Laboratory of Cellular Neurophysiology

Department of Cellular and Network Neurobiology
Head of Momentum-supported Laboratory:
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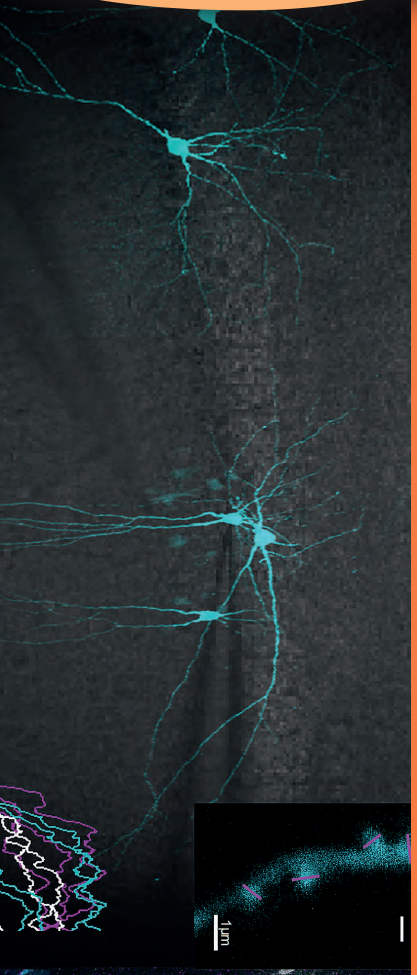

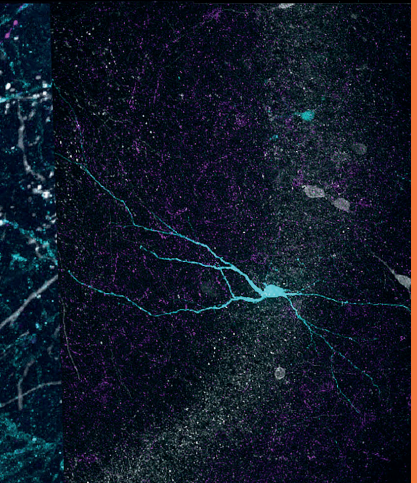

Mission statement

The most fundamental function of nerve cells is the integration of their synaptic inputs to generate their propagating output signal, the action potential. *The major aims of Dr Nusser's laboratory are to understand how identified presynaptic nerve cells release neurotransmitters; how the released transmitter molecules activate their postsynaptic receptors; and how the generated postsynaptic potentials are integrated to generate an action potential.* The Laboratory of Cellular Neurophysiology focuses on four major project areas using a variety of molecular, neuroanatomical, *in vitro* electrophysiological, imaging and *in silico* modeling approaches:

1. Understand the role of identified nerve cells in olfaction. Pharmaco- and opto-genetic approaches are used to modify the activity of nerve cells of the olfactory pathway while the animals perform odor discrimination tasks.
2. Reveal the molecular, structural and functional heterogeneity of cortical excitatory and inhibitory synapses. Determine the molecular specializations underlying the functional and structural diversity of synapses, such as the probability and short-term plasticity of transmitter release, and the extent of postsynaptic receptor activation. *In vitro* electrophysiology, two-photon imaging, light- and electron microscopic immunolocalization are combined to address these issues.
3. Create a molecular map of the neuronal surface by determining the location and density of various voltage- and ligand-gated ion channel subunits in defined subcellular compartments of identified nerve cells, using quantitative light- and electron microscopic immunolocalization. Perform multi-compartmental modeling to generate

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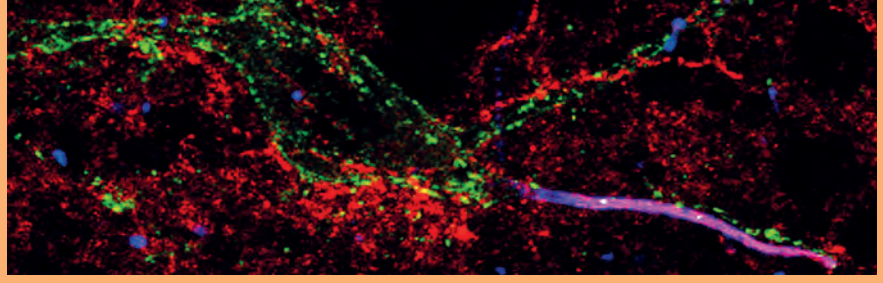
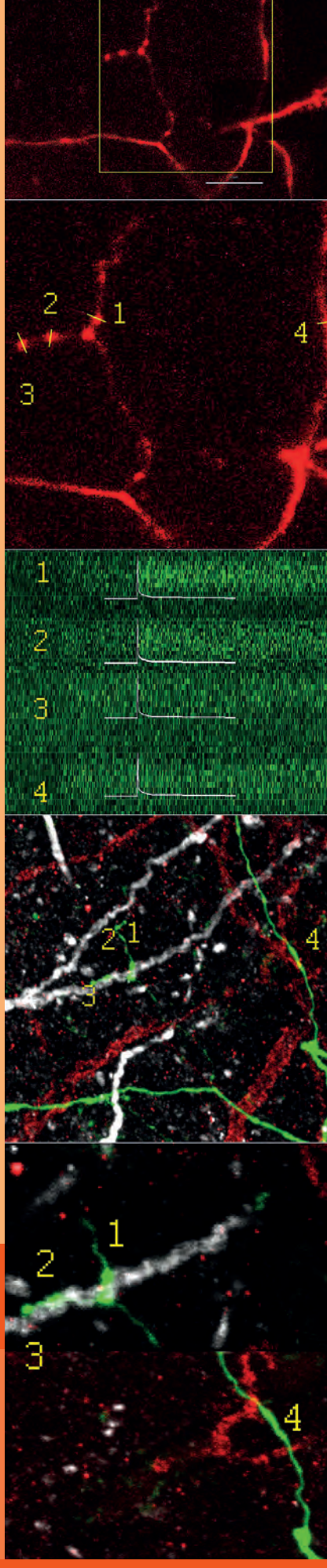


functionally testable predictions of the functional consequences of specialized ion channel distributions. *In vitro* electrophysiology and imaging approaches are used to test the functional predictions of our models.

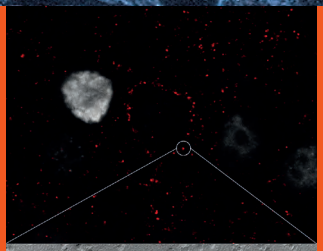
4. Provide a quantitative description of the microcircuit of the cerebellar cortex with special focus on electrically coupled GABAergic interneurons and their roles in network operations.

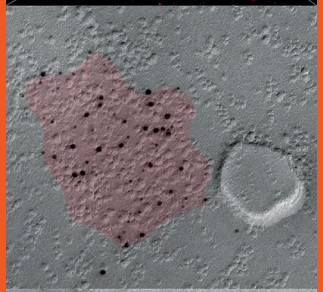
Understand the role of identified nerve cells in olfaction

The extraordinary diversity of nerve cells was already recognized over a century ago. It is now widely accepted that within most brain regions, including the main olfactory bulb (MOB), glutamatergic principal cells are rather homogeneous, whereas GABAergic interneurons (INs) form a diverse cell population. The laboratory of Cellular Neurophysiology has identified novel GABAergic IN subtypes in the MOB, which showed unique connectivity patterns. An interesting issue regarding the diversity of INs is identifying the role individual cell types might play in olfaction. Dr Nusser's laboratory aims to reveal the role of distinct cell types of the MOB and other olfactory brain areas using pharmaco- and opto-genetic approaches. Currently, three different pharmaco-genetic methods are used in the laboratory: 1) a method to employ zolpidem to selectively potentiate $GABA_A$ receptor-mediated synaptic currents in a specific subset of neurons (Wulff et al., 2007, Nat Neurosci), 2) designer receptors engineered from human M3 muscarinic receptors mutated to be exclusively activated by the drug clozapine-N-oxide (CNO; Alexander et al., 2009, Neuron), 3) a mutated nAChR and GlyR chimera, which is selectively activated by a designer drug (Magnus et al., 2011, Science). These designer receptors and the wild-type $GABA_A R\gamma 2$ subunit will be delivered to the brain with stereotaxic injection of adeno-associated viruses either in a cell type-specific or a non-specific manner. Two to four weeks after the injections, the animals will be trained in odor discrimination tasks either in a freely moving or in a head-restrained way. The effects of pharmacological silencing of certain neuronal populations will be tested on the behavioral performance of the animals.



Back row, from left: Mark D. Eyre, Miklós Szoboszlay, Zoltán Nusser, Bence Kókay, Máté Sümegi
Middle row: Tímea Éltes, Andrea Lőrincz, Dóra Bánsághiné Rónaszéki, Noémi Holderith
Front row: Nóra Lenkey, Éva Dobai, Katalin Kerti-Szigeti, Tekla Kirizs





In the head-restrained behavioral task, the cells will be also silenced by the use of light-activated archaerhodopsin.

Understanding the functional consequences of the molecular and ultrastructural diversity of GABAergic and glutamatergic hippocampal synapses

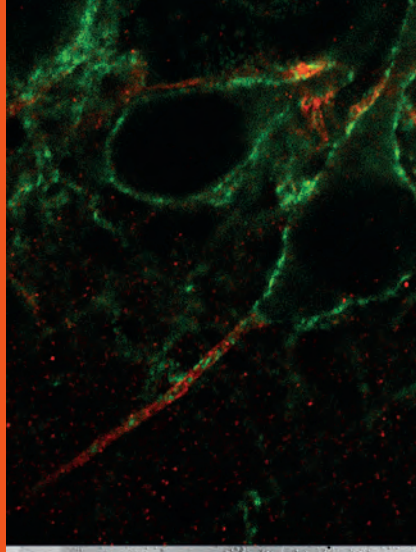
Understanding chemical synaptic neurotransmission in the CNS has been in the spotlight of neuroscience for many decades. A tremendous amount of information has been gathered regarding the molecular events leading to the release of neurotransmitter from synaptic vesicles, the diffusion of neurotransmitter molecules to their postsynaptic receptors and the activation of these receptors. At the same time, ultrastructural analysis of synapses revealed an enormous diversity in the shape and size of pre- and postsynaptic structures among central synapses. Recently, molecular approaches also revealed a large number of molecules involved in pre- and postsynaptic function, and the molecular diversity of many key players. However, it is still unknown how functional heterogeneity of synapses is generated and how alterations in synaptic geometry and molecular content affect synaptic function.
The Laboratory of Cellular Neurophysiology combines *in vitro* electrophysiology and two-photon Ca^{2+} imaging with electron microscopic analysis of hippocampal GABAergic and glutamatergic synapses to address how diversity in the release probability and short-term plasticity of release from distinct synapses correlate with the size and shape of the presynaptic active zones. Furthermore, the laboratory also employs quantitative electron microscopic freeze-fracture replica immunogold localizations to reveal the molecular composition of structurally and functionally distinct active zones. The results shed new light on the structure-function relationship of central synapses and provide a molecular explanation of why a larger active zone confers a higher release probability at hippocampal CA3 pyramidal cell synapses. These results also test the hypothesis that the ultrastructural differences among synapses that belong to the same population (e.g. hippocampal CA3 pyramidal cell to CA1 pyramidal cell connections) are the reflection of molecular specializations that confer distinct functional properties. Another area of interest in the laboratory is to gain an understanding of the precise mechanism(s) of regulation of neurotransmitter release by presynaptic neuromodulators. New combined imaging, electrophysiological and molecular neuroanatomical approaches are used to quantitatively characterize the presynaptic receptor content of axon terminals that have been functionally imaged in acute slices. Another aim is to determine how the molecular identity, number and density of postsynaptic neurotransmitter receptors (e.g. AMPA, NMDA, $GABA_A$) affect postsynaptic receptor occupancy and open probability at identified hippocampal synapses.

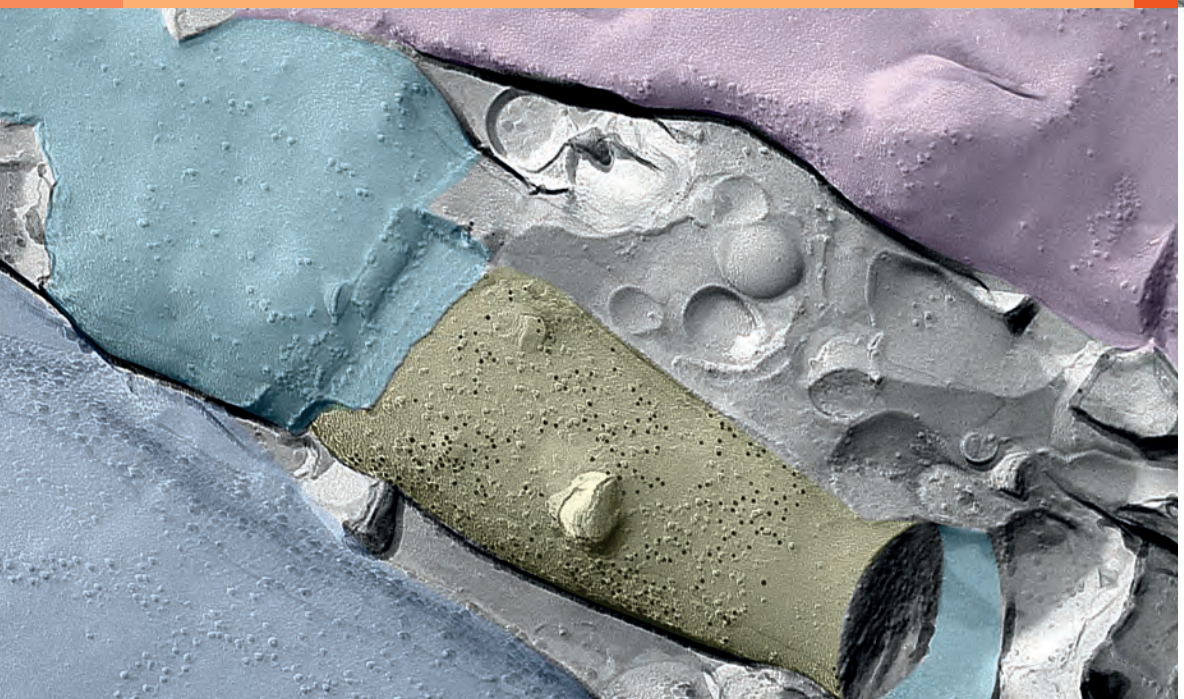
Creating a molecular map of the neuronal surface

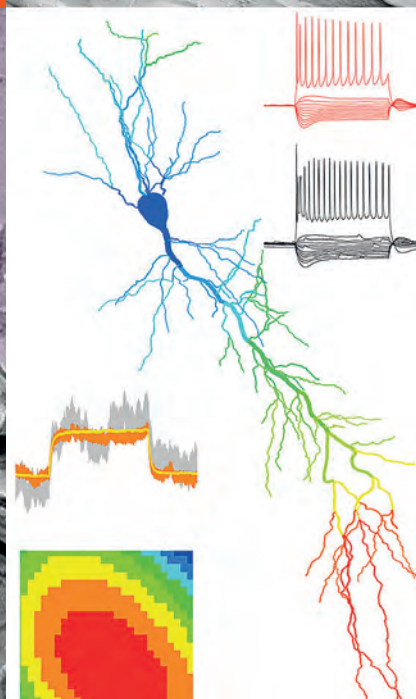
The most fundamental function of nerve cells is the integration of their synaptic inputs to generate action potentials (APs). It is a generally accepted view that APs are generated in the axon initial segment (AIS). However, input synapses are usually distributed over a large dendritic tree. Because of this spatial arrangement, the distance between a synapse and the site of output generation varies to a tremendous extent, resulting in differential filtering of postsynaptic responses by the dendrites. Thus, if dendrites were

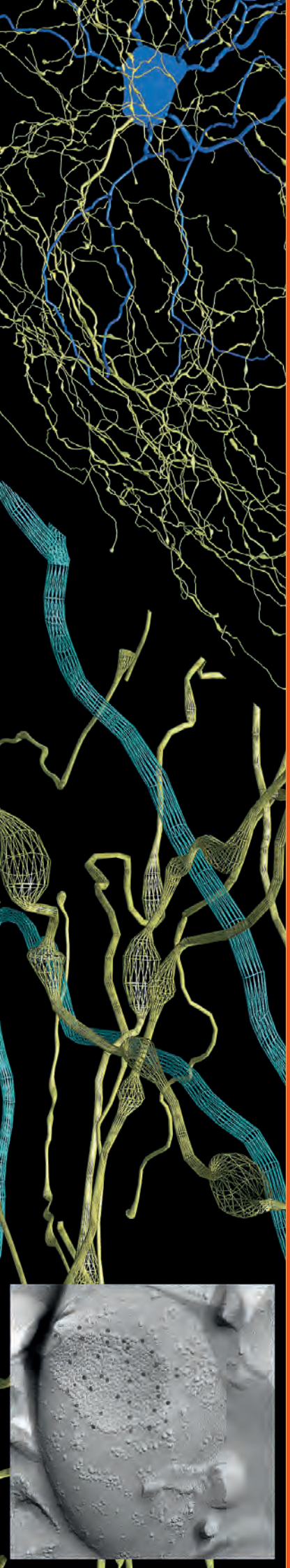
passive, the effect of a synapse on output generation would depend on its dendritic location. However, in the past decade, it has become apparent that dendrites of most nerve cells are not passive, but contain a large number of voltage-dependent conductances, which endow dendritic trees with an unanticipated computational power. The molecular identity, exact location and density of voltage-gated ion channels in small subcellular compartments on the axo-somato-dendritic surface determine their roles in synaptic integration and output generation.

Many investigations focusing on the distribution of voltage-gated ion channels in central neurons reached generalized conclusions such as 'the voltage-gated K^+ channel subunit Kv1.1 is axonal' or 'the HCN1 subunit is somato-dendritic'. Our work using high resolution immunolocalization clearly demonstrated that many ion channel subunits show different cell surface distribution patterns in distinct neuron types (*cell type-specific distributions*). The fact that the 'axonal' and 'somato-dendritic' domains contain many functionally relevant compartments introduces an additional level of complexity. The 'axonal domain' can be divided into an AIS, nodes of Ranvier, myelinated axon segments, preterminal non-myelinated axon segments, axon terminals and presynaptic AZs - compartments that have specific functional roles that require different ion channels (and many other molecules). Our recent results lead to another important conclusion; the ion channel content of distinct subcellular compartments is highly specific (*subcellular compartment-specific distributions*).
The laboratory of Dr Nusser has studied the subcellular distribution of a variety of voltage-gated ion channel subunits (HCN1, Kv4.2, Kv1.1, Kv2.1, Nav1.6) using light- and electron microscopic immunohistochemical approaches and reached the conclusion that, so far, all examined ion channel subunits have different axo-somato-dendritic distributions on the surface of hippocampal CA1 pyramidal cells. In future experiments, the laboratory aims to extend these investigations to other voltage-gated ion channels. In addition, *in silico* multi-compartmental modeling is being used to create functionally testable predictions of the functional consequences of different ion channel distributions. *In vitro* patch-clamp electrophysiology and two-photon imaging will be carried out to test the model predictions.









Provide a quantitative description of the microcircuit of the cerebellar cortex with special focus on electrically coupled GABAergic interneurons and their roles in network operations

The cerebellum plays a crucial role in a large number of diverse functions, such as coordination and timing of limb and eye movements, vestibular control, and might also play a role in higher cognitive functions. The cellular elements and the basic synaptic connectivity of the cerebellar cortex has been the subject of intense research for several decades, resulting in a wealth of information and a qualitatively well-described synaptic circuit. The cerebellar granule cell layer, the input layer of the cerebellar cortex, receives excitatory synaptic inputs from mossy fibres, which innervate the glutamatergic granule cells (GC) and the local GABAergic interneurons, the Golgi cells (GoC). The laboratory of Cellular Neurophysiology has a long-standing collaboration with Prof. Angus Silver's laboratory at University College London to elucidate the computational properties of the GC layer. Their approach is to reconstruct a realistic, *in silico* model of the granule cell layer with anatomically and physiologically constrained properties of the synapses and cell types. Prof. Silver has a long history in investigating the fine biophysical properties of mossy fibre to granule cell synapses, resulting in detailed information regarding the receptor mechanisms, conductance kinetics, synaptic vs. non-synaptic (spillover) activation of postsynaptic receptors and the short-term plasticity of transmission. The GCs can be considered electrically as a single compartment cell; the passive and active properties are also well characterized. However, the electrical properties of the remaining cell type of the input layer, the GoCs remained more elusive. To elucidate the passive and active properties of GoCs and the characterize their synaptic networks, the two laboratories joined forces to perform combined *in vitro* physiological and *post hoc* neuroanatomical and *in silico* modeling experiments. The passive nature of the dendrites of GoCs was revealed by dendritic patch-clamp recordings and high-resolution immunolocalization of voltage-gated ion channel subunits. A novel function of dendritically located electrical synapses or gap junctions was also revealed, demonstrating that they play a role in distributing the synaptic charge among electrically connected GoCs in order to compensate sublinear synaptic integration of EPSPs. The electrically-connected GoC network shows spontaneous synchronized activity. However, when out-of-phase excitatory synaptic inputs arrive, the network transiently desynchronizes, the mechanisms of which was also the subject of investigation. Combined modeling, physiological and anatomical experiments revealed that electrical connections have different strengths, which is primarily the consequence of the different number and dendritic location of gap junctions between the connected GoCs. This differential electrical connectivity strength could explain the transient network de-synchronization upon out-of-phase excitation. In the most recent collaboration, quantitative neuroanatomical approaches were used to determine the synaptic connectivity within the GC layer and this information, together with physiological data, was used to construct both simple and biologically detailed network models. Analysis of these networks with novel information-theoretic approaches revealed that the small number of mossy fibre inputs per GC and a high spike threshold provides an optimal trade-off between information transmission (without loss) and sparse coding, which is required for pattern separation. These discoveries are being extended by incorporating experimentally-constrained GoC models into the network, with which we will test whether the tonic and

phasic inhibition present in the GC layer sets the spike threshold to a level that is optimal for lossless sparse encoding.

15 most important publications from the last 10 years:

- Szabó G. G., Lenkey N., Holderith N., Andrási T., Nusser Z. & Hájos N. (2014) Presynaptic calcium channel inhibition underlies CB_1 cannabinoid receptor-mediated suppression of GABA release, J Neurosci, 34, 7958-7963.
- Holderith N., Lorincz A., Katona G., Rózsa B., Kulik A., Watanabe M. & Nusser Z. (2012), Release probability of hippocampal glutamatergic terminals scales with the size of the active zone, Nature Neurosci, 15, 988-997.
- Vervaeke K., Lorincz A., Nusser Z. & Silver R. A (2012). Gap Junctions compensate for sub-linear dendritic integration in an inhibitory network. Science, 335, 1624-1628.
- Eyre M. D., Renzi M., Farrant M. & Nusser Z. (2012). Setting the time course of inhibitory synaptic currents by mixing multiple $GABA_AR$ α subunit isoforms. J Neurosci, 32, 5853-5867.
- Vervaeke K., Lorincz A., Gleeson P., Farinella M., Nusser Z. & Silver R. A (2010). Rapid desynchronization of an electrically coupled interneuron network with sparse excitatory synaptic input. Neuron, 67, 435-451.
- Lorincz A. & Nusser Z. (2010). Molecular identity of dendritic voltage-gated sodium channels. Science, 328, 906-909.
- Watt A. J., Cuntz H., Mori M., Nusser Z., Sjostrom P. J. & Hausser, M. (2009). Traveling waves in the developing cerebellar cortex mediated by directed synaptic connections between Purkinje cells. NatureNeurosci, 12, 463-473.
- Lorincz A. & Nusser Z. (2008). Cell type-dependent molecular composition of the axon initial segment. J Neurosci 28, 14329-14340.
- Lorincz A. & Nusser Z. (2008). Specificity of immunoreactions: The importance of testing specificity in each method. J Neurosci 28, 9083-9086.
- Eyre M. D., Antal M. & Nusser Z. (2008). Distinct deep short-axon cell subtypes of the main olfactory bulb provide novel intrabulbar and extrabulbar-GABAergic connections. J Neurosci 28, 8217-8229.
- Biro A. A., Holderith N. B. & Nusser Z. (2006). Release probability-dependent scaling of the postsynaptic responses at single hippocampal GABAergic synapses. J Neurosci 26, 12487-12496.
- Kollo M., Holderith N. B. & Nusser Z. (2006). Novel subcellular distribution pattern of A-type K^+ channels on neuronal surface J Neurosci, 26, 2684-2691.
- Cathala L., Holderith N. B., Nusser Z., DiGregorio D. A. & Cull-Candy S. G. (2005). Changes in synaptic structure underlie the developmental speeding of AMPA receptor-mediated EPSCs. Nat Neurosci, 8, 1310-1318.
- Farrant M., & Nusser Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. Nat Rev Neurosci, 6, 215-229.
- Biro A. A., Holderith N. B. & Nusser Z. (2005). Quantal size is independent of the release probability at hippocampal excitatory synapses. J Neurosci 25, 223-232.