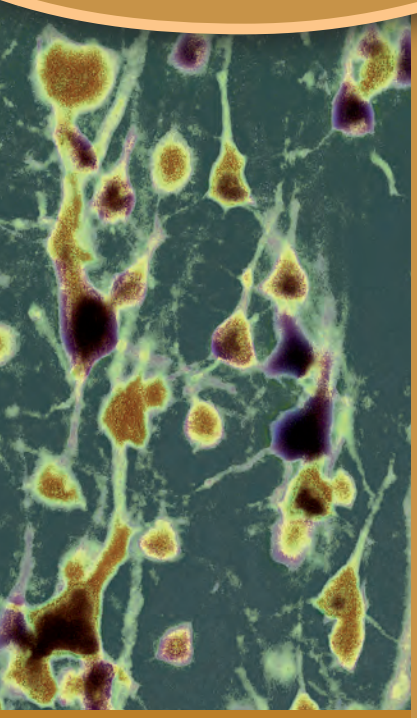
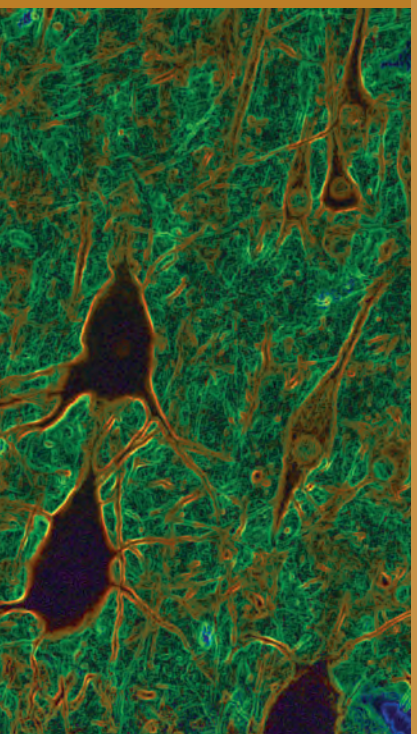
Tamás F. Freund

Laboratory of Cerebral Cortex Research

Department of Cellular and Network Neurobiology

Head of Laboratory: Tamás F. Freund, PhD

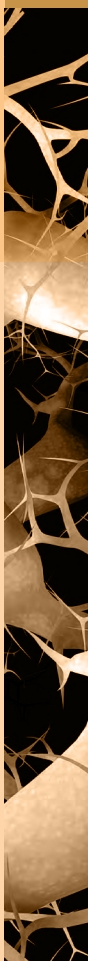




Mission statement

The cerebral cortex consists of billions of cells, which create millions of functional units called neuronal assemblies that operate in a highly sophisticated and organised manner. The concerted action of these cell assemblies or microcircuits form the basis of those neuronal operations that result in the highest level brain functions, including mental operations such as conscious perception, memory, or the generation of thoughts. Studies of Tamas Freund's laboratory over the past 25 years in this Institute represent conceptually novel steps towards uncovering: 1) new molecular pathways in the communication of nerve cells, 2) the identity and principles of connectivity of the nerve cells that build up the circuitry, and 3) the generation of network activity patterns by these circuitries that underlie various stages of information processing and storage in the brain. These findings shed new light not only on the normal operations of the cerebral cortex, but also on several of its disorders at the molecular, cellular or network levels, including epilepsy, schizophrenia, anxiety and ischemic cell death.

In recent years the laboratory has been focusing on the generation of behaviour-dependent population discharge patterns, with particular attention to the theta and gamma oscillations, and hippocampal sharp waves. In addition, we also focus on describing new signaling mechanisms at cortical synapses. Anatomical, *in vitro* and *in vivo* electrophysiological, optogenetic, pharmacological, molecular and modeling techniques are combined to elu-



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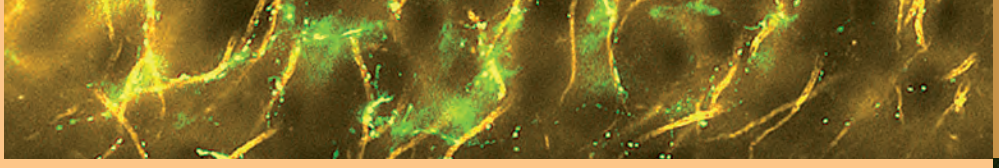
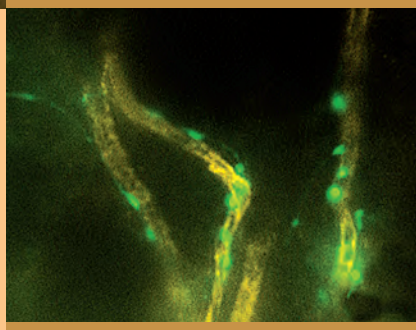


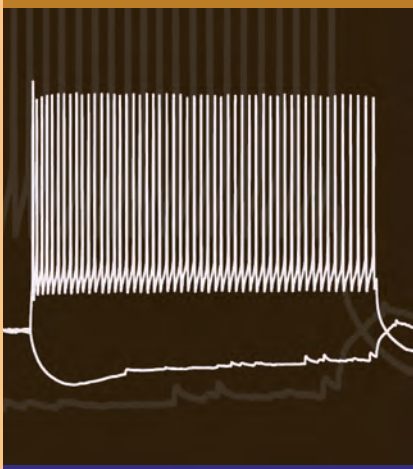
Chandelier cell terminals targeting axon initial segments.

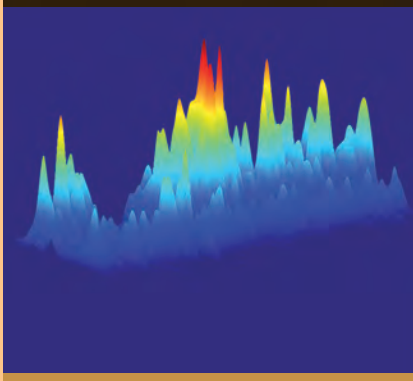


cidate the functional roles of inhibitory cell types in the control of population synchrony and synaptic plasticity in the hippocampus, their local as well as subcortical modulation via selective afferent pathways - including GABAergic and cholinergic septal, as well as serotonergic raphe input - and their pre- or postsynaptic receptors.

Inhibitory circuits and oscillations in the hippocampus

Combined electrophysiological and connectivity studies complemented by pharmacological and immunohistochemical techniques led to significant discoveries regarding the structure and function of cortical microcircuits, with particular attention to their GABAergic inhibitory components, and their relationship to cortical slow and fast oscillations that underlie different stages of memory formation.
A synthesis of data about perisomatic inhibitory neurons led to the hypothesis that CCK-containing interneurons, expressing presynaptic CB1 receptors, play a key role in anxiety-like behaviours. Genetic or pharmacological interruption of CB1 receptor-mediated actions leads to anxiety, whereas blocking the new cannabinoid-sensitive receptors on glutamatergic terminals has an anxiolytic effect. The particular expertise and history of this laboratory in the morpho-functional analysis of hippocampal circuits represent sufficient grounds for a continued effort - involving new molecular and behavioural approaches - to unravel the cellular and molecular bases of network operations involved in oscillations or different functional brain states, as well as in pathological activity including epilepsy and anxiety.
They demonstrated that fast-spiking basket cells are key players in the generation of gamma oscillations and sharp-wave ripples *in vitro*. In the same preparation, they identified the mechanism by which opiates interfere with oscillations through actions on the same basket cell type. Epileptic events evolve, because under pathological conditions the inhibitory transmission mediated by fast-spiking basket cells breaks down at several points and uncontrolled activity builds up in the network.

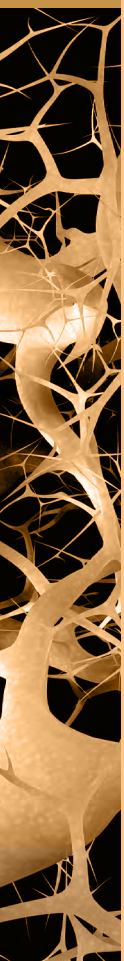


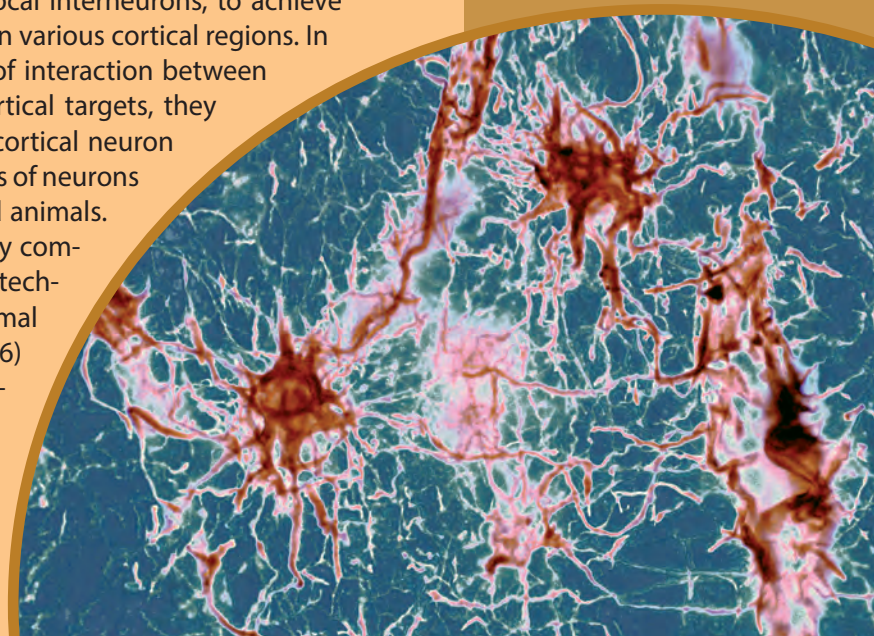


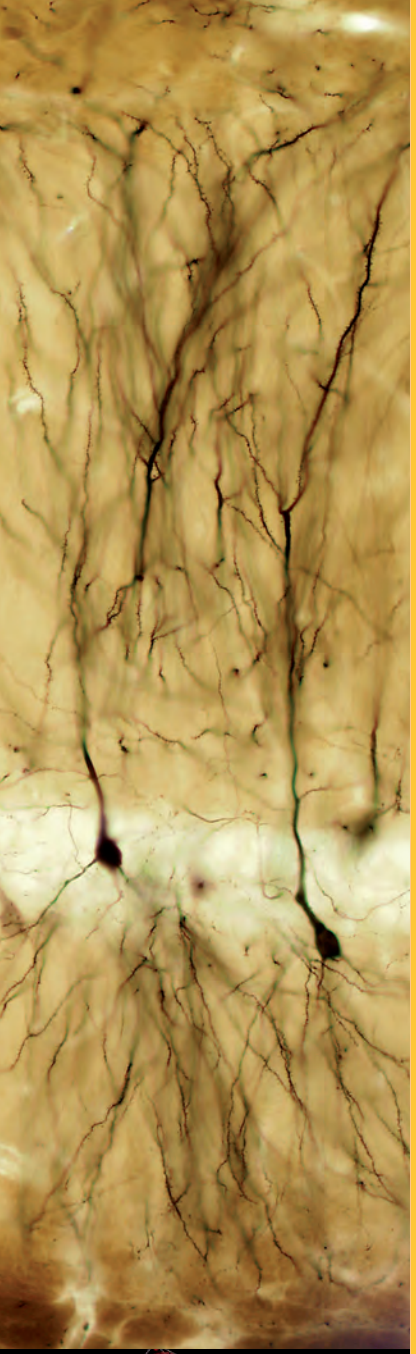
Glial cells in epileptic tissue

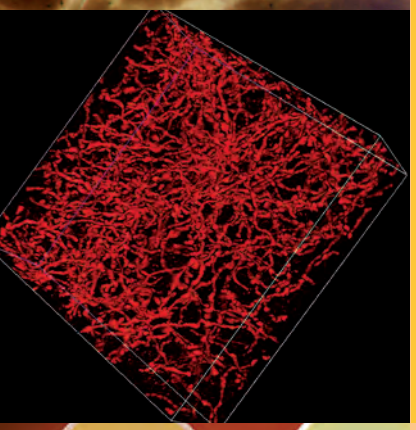
Subcortical control of hippocampal microcircuits

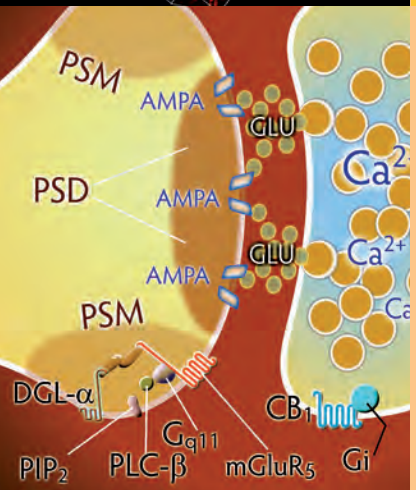
Since the early 1990's the Freund group made major discoveries related to the selective innervation of hippocampal interneurons by GABAergic pacemaker neurons in the medial septum, and the role of this connection in the formation of hippocampal theta oscillations. They showed that other subcortical pathways such as the serotonergic raphe-hippocampal projection use the same strategy, the innervation of local interneurons, to achieve control over population discharge patterns in various cortical regions. In order to decipher the intricate complexity of interaction between subcortical regulatory centers and their cortical targets, they combine the selective manipulation of subcortical neuron groups with the registration of large numbers of neurons in freely behaving as well as in anesthetized animals. This complex approach was implemented by combining the recently developed optogenetic techniques with multi-camera monitoring of animal behavior and high channel-count (up to 256) recording of neuronal activity. High resolution anatomical techniques are deployed to unravel the structural background of subcortical modulation.











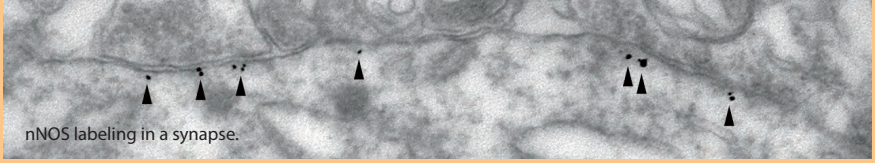
The group investigates the GABAergic feedback from the hippocampus to the medial septal pacemaker circuitry by selectively manipulating the somatostatin-expressing hippocampo-septal (HS) fibers with the optogenetic approach, and record the response of medial septal neurons both in urethane-anesthetized and in freely behaving mice. They revealed that activation of the HS feedback robustly and differentially alters the firing of MS neurons during spontaneous versus evoked theta or non-theta in urethane, or in freely behaving mice. These results shed new light on the role of the reciprocal inhibitory circuitry in generating theta oscillations.
The group's investigations of the raphe-hippocampal serotonergic projection led to an important discovery published in Science. This pathway exerts a powerful emotional/motivational state-dependent control of cortical activity patterns, yet the mechanism was unknown. In collaboration with researchers from HHMI Janelia Farm Research Campus, they presented direct evidence of a strong, spatiotemporally precise excitatory input from serotonergic median raphe neurons - that also use glutamate as a transmitter - to hippocampal interneurons. At the network level, this subcortical drive was manifested as a pattern of effective disynaptic GABAergic inhibition that spread throughout the circuit. These results fundamentally alter our view about the neurobiological bases of depression and related disorders connected to the serotonergic modulation of cortical function.

Epilepsy studies

The Freund group has studied circuit reorganization and neuronal vulnerability in various animal models of epilepsy, as well as in the temporal lobes of human epileptic patients that have undergone surgical removal of their epileptic foci. These data provided direct evidence that inhibition in the perisomatic region of pyramidal and granule cells - which is responsible for the control of synchronous firing - remains normal, or even enhanced. On the other hand, dendritic inhibitory neurons, which control the efficacy and plasticity of incoming excitation of non-principal cells, are already damaged at an early stage of epileptogenesis. Furthermore, the synchrony in their activity is also impaired due to the loss of interneuron-selective cells. These together may result in an impaired dendritic inhibition and an enhanced plasticity of excitatory inputs. This suggests that the early loss of these interneurons lead to conditions that allow interictal spiking to generate hyperexcitable circuits during the latent phase of epileptogenesis. These are the events that ultimately lead to the chronic phase, which is characterized by spontaneous seizures.

Schizophrenia research in human patients and animal models

The nature of neural alterations associated with schizophrenia is still to be clarified. A partnership began with the St. Borbála Hospital in 2011 with the aim to investigate the post-mortem brains of schizophrenic patients. Brains perfused with a post-mortem delay of 4 hours or less are used for immunocytochemical studies at the light an electron microscopic levels. Areas of particular interest are cortical regions (prefrontal, temporal, primary motor, visual, cingulate and insular cortices), as well as the hippocampus and parahippocampal gyrus. Current results suggest that the samples are suitable even for quantitative immunohistochemical analysis. Evidence has been found for a marked difference between schizophrenic and control subjects in the cytoarchitecture of the primary motor cortex. The anatomical data will be correlated with results from high resolution (256 channel) EEG re-

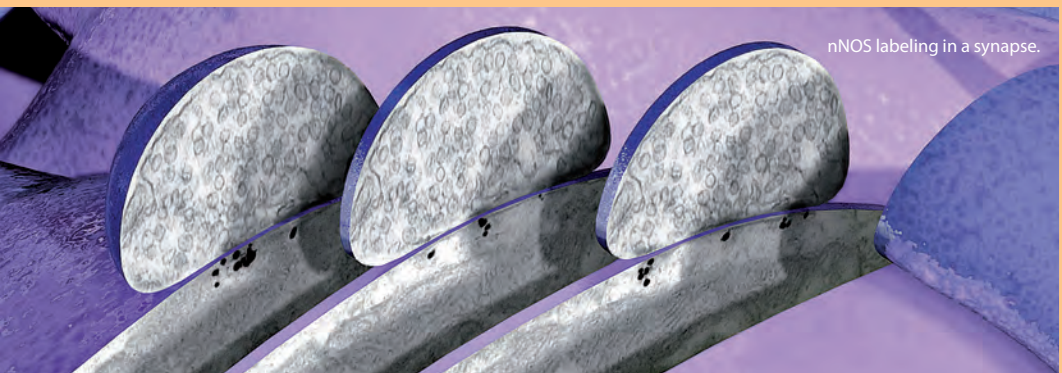


nNOS labeling in a synapse.

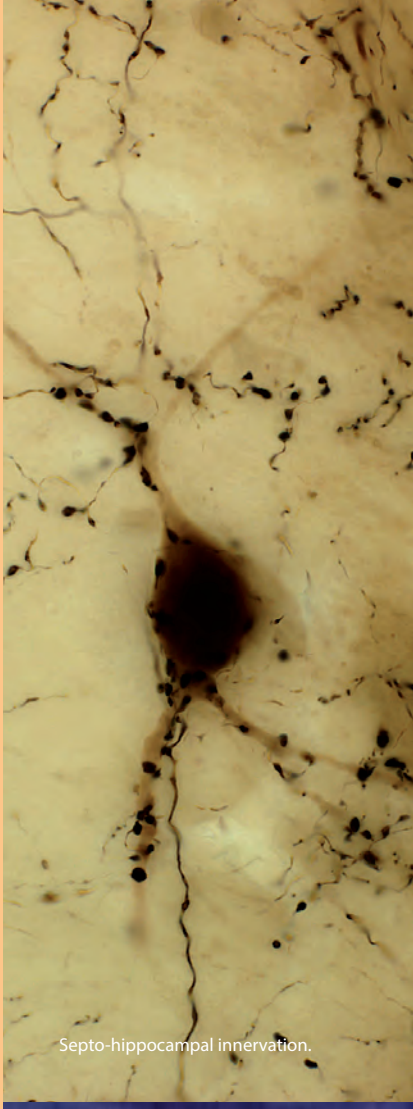
cordings that will take place at the Psychiatry Clinic of Semmelweis University Medical School. In addition, schizophrenia is investigated also in model animals. Polymorphisms at G72 genetic locus are considered to show one of the most robust associations with the disease. Transgenic mice that express the human gene G72 show behavioral changes that are expected in an animal model of schizophrenia. Physiological data suggest changes in the plasticity of synapses of the dentate gyrus in these animals, therefore, they investigate the morphological changes behind the alterations seen in physiological measurements. Using both pre- and post-embedding methods, excitatory synapses are examined in the electron microscope, and stereological measurements using both electron and light microscopy are also carried out. These studies could reveal some of the molecular changes responsible for schizophrenic phenotype caused by G72 gene and possible treatment options will also be investigated to reverse the adverse changes.

Discoveries of new signaling mechanisms at cortical synapses

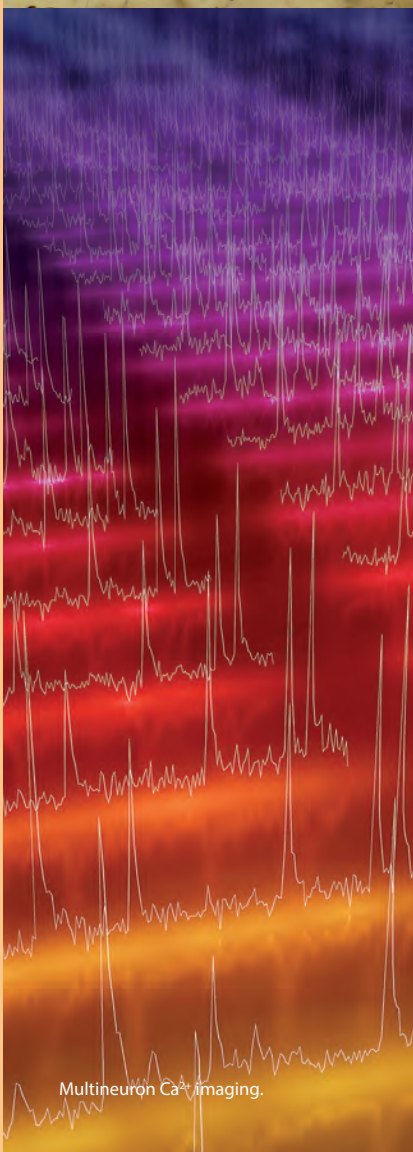
The Freund group described new signaling mechanisms at GABAergic synapses including nitric oxide and glutamate receptors. They challenged the classical views of retrograde endocannabinoid signaling by the demonstration of the dependence of this process on an intact nitric oxide pathway, and the selective localization of NO synthase within the synaptic active zone of GABAergic synapses. Using multiple immunocytochemical labeling, as well as freeze fracture replica immunolabeling, they demonstrated that NMDA receptors are also present in hippocampal GABAergic synapses, and nNOS coexist in GABAergic basket cell synapses, that NMDA receptors could be a source of calcium entry at these synapses, and that this calcium influx can lead to postsynaptic NO production and subsequent cGMP synthesis presynaptically. Activation of NMDA receptors was shown to induce NO-dependent cGMP production in these basket cell terminals, an effect absent in nNOS knock-out mice, and pharmacologically inhibited by postsynaptic NMDA receptors or nNOS blockers, as well as by a presynaptic NO receptor blocker. This novel control machinery of GABAergic transmission may be implicated in memory functions as well as anxiety-like behavior. In addition, they demonstrated that the nitric oxide signaling pathway is also important in the early development of the hippocampus. They described new sites of neuroligin 2 expression in cortical synapses. Neuroligin 2 is a postsynaptic protein that plays a critical role in the maturation and proper function of GABAergic synapses. They found that besides GABAergic synapses, neuroligin 2 is also present in the postsynaptic membrane of cholinergic synapses everywhere in the brain. Several cholinergic contact sites were identified strongly labeled with neuroligin 2 that did not resemble typical synapses, suggesting that cholinergic axons form more synaptic connections than it was recognized previously. The data indicate that mutations in human neuroligin 2 gene and genetic manipulations of neuroligin 2 levels will potentially cause severe alterations in cholinergic transmission as well. They showed an unexpected



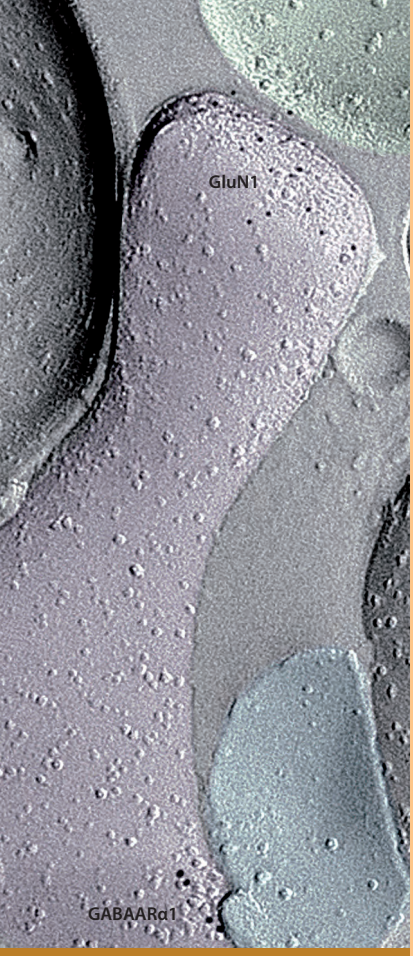
nNOS labeling in a synapse.



Septo-hippocampal innervation.



Multineuron Ca^{2+} imaging.



Freeze facture replica labeling.

Standing, from left: Viktor Varga, Zsófia Maglóczky, Andor Domonkos, András Szőnyi, Zsolt Kohus, Márton Mayer, Panna Hegedűs, Dániel Gémes, Katalin Iványi, Péter Szocsics, Emőke Szép Simon, Katalin Lengyel, Győző Goda, Dávid Burka, Dániel Schlingloff, Csaba Cserép, Eszter Katalin Sós, Balázs Pósfai, Nándor Kriczky, tamás Laszlovszky, NikitidouLitsa
Sitting: Tamás Freund, Szabolcs Káli, Attila Gulyás, Gábor Nyíri, Georgina Vig, Sára Sáray, Dóra Csordás
Crouching: Ágoston Nagy, Vivien Heiner, Péter Papp, Estilla Tóth

abundance of glutamate receptors (both AMPA- and NMDA-type) in the serotonergic ascending system. They also found that the majority of the median raphe cells that project to forebrain areas are glutamatergic and that there is a significant population of cells in the median raphe region that project to at least two forebrain areas simultaneously. The activation of key GABAergic cells in the forebrain via excitatory inputs form the median raphe would be highly effective in the modulation of their synchronous activity patterns and cooperation, while such a strong glutamatergic component in a classically serotonergic pathway was unexpected in the brain. These results may pave the way to develop new strategies for pharmacological intervention for depression and related disorders.

Implications for clinical or pharmaceutical research in anxiety

Although primarily basic research in nature, the studies of the Freund group are continuously supplying data that are of clinical relevance, and feed into applied research in the pharmaceutical industry. Using comparative expression profiling of the molecular components of the endocannabinoid system, they demonstrated that this signaling pathway is robustly down-regulated in hippocampal glutamatergic synapses of temporal lobe epilepsy patients. Thus, malfunctioning of the circuit breaker may partly explain excessive glutamate release and runaway excitation during seizures. On the other hand, CB1 receptors located on the GABAergic axon terminals of a select subset of interneurons was shown to be relevant for anxiety-like behaviour. The Freund group, in collaboration with the behavioural neuroscience laboratory of Jozsef Haller in the institute, provided evidence that impaired CB1 receptor function plays a central role in anxiogenesis. The Freund laboratory described differences between major basket cell types, one operating as a clockwork for

oscillations (the parvalbumin-containing cells), and the other as a fine tuning device (the CCK-containing neurons). CCK-containing cells were found to express several receptors and to receive afferent inputs that are all involved in anxiogenesis, which led to the conclusion that this cell type itself may represent a novel target for pharmacotherapy.

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- Cserép C., Szabadits E., Szőnyi A., Watanabe M., Freund T. F., Nyiri G. NMDA receptors in GABAergic synapses during postnatal development. PLoS One. 7(5):e37753, (2012)
- Takács V. T., Klausberger T,. Somogyi P,. Freund T. F., Gulyás A. I. Extrinsic and local glutamatergic inputs of the rat hippocampal CA1 area differentially innervate pyramidal cells and interneurons. HIPPOCAMPUS 22(6):1379-91. (2012)
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- Gulyás A. I., Szabó G. G., Ulbert I., Holderith N., Monyer H., Erdélyi F., Szabó G., Freund T. F., Hájos N. Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. J NEUROSCI. 30(45):15134-45. (2010).
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- Makara J. K., Katona I., Nyiri G., Nemeth B., Ledent C., Watanabe M., de Vente J., Freund T. F., Hajos N. Involvement of nitric oxide in depolarization-induced suppression of inhibition in hippocampal pyramidal cells during activation of cholinergic receptors. J NEUROSCI 27:10211-10222 (2007)
- Freund T. F., Katona I. Perisomatic inhibition. NEURON 56:33-42 (2007)
- Makara J. K., Mor M., Fegley D., Szabó S. I., Kathuria S., Astarita G., Duranti A., Tontini A., Tarzia G., Rivara S., Freund T. F., Piomelli D. Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. NAT NEUROSCI 8: 1139-1141 (2005)
- Maglóczky Zs., Freund T. F. Impaired and repaired inhibitory circuits in the epileptic human hippocampus. TRENDS NEUROSCI 28: 334-340 (2005)