Laboratory of Molecular Neurobiology

Department of Gene Technology and Developmental Neurobiology
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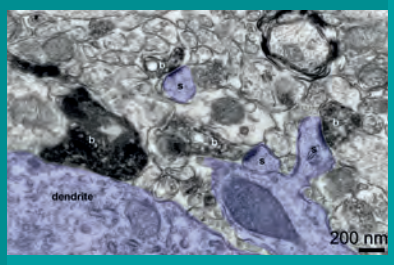
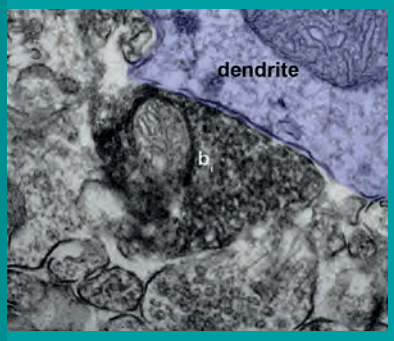
Mission statement

The central concept of communication between nerve cells in the brain involves the synaptic junction as the major site where messenger molecules convey information from presynaptic nerve cells to their postsynaptic partners. The efficacy of synaptic transmission is not uniform in time and space; instead, its plasticity is a fundamental process underlying information storage and adaptation to environmental stimuli. To accomplish their essential functions, synapses exploit a plethora of signaling molecules integrated into sophisticated pathways. A major objective of István Katona's laboratory is to identify novel signaling pathways regulating synaptic transmission and its plasticity.

Endogenous cannabinoid molecules are prime examples of principal modulators of synaptic activity. Our previous work extensively contributed to the discovery that these lipid mediators serve a key physiological role in the regulation of neurotransmitter release as retrograde messengers. Despite its pivotal importance, key aspects of endocannabinoid signaling have remained elusive or even controversial. Recent advances in lipidomic approaches revealed an unexpected number and diversity of endocannabinoid-like bioactive lipid molecules, whereas activity-based proteomic profiling uncovered a surprisingly high number of synthesizing and degrading enzymes, which can metabolize components of the brain endocannabinoid metabolome. It is conceivable that these various molecular players evolved to fulfill specific requirements of neuronal activity and may regulate several different aspects of synaptic transmission. Thus, re-





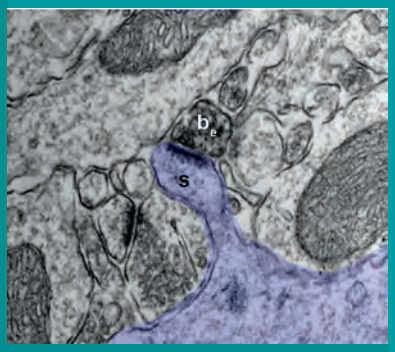


Figure 1.
These electron micrographs demonstrate the improved sensitivity for CB_1 receptors of a polyclonal antibody which was raised in FcRn overexpressing transgenic rabbits. Note the highly concentrated accumulation of the electron dense black DAB precipitate in both excitatory (b_e) and inhibitory (b_i) axon terminals. In contrast, postsynaptic dendrites and spines (s) shown in purple shades are devoid of CB_1 receptors.

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search activity in the Katona laboratory is focusing on the characterization of the molecular architecture of these novel lipid pathways and to identify their cell type- and synapse-specific functions in the brain.

Presynaptic CB_1 cannabinoid receptors

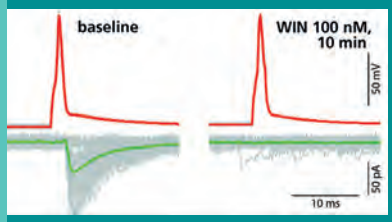
Our work during the last decade has revealed that presynaptic CB_1 receptors are ubiquitously distributed at most synapse types throughout the brain. These G-protein coupled receptors are among the most important presynaptic regulators of neurotransmitter release and mediate several forms of short-term and long-term synaptic plasticity. Importantly, the synaptic level and distribution of cannabinoid receptors is not uniform and constant across synapses. However, the biological principles underlying the synapse-specific functional heterogeneity and pathophysiological changes in the quantity and subcellular localization of CB_1 receptors remained largely enigmatic.

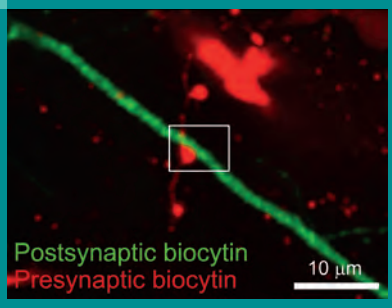
To facilitate quantitative studies on CB_1 receptors in brain circuits, we aim to improve the two most important tools of molecular neuroscience research, antibodies and microscopes. First, in collaboration with Prof. Imre Kacskovics (Immunogenes Kft), we employed the transgenic FcRn rabbit and mouse technology to produce new antibodies against the CB_1 receptor with superior sensitivity. Our efforts recently led to the generation of several polyclonal and monoclonal antibodies, which exhibit better sensitivity than any other previously described CB_1 antibody, and whose specificity could be validated in CB1 knockout mice. These antibodies will be ideal tools to study even subtle molecular changes associated with physiological or pathophysiological plasticity processes, and also in those axon terminals where only a low copy number of CB_1 is present, e.g. in glutamatergic excitatory terminals *(Figure 1)*.

The abundance and spatial localization of synaptic proteins are dynamically adjusted in a cell type- and synapse-specific manner. For example, we discovered in 2008 that CB_1 levels are selectively decreased at glutamatergic, but not at GABAergic, synapses in human epileptic samples. We also uncovered that a ~100 nm shift in the perisynaptic position of an endocannabinoid-synthesizing enzyme occurs selectively at glutamatergic synapses, but not at GABAergic synapses, in a model of Fragile X syndrome. These findings highlighted the need for a cell type- and synapse-specific approach to make molecular analysis at the nanoscale level easily feasible. Therefore, our lab recently developed a novel approach based on STORM super-resolution microscopy, which enables the correlation of physiological and anatomical data with the underlying molecular parameters at identified connections between individual target neurons in intact brain circuits. This new methodology is now extensively used in the lab to study physiological and pathophysiological changes in synaptic CB_1 receptor-mediated signaling *(Figure 2)*.

Endocannabinoid-metabolizing enzymes

Interestingly, at least two endocannabinoid molecules, anandamide and 2-arachidonoyl-glycerol (2-AG) can both serve as mediators of endocannabinoid signaling, although their spatial and functional division of labor





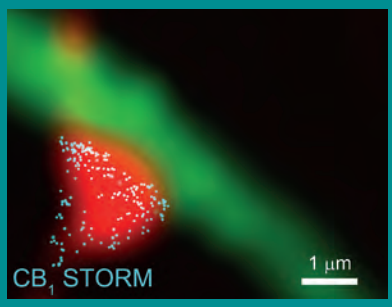


Figure 2.
The upper image shows that a CB_1 receptor agonist (WIN) readily inhibits action potential (red)–evoked inhibitory postsynaptic currents (green) in whole-cell patch-clamp paired recording. As seen in the middle image obtained by confocal microscopy, the axon (red) of the presynaptic GABAergic interneuron formed a single connection with the proximal dendrite of the postsynaptic CA1 pyramidal neuron (green). STORM super-resolution imaging then revealed the distribution of presynaptic CB_1 receptors at the nanoscale level at the very same identified connection.

in the brain is not fully understood. Moreover, the levels of endocannabinoids are regulated by multiple serine hydrolases, but the specific contribution of these enzymes to different forms of endocannabinoid signaling has remained ambiguous.

To address these issues, we first generated novel antibodies for several of these enzymes (in collaboration with Prof. Imre Kacskovics, Prof. Ken Mackie and Prof. Masahiko Watanabe) and validated their specificity in knockout animals. We now use these antibodies to reveal the regional, cellular and subcellular distribution of the endocannabinoid-synthesizing and degrading enzymes in several brain circuits, and also in the spinal cord dorsal horn pain circuits, by exploiting immunoperoxidase light microscopy *(Figure 3)*, immunofluorescence confocal and super-resolution microscopy and immunogold electron microscopy. The functional predictions obtained from the anatomical position of these enzymes are tested by using a combination of paired whole-cell patch-clamp recording and liquid chromatography/tandem mass spectrometry. Gain-of-function and loss-of function perturbations in the signaling function of these enzymes are achieved by pharmacological and genetic tools, as well as by employing *in utero* electroporation *(Figure 4)*.

Selected publications from the last 10 years:

- Katona I, Urbán GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K and Freund TF (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *The Journal of Neuroscience*, 24:5268-5237.
- Ludányi A, Erőss L, Czirják S, Vajda P, Halász P, Watanabe M, Palkovits M, Maglóczky Z, Freund TF and Katona I (2008) Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *The Journal of Neuroscience*, 28:2976-2990.
- Nyilas R, Dudok B, Urbán GM, Mackie K, Watanabe M, Cravatt BF, Freund TF and Katona I (2008) Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. *The Journal of Neuroscience*, 28: 1058-1063.
- Katona I and Freund TF (2008) Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nature Medicine*, 14:923-930.
- Pernia-Andrade AJ, Kato A, Witschi R, Nyilas R, Katona I, Freund TF, Watanabe M, Filitz J, Koppert W, Schüttler J, Ji G, Neugebauer V, Marsicano G, Lutz B,

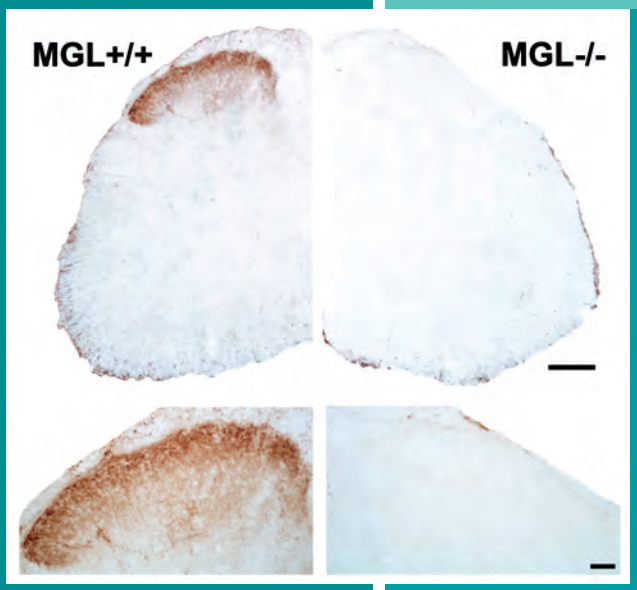


Figure 3.
Inhibition of monoacylglycerol lipase (MGL), a key regulator of endocannabinoid and prostaglandin signaling produces potent analgesia, but the anatomical location of its antinociceptive effects were unknown. These light micrographs demonstrate that MGL (brown DAB precipitate) is highly concentrated in the pain circuits of the spinal dorsal horn. Note that the specificity of the immunostaining was validated in MGL knockout (-/-) mice. For further reading see Horváth et al, 2014, EJN.

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- Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, *Katona I, *Piomelli D and *Manzoni OJ (2012) Uncoupling of the endocannabinoid signaling complex in a mouse model of fragile X syndrome. *Nature Communications*, 3:1080.
- Katona I and Freund TF (2012) Multiple functions of endocannabinoid signaling in the brain. *Annual Review of Neuroscience*, 35:529-558.
- Péterfi Z, Urbán GM, Papp OI, Németh B, Monyer H, Szabó G, Erdélyi F, Mackie K, Freund TF, Hájos N and Katona I (2012) Endocannabinoid-mediated long-term depression of afferent excitatory synapses in hippocampal pyramidal cells and GABAergic interneurons. *The Journal of Neuroscience*, 32:14448-14463.
- Horváth E, Woodhams SG, Nyilas R, Henstridge CM, Kano M, Sakimura K, Watanabe M and Katona I (2014) Heterogeneous presynaptic distribution of monoacylglycerol lipase, a multipotent regulator of nociceptive circuits in the mouse spinal cord. *European Journal of Neuroscience*, 39:419-34.
- Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I and Patel S (2014) Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron*, 81:1111-1125.

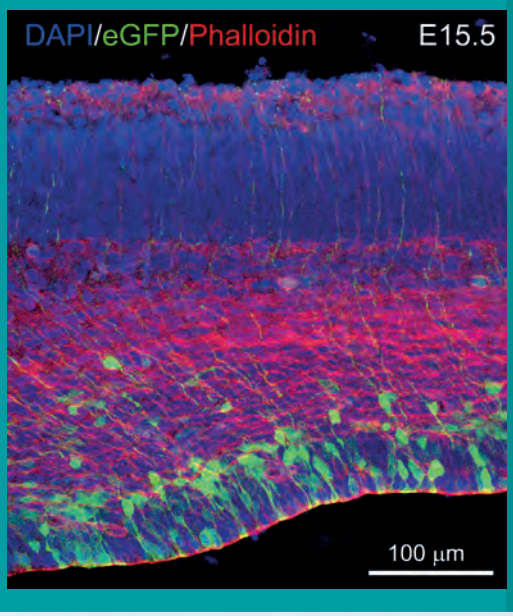

Figure 4.
Impaired migration of cortical cells (green) after *in utero* electroporation of a construct overexpressing an endocannabinoid-synthesizing enzyme reveals a new function of endocannabinoid signaling in the developing brain.

from left: Steve Woodhams, László Barna, Benjámin Barti, Erika Tischler, Ashley Dorning, István Katona, Emese Kovács, Zsófia László, Kata Kenesei, Christopher Henstridge, Vivien Miczán, Gyula Balla, Zsolt Lele, Barna Dudok, Balázs Pintér