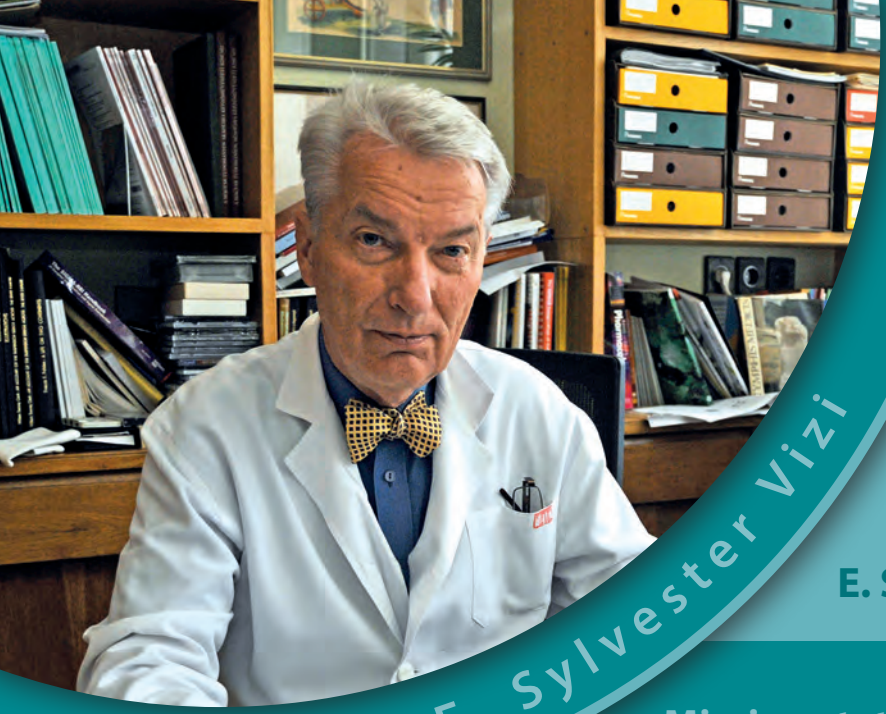


Laboratory of Drug Research

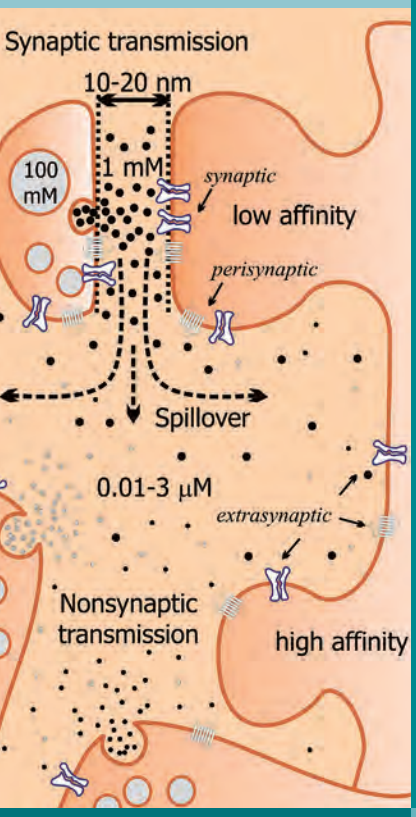
Department of Pharmacology

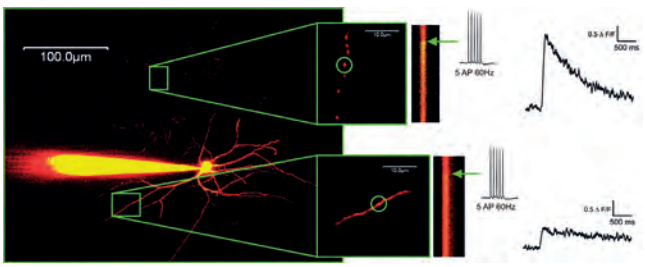
Head of Laboratory:
E. Sylvester Vizi MD, PhD

Mission statement

Chemical transmission at the synapse is the primary form of conveying a message from one neuron to another. In addition to synaptic transmission, nonsynaptic interactions exist between neurons, as theorised and demonstrated by Vizi (cf. "Non-synaptic Interactions Between Neurons: Modulation of Neurochemical Transmission: Pharmacological and Clinical Aspects." John Wiley and Sons, Chichester, New York, 1984). Since 1986, nonsynaptic information processing has been extended and also termed "volume transmission". Compelling neurochemical, functional and morphological evidence has revealed the significance of nonsynaptic interactions between neurons; transmitters are released into the extracellular space (12%–18% of the brain's volume), and they diffuse over large distances to reach remote receptors and tonically influence the activity of other neurons by stimulating extrasynaptic metabotropic and ionotropic receptors. These receptors are primarily high-affinity and are targets for low-dose drugs in several instances of medical therapy (Vizi et al., Br. J. Pharmacol. 160:785-809, 2010). The majority of transporters are high-affinity and are located extrasynaptically. Nonsynaptic transmission operates at a slower time-scale than synaptic transmission and is responsible for tonic changes in brain activity. We have further provided evidence that nonsynaptic communication is additionally a fundamental element of neuro-endocrine (Vizi et al., J. Endocrinol 135:551-556, 1992; Vizi et al., J. Endocrinol. 139:213-226, 1993) and neuro-immune (Vizi et al., Neuroscience 68:1263-1276, 1995; Haskó et al., Trends Immunol. 30:263-270, 2009) interactions. Our review (Elenkov et al., Pharmacol. Rev. 52:595-638, 2000) of this discovery is on the journal's list of the top 10 most highly cited papers.

There is a perpetual need in physiology to record spatially distributed and rapid cellular processes, especially in the swiftly developing field of neuroscience. Since 1998, my laboratory has made an effort to intro-

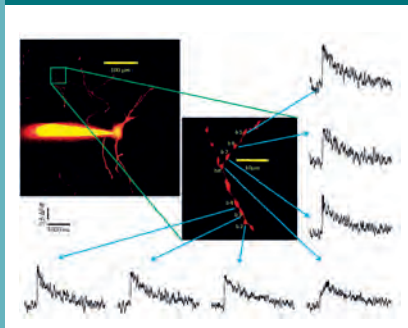


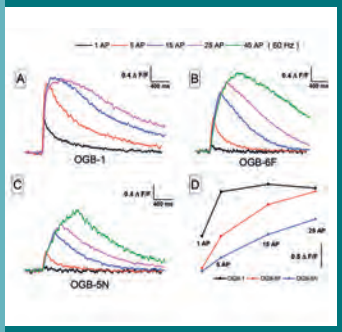


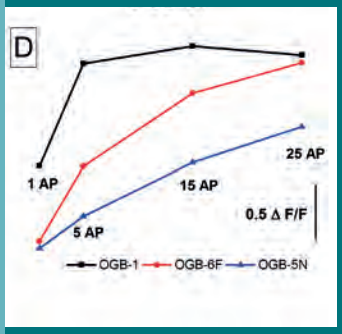
Senior scientists: Tibor Zelles MD, PhD, Gabriella Zsilla PhD
Ph.D. students: Máté Kisfali, Tibor Lőrincz , Viktória Humli , Gábor Polony MD, Zoltán Borbély
Graduate research assistant: Judit Szepesy
Undergraduate research assistants: Lakatos Marcell, student of Semmelweis University
Technicians: Anita Bagó, Judit Őszi, Katalin Windisch
Secretary: Judit Csek

duce the technology of two-photon laser scanning microscopy to the study of subcellular events at a high resolution. Dr. Lendvai learnt this method in Dr. Svoboda's laboratory at Cold Spring Harbour (Lendvai et al., Nature 404:876-881, 2000) and introduced it to our laboratory. After publishing several papers on Ca^{2+} signalling in dendrites (Rózsa et al., Eur. J. Neurosci. 27:364-377, 2008; Katona et al., Proc. Natl. Acad. Sci. USA 108:2148-2153, 2011), we compared Ca^{2+} transients evoked by somatic backpropagation and orthodromic stimulation in the dendrites and boutons of the same GABAergic interneuron (Kisfali et al., J Physiol. 591:5541-53, 2013). To date, no attempt has been made to monitor the Ca^{2+} dynamics in detail and estimate the $[Ca^{2+}]_i$ in individual anatomically identified hippocampal GABAergic boutons. GABAergic interneurons generate oscillatory activity, synchronise the activity of pyramidal cells and set time windows for synaptic integration; therefore, it is important to study Ca^{2+} transients and develop the ability to estimate $[Ca^{2+}]_i$. Several aspects of presynaptic Ca^{2+} signalling have been studied exclusively with respect to glutamatergic terminals; therefore, an important outcome of our work is to provide, for the first time, a detailed analysis of presynaptic Ca^{2+} dynamics in an important inhibitory system- the GABAergic interneurons that originate from the stratum radiatum in the CA1 area of the hippocampus. GABAergic interneurons play a strategic role in controlling the output of the prefrontal cortex and the hippocampus. Moreover, the hypofunction of NR1 NMDA receptors (located on these interneurons) is involved in the development of schizophrenic symptoms. This warrants closer examination of GABAergic transmission and its modulation. We use two photon laser scanning microscopy to study presynaptic events modulated by different factors that are believed to affect the presynaptic release machinery.

In addition we have a joint project (Hearing and Deafness: Molecular mechanisms and therapeutic approaches) with Prof. Christine Petit, Institut Pasteur, Paris and Semmelweis University, Dept. of Oto-Rhino-Laryngology Head and Neck Surgery. We study the neuronal physiology and pathophysiology of hearing at the cellular and subcellular level.



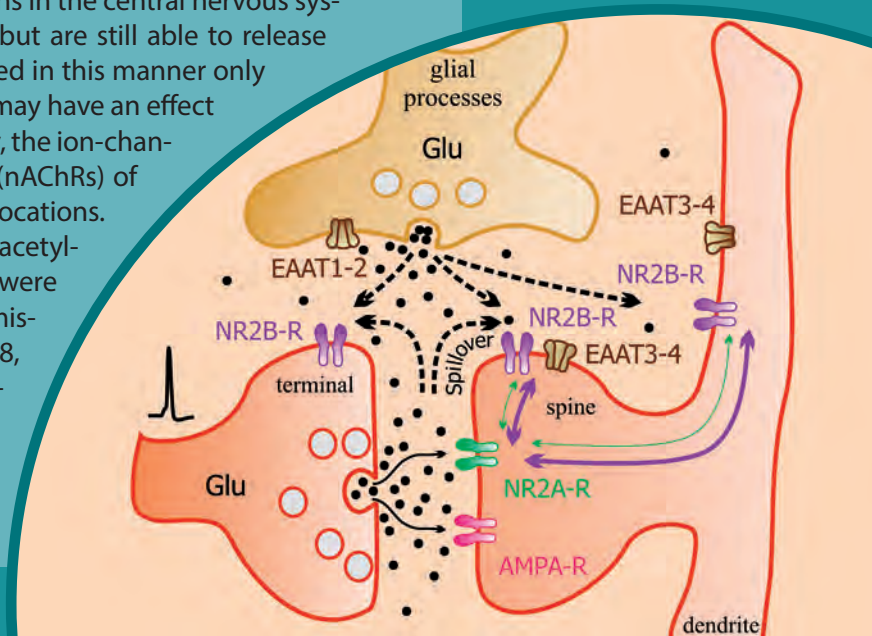


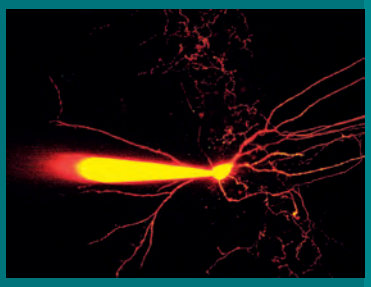


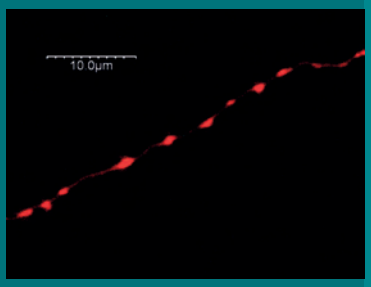
Nonsynaptic receptors and transporters as targets for drug treatment

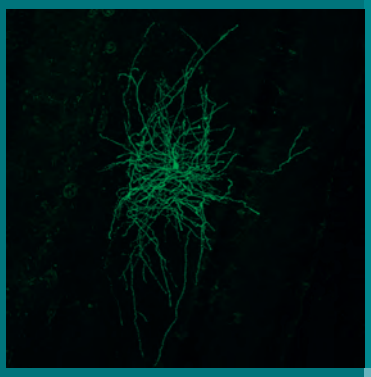
Presynaptic, preterminal axonal receptors are responsible for the local modulation of depolarisation-release coupling, including the propagation of action potentials (APs) and the modulation of release probability. Recently, strong evidence was obtained demonstrating that a high proportion (86%–93%) of cholinergic boutons in the central nervous system (CNS) do not make synaptic contacts, but are still able to release ACh into the extracellular space. ACh released in this manner only reaches low concentrations (0.1–2 μM) and may have an effect on high-affinity receptors alone. Surprisingly, the ion-channel gated nicotinic acetylcholine receptors (nAChRs) of the CNS are primarily found at nonsynaptic locations. Nonsynaptic NR2B glutamate and nicotinic acetylcholine (ACh) ionotropic receptors (nAChRs) were also found to be involved in signal transmission (Vizi et al., Eur. J. Pharmacol. 500:499-508, 2004; Rózsa et al., Eur. J. Neurosci. 27:364-377, 2008; Lendvai and Vizi, Physiol. Rev. 88:333-349, 2008).

Although a significant amount of evidence









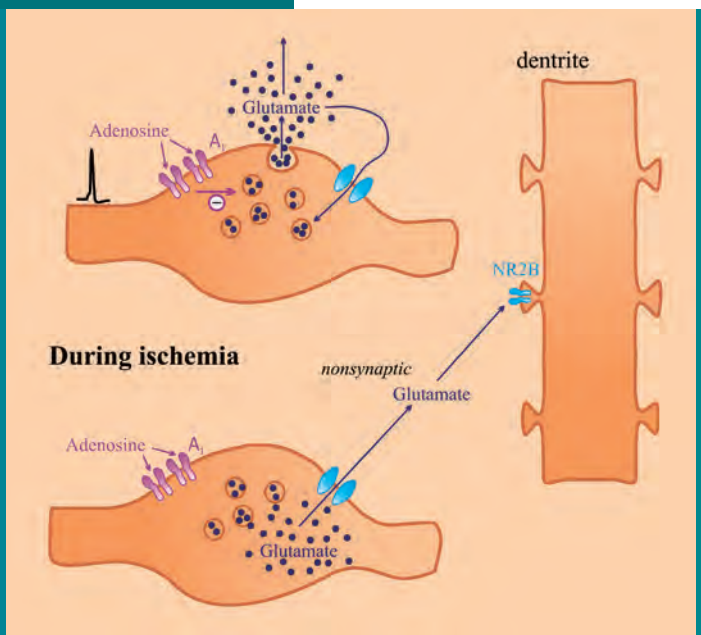
indicates that nAChRs expressed in the hippocampus are involved in synaptic plasticity and cognitive function, little is known about how this regulation occurs, particularly in brain regions known to be important for cognition. Stratum radiatum interneurons, unlike pyramidal cells, are rich in nAChRs. Using two-photon laser scanning microscopy, we determined that the activation of these extrasynaptic α7-nAChRs by cholinergic agonists either facilitated or depressed back-propagating action potentials, depending on the timing of the α7-nAChR activation (Rózsa et al., Eur. J. Neurosci. 27:364-377, 2008). Our results suggest a new mechanism for the cholinergic switch in memory encoding and retrieval. Furthermore, we obtained evidence (Szabo et al., Neuropharmacol. 81:42-54, 2014; Pesti et al., Neuropharmacol. 81:101-115, 2014) that positive allosteric modulation of nAChRs alters the affinity for agonists involved in cognitive processing.

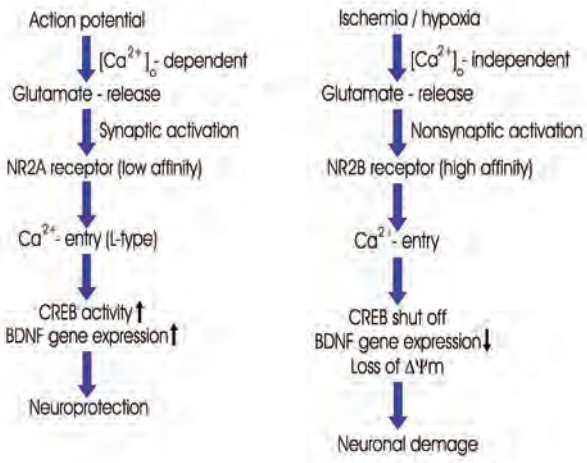
The NR2B glutamate receptor subtype is another nonsynaptic ionotropic receptor. Our laboratory intends to study the release of transmitters (e.g., glutamate) under ischaemic conditions. Extrasynaptic glutamate may activate nonsynaptic NR2B receptors, producing excitotoxicity. Using whole-cell patch-clamp recording, we have shown that fluoxetine, the most selective serotonin reuptake inhibitor, is an NR2B antagonist (Szasz et al., Biol. Psychiatry 62:1303-1309, 2007). This effect indicates that this compound may have a neuroprotective function (Vizi et. al. Brain Res. Bull. 93: 355-367, 2013).

In collaboration with Dr. Freund's and Dr. Sperlágh's groups, we demonstrated that CB1 cannabinoid receptors, the major target of cannabis, are localised presynaptically and their localisation is nonsynaptic. We provided, for the first time, evidence that the activation of CB1 cannabinoid receptors on GABAergic terminals in the nucleus accumbens results in an inhibition of GABA release and a subsequent increase in dopamine release (disinhibition) from the ventral tegmental projection (Sperlágh et al., Neurochem. Int. 54:452-457, 2009), which is controlled tonically via nonsynaptic $GABA_A$ receptors.

Effect of ischaemia on transmitter release

For several years, we have studied the effects of glucose and oxygen deprivation on transmitter release. We have shown that oxidative stress





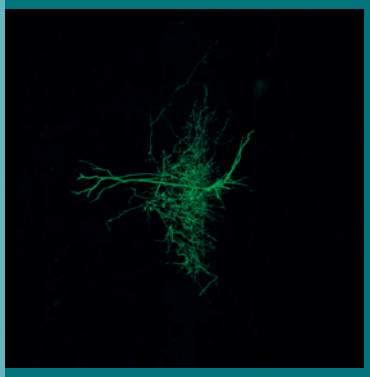
and ischaemic conditions result in $[Ca^{2+}]_o$-independent release of dopamine (Milusheva et al., Neuropharmacol. 58:816-825, 2010).

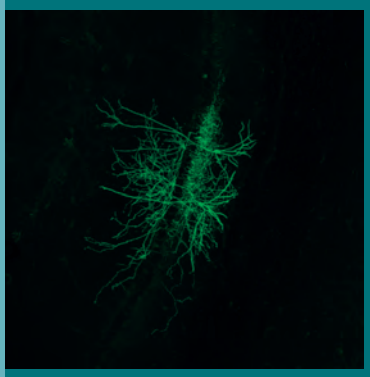
Mode of action of drugs of abuse

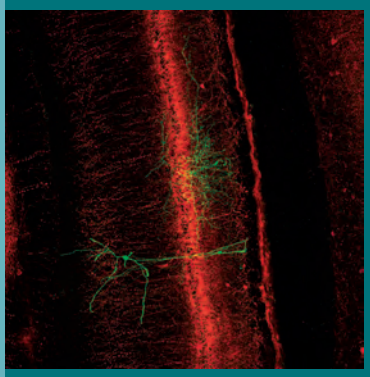
Recent investigations by the group have determined the mode of action of drugs of abuse. We provided neurochemical and pharmacological evidence that mephedrone, which is one of the most popular street drugs and functions in a similar manner to ecstasy (3,4-methylene-dioxymethamphetamine), triggers the release of ^{3}H-dopamine (^{3}H-DA) at rest from acute slice preparations of the nucleus accumbens (Nac). In contrast, methylphenidate (MPH) does not affect the release of [^{3}H]DA at rest but increases the release in response to axonal activity in NAc. Mephedrone, ecstasy and MPH biphasically inhibited [^{3}H]-DA uptake in cortical and striatal P_2 synaptosomal preparations, over a wide concentration range, by binding to high- and low-affinity sites. Lowering the temperature or administering a selective DA transporter (DAT) antagonist (GBR 12909) prevented carrier-mediated release induced by DAT substrates (DA, mephedrone, and ecstasy).

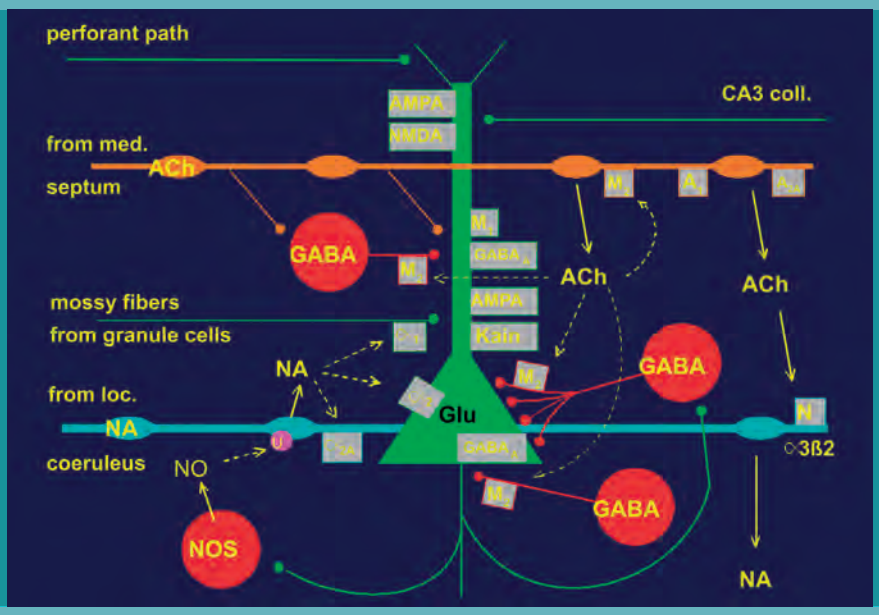
Study of Ca^{2+}-dynamics in hippocampal GABAergic varicosities. The role of presynaptic modulation and mitochondrial Ca^{2+} buffer systems

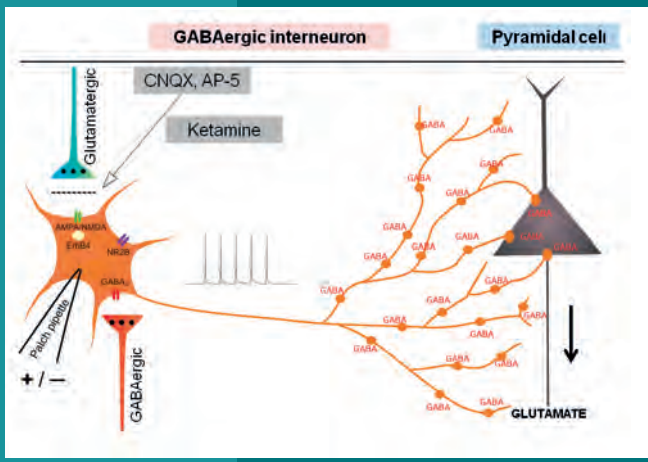
In the hippocampus, relatively uniform excitatory pyramidal cells are innervated by more than 21 different types of GABAergic interneuron. In contrast to electrophysiological studies, where the responses of the target principal cell to GABA released from the interneurons are recorded, we are able to measure somatic stimulation-evoked Ca^{2+} transients, the prerequisite of GABA release at a single bouton. Two-photon laser scanning microscopy and the unique anatomy of GABAergic interneurons in rat hippocampal slices provide an ideal combination to study the presynaptic and extrasynaptic modulation of GABAergic inhibitory transmission.
Using femtosecond two-photon laser microscopy, we recorded Ca^{2+} transients evoked by somatic current stimulation in the varicosities of various hippocampal GABAergic interneurons of the CA1 region, on a millisecond time scale with high resolution, in acute slice preparations that maintain anatomical and functional integrity. Using a patch pipette to deliver the high- (OGB-1) or low-affinity (OGB-6F) Ca^{2+} indicator dye depending on the stimulation parameters, we electrically stimulated CB1-positive, non-fast-

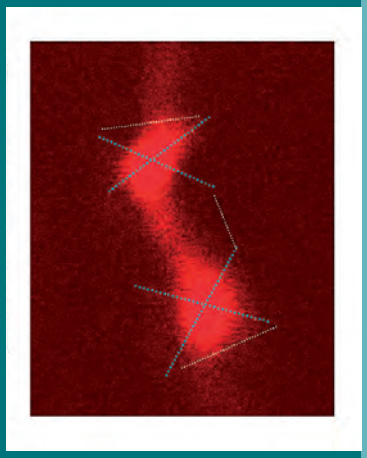


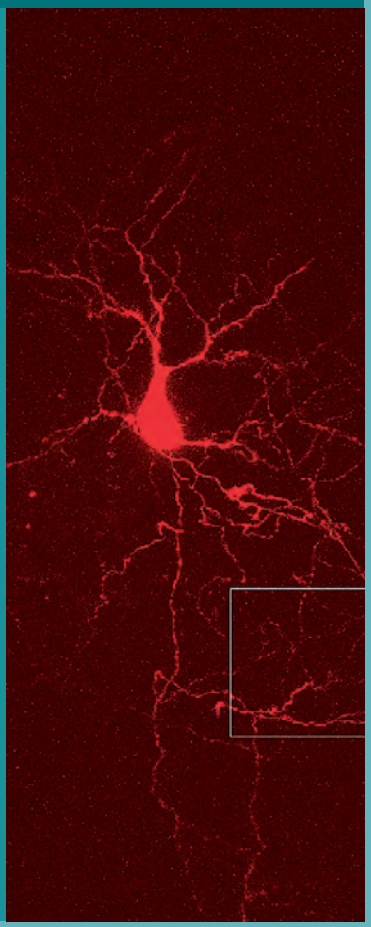












spiking (<50 Hz) and parvalbumin-positive fast spiking (>50 Hz) GABAergic interneurons present in the stratum radiatum and stratum oriens/pyramidale, respectively. No failures in Ca^{2+} transients were observed when the cell body was repeatedly stimulated, indicating reliable AP propagation from the soma into the axonal arbour. In response to an AP, all varicosities were recruited, and the response did not depend on the frequency of the firing rate.
In conclusion, our findings reveal remarkable interneuron-type-specific characteristics of axon terminals in the highly diverse hippocampal GABAergic interneuron population underlying their different functional roles.

Development of a 3-D, real-time multi-photon laser scanning microscope

Despite several attempts, imaging techniques tested to date have failed to obtain the required spatial and temporal resolutions necessary to detect axonal and dendritic events (Rózsa et al. Appl. Opt. 46, 1860-1865, 2007; Globel et al., Nat. Methods 4, 73-79, 2007). To solve this issue, we developed (c. 2000-2010) a two-photon 3D laser-scanning microscope with a millimetre z-dimension scanning range and sub-millisecond temporal resolution (Katona et al., Nat Methods 9:201-208, 2012).
This novel 3D two-photon microscope system is suitable for real-time investigation of neuronal microcircuits, cortical firing patterns, and the activity of large neuronal populations (Katona et al., Nat Methods. 9:201-8, 2012) (Hungarian Patent: P0500143, US Patent: 06710202.0-2217; 11/814,917). Dr. Rózsa has independently continued his career and established his own laboratory.

Nonsynaptic signalling of the immune system

Immune cells are equipped with numerous types of receptors. We have shown that adenosine and purine nucleoside signalling molecules originating from ATP are present in the extracellular space and are able to differentially regulate IL-10, TNF-α and nitric oxide production (Haskó et al., J. Immunol. 157:4634-4640, 1996; Trends Immunol. 30:263-270, 2009; Csóka et al., J. Immunol. 185:542-550, 2010) via four G-protein-coupled receptors (Himer et al., FASEB Journal 2010). We have described the role of enzyme activities of CD39 and CD73 in shifting a proinflammatory environment to an anti-inflammatory milieu (Antonioli et al., Trends in Mol. Med. 19:355-367, 2013). After a long and fruitful collaboration with the New Jersey Medical School (Newark, NY) and Dr. G. Haskó, we have decided to close down this section of our laboratory.

Sensorineural hearing losses (SNHLs)

Excitotoxicity and imbalance of the redox system form the pathophysiological basis of all forms of SNHLs (e.g. presbycusis, noise- or drug-induced hearing losses). The laboratory investigates the function and modulation of an endogenous protective system (lateral olivocochlear efferents, LOC), the mechanism of cellular damage and production of reactive oxygen species in the cochlea and test different compounds with multitarget action (LOC activator, neuroprotective and antioxidant) in hearing loss models in vivo. (Polony et al. Neuroscience, 265, 263-273, 2014).

Selected publications from the last 10 years:

- Vizi E. S., Zsilla G., Caron M. G., Kiss J. P. Uptake and release of norepinephrine by serotonergic terminals in norepinephrine transporter knock-out mice: implications for the action of selective serotonin reuptake inhibitors. J Neurosci 24: 7888-7894 (2004).
- Rózsa B., Zelles T., Vizi E. S., Lendvai B. Distance-dependent scaling of calcium transients evoked by backpropagating spikes and synaptic activity in dendrites of hippocampal interneurons. J Neurosci. 24: 661-670 (2004).
- Lőrincz A., Rózsa B., Katona G., Vizi E. S., Tamás G. Differential distribution of NCX1 contributes to spine-dendrite compartmentalization in CA1 pyramidal cells, Proc. Natl. Acad. Sci. 104:1033-1038 (2007).
- Lendvai B., Vizi E. S. Nonsynaptic Chemical Transmission Through Nicotinic Acetylcholine Receptors. Physiol. Rev. 88: 333-349, (2008).
- Vizi E. S., Fekete A., Karoly R., Mike A., Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. Br. J. Pharmacol. 160:785-809 (2010).
- Csóka B., Németh Z. H., Rosenberger P., Eltzschig H. K., Spolarics Z., Pacher P., Selmeczy Z., Koscsó B., Himer L., Vizi E. S., Blackburn M. R., Deitch E. A., Haskó G. A(2B) adenosine receptors protect against sepsis-induced mortality by dampening excessive inflammation. J Immunol. 185:542-50 (2010).
- Katona G., Szalay G., Maák P., Kaszás A., Veress M., Hillier D., Chiovini B., Vizi E. S., Roska B., Rózsa B. Fast two-photon *in vivo* imaging with three-dimensional random-access scanning in large tissue volumes Nat Methods. 9:201-8 (2012).
- Vizi E. S., Kisfali M., Lőrincz T. Role of nonsynaptic GluN2B-containing NMDA receptors in excitotoxicity:evidence that fluoxetine selectively inhibits these receptors and may have neuroprotective effects. Brain Res. Bull. 93: 32-8 (2013).
- Antonioli L., Pacher P., Vizi E. S., Haskó G. CD39 and CD73 in immunity and inflammation. Trends in Molecular Medicine, 19: 355-367 (2013).
- Kisfali M., Lorincz T., Vizi E. S. Comparison of Ca^{2+} transients and $[Ca^{2+}]i$ in dendrites and boutons of non-fast-spiking GABAergic hippocampal interneurons using two-photon laser microscopy and high- and low-affinity dyes. J Physiol. 591: 5541-53 (2013).

from left: Katalin Horváth Windisch, Gabriella Zsilla, E. Sylvester Vizi, Máté Kisfali, Gáborné Bagó, Tibor Zelles, Judit Őszi, Tibor Lőrincz