC2) in neurons within the superficial spinal dorsal horn of rats

Muszil Dóra[1]*; Shigemoto, Ryuichi* [2]*; Antal Miklós*[1]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Hungary
[2]Division of Cerebral Functions, NIPS, Okazaki, Japan
antal@chondron.anat.sote.hu

GABA and glycine mediated hyperpolarizing inhibition is associated with a chloride-influx that depends on the extrusion of chloride from the cytosol. In neurons, the inwardly directed chloride electrochemical gradient primarily depends on the expression of an isoform of potassium-chloride co-transporters, KCC2. Here, we investigated the cellular distribution of KCC2 in the superficial spinal dorsal horn of adult rats by using immunocytochemical methods both at the light and electron microscopic level. We demonstrated that perikarya and dendrites widely expressed KCC2, but axon terminals of nociceptive primary afferents and spinal neurons proved to be negative for KCC2. Studying the somato-dendritic expression and distribution of KCC2 we found that it varied in a wide range. Somatic and dendritic fragments with high, medium and low level of KCC2 expression were equally recovered. Moreover, we also observed dendritic segments that were negative for KCC2. Investigating KCC2 expression on NK1 immunoreactive neurons, that allowed us to study a large part of the somato-dendritic compartment, we found that KCC2 presented a quite homogeneous distribution along the somato-dentritic membrane of individual neurons. However, the density of KCC2 expression varied from neuron to neuron. We have recovered neurons with high and also with low, if any, KCC2 expression. The results suggest that GABAA receptor mediated synaptic mechanisms may evoke depolarization in axo-axonic synaptic contacts in the superficial spinal dorsal horn. In addition, the postsynaptic effect of GABAA receptor activation on the somato-dendritic membrane may also vary from neuron to neuron in laminae I-II of the spinal gray matter. This work was supported by: OTKA 46121, ETT 079/2006 and MTA-TKI 242.

Changes of EEG during processes of nonverbal (visual) creative thinking

Nagornova Zhanna
Institute of the Human Brain, Russian Academy of Sciences, Moscow, Russia

nagornova_zh@mail.ru

Methods: 30 healthy subjects (16 males, 14 females, 18-27 years, mean age 20 years) took part in our study. We used 2 creative tasks and 2 control (noncreative) tasks. In the first creative task (Cr1) volunteers were asked to draw any original, creative pictures on their choice; In the second creative task (Cr2) volunteers were asked to draw originally a face, a house and a clown; In the first noncreative (control) task (N_Cr1) volunteers were asked to draw picture, which was presented to them before; In the second noncreative (control) task (N_Cr2) volunteers were asked to draw geometric figures without any system. Subjects performed all tasks using geometrical figures: rectangle, triangle, circus and semicircle. During task performance an EEG was recorded from 19 scalp electrodes according to the 10-20 international system. Mathematical analysis of the parameters of the EEG in 7 frequency bands: delta δ (1.5-3.5 Hz), theta θ (4-7 Hz), alpha1 α1 (7.5-9.5 Hz), alpha2 α2 (10-12.5 Hz), beta1 β1 (13-18 Hz), beta2 β2 (18.5-30 Hz), gamma γ (30.5–40 Hz) was performed using program packet WinEEG (Ponomarev V.A, IHB RAS). For statistical analysis we used two-way ANOVA for interaction of factors "TASK" x "LOCALIZATION" with Greenhouse-Geisser correction.
Results: We obtained significant coherence difference ($p<0.05$) in all considered frequency bands. EEG coherence in α1 and α2 rhythms was higher during creative tasks in comparison with the noncreative tasks in derivations from most pairs of electrodes (Cr1-N_Cr1, Cr1-N_Cr2, Cr2-N_Cr1, Cr2-N_Cr2). Some authors consider a high level of α rhythm as an index of low cortical activation during creative thinking (Martindale, Hasenfas, 1978; Molle et al, 1996, 1999; Jausovec et al., 2000). We also found a long-distance coupling between frontal and occipital regions in δ and θ bands at creative tasks in comparison with noncreative task N_Cr2 and long-distance coupling between temporal regions in θ band in comparison of data obtained at creative tasks to the noncreative N_Cr1. This suggests, that changes of coherence in lower frequency bands reflect concentration of attention mostly to internal processing (Bhattacharya J., Petsche H, 2002).
Creative versus noncreative visual thinking was characterized by long-distance desynchronization between hemispheres in higher frequency bands (β1, β2, γ), and synchronization within right and left hemispheres in these frequency bands.
Thus, we suggest that lower and upper frequency bands may play a different functional role in processes of nonverbal (figurative) creative thinking. Changes of coherence in δ and θ rhythms may reflect total level of cortical activation and changes of coherence in β2 and γ rhythms may reflect cognitive processes specific for visual creative thinking. Decoupling between hemispheres may reflect reduction of influence of the left hemisphere during visual creative thinking (Rotenberg, 2004).
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Sensory properties of neurons in the pathway connecting the superior colliculus and the basal ganglia

Nagy Attila; Paróczy Zsuzsanna; Márkus Zita; Berényi Antal; Benedek György
Department of Physiology, University of Szeged, Szeged, Hungary
benedek@phys.szote.u-szeged.hu

The existence of a multisensory pathway to the basal ganglia that includes neurons in the intermediate and deep layers of the superior colliculus (SC), in the thalamo-cortical circuits between the suprageniculate nucleus (Sg) and the cortex along the anterior ectosylvian sulcus (AES) was earlier described. In this presentation, we summarize the visual, auditory,

somatosensory and multisensory response properties of neurons along this pathway. Extracellular microelectrode single-cell recordings were performed in the SC, Sg, AES cortex, substantia nigra and caudate nucleus of anaesthetized, immobilized, artificially ventilated cats. The neurons in each structure demonstrated rather uniform receptive field properties. The visual and auditory receptive fields covered the majority of the physically approachable sensory field, while the somatosensory receptive fields covered the whole body surface of the animal. A substantial portion of the neurons revealed multisensory properties. The visual neurons possessed unique spectral spatio-temporal properties; they preferred low spatial and high temporal frequencies and displayed narrow spatial and temporal frequency tuning. A large proportion of the neurons exhibited the ability to provide information via their discharge rate on the site of the stimulus within their receptive field. This suggests that they can serve as panoramic localizers. Further, the sites of maximal responsivity of the panoramic neurons are distributed over the whole extent of the large receptive fields. The absence of classical topographical organization and the panoramic localization ability of the neurons suggest the existence of a distributed population code of multisensory information in the tectal extrageniculate system. A majority of the investigated multisensory units displayed significant multisensory cross-modal interactions. The multisensory response enhancements were either additive or superadditive; multisensory response depressions were also detected. The physiological properties summarized above are uncommon along the unimodal sensory pathways, i.e. the geniculo-striate, the primary auditory or somatosensory pathways, and ideal to provide sensory feedback for motion control during self-movements. We suggest that the sensory information essential for the sensory-motor function of the basal ganglia is processed through the tecto-suprageniculo-AES cortex pathways.

Neuronal systems involved in the innervation of the thymus

Netzer, Niv; Palkovits Miklós; Gallatz Katalin
Department of Anatomy, Histology and Embryology, Neuromorphological and Neuroendocrine Research Laboratory, Semmelweis University, Budapest, Hungary
palkovits@ana.sote.hu

The thymus is a primary lymphoid organ that is the site of the proliferation and maturation of the T lymphocytes. Few endocrine cells, producing hormones (thymosin, insulin ACTH) are a present in the parenchyme and the thymus is also a target for the effects of several hormones. It is known from the earlier retrograde tracing studies, that the thymus receives parasympathetic and sympathetic innervation. The preganglionic neurons are present in the lower brain stem and in the intermediolateral cell column of the thoracic spinal cord. In the thymus, peptidergic cholinergic and catecholaminergic nerve terminals can be observed. However, there are no data about the premotor neurons and the neuronal pathways that may regulate parasympathetic and sympathetic outflow to the thymus. In this study, retrograde, transneuronal tract tracing technique was applied to identify those premotor nuclei of the CNS that are involved in these neural connections. Neurotropic pseudorabies viruses (BDG) were injected into the thymus. After different survival times the animals were perfused, 50 um thick serial coronal sections were cut on a freezing microtom from the forebrain, from the brainstem and from the cervical and thoracic segments of the spinal cord. Immunofluorescence and immunohistochemical methods were perfomed to demonstrate the viral labeling. Primary, secondary and terciary infected cells appeared 3-4 days after virus injection within nuclei of the medulla oblongata, pons, hypothalamus and in the cervical and thoracic spinal cord. Especially strong labeling was observed in the parvicellular part of the paraventricular nucleus. This data indicate that complex neuronal pathways participate in the

modulation of the CNS-thymus interaction. Further experiments are in progress to map the labeled neurons in the entire neuroaxis including the spinal cord. Transsection of the vagus nerve, sympathectomy and light and elektronmicroscopical immunocytochemistry are necessary to understand the exact route and termination of the pathways which may have important role in the regulation of the functional activity of the thymus.
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Reciprocity substantiates information funneling in the cerebral cortex

Négyessy László[1]; Nepusz Tamás[2]; Zalányi László[2]; Bazsó Fülöp[2,1]
[1]Neurobionics Research Group, Hungarian Academy of Sciences, Budapest, Hungary
[2]Research Institute for Particle and Nuclear Physics of the Hungarian Academy of Sciences, Budapest, Hungary
negyessy@ana.sote.hu

Areas of the cerebral cortex form a typical feedback network characterized by the dominance of mutual, reciprocal connections. Reciprocity and the dense connectedness of the cortex present large obstacles in deciphering how information is propagated in such a complicated network. We analysed the potential of information convergence and divergence through the connections between the cortical areas of the macaque and cat brain by introducing the new index convergence degree (CD). The data show that the potential of convergence and divergence are complementary, reciprocated properties of the connections. The arrangement of the convergent and divergent connections apparently reflects the hierarchical organization of the cortical network. We found that convergence/divergence properties of the cortical areas strongly correlate with their measure of centrality in the network. Studies on computer-generated graphs confirmed this observation and showed that the cortical-like pattern of convergence/divergence requires a high level of reciprocity in the network. Interestingly, cortical networks seem to have a definite level of reciprocity (70%-80%) and a portion of connections remains non-reciprocated. Altogether, our study explored a new organizational feature of the networks, which is characteristic of the cerebral cortex dominated by recurrent connections.

Embryonic spinal cord grafts promote the regeneration of injured host cervical motoneurones

Nógrádi Antal[1]; Szabó András[1]; Borda Stefánia[2]; Anderson N. Patrick[3]
[1]Department Ophthalmology, University of Szeged, Hungary
[2]Hód-Prodent BT, Hódmezővásárhely, Hungary
[3]Department Anatomy and Developmental Biology, University College London, UK
nogradi@opht.szote.u-szeged.hu

Spinal cord injury usually results in irreversible loss of function and morphological structures of the cord. Injury affecting the cervical segments of the cord induces tetraplegia and denervation of vital respiratory muscles. Therefore in this study we intended to restore lost spinal cord function following a spinal hemisection injury. The right cervical 6th spinal segment was hemisected, the 6th ventral root avulsed and an embryonic spinal cord graft enriched in motoneurones was placed into the hemisection cavity. Then the avulsed spinal root was reimplanted into the graft. Control animals underwent the same operation but received no graft. After 3 months survival the corticospinal and rubrospinal tracts were labelled anterogradely with BDA, the 6th spinal nerve was labelled retrogradely with Fast

Blue and various immunhistochemical markers were detected. The C6 ventral root was reinnervated by axons of host motoneurones and neurones of the lateral spinal nucleus. Grafted cells did not contribute in considerable number to the reinnervation of the ventral root. However, grafts promoted the regeneration of host neurones as significantly more neurones were retrogradely labelled in grafted animals than in control ones. Based on CatWalk analysis and tension recording data the grafted animals showed improved functional reinnervation in their affected forelimbs as compared to that of controls. The grafts received various supraspinal inputs: serotonergic fibres and rubrospinal fibres readily entered the graft and established functional synapses on the grafted neurones, while corticospinal fibres avoided the territory of the graft. These results suggest that embryonic spinal cord grafts act as relay bridges between the injured spinal cord stumps and promote the regeneration of the host neurones rather than contributing directly to the reinnervation of denervated forelimbs. This work was supported by the Wellcome Trust

Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals

Nyilas Rita[1]; Dudok Barna[1]; Urbán M. Gabriella[1]; Ken Mackie[2]; Masahiko Watanabe[3]; Benjamin F. Cravatt[4]; Freund F. Tamás[1]; Katona István[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Department of Psychological and Brain Sciences, Indiana University, Bloomington
[3]Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan
[4]The Scripps Research Institute, La Jolla, USA
nyilasr@koki.hu

Endocannabinoids are regarded as retrograde signaling molecules at various types of synapses throughout the central nervous system. The lipid derivatives anandamide and 2-arachidonoylglycerol (2-AG) are generally thought to be the key molecular players in this process. Previous anatomical and electrophysiological studies provided compelling evidence that the biosynthetic enzyme of 2-AG is indeed localized in the postsynaptic plasma membrane, whereas its target, the CB1 cannabinoid receptor and the enzyme responsible for its inactivation are both found presynaptically. This molecular architecture of 2-AG signaling is a conserved feature of most synapses and support the retrograde signaling role of 2-AG. Conversely, the molecular and neuroanatomical organization of synaptic anandamide signaling remains largely unknown. In contrast to its predicted role in retrograde signaling, here we show that NAPE-PLD, a biosynthetic enzyme of anandamide and its related bioactive congeners, the N-acylethanolamines (NAEs), is concentrated presynaptically in several types of hippocampal excitatory axon terminals. Furthermore, high-resolution quantitative immunogold labeling demonstrates that this calcium-sensitive enzyme is localized predominantly on the intracellular membrane cisternae of axonal calcium stores. Finally, the highest density of NAPE-PLD is found in mossy terminals of granule cells, which do not express CB1 receptors. Taken together, these findings suggest that anandamide and related NAEs are also present at glutamatergic synapses, but the sites of their synthesis and action are remarkably different from 2-AG, indicating distinct physiological roles for given endocannabinoids in the regulation of synaptic neurotransmission and plasticity.

Functional reinnervation of denervated hindlimb muscles induced by grafted neuroectodermal stem cells

Pajer Krisztián[1]; Szabó András[1]; Nógrádi Antal[1]

[1]Laboratory of Neuromorphology, Department of Ophthalmology, University of Szeged, Hungary
pajer.krisztian@stud.u-szeged.hu

Spinal cord motoneurons are severely injured and destined to die in cases of spinal cord injury and diseases affecting the motoneuron pools. Stem cells transplantation is a possible way to replace injured motoneuron populations or enhance their regeneration. In this study we examined whether it is possible a) to replace the damaged motoneurons by grafted murine neuroectodermal stem cells and b) to improve the regeneration of injured spinal motoneurons by grafted stem cells. In our experimental model the left lumbar 4 (L4) ventral root of spinal cord was avulsed, stem cells were grafted into the L4 segment of the spinal cord and the avulsed ventral root was reimplanted. In control animals only the L4 ventral root was avulsed and reimplanted without stem cell transplantation. After 3 months survival the L4 spinal nerve was labelled with Fast Blue and the transplanted cells were detected by immunhistochemical markers. The functional reinnervation was established with CatWalk analysis and muscle force measurements. The majority of the grafted cells survived and differentiated. The transplanted cells settled in the dorsal horn and in the intermedier gray matter and to a least extent in the ventral horn. On the other hand, both stem cell-derived astrocytes and neurons were found on the pial surface of the cord, although they appeared to be less differentiated cells. If low numbers of stem cells (n<50000) were implanted, the transplanted cells differentiated to neurons and astrocytes and induced a limited survival and regeneration of injured host motoneurons. Transplantation of higher number of cells (n>100000) induced greater numbers of motoneurons to reinnervate the reimplanted ventral root. The grafted animals showed improved locomotion pattern and functional reinnervation in their operated hindlimbs as compared to that of controls. Our results have presented evidence that stem cells are able to promote the survival and regeneration of the host motoneurons and the denervated hindlimbs were reinnervated by regenerated host motoneurons

Orexin and MCH regulating the visceromotor functions of the antrum and the proximal duodenum

Papp Sugárka Rege; Kirschenbaum Daniel; Kurucz Péter; Renner Éva; Palkovits Miklós
Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
regepapp@yahoo.com

Two distinct groups of orexinergic neuropeptides, orexin and melanin-concentrating hormone (MCH), have been localized in the dorsolateral hypothalamus (DLH). Fibers of these cell groups are widely distributed throughout the brain and the spinal cord. Thus, fine orexin- and MCH-containing varicose fibers were found in the nucleus of the solitary tract (NTS), dorsal motor vagal nucleus (DMV) and intermediolateral cell column of the thoracic spinal cord (IML). After virus injection into two ulcer-sensitive regions of the rat gastrointestinal tract: the antrum and the proximal duodenum, retrogradely labeled cells were found first order in the DMV, NTS and IML. Praemotor neurons projecting to the vagal complex were traced in the DLH. After bilateral subdiaphragmatic vagotomy, labeled cells were found first order in the IML, second order in the NTS, and tertiary ones by 96 hours in the DLH. We demonstrated that there is an overlap between virus-infected and orexin- or MCH-containing neurons in the DLH. We showed orexin- and MCH-immunopositive fibers in juxtaposition to the virus-labeled cells in the vagal complex, as well as in the IML. The density of fibers immunostained for orexin or MCH in the medulla and the spinal cord has been investigated

after unilateral transections of the descending hypothalamic pathway at the level of the pontine-medullary junction. The density of orexin- and MCH-immunoreactive fibers were decreased caudal to the transaction. Based on these results, orexin and MCH are suggested to regulate the vagal and intermediolateral nuclei which innervate the antrum and the proximal duodenum.
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Prolactin releasing peptide expressing neurons in the dorsomedial nucleus of the hypothalamus influence the orexinergic system via direct connections with MCH and orexin producing cells

Papp Sugárka Rege[3]; Kirilly Eszter[1]; Kitka Tamás[1]; Bagdy György[1]; Szabó-Meltzer Kinga[3]; Tóth Zsuzsanna E.[1,2]
[1]Department of Pharmacology and Pharmacotherapy and [2]Department of Anatomy, Histology and Embryology, Semmelweis University Budapest, Hungary [3]Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
regepapp@yahoo.com

Prolactin-releasing peptide (PrRP) is expressed in catecholaminergic cell groups of the caudal medulla oblongata and in non-catecholaminergic cells in the dorsomedial nucleus of the hypothalamus. It is involved in many functions like in regulation of the energy balance, in the thermoregulation, in the sleep-wake regulation and in organization of stress response. PrRP immunohistochemistry showed dense fiber network in the hypothalamus, especially in the lateral hypothalamic area and in the perifornical nucleus, where many orexin- and melanin-concentrating hormone- (MCH) producing neurons were located. Since both orexin and MCH have been implicated in several functions as PrRP, we aimed to investigate if there is any morphological basis of possible functional interactions between PrRP and orexin, as well as PrRP and MCH signaling. At light microscopic level our results demonstrate that 28% of the MCH- and 49% of the orexin-immunopositive cells are in close contacts with PrRP-positive terminals. Many MCH- and orexin-positive cells are bearing the PrRP receptor, GPR10. Confocal microscopy reveals that 1) PrRP-positive terminals contacting MCH/orexin cells are mainly not catecholaminergic, 2) synaptophysin, a postsynaptically located membrane protein, is present at the PrRP-positive nerve terminals showing close contacts with MCH or orexin positive neuronal elements. Paradoxical sleep deprivation (PSD) for 72 h induced c-fos expression in a number of orexin, but not MCH neurons, while 3h rebound after PSD had opposite effect. Food intake and body weight of the rats decreased during PSD. Cold stress for 12h did not activate MCH or orexin neurons, but 1h recovery at room temperature following the cold activated a lot of orexin-immunopositive neurons. Based on the results, PrRP is suggested to effect sleep-wake regulation and the reaction to cold stress through MCH and orexin neurons in the hypothalamus.
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Unidirectional propriospinal interactions between the medial and lateral aspects of laminae I-IV of the spinal dorsal horn

Petkó Mihály; Szabó Zoltán; Veress Gábor; Antal Miklós
Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

petko@chondron.anat.dote.hu

In one of our earlier studies (J Comp Neurol 422:312-325, 2000), we have demonstrated that the medial aspects of laminae I-IV of the spinal gray matter project extensively to the lateral areas of the same laminae of the spinal dorsal horn. In contrast to this, the lateral areas of laminae I-IV, with the exception of a few fibers at the segmental level do not project back to the medial territories. In the present experiments, injecting Phaseolus vulgaris leucoagglutinin (PHA-L) into the medial areas of laminae I-IV at the level of the lumbar spinal cord, and revealing vesicular glutamate transporter (VGLUT1, VGLUT2), glutamic acid decarboxylase (GAD65/67), and glycine transporter (GLYT2) immunoreactivities of the labeled axon terminals we have investigated the synaptic relations and neurochemical properties of this unidirectional medio-lateral propriospinal projection system of the spinal dorsal horn in rats. A large number of terminals anterogradely labeled with PHA-L at the segmental level of the injection site were revealed in the lateral aspects of laminae IIi, III, and IV, where they formed synaptic contacts with dendrites of various diameters. Some of the labeled boutons received synaptic contacts from unlabeled axon terminals. About two thirds of the labeled terminals showed immunoreactivity for VGLUT1/2, and one third of them proved to be immunoreactive for GAD65/67 and/or GLYT2. The results suggest that the medio-lateral propriospinal projection system of the spinal dorsal horn transmits both excitatory and inhibitory signals to their postsynaptic targets, and these inputs are partially controlled by presynaptic mechanisms. Support: OTKA T043378 and T046121; ETT 079/2006; MTA-TKI-242.

Multichannel recording of medial prefrontal cortex neurons during glucose solution intake in freely moving rats

Petykó Zoltán[1]; Tóth Attila[2]; Szabó Imre[3]; Gálosi Rita[4]; Lénárd László[1]
[1]Institute of Physiology and Neurophysiology Research Group, Hungarian Academy of Sciences, University of Pécs, Pécs, Hungary
[2]Analogical and Neural Computing Systems Laboratory, Computer and Automation Research Institute, Hungarian Academy of Sciences
[3]Institute of Behavioral Sciences, University of Pécs, Faculty of Medicine, Pécs, Hungary
[4]Institute of Physiology, Faculty of Medicine, University of Pécs, Pécs, Hungary
zoltan.petyko@yahoo.com

The prefrontal cortex is associated with the temporal organization of behavior, rewarding mechanisms, hedonic evaluation of nutrients and glucose preference. There are only a few data, however, about the single neuron activity in the medial prefrontal cortex (mPFC) related to consumption of high palatable foods such as glucose solution. In the present experiments multiple unit recordings were made in the mPFC of freely moving wistar rats during consumption of sugar solution. Eight tetrodes with multichannel PCB microdrive were chronically implanted and a tetrode selector was applied. During the 2 hour test period animals had free access to 5 % glucose solution or water. Multiple unit activity was continuously recorded and behavioral actions (drinking periods and other behavioral patterns such as sniffing, rearing, grooming) were recorded with a video camera. Two tetrodes were selected for data sampling. Single unit activity was separated offline with principal component analisis. The mPFC neurons exhibited the following response patterns: Excitation at the beginning of drinking periods, excitatory responses 1-3 seconds before the rats began to drink glucose solution, inhibition during glucose consumption. A distinct group of neurons exhibited decreased responses at the end of the test period. Our results suggest that the mPFC

is involved in the hedonic evaluation of glucose consumption in a complex manner. (Supported by ETT 317/2006, RET-0008/2005 MEDIPOLIS and HAS, NKTH-OTKA K68431)

Central alpha-MSH infusion and parameters of energy balance in young and old rats

Pétervári Erika; Balaskó Márta; Garami András; Pákai Eszter; Soós Szilvia; Székely Miklós
Department of Pathophysiology and Gerontology, Faculty of Medicine, University of Pécs, Pécs, Hungary
erika.petervari@aok.pte.hu

Hypothalamic melanocortins (MC-s) are important in the long-term regulation of energy balance: they suppress food intake and enhance energy expenditure, consequently body weight (BW) declines. The endogenous agonist of MC3 and MC4 receptors is α-MSH. Since BW and factors of energy balance change with advancing age, α-MSH possibly participates in the age-related regulatory alterations. In the present experiments, the effects of central α-MSH infusion on parameters of energy balance were studied in young and old animals. Male Wistar rats aged 4 or 24 months were used. They were kept individually in special cages in a room with lights on from 6 a.m. to 6 p.m., where powdered chow and water were available ad libitum. Transmitters (Minimitter) for telemetric recording of body temperature (Tc), spontaneous activity (ACT) and heart rate (HR, a correlate of metabolic rate) were surgically implanted. The cage was also equipped with infrared sensor to record feeding frequency (FF) and feeding duration (FD). Besides, total food intake (FI) and BW were measured on a daily basis. About 7-10 days after this implantation an osmotic minipump (Alzet) was implanted into a lateral cerebral ventricle providing a 7-day-long α-MSH infusion (1 μg/μl solution) at a rate of 1 μl/h. In both age groups, the daytime nadirs (but not the nighttime peaks) of the circadian Tc changes were elevated during the first infusion days, exhibiting a gradual return from the second day, despite the ongoing infusion. ACT was much greater in young than in old rats and in young ones the nighttime peaks were reduced during the early days of the infusion. HR also showed a circadian pattern; in old animals, it was much smaller and α-MSH induced a significant tachycardia for ca. 4 days. The daily FI was smaller in old than in young rats. In young rats, the α-MSH-induced anorexia was over by about the fourth day, while in old ones the normalization was slow and incomplete. BW was suppressed for 4 days in young rats, but later their BW-gain rate ran parallel with that of the controls; in old ones, BW went on falling throughout the experiment. Both FF and FD were smaller in old than in young rats and α-MSH caused greater and more lasting suppression in the old ones. In conclusion, parameters connected with feeding (but not all parameters of energy balance) are particularly sensitive to MC-s in the aged rat.

Convergence of ascending and descending driver afferents on the same thalamic relay cells in the rat

Plattner Viktor M.[1]; Bokor Hajnalka[1]; Slézia Andrea[1]; Bodor Ágnes L.[1]; Hangya Balázs[1]; Deschenes Martin[2]; Acsády László[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]CRULRG, Laval University, Québec City, Canada
Plattner.Viktor@hallgato.szie.hu

A central dogma of thalamic information transfer is that a single thalamocortical neuron relays one type of driver message. In the present study using anatomical and physiological

methods we demonstrate that a single thalamocortical relay cell may integrate driver inputs arising from completely different sources i.e. from the neocortex and from the trigeminal complex. In the higher order somatosensory nucleus posterior (Po) large cortical terminals originating in layer V of the S1 somatosensory cortex labeled by anterograde tracing were mapped together with large subcortical terminals visualized by their selective marker, vesicular glutamate transporter 2 (vGLUT2). Light microscopic analysis demonstrated that giant cortical terminals from S1 partly overlapped with Po regions rich in large vGLUT2 terminals. At the electron microscopic level vGLUT2-immunostained and large cortical terminals were found to establish synapses on the same relay cell. Intracellular recording was performed in Po and in the first order ventral posteromedial (VPM) nuclei in anaesthetized rats with S1 cortex EEG recording and air jet whisker stimulation. Recordings in Po demonstrated fast rising, large amplitude excitatory postsynaptic potentials (EPSPs) coherent with slow cortical oscillation. These EPSPs disappeared following cortical inactivation, demonstrating their cortical origin. Cortical EPSPs showed similar characteristics to peripheral, so called driver type EPSPs, evoked by whisker stimulation. In VPM cells, which lack cortical driver input, cortical upstates were not associated with fast rising, large amplitude EPSPs. Large amplitude driver EPSPs from cortical and peripheral origin were recorded from the same Po cell, such Po units were contacted by giant vGLUT2-positive terminals as well. Complete and transient cortical inactivity during CSD abolishes spiking activity in most Po but not in VPM cells. Po cells showed more precise phase locking with the cortical LFP than VPM. The convergence of peripheral and cortical drivers on the same cell suggests that higher order relays may serve as AND gates with output determined by the temporal coincidence of cortical and peripheral inputs. The integration of the activity of two drivers of different origin would assign a conceptually new function to thalamocortical cells.

Spatial organization of actin binding proteins: cytoskeletal architecture underlying hippocampal spine-morphing

Rácz Bence[1]*; Weinberg, Richard J.*[2]
[1]Dept Anatomy and Histology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary
[2]Dept Cell & Developmental Biology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA
racz.bence@aotk.szie.hu

Among the actin binding proteins (ABPs) known to contribute to actin reorganization in model systems are alfa-actinin, cortactin, cofilin, and the Arp2/3 complex. These occur in a wide range of organisms from yeast to mammals. However, despite their crucial role in actin remodeling, we have limited knowledge about their organization in brain. Synaptic plasticity is associated with morphological changes in dendritic spines. The actin-based cytoskeleton plays a key role in regulating spine structure, and actin reorganization in spines is critical for the maintenance of long term potentiation. Here we performed immunoelectron microscopy to elucidate the subcellular distribution of four actin binding proteins that mediate reorganization of the actin cytoskeleton. We provide direct evidence that cofilin in spines avoids the core, and instead concentrates in the shell and within the postsynaptic density (PSD) along with alfa-actinin. However, cortactin resides in the core of the spine, away from the PSD. The Arp2/3 complex concentrates away from the non-synaptic membrane, but also away from the geometric centre of the spine. Arp2/3 plays a pivotal role in reorganizing actin filaments, filament branching and polymerization.

Distribution of extracellular matrix in the vestibular nuclear complex of the frog

Rácz Éva[1]; Gaál Botond[1,2]; Hunyadi Andrea[1]; Székely György[1,2]; Matesz Klára[1]
[1]Department of Anatomy, Histology and Embryology, University of Debrecen, Medical and Health Science Center
[2]Neuroscience Research Group, Hungarian Academy of Sciences, Debrecen, Hungary
eva@chondron.anat.dote.hu

Recent biochemical and histochemical analyses explored a number of ECM components in the developing and adult nervous system. In vitro studies revealed that these molecules may have either permissive or non-permissive roles in the regeneration of nerve fibers. The possible function of the ECM during the regeneration can be best studied in lower vertebrates because contrary to the mammalian species their lesioned vestibulocochlear nerves are capable of regenerating. In our earlier study we have demonstrated that the vestibular axotomy of the frog is accompanied by the changing in the expression pattern of hyaluronan (HA), one of the molecules of the ECM. Since the changes were different in the vestibular nuclei and in the entry zone of the nerve, we suppose an area dependent molecular mechanism of the HA during the regeneration. In order to study the role of the other ECM molecules in the regenerative process of the vestibular nerve, a detailed map is required about their normal distribution. Experiments were performed on adult frogs. In anesthetized animals the cranial cavity was opened and the brainstem with the attached vestibulocochlear nerve was excised and immersed into Sainte-Marie's fixative. The paraffin embedded specimens were cut in horizontal plane and the following reactions were performed on the slides containing the vestibular nuclear complex. Wisteria floribunda agglutinin (WFA) and the anti-chondroitin sulfate proteoglycan (CSPG) were used as the general marker of various CSPGs. All the vestibular nuclei were positive for WFA. The perineuronal net (PN), the special ECM-rich coat around the neurons, showed a very strong positivity in the lateral vestibular nucleus (LVN) and the anti-CSPG reaction was also positive in the LVN. Subtypes of the CSPGs were detected by using anti-versican and anti-neurocan and both of them displayed similar reaction pattern than that of the WFA reaction. At the transitional zone of the vestibulocochlear nerve all the reactions were positive with different intensity of staining. Our results indicate that different molecules of the ECM are detected in the vestibular nuclear complex showing regional differences in the individual vestibular nuclei. This work was supported by OTKA K 67641 and MTA-TKI 242.

The role of neuropeptide-containing fibers in the c-fos activation of neurons in the dorsomedial hypothalamic nucleus in response to refeeding of fasted rats

Renner Éva; Puskás Nela; Tóth Zsuzsanna E.; Palkovits Miklós
Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
evarenner@yahoo.com

In an attempt to identify brain centers involved in the central regulation of food intake, we examined *c-fos* activation in refed rats, which were previously fasted for 48 hours. *C-fos* expressing neurons were detected in the ventral subdivision of the dorsomedial hypothalamic nucleus (DMH) by *in situ* hybridization histochemistry, as well as by immunolabeling. We found four neuropeptides: galanin, neuropeptide Y, glucagon-like peptide-1 (GLP-1), and prolactin-releasing peptide (PrRP) present in nerve terminals in the ventral subdivision of the DMH. As reported previously and confirmed in the present study, PrRP- and GLP-1-

containing fibers in the dorsomedial hypothalamic nucleus originate in the caudal portion of the nucleus of the solitary tract in 2 separate cell groups. To investigate the role of PrRP- and GLP-1 in the *c-fos* activation in response to refeeding, we transected ascending pathways from the nucleus of the solitary tract to the hypothalamus at the level of the pontine-medullary junction, uni- and bilaterally. The transection of PrRP- and GLP-1-containing fibers was demonstrated by accumulation of immunoreactivities caudal to the transection. The density of PrRP-immunoreactive fibers decreased dramatically rostral to the transaction, especially in the dorsomedial nucleus and a decrease in the density of GLP-1 fibers was also detectable. Ipsilateral disappearance of fibers following unilateral transections suggests ipsilateral projections. The concomitant decrease in the level of refeeding-induced *c-fos* expression in the DMH, uni- or bilaterally depending on the transection, suggests that PrRP- and GLP-1 fibers originating in the nucleus of the solitary tract might contribute to the activation of DMH neurons during refeeding. In conclusion, we demonstrated a new element of the brain circuitry of food intake regulation in the ventral subdivision of the dorsomedial hypothalamic nucleus and implicated neuropeptides in the activation of its neurons.
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Neuroprotective effects of L-Kynurenine sulfate in combination with Probenecid in the ischemic rat brain

Robotka Hermina[1]; Sas Katalin[2]; Ágoston Márta[3]; Rózsa Éva[1]; Szénási Gábor[3]; Vécsei László[2]; Toldi József[1]
[1]Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary
[2]Department of Neurology, University of Szeged, Szeged, Hungary
[3]Division of Preclinical Research, EGIS Pharmaceuticals PLC, Budapest, Hungary
hermina.robotka@gmail.com

Kynurenic acid (KYNA) is a selective antagonist at the glycine coagonist site of the N-methyl-D-aspartate receptors, and it might therefore be regarded as a neuroprotective agent. However, KYNA provides neuroprotection only at very high doses as it is practically unable to cross the blood-brain barrier. The neuroprotective effect of L-kynurenine sulfate (KYN), a precursor of KYNA, was therefore studied because KYN readily crosses the blood-brain barrier. The present study evaluated the effects of KYN (300 mg/kg i.p.) and Probenecid (PROB) (200 mg/kg i.p.) administration, applied both before and after the ischemic insult, on the hippocampus and cortex of the global ischemic (transiently four-vessel-occluded: 4VO) rat brain. The statistical evaluation revealed that KYN + PROB pretreatment appreciably decreased the number/mm2 of injured cells (Fluoro Jade B - labelled neurons) in the pyramidal layer of the CA1 region of the hippocampus (to 60%, $p<0.001$). KYN + PROB administration after 4VO intervention also resulted in a decrease in the number of injured cells, but not on so high level of significance. On the other hand KYN + PROB administration beneficially decreased the number of neurones injured per mm2 in the cortex, not only in pretreated animals, but also in those which received KYN + PROB after the ischaemic insult. These results were supported by the findings of NeuN immunohistochemistry, with which the intact cells were visualized in the controls, in 4VO animals and in KYN + PROB-treated 4VO animals. It is concluded that the administration of KYN + PROB might possibly elevate the brain concentration of KYNA to neuroprotective levels, suggesting its potential clinical usefulness for the prevention of neuronal loss.

Spike timing-dependent long-term depression in the CA1 region of the hippocampus

Rodriguez Moreno, Antonio; Ole Paulsen
Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK
rodriguezmoreno@dpag.ox.ac.uk

Spike timing-dependent plasticity (STDP) is a model of synaptic plasticity processes that may underlie learning and memory. The aim of our research was to determine the signalling pathway for the induction of long-term depression (LTD) using STDP protocols. We conducted experiments in the CA1 region of hippocampal slices prepared from P9-P16 mice using the whole-cell configuration of the patch clamp technique. Two afferent pathways were activated alternately by extracellular stimulation. We found that a post-before-pre pairing protocol (pairing EPSCs with postsynaptic action potentials at 0.2 Hz) produced robust LTD (75 ± 5 % of control, n = 9). The induction of this form of LTD was completely blocked by D-AP5 (50 µM, 118 ± 7 %, n = 7) and by the broad spectrum antagonist of mGluRs MCPG (500 µM, 115 ± 9 %, n = 7). This form of LTD was also completely blocked by antagonists of the Group I mGluRs selective for mGluR 1 (MPEP, 20 µM, 99 ± 10 %, n = 10) and mGluR5 (LY367385, 100 µM, 102 ± 6 %, n = 7). The inhibition of the phospholipase C (PLC) with U73122 (10 µM), also blocked completely the LTD-induction (115 ± 9 %, n = 6). The application of the CB1 receptor antagonist AM251 (2 µM) resulted in the block of the LTD induction (96 ± 6 %, n = 9). The blockade of postsynaptic NMDARs by MK-801 (1 mM) through the patch pipette abolished the induction of STD-LTP (100 ± 6 %, n = 6, vs 144 ± 6 % in control experiments, n = 10 similar to D-AP5, 102 ± 6 %, n= 5. The LTP was induced using a pre-before-post pairing protocol), but not the induction of the LTD (82 ± 6 %, n = 9). These results indicate that STD-LTD requires the activation of Group I mGluR and involves the participation of PLC and endocannabinoids. These results also show that whereas the STD-LTP depends on postsynaptic calcium influx through NMDARs, LTD induction is independent of postsynaptic activation of NMDARs

Sensory neurogenic meningeal vascular responses mediated by the activation of proteinase-activated receptor-2

Rosta Judit; Dux Mária; Jancsó Gábor
Department of Physiology, Faculty of Medicine, University of Szeged, Hungary
rostaj@phys.szote.u-szeged.hu

Recent findings from our laboratory demonstrated that a significant population of the sensory fibres of the rat dura mater express the transient receptor potential vanilloid type 1 receptor (TRPV1; capsaicin receptor). Topical application of capsaicin elicited a marked meningeal vasodilatatory response which was inhibited by capsaicin pretreatment and by the competitive capsaicin receptor antagonist capsazepine. Pharmacological and neurochemical evidence indicated that this neurogenic vasodilatatory response was mediated by the release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive afferent nerves. Previous studies demonstrated that proteinase-activated receptor-2 (PAR2) is localized on and modulate the function of cutaneous capsaicin-sensitive afferent nerves. Since cutaneous and meningeal capsaicin-sensitive afferents share similar functional properties, the present study examined the possible role of PAR2 in neurogenically mediated vascular responses of the rat dura mater. Laser Doppler flowmetry was used to measure blood flow in branches of the medial meningeal artery in an open cranial window preparation. Topical application of trypsin, a PAR2-activator or SLIGRL-NH2 (10-6- 10-5 M), a selective PAR2 agonist peptide resulted in a dose-dependent increase in meningeal blood flow. This vasodilatatory response

was significantly reduced by prior application of hCGRP8-37 (10-4 M), a CGRP receptor antagonist, and L-NAME (10-4 M), an inhibitor of NO production. In addition, prior application of SLIGRL-NH2 significantly enhanced the vasodilatatory response elicited by capsaicin. These findings indicate that activation of PAR2 induces vasodilatation of meningeal arteries through the release of CGRP and NO from sensory nerves and vascular endothelium, respectively. It is suggested that activation and modulation by PAR2 of neurogenic sensory vascular responses and meningeal C-fiber nociceptor function may bear of significance as regards the pathomechanism of headaches. Supported by OTKA T 46469, OTKA K 63663, ETT 193/2006 and RET-08/2004.

Drivers of the primate thalamus first order, higher order and basal ganglia recipient nuclei

Rovó Zita[1]; Bodor Ágnes Lívia[1]; Ulbert István[2]; Giber Kristóf[1]; Acsády László[1]
[1]Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
rovozita@gmail.hu

One of the keys to understand thalamic function is the driver concept. According to this, the activity of a thalamic relay cell is determined by giant excitatory terminals contacting its proximal dendrites via multiple active zones. The origin of these drivers defines the thalamic nucleus as first or higher order innervated by ascending subcortical or descending cortical drivers, respectively. In the present study we mapped the distribution of cortical and subcortical drivers using vesicular glutamate transporter (vGLUT) immunocytochemistry and anterograde tracing from the cortex in the thalamus of rhesus macaques. The two types of vGLUTs, vGLUT1 and vGLUT2, are selective markers of cortical and subcortical excitatory inputs, respectively. Based on their distribution, vGLUT2 immunostaining labeled the terminals of all major known subcortical afferents (retinal, lemniscal, cerebellar and from the inferior colliculus and mammilary body) in the first order nuclei, where large vGLUT1-positive terminals were very rarely observed. Conversely, large territories of the higher order nucleus pulvinar received numerous, large, vGLUT1-positive boutons but were entirely devoid of giant vGLUT2-immunoreactive terminals. Co-occurance of vGLUT1 and vGLUT2-positive large terminals were observed in the mediodorsal, laterodorsal and pulvinar nuclei. Close appositions (within 10 microns) of vGLUT1 and vGLUT2-positive large terminals were also encountered, suggesting that the two types of drivers may converge on the same relay cells. The unique feature of basal ganglia (BG) recipient thalamic nuclei was the lack of both vGLUT1 and vGLUT2-positive giant terminals. Electron microscopic examination of BG recipient nuclei confirmed the paucity of large GABA-negative and vGLUT1-positive terminals. Anterograde tracing from the motor cortex confirmed the lack of large cortical terminals in basal ganglia recipient nuclei and their presence in the pulvinar and the mediodorsal nucleus. Our data confirms that first order nuclei are driven by subcortical drivers and in large higher order territories the driving excitatory information originates exclusively in the cortex. In certain regions relay cells may integrate subcortical and cortical driver inputs whereas in BG recipient nuclei giant excitatory drivers are replaced by large GABAergic terminals of BG origin.

Which conformational state is preferred by use-dependent sodium channel inhibitor drugs?

Sas Balázs; Lenkey Nóra; Mike Árpád; Károly Róbert; Vizi E.Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
sas.balazs@doboka.hu

While all use-dependent inhibitors of sodium channels share certain properties of inhibition, we have recently provided evidence that this kind of inhibition can emerge on the basis of different mechanisms of action. One important aspect of exploring the mechanism is to find the states that are preferred by the drug. We developed voltage protocols, which were intended to maximalize the percentage of sodium channels in one of the following conformational states: resting / open / fast inactivated / slow inactivated. Drugs were applied during all four protocols (for a duration of 16 s), and the degree of inhibition was compared. We have tested several drugs, including antidepressants, anticonvulsants, Class I antiarrhythmics, antipsychotics, and neuroprotectants. The most evident examples are crobenetine, and chlorpromazine for an especially high affinity to open state, flunarizine for the fast inactivated state, and amitryptiline, and sertraline for the slow inactivated state. Our results confirm that use-dependent sodium channel inhibitors are heterogenous as regards their affinity to different conformational states of the channel.

Depletion of the lipid raft component GM1 ganglioside impairs NGF mediated regulation of the capsaicin sensitivity of nociceptive primary sensory neurons

Sántha Péter; Oszlács Orsolya; Dobos Ildikó; Jancsó Gábor
Department of Physiology, Faculty of Medicine, University of Szeged, Hungary
santha@phys.szote.u-szeged.hu

Capsaicin sensitivity of nociceptive primary afferent neurons (PSNs) is conferred by the expression of the transient receptor potential receptor vanilloid type 1 (TRPV1). Nerve growth factor (NGF) regulates capsaicin sensitivity via different mechanisms: first, NGF maintains the expression of the TRPV1 protein and, second, upon acute exposure NGF induces sensitisation of TRPV1 to thermal and chemical stimuli. Previously we have demonstrated an axonal injury-induced accumulation of the membrane raft component GM1 ganglioside in TRPV1 expressing PSNs. Since lipid rafts play an important role in NGF signalling, we investigated the effects of perturbations in the GM1 synthesis on the NGF mediated regulation of capsaicin-sensitivity in PSNs. Cultured PSNs obtained from dorsal root ganglia of adult rats were kept in F12 – HAM medium supplemented with 50 ng NGF and 0 (control), 10, 20 or 50 μM D-PDMP (D-threo- phenyl-decanoylamino-morpholino-propanol), an inhibitor of ceramide synthase. Capsaicin sensitivity and TRPV1 expression were assessed on the 3rd-5th days after plating by using the cobalt uptake method and immunohistochemistry. Chronic exposure to D-PDMP resulted in a gradual, dose-dependent decrease in the intensity of neuronal Co2+ labelling and a reduction by about 40% in the proportion of capsaicin-sensitive neurons. Immunohistochemistry revealed a similar reduction in the proportion of TRPV1-positive neurones. In addition, D-PDMP pretreatment almost completely abolished the acute sensitising effect of NGF (100 ng/mL) as assessed with the cobalt assay. Our results indicate a pivotal role of lipid raft components, in particular GM1 ganglioside in the NGF-mediated regulation of neuronal capsaicin sensitivity and nociceptive function and suggest that pharmacological perturbation of neuronal ganglioside metabolism may offer new possibilities in the modulation of pain. Supported by OTKA T 46469, OTKA K 63663, ETT 193/2006 and RET-08/2004. P. Sántha was supported by the Bolyai János fellowship of the H.A.S.

Neurotoxic effects in rats following subacute manganese exposure in various forms

Sárközi Leila; Szabó Kinga; Hornyik Tibor; Horváth Edina; Oszlánczi Gábor
Department of Public Health, Faculty of Medicine, University of Szeged, Szeged, Hungary
sarkozi@puhe.szote.u-szeged.hu

Chronic human exposure to manganese induces neurological and respiratory problems. Neurotoxicity due to excess Mn (such as a Parkinson-like syndrome) has been reported after occupational and non-occupational inhalation exposure to Mn. The consequences of airways exposure by Mn-containing dust is highly influenced by the particle size. Nanoparticles, in the submicron range, are a major part of air pollutants, and their effects and movement within the organism can be distinct from those of larger grains. In this work, manganese was given to adult male Wistar rats (ca. 250 g body weight) in nanoparticle and dissolved form, and the effects on cortical electric activity were observed. In the first experiment, the rats received intranasal instillation of dissolved MnCl2 or a suspension of MnO2 nanoparticles (30 nm diameter), both equal to 2.53 mg Mn per rat in a viscous medium for 6 weeks, 5 day a week. In the second experiment, MnO2 nanosuspension in saline was instilled in the rats' trachea in 2.63 or 5.26 mg Mn/kg b.w. dose. Controls were vehicle treated. After the treatment period, the rats were anesthetized with urethane, the skull was opened and the left hemisphere exposed. Spontaneous and stimulus-evoked cortical ,activity was recorded from the primary somatosensory, visual and auditory areas with silver surface electrodes. In the spontaneous cortical activity, the slow frequencies decreased and the fast frequencies increased in the first experiment, and changed oppositely in the second, but mostly non-significantly. Evoked cortical activity was. however, significantly altered in both experiments. Typically, the latencies were lengthened and the dependence of the response parameters on stimulation frequency increased. Nanoparticles, a newly recognized form of pollution, may be important environmental neurotoxicants.

Beyond physical boundaries: coding of two types of illusory contours in the macaque inferotemporal cortex

Sáry Gyula[1]; Tompa Tamás[1]; Köteles Károly[1]; Chadaide Zoltán[2;] Benedek György[1]
[1]Department of Physiology and [2]Department of Neurology, University of Szeged, Szeged, Hungary
sary@phys.szote.u-szeged.hu

Illusory contours (ICs) are perceived boundaries without physical differences between the shape and the background. They activate the early processing stages of the macaque visual pathway in a similar way to real contours (RCs). It is not known, however, how these contours are processed further in the higher visual areas. Another question is if ICs defined in different ways will trigger different neuronal responses in these areas.
We tested how the responses of inferior temporal cortical (IT) neurons of macaque monkeys change in relation to figures with RCs and two types of ICs. One set of ICs was defined by phase shifted dark and light lines, the others were induced by pacmen (Kanizsa type). ICs and their corresponding controls were presented to awake, fixating rhesus monkeys while recording single-cell activity in the anterior part of the IT.
Most of the neurons responsive to RCs were also responsive to the same shapes presented as ICs. The average net firing rates, however, were significantly lower for the illusory stimuli than for the stimuli in the RC conditions which might reflect their different visibility. The latency of the responses was significantly longer for the ICs than for the RCs, suggesting the

involvement of possible feedback mechanisms in processing ICs. For the two kinds of ICs there were no differences between the firing rate and the latency.
The shape selectivity was found to be different for the RCs and ICs, and we found a slight difference between the differently defined ICs too.
Our earlier results suggested different modes of processing of RCs and phase shifted ICs in the IT, which might explain the differences in their perception. The same might apply to the two kinds of ICs. The different way of inducing ICs parallels the role of lines and surfaces as shape defining cues.

Extracellular matrix components demonstrated by peanut lectin labeling and immunohistochemistry in the adult and developing lymnaea nervous system

Serfőző Zoltán[1]; Fekete Zsuzsanna N.[1]; Kőszegi Zsombor[2]; Elekes Károly[1]
[1]Department of Experimental Zoology, Balaton Limnological Research Institute, Hungarian Academy of Sciences
[2]Department of Experimental Zoology and Neurobiology, University of Pécs, Hungary
serfozo@tre.biki.hu

Extracellular matrix (ECM) has a pivotal role in the formation and proper function of neuronal connections. However, in the snail nervous system, which is an experimental model of studying neuronal background of simple behaviors, yet little is known about the molecular composition of the ECM. In the present study, using different lectin probes and antibodies raised against different mammalian ECM proteins (chondroitin sulfate proteoglycan [CSPG], fibronectin, phosphacan, tenascin, laminin), respectively, the distribution of ECM molecules was investigated in the nervous system of the developing and adult pond snail, Lymnaea stagnalis. Out of 14 lectins, only peanut agglutinin (PNA) was found to label peripheral sensory fibers entering the CNS and running along the ganglionic ring, then arborizing in the neuropil of the cerebral, pedal and buccal ganglia. Perikaryonal baskets in the visceral ganglion and a pair of small groups of neurons located symmetrically in the cerebral ganglia also displayed PNA reactivity. After metamorphosis, from E60% embryonic stage, PNA-labeled peripheral neurons appeared in the foot and projected to the neuropil of the cerebral and pedal ganglia. In the pedal ganglia, a symmetric pair of neural perikarya showing transient PNA reactivity could be detected at early (P1) postembryonic development. The sensory epithelium of the eye and the optic tract displayed also transient PNA labeling from E70% embryonic to P3 mid-postembryonic stages. By an antigen retrieval technique, a successful labeling with anti-CSPG, anti-fibronectin and anti-phosphacan antibodies could be achieved in the CNS. The cytoplasm of discrete cell populations in the cerebral ganglia and elements of the periganglionic sheath displayed CSPG-like immunoreactivity. Fibronectin-like-IR structures were found on the surface of numerous perikarya and in the neuropil. An intensive phosphacan-like immunoreactivity was detected throughout in the CNS, labeling neuronal tracts connecting different ganglia, and neuropils. Our findings indicate that PNA, labeling cell surface glycoproteins and glycolipids, is the exclusive lectin marker of specific sensory pathways in Lymnaea. Results obtained with immunolabeling suggest a similar ECM composition in the invertebrate (snail) and vertebrate

Are there any EEG differences between verbal tasks of various subjective complexity levels?

Shemyakina N. V.; Danko S. G.; Starchenko M.G.; Zhanna V. Nagornova; Bechtereva N. P.
Institute of the Human Brain, Russian Academy of Sciences, Moscow, Russia

nagornova_zh@mail.ru

Our main hypothesis was that as the brain mechanism, processes of creative thinking and noncreative thinking of the comparable level of complexity may differ. This problem seems to be really new for neurophysiology of creativity, as we didn't find any direct data in literature. We found out, that creative and non creative tasks of the same subjective complexity level differ in their EEG spectral power correlates. Creative task was characterized by generalized increase of spectral power in high frequency bands: beta1, beta2, gamma. Observed increase of EEG spectral power seems to be distinguishing feature for creative task fulfillment, thus in comparison: creative task versus much more easier one - memory retrieval task, it was also observed. Increase of spectral power in beta and gamma frequency bands, obtained for creative task on new subject's group of present study confirms our previous published results (Bechtereva et al., 2004, 2006, 2007). We also found out, that two noncreative tasks of different subjective complexity levels in comparison with easiest one memory-retrieval task revealed isolated and not well pronounced increase and decrease of EEG spectral power. It seems that those memory retrieval processes don't differ too much because of the different subjective complexity levels of the tasks.
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Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3-CA1 connections in the hippocampus
Sivakumaran, Sudhir
Cherubini Lab, Neurobiology Sector, SISSA-ISAS, Trieste, Italy
siva@sissa.it

During development, correlated neuronal activity is thought to exert a critical control on functional and structural refinement of synaptic connections. In the hippocampus, between postnatal day 2 (P2) and P6, network-driven giant depolarizing potentials (GDPs) are generated by the synergistic action of glutamate and GABA that is depolarizing and excitatory. The rising phase of GDPs was used to trigger Schaffer collateral stimulation in such a way that synchronized network activity was coincident with presynaptic activation of afferent input. This procedure produced a persistent increase in spontaneous and evoked AMPA-mediated glutamatergic currents, an effect that required calcium influx through postsynaptic L-type calcium channels. No potentiation was observed when a delay of 3 s was introduced between GDPs and afferent stimulation. Pairing-induced potentiation was prevented by scavengers of endogenous BDNF or TrkB receptors antagonists. Blocking TrkB receptors in the postsynaptic cell did not prevent the effects of pairing, suggesting that BDNF, possibly secreted from the postsynaptic cell during GDPs, acts on TrkB receptors localized on presynaptic neurons. Application of exogenous BDNF mimicked the effects of pairing on synaptic transmission. In addition, pairing-induced synaptic potentiation was blocked by ERK inhibitors, indicating that BDNF activates the MAPK/ERK cascade, which may lead to transcriptional regulation and new protein synthesis in the postsynaptic neuron. These results support the hypothesis that during a critical period of postnatal development, GABAA-mediated GDPs are instrumental in tuning excitatory synaptic connections and provide new insights into the molecular mechanisms involved in this process.

Influence of extrareticular GABAergic inhibition on higher order thalamic relays
Slézia Andrea[1]; Hangya Balázs[1]; Ulbert István[2]; Bokor Hajnalka[1]; Acsády László[1]

[1]Department of Cellular and Network Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Institute of Psychology, Hungarian Academy of Sciences, Budapest, Hungary
acsady@koki.hu

GABAergic inhibition has a major role in shaping network activity in the thalamocortical system. Up to now this has largely been attributed to the reticular thalamic nucleus (nRT). Recently, a novel GABAergic system has been described, which exerts strong control over the relay cells via specialized giant terminals. This extrareticular pathway arises in the anterior pretectal nucleus (APT) and selectively innervates higher order (HO) thalamic relays. In order to characterize the responses of HO relays to the chemical activation or inactivation of APT in vivo we injected bicucculine (BIC) to the APT, while recording neuronal activity simultaneously in the APT and the HO, somatosensory, posterior nucleus of thalamus (Po) together with local field potential (LFP) in the primary somatosensory cortex (S1) of urethane anaesthetized rats. Normalized autocorrelograms, spike triggered cortical LFP averages (STA) and phase histograms were constructed from the respective spike trains to examine the rhythmicity and the coupling of cortical LFP and the action potentials. Putative low threshold spikes were selected by a burst detecting algorithm and burst- and single spike STA-s were generated separately. Following Hilbert transformation of the cortical LFP signal, the phase distribution of the spikes were examined by comparing the vector strength of the phase distribution to judge the jitter of action potential timing during the slow oscillation in Po cells. BIC caused strongly elevated firing in the APT that manifested as 200-300 ms long, rhythmically recurring bouts of multiunit activity. The most consistent changes with a monosynaptic inhibitory input at the peak of the upstate are increase in burstiness; phase shift of unit firing; increase of phase consistency, however increased burstiness sometimes was not coupled with changes in the phase consistency and burstiness can show no change (or even decrease) coupled with changes in the phase consistency. In certain neurons no change is observed, which serves as a control for drug delivery. We found heterogeneous responses, however, GABAergic input is expected to increase burstiness via the rebound burst mechanism and cause phase shift by inhibiting firing at the peak of the upstate. Thus, in certain cases burstiness and phase change independently.

Spatio-temporal spike dynamics: Localization of single cell currents based on extracellular potentials patterns

Somogyvári Zoltán[1]; Ulbert István[2]; Érdi Péter[1,3]
[1]Department of Biophysics, Research Institute for Particle and Nuclear Physics, Hungarian Academy of Sciences, Budapest, Hungary
[2]Institute for Psychology, Hungarian Academy of Sciences, Budapest, Hungary
[3]Center for Complex System Studies, Kalamazoo, Michigan, USA
soma@sunserv.kfki.hu

Traditional current source density calculation method (CSD) method allows calculation of current source distribution from the extracellular potential patterns, thus provides important information for neurophysiology. The traditional CSD method is based on strong physical foundations, but uses some assumptions, which can not hold for single cell activity. By this reason, traditional CSD method gives false results for single cell activity. A new, spike CSD (sCSD) method have been developed, directly designed for revealing current source density distribution of a single cell, during firing. This new method is based on the inverse solution of the Poisson-equation and were applied on extracellular spatial potential patterns of spikes.

The spikes were measured in cat primary auditory cortex with a 16 channel chronically implanted linear probe in vivo. Using our new method, many fine details of the spatio-temporal dynamics of spikes were uncovered. Dendritic back propagation was proven to be much more frequent than it was known before, it was observable in every cell. The speed of back propagation was typically different in the apical and basal directions. In contrast to the literature, forward propagation preceding the spikes was also observable. In perspective, this new method raises the possibility of identifying synaptic inputs, which causes a cell fire.

Comparison of energetics of short-term cold-acclimation in the rat and mouse biotelemetric studies

Solymár Margit[1]*; Garami András*[1]*; Pákai Eszter*[1]*; Szelényi Zoltán*[2]
[1]Department of Pathophysiology and Gerontology, Faculty of Medicine, University of Pécs, Pécs, Hungary
[2]Department of Applied Physiology, Faculty of Health Sciences, University of Pécs, Pécs, Hungary
margit.solymar@aok.pte.hu

Cold-acclimation in rats is characterized by a rise of non-shivering thermogenesis and is supported by increased food intake. Mice can also develop cold-acclimation with the help of similar heat-producing mechanism, but energy balance may change more markedly because of the small body mass of that species. Changes in the dynamics of core temperature and locomotor activity during cold-acclimation in unrestrained rodents has not been studied so far. Minimitter biotelemetry transmitter (series 4000) was implanted into the abdominal cavity of Wistar rats and C57BL6 mice under general anaesthesia. After a week at neutral ambient temperature (23-25 oC) the animals were transferred to a cold room (4 oC) and held there for 1 to 2 weeks. Changes of core temperature and activity induced by cold exposure were analysed using Vitalview and Actiview softwares. In a separate group a mice cold-exposure was carried out under the condition of daily injections of methylprednisolone. Rats responded to cold exposure by a few tenths of degree fall a 24-hour average core temperature with a minor trend to increased activity observed in individual animals. Acclimation to cold developed within a day both in terms of core temperature and activity with daily changes of both parameters remaining practically unchanged. In mice a more robust fall of maximal (night) values of core temperature developed during the first three days in cold with no change of minimal (day) values. The 24-hour average of general activity in mice showed a tendency to rise becoming significant from day 4 onwards. About a week was needed for the re-establishment of nocturnal type core temperature and activity oscillations. In mice on chronic steroid treatment the course of body temperature in cold was similar to that of the controls, but – opposite to them – activity showed a slight fall during the first days in cold. Circadian body temperature in these mice remained unchanged even on the first days of cold exposure. The three days long reduction of night temperature observed in mice may be caused by a relatively slow adaptation of increased food intake needed to counterbalance enhanced cold-load. In fact, during the initial stage of cold-acclimation food intake behaviour stretches over the whole 24 period returning gradually to a night-dominance of energy intake characteristic of mice under neutral environmental conditions. Consequently, nocturnal type of activity reappears again. Chronic methylprednisolone treatment allowed a rapid adaptation to cold in terms of maintainance of circadian body temperature and activity rhythms. Changes of core temperature and activity should be studied also under conditions of inhibition of shivering and/or non-shivering thermogenesis to learn their possible role in the first stage of

cold-acclimation process in freely moving rodents. Supported by the Hungarian National Science Fund (OTKA-T62598) and by an European grant (GVOP-3.2.1-2004-0271/3.0).

20 Minutes G3 mobile phone irradiation decreases the amplitude of P300 ERP component in auditory oddball paradigm

Stefanics Gábor[1]; Thuróczy György[2]; Kellényi Lóránd[3]; Hernádi István[3]
[1]Department of Experimental Zoology and Neurobiology, University of Pécs, Hungary Institute of Psychology, Hungarian Academy of Sciences
[2]Department of Non-ionizing Radiation, National FJC Research Institute for Radiobiology and Radiohygiene, Hungary
[3]Department of Experimental Zoology and Neurobiology, University of Pécs, Pécs, Hungary
hernadi@ttk.pte.hu

In our study we investigated the potential effects of 20 min irradiation from new generation 3G mobile phones on human event-related brain potentials in auditory oddball paradigm. Before and after irradiation subjects were presented with a random series of 50ms tone burst (frequent standards: 1 kHz, p=80%, rare deviants: 1.5 kHz, p=20%) at a mean repetition rate of 1500 ms while EEG was recorded. The subjects' task was to silently count the appearance of target tones. In two separate sessions the irradiation was either genuine or sham. The amplitude of the N100, N200, P200 and P300 components for targets were analyzed in ten subjects. We found a significant decrease in the P300 amplitude after 20 min genuine irradiation in the 280-380 ms time window which was not observable after sham irradiation. The amplitude of the P300 event-related potential component is believed to index brain activity that underlies the maintenance of working memory when the mental model of the stimulus context is updated in a cognitive task. P300 amplitude from a given task situation reflects how the processing system provides general attentional resources in the evaluation of incoming stimuli. A common finding related to decreased attention in auditory tasks is a reduced P300 component. Our results so far suggest that 20 min irradiation of 3G mobile phones may disrupt attention processes which may lead to a decrease in P300 amplitude.

Cervical and lumbar motoneurons are morphologically and electrotonically different in frog (Rana esculenta)

Stelescu András; Vida Edith; Wéber Ildikó; Birinyi András; Wolf Ervin
Department of Anatomy, Histology and Embryology, University of Debrecen, Debrecen, Hungary
stelescu@gmail.com

Hind limb and lower limb muscles provide different forms of movements in terrestrial and in water locomotion of frogs. We morphologically and electrotonically compared the cervical and lumbar motoneurons (MN), responsible for controlling and driving activity of these muscles. Eight cervical and nine lumbar MNs were filled up iontophoretically by neurobiotin and MNs were three-dimensionally reconstructed from serial sections. Shrinkage and optical corrections were applied to increase accuracy of morphological data. To describe the morphology of MNs, quantitative data of soma (roundness, surface), of stem dendrites (number and sum of diameters), and of dendritic trees (number of segments, maximum order, total length and surface, mean distance along the dendrites from soma to branch points and end points, mean parent length, max. distance to end points) were measured. Discriminant analysis proved that cervical and lumbar MNs are completely different morphologically in

frogs (Wilks' lambda, $p<0.03$). We used computer modelling to investigate if morphological difference was preserved functionally too. MNs were morphoelectrotonically transformed to represent attenuations of postsynaptic potentials from dendritic synapses to the soma as lengths of dendrites. Discriminant function classified morphoelectrotonic transforms of cervical and lumbar MNs at least in 90% of cases correctly (Wilks' lambda, $p<0.02$). This functional difference was maintained independently of the neuron input resistance and the extent of membrane inhomogeneity. We did not find any substantial overall difference between the functional and morphological representations of the same MN dendrites. However, we identified those regions which differed electrotonically the most from their morphological counterpart. These territories were spread all over the dendritic trees and did not vary with changes in strength of background synaptic activity. We conclude that cervical and lumbar motoneurons are different morphologically in frogs and this difference is preserved electrotonically since morphoelectrotonic transformation, unlike in many other types of neurons, did not change the proportions of dendrites significantly. The authors are grateful to Dr. Dityatev for dye filling of neurons and the grants ETT 025/2006 and OTKA K67747 for financial support.

Ischemia-induced damage of cerebrocortical microvessels in apolipoprotein B-100 overexpressing transgenic mice fed with a cholesterol-enriched diet

Süle Zoltán[1]; Sántha Miklós[2]; Bereczki Erika[2]; Farkas Eszter[1]; Bari Ferenc[3]
[1]Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary
[2]Animal Genetics and Neurobiology Group, Institute of Biochemistry, Biologial Research Center, Szeged, Hungary
[3]Department of Physiology, School of Medicine, University of Szeged, Szeged, Hungary
sulez@anatomy.szote.u-szeged.hu

Apolipoprotein B (ApoB) deposited in vascular walls and high serum cholesterol level have been implicated in the pathogenesis of cerebrovascular disorders. The aim of our study was to investigate whether the ischemia-related injury of cerebral microvessels was augmented by a high cholesterol diet and/or the overexpression of the human ApoB-100 in transgenic mice. Cerebral ischemia was induced by the unilateral occlusion of either common carotid artery in TgApoB+/+ and wild type mice (n=49). Half of the mice were supplied with a diet enriched with 2% cholesterol, while the other half of the animals received standrad chow for 18 weeks. Samples from the ipsilateral and the contralateral frontoparietal cortices were collected and routinely embedded for electron microscopic investigation. The ratio of capillaries with astrocytic swelling, capillaries with endothelial processes, the lumen diameter and the vascular density were measured. The overexpression of ApoB-100 coincided with a significant decrease in cortical microvascular density (from 210.04 ± 16.99 vessels/mm2 to 184.22 ± 14.30 vessels/mm2) and an increase in the diameter of capillaries (from 2.95 ± 0.10 µm to 3.25 ± 0.06 µm), which were uneffected by the diet. The induced ischemia considerably promoted the swelling of the perivascular astrocytic endfeet (from 26.69 ± 4.17% to 59.70 ± 7.14%) and the incidence of endothelial processes (from 14.44 ± 2.70 % to 18.76 ± 2.89 %), and decreased the ratio of intact capillaries (from 65.23 ± 3.66 % to 32.06 ± 5.59 %). The injury was not worsened by the overexpression of ApoB-100 or cholesterol-enriched diet. In conclusion, the overexpression of human ApoB-100 alters the density of the microvascular network and the diameter of capillaries, but does not exacerbate the ischemia-related microvascular damage in the mouse brain.

An in vitro screening method for optimal affinity tagging neurotransmitter receptors

Sümegi Máté[1,2]; Fukazawa, Yugo[1]; Matsui, Ko[1]; Nusser Zoltán[2]; Shigemoto, Ruychi[1]
[1]Division of Cerebral Structure, National Institute for Physiological Sciences, Myodaiji, Okazaki, Japan [2]Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
sumegi@koki.hu

Genetically encoded affinity tags are useful for investigating the subcellular location, assembly and trafficking of proteins when specific primary antibodies are not available. However, the insertion of a tag coding sequence into a gene might interfere with the detection of the tag itself or the subcellular distribution or the function of the target protein. To reduce effort spent on producing multiple transgenic animals for testing different tags and tag locations, we developed an in vitro system to generate, express and screen for tagged GABAA receptor γ2 subunits in cultured neuronal cells. We introduced a new PCR based cloning strategy for precise and site-independent tagging by inserting a type IIs restriction endonuclease recognition sequence into the target region that can be swapped with different tag coding sequences. We also established a method to genotype and culture cortical neurons obtained from GABAA γ2-/- embryos. We generated six (AU1, bungarotoxin binding site, Flag, 3XFlag, HA, StrepII,) N- and six C-terminal tagged γ2 subunits, followed by lentivirus-mediated transgene expression in γ2-/- neuronal cultures. We found that only one of the twelve variants, the N-terminal AU1 tag (inserted between the 4th and 5th amino acid residues) resulted in excellent immunodetection and unimpaired colocalisation with the GABAA receptor β2/3 subunits when compared to native γ2 subunit in WT cells or untagged γ2 subunit in γ2-/- cells. The electrophysiological and pharmacological properties of the AU1 tagged γ2 subunit-containing GABAA receptors were similar to receptors with untagged γ2 subunit expressed in γ2-/- cells and indistinguishable from the native γ2 subunit containing receptors in WT cells. Our results represent an efficient in vitro screening method for selecting optimal affinity tags and tagging locations. Importantly, this method also includes a patch-clamp electrophysiology-based verification of the lack of unwanted effects of tag insertion on the functional properties of the receptors.

The neuroprotective effects of urocortin in bilateral common carotid artery occlusion induced retinal degeneration

Szabadfi Krisztina[1]; Reglődi Dóra[2]; Kiss Péter[1]; Tamás Andrea[1]; Babai Norbert[1]; Hamza Levente[1]; Gábriel Róbert[2]; Fekete Éva[1]; Zorrilla EP[1]; Atlasz Tamás[1]
[1]Department of Experimental Zoology and Neurobiology, University of Pécs, Hungary
[2]Department of Anatomy, Faculty of Medicine, University of Pécs, Pécs, Hungary
kriszta.szabadfi@gmail.com

Urocortin (Ucn), a corticotropin-releasing factor related peptide found in bony fish, amphibians, birds and mammals, have unique phylogenies, pharmacologies, and tissue distributions. Ucn may be clinically relevant in the pathogenesis or management of many conditions, including congestive heart failure, hypertension, gastrointestinal and inflammatory disorders. Recent data have shown neuroprotective effects of Ucn in some neuronal pathological conditions. The aim of the present study was to investigate the effects of Ucn on retinal degeneration induced by bilateral common carotid artery occlusion (BCCAO). Two-month-old rats were subjected to BCCAO and their retinas were processed for histology after 3 weeks survival. We measured the ganglion cell number/100 μm, and the thickness of outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer. Outer limiting

membrane-inner limiting membrane distances in the cross section of the retina were also measured. In the present study, neuroprotective effects of Ucn were observed in BCCAO-induced degeneration. BCCAO resulted in severely reduced thickness of retinal layers three weeks after ligation. Intraocular Ucn treatment (2 nmol in 5 µl saline) following BCCAO led to a nearly intact appearance of the retinal layers. Our results may have clinical implications in reducing ischemic retinal degeneration in ophthalmic diseases. Immunocytochemical experiments are in progress to reveal the cell type-specific effects of the degeneration models and the neuroprotective treatments. Supported by OTKA T061766, T046589, F67830, Richter Gedeon Ltd, ETT439/2006

Reinnervation of the denervated forelimb muscles following brachial plexus injury

Szabó András[1]; Pintér Sándor[2]; Nógrádi Antal[1]
[1]Department of Ophthalmology, Laboratory of Neuromorphology
[2]Department of Traumatology, Faculty of Medicine, University of Szeged, Szeged, Hungary
asza@usa.net

The influence of systemic administration of riluzole on injured cervical motoneurons and various surgical procedures to restore the lost forelimb functions following avulsion injury was studied in rats. The right C6 ventral root of adult animals was avulsed. The avulsed root was reimplanted into the spinal cord (n=4), or reimplantion was combined with riluzole treatment [4mg/kg i.p for three weeks (n=4)]. In other four animals a peripheral nerve graft was used to connect the C6 segment following avulsion to the surrounding neck muscles. In further five animals avulsion was performed without reimplantation while another four animals remained untreated. Three months after the operation the flurescent dye Fast Blue was applied to the proximal end of the cut ventral ramus of the C6 spinal nerve to retrogradely label reinnervating neurons. Three days later the spinal cords were processed for counting the retrogradely labelled cells and ChAT immunohistochemistry. Animals with avulsion only injuries had their C6 spinal nerve prelabelled 3 days before the operation. In the avulsed group there were 65.0±7.5 (SEM) retrogradely labelled neurons. The number of retrogradelly labelled motoneuron increased in the animals where the ventral root was reimplanted (211.3±14.8 SEM). The number of surviving motoneurons was significantly higher when riluzole treatment was applied (573.5±8.6 SEM) commencing immediately after the surgical procedure. There was no significant difference in the numbers of retrogradely labelled motoneurons between the ventral root reimplanted (211.3±14.8 SEM) and the autologus peripheral nerve graft implanted series (274.8±27.8 SEM). This work was supported by the Wellcome Trust

Nicotine increases excitability and evoke Ca2+ responses in dendrites and spines of CA1 pyramidal neurons and stratum radiatum interneurons

Szabo I. Szilárd; Zelles Tibor; Vizi E. Szilveszter; Lendvai Balázs
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
szilard.szabo@koki.hu

Nicotinic acetylcholine receptors (nAChRs) of the hippocampus have been thought to contribute to the cognitive effects of cigarette smoking. To explore the unitary actions of nicotine on functions important in dendritic signal integration, we measured Ca2+ transients in dendrites of hippocampal pyramidal neurons and stratum radiatum interneurons using two-photon laser scanning microscopy. Rapid application of high-dose nicotine could induce

firing through nAChRs within the time window of desensitization. However, the mechanisms of action were different: while Ca2+ responses can be induced by local nAChRs in dendrites of CA1 interneurons, pyramidal neurons can only be activated by presynaptic nAChRs. We observed a minute-scale enhancement of response amplitudes during pressure application of nicotine and choline. In addition, low-dose nicotine (1 microM) enhanced backpropagating action potential-evoked Ca2+ transients in spines of pyramidal neurons via activation of presynaptic nAChRs of innervating terminals. Most likely these axons that give synapses on spines of basal dendrites originate from local pyramidal neurons. Our data revealed the interaction between synaptic Ca2+ transients and nicotinic agonist-evoked Ca2+ responses in dendrites of CA1 interneurons. On top of the long-lasting elevation caused by agonist puff fast synaptic Ca2+ responses also appeared. This interplay between extrasynaptic and synaptic receptors could be one important element of the dendrite's encoding mechanisms.

Dopamine induced alterations in the subthreshold oscillations of the striatal cholinergic interneuron: a modelling study

Szalisznyó Krisztina; Müller László
Department of Biophysics, Research Institute for Particle and Nuclear Physics, Hungarian Academy of Sciences, Budapest, Hungary
szali@sunserv.kfki.hu

In this modelling study we examined the dopamine induced single cell activity alterations in the striatal cholinergic interneuron (AchN). AchN-s are key elements of the striatal circuitry and play a crucial role in associative learning, controlling and planning movements. AchN-s are tonically active in vivo and in vitro as well, are able to fire in the absence of synaptic inputs, and respond to sensory stimuli and sensorimotor learning by transiently suppressing their firing activity. The regular spiking, as well as the pause response are influenced by the inwardly rectifying outward G_{kir}, by a slow hyperpolarization activated noninactivating G_h, and calcium dependent potassium conductances. The pause in tonic firing is dependent on dopaminergic activity; however the cellular mechanisms of this effect remained unclear. Recent experimental evidence showed that dopaminergic inhibition of G_h is involved in modulating the pause response. Further experimental studies demonstrated dopamine evoked increased G_{kir} in other striatal neurons. Given these motivations, we constructed a single cell point neuron model and restricted our analysis to the subtreshold membrane potential range. We have implemented and analyzed the dopaminergic effect on both of G_h and G_{kir}. Our simulation results predict that the G_{kir} and the half activation voltage of the G_h have very little effect on the amplitude of the subthreshold membrane potential oscillations at a certain injected parameter value. However the width of the dynamic regime is influenced by the both. We have found that the intrinsic amplification of hyperpolarizing synaptic events is dependent on the synaptic currents' parameters and on the timing of the events during the subthreshold oscillation cycle. We conclude that in agreement with experimental findings the EPSP-s are able to precisely pattern the action potential timing of the AchN-s with dynamically interacting the intrinsic hyperpolarization activated conductances. On the other hand the simulation results suggest that the IPSP-s influence the precise timing, and not just the overall firing rate of the AchN-s. We predict that the subtreshold conductances allow the dopamine to switch the AchN between oscillatory and stable fixed point behaviours.

Resource competition: a new model to study anxiety and aggression

Szemerszky Renáta; Elek Szilvia; Bárdos György

Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
rszemerszky@gmail.com

A new method to study social anxiety and/or aggression is proposed: „the resource competition test". In this test situation, rats should compete for a limited resource - in our case for drinking water - in a setting identical to their home-cage. To validate the test, we studied male Wistar rats deprived of water for 48 hours in three different settings. The experimental subject and an unfamiliar control rat were placed in the same cage and were allowed to compete for the water-source for 10 minutes. Anxiety was generated by 4 days isolation (and rats were tested against a bigger resident rat) in the first series; by a single exposure to electric shocks (and tested on Day 1 and Day 5 afterwards) in the second series; whereas the experimental rat was resident to the cage isolated for 10 days and a female rat was placed in the cage 2 days before testing to enhance territorial aggression in the third series. While a bigger mate has not generated anxiety, electric shocks did. Shocking reduced competitiveness, these rats had got less access to and spent less time with drinking than the controls. This effect lasted more than 5 days and had seemed not to diminish even if tested repeatedly. Locomotor activity remained unaffected. Finally, aggressive rats contended more for the resource with their control mates than control vs. control rats and spent also more time with drinking. We suggest that the model can be used for studying the effects of various compounds and treatments on anxiety and aggression. The pharmacological validation of the test with anxiolytic, anxiogenic and anti-aggressive drugs, respectively, is in progress.

Neural substrates for differential descending control of motoneurons innervating trunk and hindlimb muscles

Szokol Karolina; Glover Joel C.; Marie-Claude Perreault
Department Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
karolina.szokol@medisin.uio.no

To better understand how the brainstem reticular formation controls and coordinates trunk and hindlimb muscle activities, we used optical recordings to characterize the functional connections between medullary reticulospinal neurons and lumbar motoneurons of the L2 segment in the neonatal mouse. In an isolated brain stem-spinal cord preparation, calcium transients induced by focal electrical stimulation of the medullary reticular formation (MRF) were visualized in individual motoneurons of the ipsilateral and contralateral medial and lateral motor columns (iMMC, iLMC, coMMC, coLMC). Stimulation of the MRF elicited differential Ca2+ responses in MMC and LMC, following a specific spatial organization. Ipsilaterally, stimulation of the medial MRF elicited responses predominantly in the ipsilateral LMC (mean iMMC/iLMC of 0.35 ± 0.12) whereas stimulation of the lateral MRF elicited responses predominantly in the ipsilateral MMC (mean iMMC/iLMC of 2.86 ± 0.72). The same reciprocal response pattern was observed on the contralateral side of the spinal cord. To assess which reticulospinal neuron populations were involved, we retrogradely labeled MRF neurons with axons coursing in different spinal funiculi, and compared the distributions of the labeled neurons to the stimulation sites. This comparison implicates specific populations of reticulospinal neurons within the gigantocellularis reticular nucleus. The existence of a mediolateral organization within the MRF, whereby distinct populations of reticulospinal neurons exert a predominant influence over trunk versus hindlimb motoneurons, provides an anatomical substrate for a relatively independent control of posture and movement during development.

Taste perception deficit after interleukin-1β microinjection into the nucleus accumbens of the rat

Takács Gábor[1]; Nagy Bernadett[1]; Szalay Csaba[1]; Fotakosz Dimitriosz[1]; Hanna Szófia[1]; Mizuno Masaharu[2]; Karádi Zoltán[2]
[1]Institute of Physiology and Neurophysiology Research Group, Hungarian Academy of Sciences, University of Pécs, Faculty of Medicine, Pécs, Hungary
[2]Kyushu Institute of Technology, Department of Brain Science and Engineering, Kitakyushu, Japan
gabor.takacs@aok.pte.hu

The nucleus accumbens (NAcc) is a key regulatory structure of the limbic forebrain. It has been shown to play important roles in the central control of feeding. In our previous studies, intra-accumbens administration of interleukin-1β (IL-1β) was demonstrated to be followed by food- and water intake suppression and metabolic disturbances. To elucidate whether alterations of gustatory perception could contribute to these anorexigenic and adipsogenic effects, bilateral IL-1β microinjection was administered into the NAcc of adult male Wistar rats, and taste reactivity tests were performed in interleukin treated and sham-operated control (CO) animals. In general, an evident deficit of taste reactivity was observed after the central cytokine administration. Pleasant taste stimuli elicited significantly weaker ingestive reactions in the IL-1β treated rats compared to CO ones. Animals with the cytokine microinjection also showed significantly poorer aversive reactions to unpleasant tastes. In contrast to the controls, the intensity of ingestive reactions evoked either by hedonically positive or negative taste stimuli proved to be similar in the IL-1β treated rats. Especially the evaluation of unpleasant tastes appeared to be disturbed in the cytokine treated animals, because the intensity of their ingestive and aversive reactivity patterns did not differ from each other. These findings indicate that gustatory deficits occurring in the cytokine treated animals are integrant parts of the complex feeding disturbances following IL-1β microinjection into the NAcc of the rat.
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Types and connectivity of hippocampal inhibitory neurons reciprocally connected with the medial septum

Takács T. Virág; Freund F. Tamás; Gulyás I. Attila
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
gulyas@koki.hu

Using anterograde and retrograde tracers we demonstrated that hippocampo-septal projection (HS) cells receive innervation from the medial septum. The density of excitatory and inhibitory inputs on HS cells was measured in serial electron microscopical sections immunostained for GABA. We could distinguish two types of HS cells according to their morphology and input properties: Cells with aspiny proximal and sparsely spiny distal dendrites in CA1-3 str. oriens, CA3 str. radiatum and less frequently in hilus received dense synaptic input of which ~14% are inhibitory; Densely spiny HS cells in str. lucidum and in hilus received a larger amount of excitatory inputs and were hardly ever innervated by inhibitory terminals (~2%). High proportion of their inhibitory inputs, especially on the somata (up to 55%) derived from the medial septum. Axon initial segments of CA1 HS cells were also innervated by septal and unlabeled inhibitory terminals and notably by GABA-

negative boutons. Using 3-D dendritic reconstructions we estimated the total number of inputs of the two cell types (~22000 in CA1 str. ori. and ~37700 in CA3 str. luc.). Local axons of CA1 HS cells innervated stratum oriens and radiatum, where they predominantly targeted thin pyramidal dendritic shafts and in smaller proportion spines and interneurons, including an HS cell. The massive synaptic input enables the HS cells to effectively sample the activity of local principal cells. Their reciprocal connection with GABAergic cells from the medial septum can play an important role in the synchronization of the two areas. The study was supported by the OTKA K60927 grant.

Effect of MPEP on wind-up of spinal dorsal horn neurons in rat in vivo

Tarnawa István; Gál Krisztina; Kocsis Pál
Gedeon Richter Pharmaceuticals Plc., Budapest, Hungary
tarnawa@richter.hu

Several lines of evidence support the role of ionotropic and metabotropic glutamate receptors (mGluR) in the pathophysiology of chronic pain. The mGluR5 antagonist MPEP has been reported to have analgesic effect in the formalin test, in mice, without affecting acute pain. Our aim was to test the effect of MPEP on windup of wide dynamic range (WDR) spinal dorsal horn neurons. Windup is a widely used electrophysiological model of central sensitization involved in chronic pain. It is a frequency dependent potentiation of neuronal responses to C-fibre stimulation, which can be blocked by NMDA receptor antagonists. We compared the effect of MPEP on windup with the NMDA antagonist ketamine and the clinically used analgesics morphine. Dorsal laminectomy was performed at the level of L3-L5 in spinalized, male SPRD rats.. A tungsten microelectrode was lowered into the deep dorsal horn for recording extracellular unit activity from WDR neurons. The sciatic nerve was stimulated at 10-min intervals using constant current pulses with intensity of 3 times of C-fiber response threshold. Windup stimuli consisted of trains of 16 pulses, delivered at 1 Hz. Drugs were administered i.v. after the responses had stabilized. Windup was calculated as the total number of C-fiber mediated action potentials for all 16 shocks minus 16 x the response to the first shock (input response). Both ketamine and morphine exerted a clear windup inhibitory effect. Morphine (1 mg/kg iv.) caused 55 % inhibition, while ketamine (10 mg/kg iv.) almost completely inhibited windup (88%). MPEP at a dose of 10 mg/kg iv. caused only slight (about 32 %) inhibition. Our results with ketamine and morphine are in line with the literature. However, the role of mGluR5 in central sensitization remains to be further clarified. MPEP is not selective for the mGlu5 receptor, its norepinephrine transporter inhibitory effect could also influence the result at the applied dose. More selective mGluR5 antagonists (e.g. MTEP) are needed to clarify the role of this receptor in central sensitization, and the therapeutic value of the mGluR5 antagonists in pain syndromes.

Spatial distribution and connectivity of OFF midget bipolar cells in marmoset retina

Telkes Ildikó[1]; Lee, Sammy C. S.[2]; Jusuf, Patricia R. [2]; Grünert Ulrike[2]
[1]Institute of Physiology, University of Pécs, Hungary
[2]National Vision Research Institute and Department Optometric and Vision Science, University of Melbourne, Australia
ildiko.telkes@aok.pte.hu

PURPOSE: Midget bipolar cells are crucial interneurons in the parvocellular pathway of the primate visual system, and are involved with high acuity and red-green colour vision. In

central retina, there is a one-to-one connectivity between cones, midget bipolar and midget ganglion cells. In peripheral retina, the connectivity of the midget pathway is less well understood. Here we studied the population of OFF midget bipolar cells across the retina of a New World monkey with respect to their connectivity with cones and midget ganglion cells. METHODS: OFF midget bipolar cells were labelled with antibodies against CD15. Cone pedicles were labelled with the lectin peanut agglutinin, and midget ganglion cells were retrogradely labelled from the lateral geniculate nucleus with dextran tetramethylrhodamine coupled to biotin and subsequently photofilled. RESULTS: In central retina (0.5 mm distance from the fovea), midget bipolar cells form a single dendritic cluster ("single headed") underneath each medium (M-) or long (L-) wavelength sensitive cone pedicle. Beyond 1 mm eccentricity single- and double-headed midget bipolar cells are found. At 3 to 4 mm eccentricity midget bipolar cells contact on average three or four cones, and in far peripheral retina (> 6 mm eccentricity) the average number of cones is five or six. The number of dendritic terminals at M/L cone pedicles does not change with eccentricity. The numerical ratio between midget bipolar and midget ganglion cells is 1:1 in central retina, and increases to 6:1 at 2 to 4 mm, and 13:1 in far peripheral retina. CONCLUSION: In marmoset, the 1:1 connectivity between cones and midget bipolar cells is limited to central retina.

Intraovarian effects of salsolinol (SAL) in intact and in polycystic ovary (PCO) syndrome in rats

Tidrenczel Zsolt[1]; Oláh Márk[2]; Székács Dániel[2]; Bodnár Ibolya[2]; Fekete Márton I. K.[2]; Nagy M. György[2]

[1]Department of Obstetrics and Gynaecology, National Health Center, Budapest, Hungary

[2]Neuromorphological and Neuroendocrine Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

tidrenc@hotmail.com

Background: Estradiol-valerate (EV) induced polycystic ovary (PCO) syndrome in rats is characterized by an increased synthesis and release of norepinephrine (NE) that is supposed to be related with the hyperactivation of the ovarian sympathetic nerves. It has been recently found that salsolinol (SAL), an endogenous dopamine (DA)-derived isoquinoline, besides its prolactin releasing activity induces a dose- and time-dependent decrease of DA concentrations in several organs, including ovary of rats that are densely innervated by the peripheral sympathetic nervous system (SNS). Therefore, our present study was designed to investigate the site- and the mechanism of action of SAL in the ovary obtained from intact as well as EV-induced PCO female rats. Methods: Virgin adult cycling Sprague-Dawley rats were used in the experiments. Experiment I: rats were treated with SAL or saline intraperitonealy (ip). Experiment II: PCO was induced by EV (2 mg i.m.). Sixty days after EV treatment rats were administered with SAL (25 mg/kg ip.) or saline 20 minutes before measurements. Several parameters were determined: plasma prolactin by RIA, catecholamine (DA, NE, SAL) concentration of the ovary by HPLC-EC, phosphorylated- (pTH) and total tyrosine hydroxylase (TH) by Western-blot analysis. Results: A single i.m. injection of EV induced a typical PCO condition in the ovary with an increase of TH immunoreactivity in the stroma and with an elevated NE level. While endogenous SAL was significantly reduced in PCO rats, administration of SAL induced a significant reduction of DA concentration in the ovary obtained either from control or from EV-induced PCO rats. SAL treatment, while not influencing total TH, can cause a decrease in level of pTH (i.e. active enzyme) of the ovary in intact rats. Conclusions: These results indicate that the pTH and DA decreasing effect of SAL and its reduced tissue level following EV treatment might be related to the increased activity

of the sympathetic nerves (NE tone) observed in this rat model of PCO syndrome. It can be hypothesised that SAL has an intrinsic effect in ovaries densely innervated by peripheral SNS. It may exert its regulatory function by influencing TH (phosphorylation level of TH) activity, consequently NE release. This work was supported by Ministry of Health (ETT) 177/2006, and Hungarian Scientific Research Fund (OTKA) 68170 to GMN.

Changes of neuropeptid gene expression pattern in the paraventricular nucleus of hypothalamus in response to metabolic challenges

Tóth Eszter; Bali Balázs; Bosca Zoltán; Ferenczi Szilamér; Kovács J. Krisztina
Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
t.eszter@freemail.hu

The hypothalamo-pituitary-adrenal (HPA) system has an important role in the metabolic- and stress-regulation. The center of hypothalamic stress regulation is the paraventricular nucleus (PVN). The PVN integrates the information from the circumventricular organs, limbic, cortical, viscero- and somatosensory areas and generates adequate hormonal and autonomic responses. Dallman et al. reported that the stress- and adrenalecotmy-induced activity of the HPA axis can be suppressed by metabolic factors, such as sucrose consumption. The aim of our study was to determine the effect of sucrose consumption on neuropeptide (AVP - arginine vasopressin, OXY - oxytocin, CRH - corticotropin releasing hormone) gene expression and neuron activation in functionally distinct cell population in the PVN.. Water-, sucrose-, saline-, and food intake were followed in rats for 3 weeks before and after adrenalectomy (ADX) or SHAM-operation. The neurons that became activated by steroid withdrawal or metabolic challenge were revealed by Fra-2. ADX increased the ACTH secretion, Fra-2 ir and CRH gene expression in the hypophyseotropic region of the PVN, and induced AVP gene expression in CRH-neurons. Sucrose consumption did not change this increase, however increased the neuronal activity and resulted in AVP gene expression in the autonomic part of the PVN. These neurons project to the dorsal vagal complex, and regulate autonomic functions. Cells in the ventral part of the PVN express melanocortin-4-receptor and have OXY-phenotype, however OXY gene expression levels remained unchanged after sucrose ingestion. Adrenalectomy and sucrose consumption result in distinct changes in neuropeptid gene expression changes in the functional different regions of the paraventricular nucleus, and these changes have an important role in the hypothalamic integration of stress and metabolism.

Comparison the effects of two antipsychotic compounds, olanzapine and aripiprazole on metabolic parameters in rats

Tóth Eszter; Darida Miklós; Földes Anna; Márkus Veronika; Gyertyán István; Kiss Béla; Kovács J. Krisztina
Laboratory of Molecular Neuroendocrinology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
t.eszter@freemail.hu

Weight gain is common in psychotropic therapy. Regulation of food intake and energy expenditure is under complex regulation of hypothalamic and brainstem autonomic centers. Antipsychotic drug-induced weight gain is a serious side effect leading to noncompliance with therapy and to exacerbation of comorbid conditions related to obesity. Olanzapine is one

of those atypical antipsychotics that have been reported to cause the most serious metabolic side effects. Weight gain, food and water intake, hormonal and metabolic parameters were followed in female Wistar rats treated by olanzapine (2 & 20mg/kg) and aripiprazole (4 & 40mg/kg). The low dose of olanzapine increases body weight gain, food intake and the retroperitoneal fat depot. During overnight fast the highest weight loss was detected in olanzapine 2mg/kg group, suggesting a metabolic imbalance in these animals. Our analysis also revealed that the higher dose of olanzapine resulted changes in metabolic parameters in an opposite way compared to the lower dose. In contrast, chronic administration of aripiprazol did not affect significantly the metabolic parameters of rats. These results confirm clinical studies, which showed no changes in body weights of schizophrenic patients following aripiprazole treatment. Intraperitoneal injection of glucose resulted in a rapid elevation of blood glucose levels, however neither the peak nor the decline of this parameter was not different among the treatment groups. We found that the basal leptin levels were not significantly different in ad libitum fed rats treated with either antipsychotic drug when compared to the control. Vehicle treated animals showed a significant reduction in leptin levels following overnight fast. In contrast, plasma leptin levels remained elevated in rats that received 2 mg/kg olanzapine for 14 days, suggesting a leptin-resistant state. These results confirm differential effect of atypical antipsychotics on the metabolic parameters and define plasma leptin level as potential indicator of metabolic imbalances induced during antipsychotic therapy.

Acylated-ghrelin microinjection into the amygdaloid body elevates blood glucose level and decreases food intake

Tóth Krisztián[1]; László Kristóf[1]; Lukács Edit[1]; Lénárd László[2]
[1]Institute of Physiology, Faculty of Medicine, University of Pécs, Pécs, Hungary
[2]Neurophysiology Research Group, Hungarian Academy of Sciences, Pécs, Hungary
krisztian.toth@aok.pte.hu

The effect of brain-gut peptide acylated-ghrelin (aGhr) is mediated via growth hormone secretagogue receptors (GHSR). I. p., i.c.v. or direct hypothalamic application of aGhr facilitates feeding and it has effects on blood glucose level. Amygdala has an important role in food intake. Projections of ghrelinergic neurons to the basolateral nucleus of the amygdala (BLA) were identified. Furthermore, our preliminary electrophysiological results showed that there are neurons in the BLA responding to aGhr. In male Wistar rats liquid food intake (Milkquick, Debrecen [136,45 kJ/100ml]) was studied under ad lib. condition and after 24h food deprivation. aGhr was used in doses of 25, 50, 100, 250 ng, respectively. Thirty ng (32.25 pM) GHSR antagonist (ANT) was applied alone or 15 min prior to 100 ng (30.16 pM) aGhr microinjections. Drugs were dissolved in sterile saline (0.15 M) and were microinjected bilaterally into the BLA in 0.4 µl volume. After microinjections food intake were measured in every 10 min for the first 30 min and at the 60th, 90th and 120th min, respectively. In ad lib. fed rats the 50 ng and 100 ng aGhr treatments caused significant decrease in food intake. The other doses were ineffective. Food intake decreasing effect was eliminated by prior ANT treatment. ANT applied in itself had no influence on feeding. After 24 h food deprivation the previously potent 50 and 100 ng aGhr had no effect. In separate experiment blood glucose level was measured 10 min before and 10, 20, 30, 50, 70, 90, 120 min after the bilateral intraamygdalar application of the previously most effective 100 ng aGhr (Glucometer Elite 2000, Bayer). Significant elevation was observed at the 10th and 20th min compared to the control treatment. Our results are the first reporting that aGhr causes food intake decrease in the BLA within a limited dose range. It can be eliminated by GHSR antagonist in the BLA.

The aGhr elevates the blood glucose level which develops before of the food intake decreasing effect. It is suggested that ghrelinergic mechanism plays a specific modulatory role in the regulation of feeding. Supported by ETT 317/2006, RET-0008/2005 MEDIPOLIS, NKTH-OTKA K 68431 and by the HAS.

The role of NK1 receptor positive neurons of the hypothalamus in the regulation of different emotion-related behaviors

Tóth Máté; Halász József; Zelena Dóra; Haller József
Department of Behavioral Neurobiology, Institute of Experimental Medicine, Budapest, Hungary
matet@koki.hu

Literature provided growing number of evidence for the involvement of substance P and neurokinin 1 (NK1) receptor in the regulation of emotional behaviors. NK1 receptor antagonists are potential targets for the treatment of depression, anxiety, and recently suggested for violence, as well. However, the region-specific action of these compounds were not clarified. The aim of our experiment was to clarify the role of neurons expressing NK1 receptors in the intermedier hypothalamus in the regulation of emotion-related behaviors in male Wistar rats. To elucidate the specific role of this neurotransmitter system we induced a selective lesion (using microinjection of saporine-conjugated substance P) of neurons expressing NK1 receptors in this area. After a week recovery, in one-week intervals, aggressive behavior, anxiety and depression-like behavior was assessed. After behavioral tests brain were removed for a detailed immunohistological analysis. In the resident-intruder test, the number of attacks -especially the number of hard bites- decreased dramatically in the lesioned group, without affecting other aggression-related behaviors. Additionally, compared to controls, the lesion induced a marked increase in the open-arm activity in the plus-maze test. In the forced swim test, the time spent with immobility/floating was lower in the lesioned group, providing further evidence for the role of SP/NK1 system in mood disorders. Our data support earlier observations outlining the importance of the NK1/substance P system in the regulation of emotional processes. In this respect, NK1 positive neurons in the intermedier hypothalamus seems to be crucial not only in the regulation of aggression, but from the aspect of anxiety and depression-like behavior, as well.

Chronic stress in cannabinoid receptor 1 knockout mice

Trzosek-Szabó Ursula; Barna István; Domokos Ágnes; Zelena Dóra
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
ula-trzosek@freemail.hu

The endocannabinoid system affects the neuroendocrine regulation of hormone secretion, including the activity of the hypothalamus-pituitary-adrenal (HPA) axis. Our previous data suggest that among basal condition and during novelty stress endogenous cannabinoids inhibit the HPA axis via centrally located CB1 receptors. Here we demonstrate the effect of repeated stimulation (daily 1h restraint) on mice lacking CB1 receptor. The behavior of the mice were examined on 11. day (10R, elevated plus maze, EPM) and HPA axis changes were examined on day 15 (14R). After 10R the animals spent more time in the open arm of the EPM but their locomotion were also enhanced. As a sign of enhanced anxiety the stressed mice showed higher risk assessment behavior and there was a tendency for more defecation. The CB1 receptor KO mice seemed to be more anxious irrespectively of stress. The wild-type (WT)

mice started to eat and drink less during repeated restraint as signs of anhedonia with similar tendencies in KO. Typical somatic changes (body weight decrease, adrenal gland hypertrophy, thymus involution) were visible at the end of 14R without influence of the lack of CB1 receptor. There was a tendency for elevated resting corticosterone plasma levels after 14R both in WT and KO mice. It is important to emphasize that the lack of CB1 receptors per se resulted an elevated resting ACTH and corticosterone secretion, suggesting a prominent role of the cannabinoid system in basal modulation of the HPA axis. We confirmed the role of CB1 receptors in inhibiton of anxiety as well as in inhibition of the basal HPA axis activity. One can hypothesise that the lifelong elevation in basal corticosterone levels in KO mice is contribute to the anxiety of these animals. The signs of chronic stress developed in this mice strain but it seems that the role of cannabinoids is principally to reduce the basal activity of HPA than to influence its reaction during stress situations.

Complex model for schizophrenia in rats for assessment of pain sensitivity

Tuboly Gábor[1]; Vezér Tünde[2]; Horváth Gyöngyi[2]; Benedek György[2]
[1]Department of Physiology and [2]Department of Public Health, Faculty of Medicine, University of Szeged, Hungary
tuboly@phys.szote.u-szeged.hu

Clinical studies have proved that schizophrenia is often accompanied by hypoalgesia. Several animal models have been introduced lately to study the pathophysiology of this disease. Unfortunately only a few of them examined the phenomenon of hypoalgesia together with specific tests of schizophrenia. We induced schizophrenia-related alterations by subchronic ketamine treatment and post-weaning social isolation in rats to examine their long-lasting effects on nociceptive responses and sensorymotor gating. Materials and Methods Wistar rats (day 21-23 of age, n=40) were either housed individually or grouped (4-5/cage) for 32 days, and were treated daily with either an increasing dose of ketamine (30, 40, 50, 60 mg/kg; increased each week) or saline. Tail-flick latencies were determined before starting the treatment and after the last drug administration, at 46, 48 (C-fiber activation) and 52 °C (affects mainly Adelta-fibers). After testing rats were rehoused. At 5th week acoustic prepulse inhibition (PPI) was tested to observe the reflex modification affected by social isolation and/or ketamine treatment. Results As regards tail-flick latencies at 46 and 48 °C, juvenile isolation but not ketamine treatment resulted in a significantly enhanced pain threshold ($p<0.005$ and 0.05. respectively), while the changes at 52 °C were not significant ($p=0,68$). Ketamine treatment by itself did not reduce the PPI, but caused a further impairment in the decrease induced by social isolation. Conclusion In summary, social isolation exerts significant effect on acute heat pain sensitivity in young animals, disturbing primarily C-fiber linked pain pathways, while subchronic ketamine administration did not influence it. However, PPI revealed a more pronounced deficit after the combination of these two treatments. Thus, our study suggests a selective disturbance in the parallel sensory pathways under these experimental conditions. This work was supported by grants of RET-08/04 OMFB-0066/2005 and OTKA, K60278.

Afferent neuronal connections of the medial paralemniscal nucleus in rat

Varga Tamás[1]; Palkovits Miklós[1]; Usdin, Ted B.[2]; Dobolyi Árpád[1,3]
[1]Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

[2]Section on Fundamental Neuroscience, National Institute of Mental Health, Bethesda, Maryland, USA
[3]Department of Anatomy, Histology, Embryology, Semmelweis University, Budapest, Hungary
varga@ana.sote.hu

We investigate the medial paralemniscal nucleus (MPL) as one of the 2 major sites of the synthesis of the recently discovered neuropeptide, tuberoinfundibular peptide of 39 residues (TIP39). We have presented the cytoarchitectonic identification of the MPL and the distribution of TIP39 neurons within the nucleus. As a next step to understand the functions of medial paralemniscal TIP39 neurons, the afferent neuronal connections of the MPL were investigated. The retrograde tracer cholera toxin B subunit was injected into the MPL of rats. A significant amount of retrogradely labeled cells were found in auditory, insular, and ectorhinal cortices, anterior cortical amygdaloid nucleus, lateral preoptic and lateral hypothalamic areas, ventromedial hypothalamic nucleus, zona incerta, subparafascicular and posterior intralaminar thalamic nuclei, periaqueductal gray, deep and intermediate layers of the superior colliculus, deep mesencephalic nucleus, medial part of the medial geniculate body, nucleus brachium of inferior colliculus, external cortex of the inferior colliculus, cuneiform, periolivari, medial vestibular, spinal trigeminal and lateral paragigantocellular nuclei. Control injections adjacent to the MPL resulted in markedly different patterns of retrogradely labeled cells confirming our previous cytoarchitectonic data on a distinct MPL. In addition, the anterograde tracer biotinylated dextran amine was injected into the auditory cortex and the ventromedial hypothalamic nucleus, 2 brain regions, which contained labeled cells following injection of retrograde tracer into the MPL. Following these injections, labeled fibers were distributed evenly in the MPL confirming that neurons of the auditory cortex and the ventromedial hypothalamic nucleus project to the MPL. Our results support a role of medial paralemniscal TIP39 neurons in auditory processing, which is consistent with our previous results of major TIP39-containing projections from the MPL to the periolivary area, external cortex of the inferior colliculus, and medial part of the medial geniculate body, as well as c-fos activation in TIP39 neurons following high-intensity acoustic stimuli. The data also suggest that medial paralemniscal TIP39 neurons receive non-auditory inputs as well implying their functional activity with multimodal inputs.
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Temporal changes in gene expression of immortalized LHRH neurons following estrogen treatment

Varjú Patricia[1]; Chang Ken C. N.[2]; Merchenthaler István[3]; Hrabovszky Erik[1]; Liposits Zsolt[1]
[1]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Women's Health Research Institute, Wyeth Research, Collegeville, Pennsylvania, USA
[3]Department of Epidemiology & Preventive Medicine, University of Maryland, Baltimore, USA
turekne.marta@koki.hu

Estrogen acts as a feed-back signaling molecule between the gonads and the brain to control mammalian reproduction. Gonadotropin secretion is tightly regulated by the pulsatile secretion of luteinizing hormone-releasing hormone (LHRH) into the hypophyseal portal circulation from a small group of neurons scattered in the preoptic area. While many estrogen

effects upon LHRH neurons are exerted through estrogen sensitive afferents to LHRH cells, recent evidence indicates that estrogen can also influence LHRH neurons directly. *In vivo* studies of estrogen-regulated genes in LHRH neurons are extremely difficult due to the scattered distribution of LHRH neurons and difficulties in sampling of their mRNA complement. Therefore, in the present studies we chose to identify the estrogen regulated genes using an *in vitro* model of LHRH neurons, the GT1-7 cell line. Gene-networks and pathways some of which may also be regulated by estrogen in LHRH cells during the estrus cycle *in vivo* were studied by a high throughput cDNA microarray technology.
In the absence of estrogen, GT1-7 cells expressed about 6000 mRNA species and the Ingenuity Pathway Analysis program identified almost 70 active canonical pathways in these cells. The analysis of the receptor profile in untreated cells showed the expression of several neurotransmitter and neuropeptide receptor subunit mRNAs, many of which were not detected previously in LHRH neurons. Estrogen-treatment of GT1-7 cells modified the expression level of 1200 transcripts; the majority of these transcripts were up-regulated at each time point investigated (0.5h, 2h, 8h, 24h and 48h). The treatment affected significantly several canonical pathways, including those for Cellular movement, Cellular growth, Cellular development, Cell death, Cell signaling and Immune response. Further, Gene Ontology analysis of regulated transcripts revealed that certain functional categories of genes (transcription factor, cytoskeletal component, CAMs, ECM components, receptors, transporters, ion channels and mitochondrial enzymes) are the main targets of estrogen action in LHRH neurons. Quantitative real-time PCR was used successfully to verify microarray data. This work was supported by grants from the National Science Foundation of Hungary (OTKA T46574 and K69127) and by NKFP (1A/002/2004). Patricia Varju and Erik Hrabovszky were supported by the Bolyai Fellowship of the Hungarian Academy of Sciences.

Electrophysiological effects of two insecticides on the isolated optic tectum of the carp
Varró Petra; Világi Ildikó
Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
pepimail@freemail.hu

Insecticides are toxic chemicals, several of them designed to act on synaptic receptors of the insects. As they are not fully selective, they can affect non-target organisms also. Aquatic animals like fish can be exposed to insecticides via pollution of their habitat. In the present experiments, the neuronal effects of two insecticides, bensultap and fipronil, were examined on an in vitro preparation of carp (Cyprinus carpio) brain. Bensultap is an insecticide of the neonicotinoid group, it acts as an antagonist of nicotinic acetylcholine receptors (nAChRs). Fipronil is a phenylpyrazole type insecticide acting as an antagonist on ionotropic GABA receptors. The optic tectum of teleosts is an integrative brain area processing visual-spatial, somatosensory and vestibular information. Due to its anatomical characteristics, it can be isolated and maintained in a brain slice chamber as a thin, hollow hemispherical structure with intact laminar organization. By stimulating the optic nerve trunk electrically, extracellular field potentials can be recorded from stratum opticum. Insecticides were applied onto the tecta using in vitro administration. Electrophysiological experiments were performed by measuring the amplitude of evoked field potentials. Changes in the basal excitability and synaptic plasticity were examined. The stimulus intensity-evoked response correlations, as well as the effects of paired-pulse and high-frequency stimulation were recorded. Both insecticides slightly decreased the amplitude of evoked potentials and they significantly inhibited LTP-induction after high-frequency stimulation. On the other hand, in the paired-pulse tests, neither of the two chemicals had any significant effect. These results were unexpected in case

of the GABA-antagonist compound fipronil. To elucidate the mechanisms we are planning to perform some more experiments with the nAChR-antagonist mecamylamine and the GABAR-antagonist bicuculline. Similar experiments studying pesticide action on non-target animals may provide useful data for the regulation of the chemical and the recognition and cure of eventual intoxications.

Factors of energy balance during central leptin infusion

Vámos Zoltán; Vinagre, Joao Marques; Solymár Margit; Balaskó Márta; Pétervári Erika; Székely Miklós
Department of Pathophysiology and Gerontology, Faculty of Medicine, University of Pécs, Pécs, Hungary
miklos.szekely@aok.pte.hu

Leptin from the fat tissue is known to be anorexigenic, but its multiple effects on various factors of the catabolic state have not been analyzed simultaneously. In the present studies the complex effects of central leptin infusion on energy balance were checked in male Wistar rats aging 3 months (body weight: 280-300 g). The rats stayed in a room with lights on between 6:00 and 18:00 h, and had powdered chow and water ad libitum. About 7-10 days prior to the leptin administration all animals underwent an operation: they had a transmitter placed into the abdominal cavity, with electrodes for heart rate measurement (Minimitter). By the help of an Alzet osmotic minipump, the animals received an intracerebroventricular infusion of leptin in a dose of 1 μg/h (1 μg/μl). The circadian changes of body temperature, spontaneous activity and heart rate were followed by the telemetric system. An attachment of the system allowed observation of feeding frequency and feeding duration. Besides, the amount of food consumed from the container, as well as the body weight was measured on a daily basis. The infusion induced a rise in the daily nadirs but not the peaks of body temperature, with a gradual decline (even below the initial value) from the third day, despite the ongoing infusion. Spontaneous activity and heart rate exhibited a similar pattern. During daytime the rats rarely turned to the food, spent minimal time with feeding and these were hardly altered by the infusion. During the dark period the feeding frequency declined significantly upon leptin infusion and there was a large (although not statistically significant) fall also in the feeding duration. The effects on feeding frequency was waning at the end of the infusion, the duration remained low throughout. The daily food intake remained significantly below the initial value throughout the infusion period although some return was seen from the fifth day. Body weight drastically declined and did not show any sign of reversal. It is concluded that at the early phase of infusion leptin induces a coordinated catabolic state with signs of elevation in resting metabolism and strong suppression in feeding behavior. Later on the food intake remains low, while the reversal of changes in heart rate, activity and body temperature suggest that some compensatory steps develop, probably due to the relative fasting state.

Possible role of PACAP in the regeneration of the VNC ganglia of the earthworm: a biochemical and immunohistochemical approach

Várhalmi Eszter[1]; Somogyi Ildikó[1]; Kiszler Gábor[1]; Pollák Edit[1]; Lammel Krisztián[1]; Lubics Andrea[2]; Reglődi Dóra[2]; Németh József[1]; Molnár László[1]
[1]Department of Experimental Zoology and Neurobiology, University of Pécs, Hungary
[2]Department of Anatomy, Faculty of Medicine, University of Pécs, Pécs, Hungary
varhalmieszter@freemail.hu

The regeneration of the ventral nerve cord (VNC) ganglia of earthworms is thought to be affected by some bioactive substances (growth factors, neurohormones and neurotransmitters) and certain cells, namely coelomocytes and neoblasts. This study focuses on the possible role of pituitary adenylate-cyclase activating polypeptide (PACAP) in the regeneration of the VNC ganglia applying radioimmunoassay (RIA), immunocytochemistry and electron microscopic immunohistochemistry. By means of RIA we have shown that PACAP expression is inducible in earthworm tissues. On the effect of segment amputation PACAP level markedly increased in the VNC ganglia, body wall and coelomocytes; however, a definitive rostrocaudal concentration gradient had been identified in each tissues investigated. Whole mount immunocytochemistry revealed that PACAP labelled nerve fibers enter into the regeneration blastema formed on the severed ganglion. In the vicinity of the labelled nerve fibers high number of mitotic figures was observed. Further in the regeneration blastema strongly stained neoblasts, thought to be stem cells of earthworms were seen. Some of them expressed PAC1 receptors as well. Close to the regeneration blastema numerous PACAP of labelled coelomocytes were detected. However, at this stage of our experiments it is not clear whether coelomocytes express and/or transport PACAP. Based on our results we conclude that PACAP has influence on the regeneration of the VNC ganglia; it could stimulate both division and differentiation of the neoblasts resulting formation of renewed tissues like muscle and neural tissues.

Toxic effects of mitochondrial complex I inhibitors (rotenon, MPTP and MPP+) on aquatic animals

Vehovszky Ágnes; Szabó Henriette; Hernádi László; Győri János; Farkas Anna
Department of Experimental Zoology, Balaton Limnological Research Institute, Hungarian Academy of Sciences, Tihany, Hungary
agnes@tres.blki.hu

Aquatic invertebrate animals, the brine shrimp (Artemia) nauplii, the freshwater amphipod Dikerogammarus villosus and the pond snail Lymnaea stagnalis were tested in rotenone, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP+ (1-methyl-4-phenylpyridiniμM). These substances known as mitochondrial complex I. inhibitors, are also used in experimental animals to selectively destroy dopaminergic neurons in the CNS, thus mimicking the behavioural and cellular symptoms of the Parkinson's disease (Beal 2001). All the tested animals showed dose-dependent mortality after rotenone treatment. Artemia and Dikerogammarus 0.26 μM,± 0.18 μM, and 0.54 ±showed similar sensitivity (48 hours LC50: 0.37 respectively) while on Lymnaea rotenone had longer lasting effect (96 hours LC50: 0.84 μM). Sublethal doses of rotenone (0.1 – 1 μM) significantly decreased the spontaneous activity of Lymnaea and Dikerogammarus in a dose-and time-dependent manner. In Artemia nauplii MPTP and MPP+ also had toxic effects (LC50: 0.21 ± 0.09 mM and 0.2 ± 0.08 mM). while 100 μM L-DOPA partly prevented the toxic effects of rotenone (LC50: 1.93 ± 0.39 μM, in rotenone plus L-DOPA). Glutathion s transferase (GST) enzyme activity was measured.in the whole body tissue homogenates of Artemia and Dikerogammarus and the isolated central nervous system of Lymnaea. Sublethal (0.1- 0.3 μM) rotenone treatment increased the GST activity up to about 50 % in Artemia and Dikerogammarus in 48 hours, while 0.5 μM rotenone increased the GST activity after 4- 6 days in the Lymnaea CNS. Our recent results show similarities with other animal models of Parkinson's evoked by mitochondrial complex I. inhibitors. All the substances are characterized by time and dose-dependent toxicity, and rotenone decreases the spontaneous locomotion of Lymnaea and Dikerogammarus. The increased level of GST activity in the tissues of rotenone-treated animals suggests oxidative

stress in the cells involved (Obata 2006). References: Obata, T. Nicric oxide and MPP+ - induced hydroxyl radical generation (2006) J. Neural Transmission 113, 1131-1144. Beal, M.F. (2001) Experimental models of Parkinson\'s disease. Nature Reviews Neuroscience, 2, 325-332. Acknowledgement: This work was supported by the Hungarian OTKA T63451 grant for A. V.and MEH ÖKTM 12002 grant for A. F.

Innervation of kisspeptin neurons by vasopressin and VIP- immunoreactive fibers in the anteroventral periventricular and preoptic periventricular nuclei of the mouse

Vida Barbara[1]; Kalamatianos Theodosis[2]; Hrabovszky Erik[1]; Carathy Alain[3]; Coen Clive W.[2]; Liposits Zsolt[1]; Kalló Imre[1]

[1]Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

[2]Biomedical and Health Sciences, King's College London, London, UK

[3] NRA/CNRS/University of Tours/Haras Nationaux, Nouzilly, France

turekne.marta@koki.hu

Gonadotropin releasing hormone (GnRH) secretion is under circadian regulation, but the mechanisms underlying this are poorly understood. A signal from the suprachiasmatic nucleus (SCN) seems essential for the proestrous GnRH surge, which leads to LH release and ovulation. Receptors for the principal peptides in SCN efferents, vasopressin (VP) and vasoactive intestinal peptide (VIP), have not been detected in mouse GnRH neurons, and no direct input has been traced onto GnRH neurons from the SCN (Wintermantel et al, 2006). However, there is evidence for expression of receptors for VP (V1a; Kalamatianos et al, 2004) and VIP (VPAC2, Kalló et al, 2004) in the rodent anteroventral periventricular and preoptic periventricular nuclei (AVPV/PeN), a region that sends projections to GnRH neurons and is essential for the GnRH surge. Kisspeptin (KP) neurons in the AVPV/PeN have been implicated in conveying the positive feedback effect of estrogen to GnRH neurons. The current study tests the hypothesis that these KP neurons also receive vasopressin and/or vasoactive intestinal peptide input. Dual-label immunohistochemistry for KP and VP or VIP was carried out on sections from female and male mice. Confocal microscopic analysis revealed that VP-immunoreactive (IR) axon varicosities are in close apposition to the perikarya and dendrites of KP neurons in the AVPV/PeN. Nearly half of the KP-IR perikarya received VP-IR innervations. In contrast, only a few VIP-IR fibers were detected abutting KP neurons in either sex. These results suggest that, in addition to their role in mediating the positive feedback effect of estrogen, KISS-IR neurons in the AVPV/PeN are integrators of VP (and possibly VIP) signals, potential mediators of circadian information from the SCN.

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Quantitiative morphological description of reticulospinal interneuron – motoneuron connections in the cervical spinal cord of frog

Vida Edith[1]; Stelescu András[1]; Wéber Ildikó[1]; Dityatev Alexander[2]; Wolf Ervin[1]; Birinyi András[1]

[1]Department of Anatomy Faculty of Medicine, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

[2]Zentrum für Molekulare Neurobiologie Hamburg, University of Hamburg, Hamburg, Germany

vida.edith@gmail.com

It is well established that interneuronal circuits in the spinal cord generate rhythmic alternating activity of spinal motoneurons. These spinal central pattern generators (CPG), however, are under the control of brainstem nuclei. Descending commands from various brainstem nuclei can influence spinal motor activity through the CPGs or through the spinal motoneurons. In the experiments presented here, a single reticulospinal axon in the ventromedial white matter and the postsynaptic motoneuron in the ventral grey matter were impaled in the cervical segment of the frog spinal cord. Following physiological records, the impaled reticulospinal interneuron - motoneuron pairs were labeled with neurobiotin and 3-dimensionally reconstructed from serial sections of the spinal cord by using Neurolucida system. The labeled premotor interneurons were located in the medial and inferior nuclei of the reticular formation. Stimulation of axons of reticular interneurons evoked 160-300 μV excitatory postsynaptic potentials in spinal cervical motoneurons. Three of these postsynaptic motoneurons were successfully labeled intracellularly and reconstructed. They had elongated cell bodies emitted 6-7 individual dendritic trees with lengths varied between 28800-48000 μm (mean: 39300 μm) and with surface area of 303300-428800 μm2 (mean: 345800 μm2). The total length and surface of motoneuron dendrites were between 29000-48000 μm and 303000-430000 μm2. The labeled reticulospinal axons established 6-7 close appositions with dendrites of the cervical motoneurons. The contact areas were distributed over a relatively wide area of the dendritic trees. These contacting boutons apposed relatively thick (average diameter: 1.56 μm) dendritic segments located at 160 - 1200 μm (mean: 520 μm) distance from the soma. This work was supported by the Hungarian National Research Fund, ETT 025/2006 and OTKA K67747.

Comparative study of myelination in the developing murine corpus callosum using immunohistochemistry, electron microscopy and conventional myelin staining

Vincze András[1]; Mázló Mária[1]; Seress László[1]; Komoly Sámuel[2]; Ábrahám Hajnalka[1]
[1]Central Electron Microscopic Laboratory and [2]Department of Neurology, Faculty of Medicine, University of Pécs, Hungary
hajnalka.abraham@aok.pte.hu

Oligodendroglial cells differ in their ultrastructural appearance depending on their myelin producing and maintaining activity. Myelin contains a major class of proteins, the myelin basic proteins (MBP) which have an important role in myelin compaction and can be detected with immunohistochemistry. In order to have a better understanding of the correlation of light and electron microscopic features of myelination, myelin formation in the corpus callosum was studied in young postnatal (3-28 days old) mice using immunohistochemical detection of MBP and routine electron microscopy (EM). In addition, conventional myelin staining Luxol Fast Blue (LFB) method was compared to MBP immunohistochemistry and EM. MBP immunohistochemistry revealed a few stained oligodendroglial cells in the lateral part of the corpus callosum at postnatal day (P) 3, and in the medio-lateral part of it at P7. MBP-immunoreactive axons appeared only on P10, and the number of the myelinated axons in all parts of the corpus callosum is increased by P14. Later, the density of MBP-immunoreactive axons gradually increased and at P28 it was comparable with the density of myelinated axons in adults. With EM, the first intermediate and dark oligodendroglial cells, which produce and maintain myelin, were observed at P7 in the medio-lateral part of the corpus callosum. The first signs of myelination were seen at P10, although numerous myelin sheaths with loosely built structure appeared only at P14. At P21, the majority of oligodendroglial cells were dark

and the numbers of myelin sheaths were increased, although they still consisted of few lammellae. At P28 thick and compact as well as loosely structured and thin myelin sheaths were observed indicating the ongoing myelination. With LFB method, myelin was first observed at P14 in the lateral part of corpus callosum and the staining became more intense until P28. Our data show that unlike LFB method, results obtained with light microscopic MBP immunohistochemistry correlates well with that of EM. Therefore both MBP immunoreaction and EM are useful, alone or in combination, for the detection of myelination, demyelination as well as remyelination processes in animal models and in humans. Supported by the Bolyai Scholarship (H. Á.).

Patterns of sharp wave-ripple complexes in the rat hippocampus in vitro

Wittner Lucia; Ulbert István
Institute of Psychology, Hungarian Academy of Sciences and National Institute of Neurosurgery, Budapest, Hungary
wittner@cogpsyphy.@

Hippocampal sharp wave-ripple complexes (SPW-Rs) consist of fast oscillations (100-200Hz) superimposed on a slow field potential transient. SPW-Rs occur during slow wave sleep and behavioural immobility and are thought to play an important role in memory formation. Rodent slice preparations are shown to exhibit spontaneous SPW-Rs, but the cellular and network properties of this rhythmic population activity remains to be elucidated. Field potential gradient (FPG) and multi unit activity (MUA) were recorded in rat hippocampal slices, using laminar multielectrodes, and current source density (CSD) and time frequency (TFR) analysis were performed. Pyramidal neurons were characterised in simultaneous intracellular records and filled with neurobiotin for subsequent anatomy. Spontaneous SPW-Rs were generated with a frequency of 1-4 Hz in the dentate gyrus, CA3 and CA1 regions of the hippocampus. A field potential transient, increased fast oscillatory activity and increased MUA characterised these synchronous population events. Two types of SPW-Rs could be distinguished in the CA3 region. The large majority of CA3 pyramidal cells showed hyperpolarization in response to both types of SPW-Rs, together with a current source in the cell body layer. During type 1 SPW-Rs, simultaneous current sink was detected in the str. lucidum, in the termination zone of mossy fibers, whereas during type 2 SPW-Rs, current sinks were present in the str. oriens and radiatum, in the dendritic zones known to receive recurrent synaptic input. Our results suggest that multiple neuronal networks are able to generate SPW-Rs in the rat hippocampus in vitro. Type 1 SPW-R was presumably generated in the dentate gyrus and conducted to the CA3 region through mossy fibers, whereas type 2 SPW-R was possibly generated within the CA3 region. The hyperpolarizing response of the pyramidal cells with an increased MUA indicate that interneurons might play an important role in the generation of SPW-Rs.

Effects of oxygen tension on the survival and differentiation of neural stem cells

Zádori Anita[1]; Ágoston A. Viktor[2,3]; Demeter Kornél[1]; Gőbl Anna[3]; Nagy Zoltán[2]; Madarász Emilia[1]
[1]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest
[2]National Institute of Psychiatry and Neurology and [3]Hyperbaric Centre, Budapest, Hungary
madarasz@koki.hu

Background: Previously we demonstrated that the host environment modified the fate of stem cells implanted into the mouse forebrain. Brain lesions, causing long-lasting ischemia (hypoxic conditions), were suspected to influence the survival and differentiation of stem cells. Therefore, we investigated the fate of GFP-4C neural stem cells in several in vitro and in vivo environments with different oxygen tensions.
Methods: The consequences of *hyperbaric oxygen* treatment (hyperoxia) were studied in vivo, on cold lesioned host animals implanted with GFP-4C cells. For lesions, a modified model of Klatzo et al. (1958) was applied. For implantation, GFP-4C neuroectodermal stem cells were used (Demeter et al. 2004). Lesioned and normal mice were treated with hyperbaric oxygen (2.5 ATA, 100% O_2 for 2x30 min), each day for one week, either before or after implantation. The host animals were sacrificed 2 and 6 weeks after the implantation.
Results: In lesioned, hypoxic cortex, GFP-4C cells survived for a long period (>2 months), but did not differentiate to neurons and only sporadically to astrocytes. In animals treated with hyperbaric oxygen, an increased rate of differentiation was detected: the implanted cells survived for an equally long period, but formed both neurons and astrocytes.
Discussion: The data suggest that neural stem cells can survive and proliferate under hypoxic conditions, but require higher O_2 tension for neural tissue type differentiation. The hypoxic environment may be partially responsible for preventing neural differentiation of implanted stem cells in lesioned brain.

Effect of high and low vasopressin levels in posterior pituitary denervated and Brattleboro rats

Zelena Dóra; Mergl Zsuzsa; Makara B. Gábor
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
zelena@koki.hu

Beside its role in the water homeostasis vasopressin (AVP) takes place in the multifactorial regulation of ACTH secretion mainly by modulating the effects of CRH in the hypophysis. Mechanical compression of the pituitary stalk results in posterior pituitary denervation (PPD) and shunting of magnocellular AVP to the portal circulation. This provides an experimental model for the traumatic damage of the pituitary stalk. Another experimental paradigm is the Brattleboro rat with genetical damage of AVP synthesis. This animal is a good model for diabetes insipidus in human. We compared the effect of sustained changes after PPD to lifelong deficits in Brattleboro rat. We investigated in vitro the adenylate cyclase activity of the adenohypophysis (AL) and NIL parallel with their β-endorphine (BE) secretion. Previously we establishes that 1 week after PPD the plasma ACTH and corticosterone levels were higher without changes in CRH content of the median eminence. The POMC mRNA levels were elevated only in intermediate lobe of the pituitary where BE is the main splicing variant. The PPD-induced decrease in AL BE secretion with the slight decrease in cAMP content could be due to the slight damage in AL (partial infarctions). The lack of AVP in Brattleboro rats did not influence the AL cAMP content although it was able to diminish the BE secretion The transient as well as the lifelong lack of AVP in NIL elevates the number and/or activity of adenylate cyclase activating receptors without influencing the number and/or activity of phosphodiesterase (the cAMP degrading enzyme). However in PPD rats the adenylate cyclase activating receptors does not seem to be CRH receptors, while in Brattleboro rats enhanced CRH receptor sensitivity can be detected. The regulatory role of AVP in the ACTH secretion was not proved by the changes in the adenylate cyclase activity in the AL. The functional significance of cAMP elevation in NIL is unclear because the BE

(as a main POMC peptid in NIL) secretion and content was not affected by any of the used treatment.

Measuring intracellular Ca2+ concentration changes in the mouse hemicochlea preparation

Zelles Tibor; Fekete Ádám; Horvát Tamás; Polony Gábor; Halmos György; Lendvai Balázs; Vizi E. Szilveszter
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
zelles@koki.hu

Ca2+ plays an important role in the intra- and intercellular signaling in the cells of the inner ear. The possibility of investigating simultaneously the Ca2+ regulation of different sensory- and supporting cells of the organ of Corti in a preparation that keeps the original tissue organization, lacks the adaptive metabolic changes typical in culture preparations and is prepared from mice with mature cochlear tissue has been missing. We have developed a method of fluorescence Ca2+ imaging in acute mouse hemicochlea preparation. Cochleae of 15-21 days old male CD1 mice were hemisected with a vibratome and bulk loaded with the Ca2+ sensitive fluorescent dye fura-2/AM. The preparations were placed under an epifluorescence microscope and continuously perfused with a HEPES based buffer. The emitted light was detected by a CCD camera-based ratio imaging system and the intracellular Ca2+ concentration ([Ca2+]i) changes of inner and outer hair cells, pillar and supporting cells of the organ of Corti were determined off-line. By the perfusion of different concentrations of K+ and ATP we could evoke reversible and repeatable Ca2+ transients in the imaged cells. The method can be used for testing potential drug candidates by comparing the Ca2+ responses evoked in the presence and absence (internal standard) of the drugs. Investigating the effect of genetic manipulations that directly or indirectly influences the regulation of [Ca2+]i in the organ of Corti is another potential application.

Studies on the interaction of type 2 deiodinase and microtubule-associated protein 1A

Zeöld Anikó[1]; Szabó Edit[1]; Zavacki Ann Marie[2]; Bianco Antonio C.[2]; Gereben Balázs[1]
[1]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[2]Thyroid Section, Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts
turekne.marta@koki.hu

The major secretory product of the thyroid gland is thyroxine (T4), a pro-hormone that has to be converted to T3 to efficiently bind to the thyroid hormone receptor (TR). The T3-TR interaction initiates thyroid hormone dependent changes in expression profiles in the brain and other tissues. The type 2 deiodinase enzyme (D2) is the key factor of T4 to T3 conversion. D2 is a highly active oxido-reductase selenoenzyme subjected to stable retention in the endoplasmic reticulum (ER) and undergoes substrate-mediated ubiquitination along the ER–associated degradation (ERAD) pathway. D2 function and consequently T3 production could be significantly affected by ER retention, but the mechanism of this process has not yet been resolved.
A yeast two hybrid (Y2H) assay was used to identify D2 interacting proteins. The assay revealed that the light chain 2 (LC2) of the microtubule-associated protein A (MAP1A) directly interacts with the carboxy-terminal end of D2, a part of the globular domain of the

enzyme that is located in the cytoplasm. MAP1A is comprised of heavy chain and LC2 and is widely distributed along the microtubules in both mature neurons and glial cells, where it seems to be involved in both the stabilization of microtubules and in the regulation of the distribution of associated molecules by linking them to the microtubule-based cytoskeleton, known to interact with the ER. V5-tagged LC2 and FLAG-tagged D2 were transiently co-expressed in Ptk2 cells for co- immunoprecipitation studies. Pull-down with anti-FLAG and consequent V5 detection indicated an interaction between the two proteins. Immunofluorescence demonstrated co-localization of the two proteins in Ptk2 cells co-transfected with V5-LC2 and FLAG-D2 in line with previous data. Transient expression of D2-FLAG in TalphaT1 cells, a thyrotroph cell-line with endogenous Map1a expression demonstrated co-localization of D2-FLAG with the tubular system. Based on these data we speculate that the D2-MAP1A interaction could be a factor in the ER retention of the enzyme with potential consequences on the post-translational regulation of the D2 protein. This work was supported by OTKA T49081.

Vasodilatory and endothelial protective effects of vasoactive intestinal peptide (VIP) against ischemia/reperfusion in the newborn brain

Zimmermann Aliz[1]; Lenti Laura[1]; Oláh Orsolya[1]; Kis Dávid[1]; Tóth Gábor[2]; Bari Ferenc[1]
[1]Department of Physiology and [2]Department of Medical Chemistry, Faculty of Medicine, University of Szeged, Hungary
zimmermannaliz@yahoo.com

VIP dilates cerebral vessels and is protective against ischemic brain damage, but the mechanisms of these actions have not been investigated in the newborn. VIP and pituitary adenylate cyclase activating polypeptide (PACAP) are structurally related neurotransmitters potentially acting on common receptors. We tested if 1) VIP-induced cerebral vasodilation is mediated by a) cyclooxygenase (COX), b) nitric oxide synthase (NOS) or c) PACAP-receptors; 2) VIP preserves cerebrovascular responsiveness after I/R. Pial arteriolar diameter was measured by intravital microscopy in newborn piglets equipped with closed cranial windows. VIP (10^{-9}-10^{-6}M) caused a significant, dose-dependent and repeatable pial arteriolar dilation that was blocked by the non-selective COX-inhibitor, indomethacin, only at 10^{-9}-10^{-8}M VIP, was unaffected by the NOS inhibitor, L-NAME, and reduced by the PACAP antagonists, PACAP 6-27 and 6-38. VIP (10^{-9}M, 20 min before I/R) preserved the endothelium-dependent response to hypercapnia, but not the neuronal-dependent reactivity to NMDA. We conclude that 1) vasodilator effect of VIP partially involves COX and common receptors with PACAP and 2) VIP protects cerebrovascular endothelial function against I/R. Supported by ETT 194042006 and DNT 25-11-OR126.