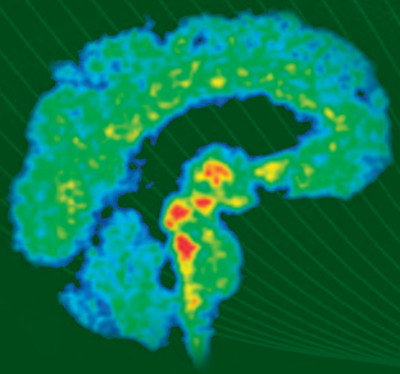
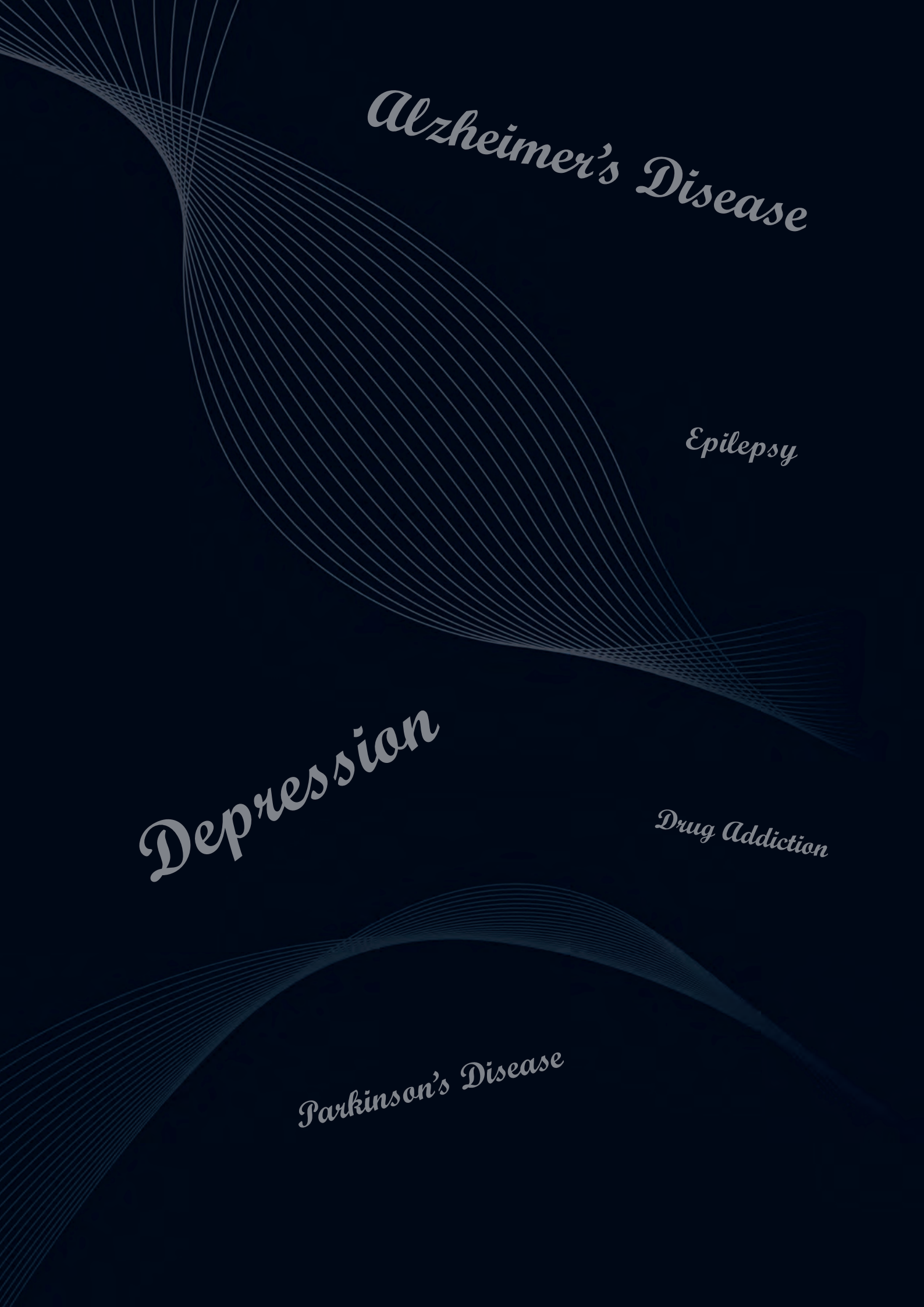
Institute of Experimental Medicine

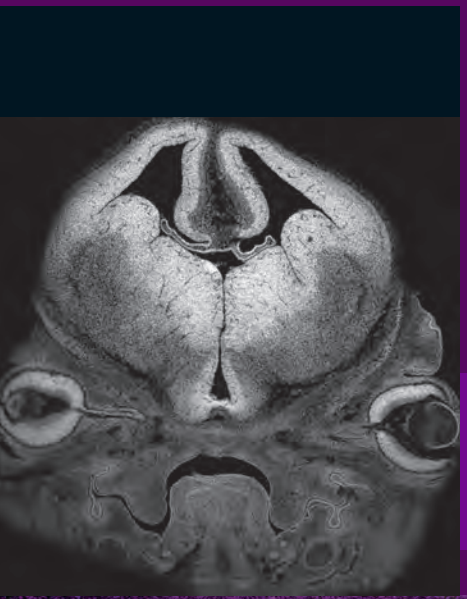
Hungarian Academy of Sciences

B U D A P E S T




Alzheimer's Disease
Epilepsy
Depression
Drug Addiction
Parkinson's Disease

Contents



Site visit in the IEM by the IBRO Scientific Evaluation Committee in 2004. Members of the Committee from left to right around the table: Jean-Claude Lacaille (Montreal), Henry Markram (Lausanne), Larry Swanson (San Diego), Marina Bentivoglio (Verona) and Roger Nicoll (San Francisco), as well as the IEM deputy directors Beata Sperlagh, Zsolt Liposits, and director Tamás Freund.

Introduction

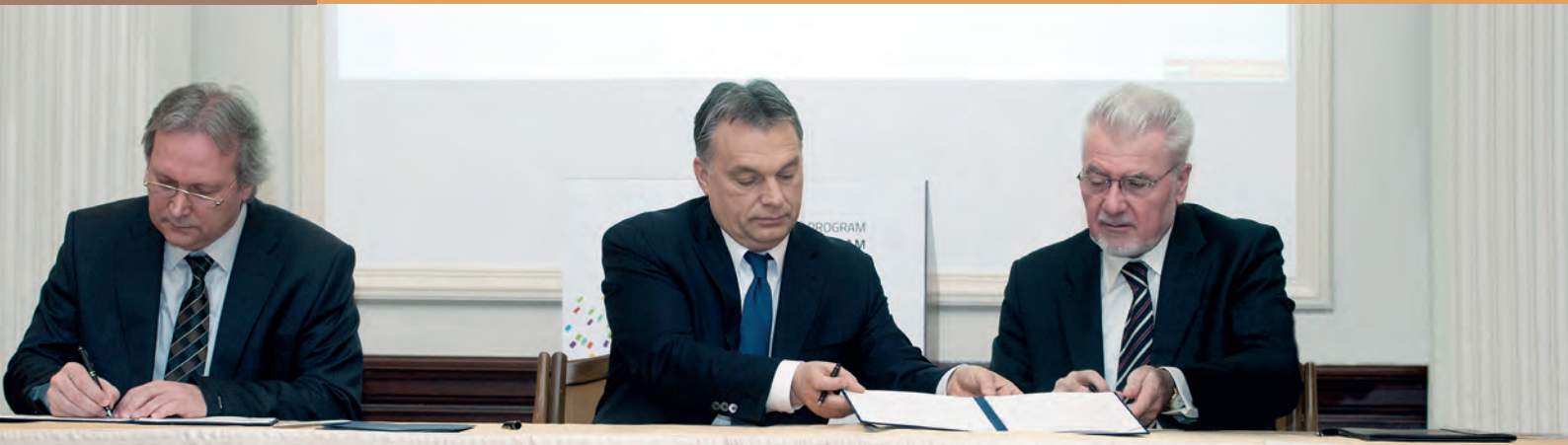
Mission of the Institute

The social burden and cost of brain disorders are enormous, and no serious progress can be expected in the treatment or in the prevention of these disorders without research and new discoveries. With this watchword the Institute of Experimental Medicine (IEM) of the Hungarian Academy of Sciences, founded in 1952, has undergone a dynamic development over the last 20 years. The IEM is dedicated exclusively to basic biomedical research in the field of neuroscience. This includes studies on neurotransmission, learning and memory, neuronal development, anxiety, depression, schizophrenia, aggressive behaviours, ischemic and epileptic brain damage, neurodegenerative disorders and the central and peripheral control of hormone secretion.

The research teams of the Institute employ multidisciplinary approaches: traditional, well-established methodologies (e.g. anatomy, electrophysiology, neurochemistry and pharmacology) are combined with novel approaches in cellular and molecular biology such as the use of transgenic animals with in vivo calcium imaging and optogenetics, as well as with super-resolution microscopy, patch clamp, 2-photon microscopy, calcium imaging techniques and behavioral technologies. International mobility of researchers - our Institute hosts 8-10 foreign researchers per year and a similar number of our own researchers visit leading labs in the world - and the adequate local as well as foreign funding (including new infrastructure in recent years) enable the Institute to stay in the international mainstream of its pursued neuroscience research.

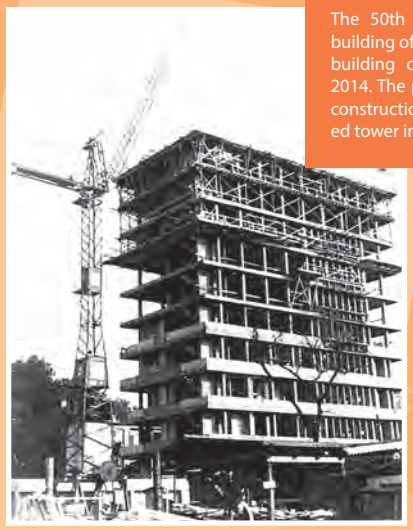
The IEM is involved in undergraduate and postgraduate training as part of a close collaboration with 3 universities in Budapest, one of which has its Neuroscience Department in the IEM, and another has a joint Graduate School with the IEM. The Institute extensively participates in education of the general public, as well as in the formation of advisory and lobby organizations to influence governmental science policy in Hungary. Its main message is that it is in the best interest of Hungary and of all European countries to consider discovery research into the mechanisms of brain disorders as major priority.

Signing of the cooperation agreement on the National Brain Research Programme at the Hungarian Academy of Sciences, 26 February 2014. From left: Tamás Freund, President of the Hungarian Brain Research Program, Viktor Orbán, Prime Minister of Hungary, József Pálinkás, President of the Hungarian Academy of Sciences.



"Centre of Excellence"

In the year 2000, the Institute was ranked second from 260 candidates in a competition for the *"Centre of Excellence"* title and won this recognition for three years by the decision of independent experts of the European Commission.



The 50th anniversary of the building of the 10-storey tower building of IEM was in July 2014. The photos illustrate the construction and the completed tower in 1964.

The tower building of the IEM and the Medical Gene Technology Unit.

Report of the IBRO Review Committee

"The IEM has also succeeded in integrating into the international neuroscience arena, not only making Hungarian neuroscience highly respectable, but placing Hungary at the center stage of world neuroscience."

"The Institute of Experimental Medicine is a world-class neuroscience research center that provides the most creative environment for brain research in Hungary."

Opening ceremony of the main "R and D" event of the Hungarian EU presidency entitled: Discovery Research in Neuropsychiatry: Depression, Anxiety and Schizophrenia in Focus. From left to right: Tamás Freund, president of the conference, Maire Geoghegan Quinn, EU Commissioner for Research and Innovation, József Pálinkás, President of the Hungarian Academy of Sciences and Zoltán Cséfalvay, Secretary of State for Economy and National Development. Assembly Hall of the Headquarters of the Hungarian Academy of Sciences, Budapest, 18 March 2011.

4 March 2011. Toast and drink together in honor of Tamás Freund, Winner of The Brain Prize 2011 of the Grete Lundbeck European Brain Research Foundation. From the left: Tamás Freund, Dezső Szabó, Zsuzsa Győrfiné Vörös, Beáta Sperlágh, E. Sylvester Vizi, Zsolt Liposits and Ferenc Oberfrank.

Hungarian Brain Research Program

The Hungarian Government launched a national research program called "Hungarian Brain Research Program" (HBRP) with a budget of 12 billion HUF (40 million €s), for a period of four years (2013-2017) of which the share of IEM is 150 million HUF (500 000 €s) annually. The government invited the Director of IEM, the Brain Prize holder, Professor Tamás F. Freund, to lead the program as President. The director of the HBRP is the executive director of IEM, Dr. Ferenc Oberfrank.

Leadership

Director: *Professor Tamás F. Freund*, is an active neuroscientist.

Board of the institute — Chaired by the Director, the IEM Board meets every week, and provides essential everyday guidance, as well as strategic planning.

Zsuzsa Györfiné Vörös - financial deputy director (finance)
Professor Beáta Sperlágh - scientific deputy director (scientific affairs)
Associate Professor Ferenc Oberfrank - executive director
Professor emeritus Dezső Szabó - scientific secretary

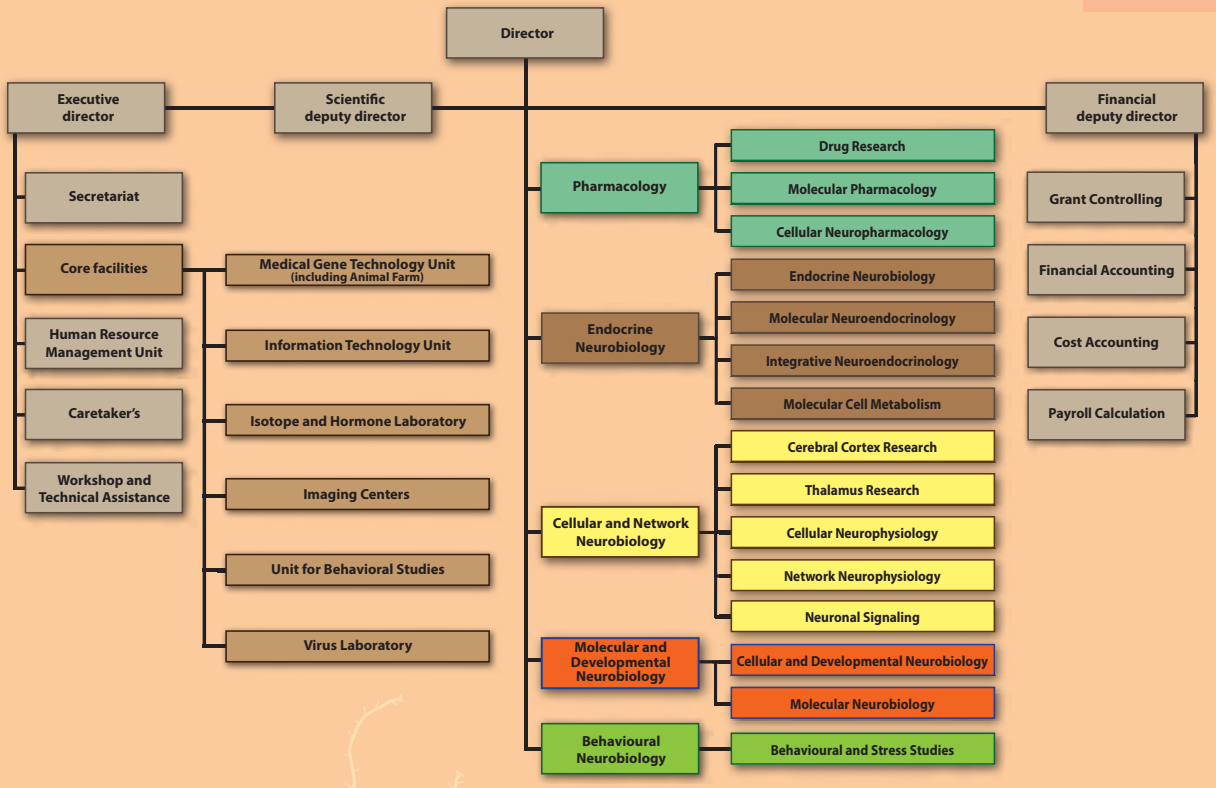
Scientific Advisory Board — The Board consists of internationally well known, leading neuroscientists from the USA, the U.K. and Hungary.

Professor György Buzsáki (Rutgers University, Newark, USA)
Professor Barry Everitt (University of Cambridge, UK)
Professor George Kunos (NIH-NIAAA, Bethesda, USA)
Professor Gareth Leng (University of Edinburgh, U.K.)
Professor István Módy (Univ. California Los Angeles, USA)
Professor János Szolcsányi (University of Pécs, Hungary), Chair

The new auditorium of the institute.

The renewed corridor of IEM. Right: The glass wall, based on a fluorescent image of the hippocampus designed by Gábor Nyíri.

The organizational structure and functioning



IEM-HAS departments, research groups and their leaders

Department of Pharmacology – *Beáta Sperlágh*
- Laboratory of Drug Research – *E. Sylvester Vizi*
- Laboratory of Molecular Pharmacology – *Beáta Sperlágh*
- Laboratory of Cellular Neuropharmacology – *János Szabadics*

Department of Endocrine Neurobiology – *Zsolt Liposits*
- Laboratory of Endocrine Neurobiology – *Zsolt Liposits*
- Laboratory of Molecular Neuroendocrinology – *Krisztina Kovács*
- Laboratory of Integrative Neuroendocrinology – *Csaba Fekete*
- Laboratory of Molecular Cell Metabolism – *Balázs Gereben*

Department of Cellular and Network Neurobiology – *Tamás F. Freund*
- Laboratory of Cerebral Cortex Research – *Tamás F. Freund*
- Laboratory of Thalamus Research – *László Acsády*
- Laboratory of Cellular Neurophysiology – *Zoltán Nusser*
- Laboratory of Network Neurophysiology – *Norbert Hájos*
- Laboratory of Neuronal Signaling – *Judit Makara*

Department of Gene Technology and Developmental Neurobiology – *István Katona*
- Laboratory of Cellular and Developmental Neurobiology – *Emília Madarász*
- Laboratory of Molecular Neurobiology – *István Katona*

Department of Behavioural Neurobiology – *József Haller*
- Laboratory of Behavioural and Stress Studies – *József Haller*

Scientific staff

Local and foreign funding enables the Institute to maintain a leading position in the international mainstream of neuroscience research. The strategies to preserve high standards of research staff include retaining top researchers by providing an optimal research environment, promoting young talents to become independent scientists, reversing "brain drain" by reclaiming and recruiting young top scientists from abroad. The Institute supports the international mobility of researchers, hosting several foreign scientists per year, and a similar number of IEM researchers visit leading laboratories in the world. Our aim is to raise the number of foreign researchers working in our research groups. The IEM encourages the involvement of university students in research. The IEM has been involved in undergraduate and postgraduate training as part of a close collaboration with universities. In most cases these students already have publications by the time of graduation, and are thereby accepted into Ph.D. programmes. Our links with universities ensure a continuous supply of the best students to our undergraduate and postgraduate research programmes, while providing top quality lecturers for the universities from among our staff.

Funding and operations

IEM's commitment to best-in-class neuroscience research remains unwavering, even in the face of a challenging funding environment. Drawing upon its own endowment, the support of the Hungarian government, as well as national, European and foreign public or private research grants, the Institute has been able to keep pace with the rapidly escalating costs associated with maintaining — and defining — the state of the art. The Institute administration is permanently focusing on streamlining operations, helping to ensure that a larger proportion of our income will directly support research and science in general.

The future for IEM's research is promising, firmly supported by the Institute's solid financial foundation and disciplined management. Going forward, major efforts, such as the steady development of the research infrastructure, the recently launched new laboratories as of behavioral and metabolic studies, the enlargement of the virus laboratory and the NIKON Imaging Center at IEM, the implementation of a cutting edge electron microscope, traditional and new partnerships, has long been a hallmark of IEM.

Several fora have been established to facilitate the exchange of ideas and information among researchers. "Laboratory Progress Reports" take place monthly, in which the research groups present and discuss their results and plans in the presence of the entire scientific community of the Institute. A two-day science workshop ("IEM Days") is organized annually at lake Balaton in the presence of members of the Scientific Advisory Board, in which the active participation of young scientists is particularly encouraged. The IEM hosts several scientific workshops, invited lectures and seminars by visiting scientists each year.

The renovated hall of IEM, with sculptures of János Szentágothai (left) and Ramon y Cajal (right), world famous neuroanatomists.

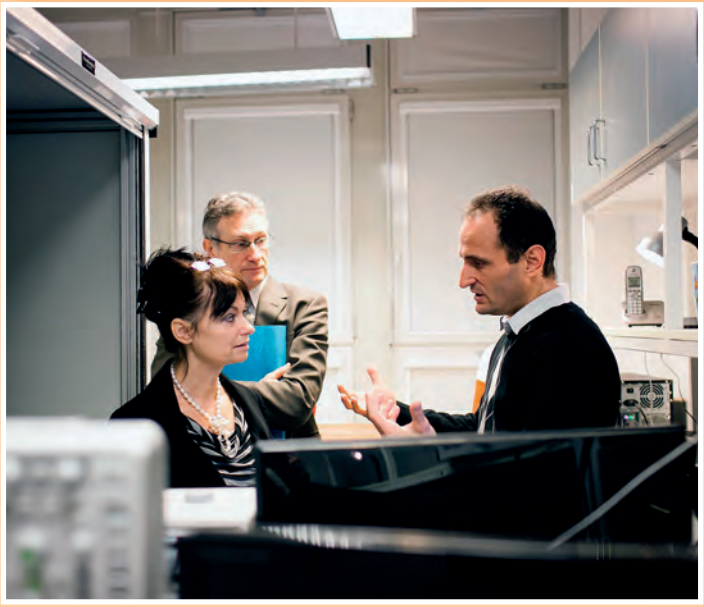
Research infrastructure, core facilities, methods and technologies in the Institute

The major equipment and facilities of the Institute represent state-of-the-art, recent technologies, and resulted from a watchful development in the last 20 years. The core facilities are accessible to every researcher, including undergraduate and Ph.D. students. Major equipment and technologies in the Institute in 2014 include the followings:

- Neurolucida: workstation for the evaluation of morphological experiments
- An integrated instrumental system for complex behavioral analysis of laboratory rodents
- In vivo metabolic phenotyping
- Confocal laser-scanning microscopes, one of them for live-cell imaging
- 2-photon microscopes
- Patch clamp electrophysiology setups for examination of in vitro slice preparations
- Patch clamp electrophysiology setup with C1 confocal head
- Chemiluminescent gel documentation system
- Mulitplex gene and protein analyzers
- Imaging microscope systems, TIRF and STORM super-resolution microscope (as parts of the NIKON-IEM Microscopy centre, from Autumn, 2010)
- Bacterium incubator
- High-pressure freezer and freeze-fracture machines for SDS-digested freeze-fracture replica labeling
- In vitro electrophysiological setups with two-photon imaging facility
- Electron microscopy with 3 electron microscopes (one of them for tomography) and 4 ultramicrotomes
- Real-time microelectrode biosensor set up for neurotransmitters and metabolites
- HPLC technologies
- Medical Gene Technological Unit: Transgenic and Animal Technology Units and Animal Facility
- In vivo electrophysiology setups for measuring neuronal activity in the intact brain of anesthetized animals
- Cell and Tissue Unit (with 3 sterile hoods and cell-analytical equipment and FACS)
- Fluorescence-aided Cell Sorter and Flow Cytometer
- Videomicroscopic Workstation for live cell monitoring
- Virus technologies for optogenetics and tracing

Ann Glover, Chief Scientific Adviser to the President of the European Commission on a lab visit in the IEM talking with Zoltán Nusser, 1 February 2013.

Opening of the enlarged NIKON Center of Excellence at the Institute of Experimental Medicine, 11 October 2013. Tamás Freund, director of KOKI and Sumio Eimori, president of Nikon Europe.



József Pálinkás, President of the Hungarian Academy of Sciences, and Tamás Freund, Director of the IEM, are cutting the ribbon at the inauguration ceremony of the new auditorium of the Institute, 26 November 2012.

Cooperations

Major collaborations in Hungary:

- Semmelweis University Medical School
- Natural Sciences Research Centre, Hungarian Academy of Sciences
- Eötvös Loránd University, Faculty of Natural Sciences
- Péter Pázmány Catholic University, Faculty of Information Technology
- Institute of Neurosurgery, Budapest
- Szent István University, Gödöllő
- Agricultural Biotechnology Center
- University of Pécs

Translational research and collaboration with pharmaceutical companies

The Institute has extensive collaborations with pharmaceutical companies, which allow the immediate exploration of the potential use of basic research results of the Institute in the development of new drugs and strategies in pharmacotherapy. Complementary to this, the new generations of some drugs developed by these companies are tested in laboratories of the Institute in various behavioral, molecular, anatomical, electrophysiological and pharmacological tests.

Considerable efforts are made to bridge the gap between the academic side and the pharmaceutical or clinical research by an increasing number of translational neuroscience projects, and a closer collaboration with pharmaceutical companies and clinical researchers.

These activities in the Institute meet the research strategies and expectations of the European Union, leading to an increased chance of funding by the EU Horizon 2020 Program, and a better integration into the European Research Area.

Main pharmaceutical and biotechnological company partners:

- Gedeon Richter Plc.
- EGIS Plc.
- Servier International
- Pfizer Hungary
- ImmunoGenes Kft.

Spin-off companies of the institute:

- Anxiofit Ltd.
- Femtonics Ltd.

Partnerships with universities

Semmelweis University Medical School – *strategic partner*

- Undergraduate and graduate training programmes
- Joint graduate school: The János Szentágothai Graduate School in Neurosciences

- Common grants
- Common facilities (hormone laboratory, transgenic facility, virus laboratory)

Péter Pázmány Catholic University, Faculty of Information Technology

- Neuroscience Department located in IEM, undergraduate training
- Szentágothai János Knowledge Center

Loránd Eötvös University, Faculty of Natural Sciences

- Joint undergraduate and graduate training programmes
- Joint PhD programmes

Invited speakers of the Biannual Conference of the Hungarian Neuroscience Society attending a reception in the Winery of Attila Gere in Villány. Standing, from left to right: Beat Lutz, Tibor Harkány, Carol Barnes, Gábor Tamás, Michael Hasselmo, Karl Deisseroth (The Brain Prize in 2013), Barry Everitt, Katalin Gere (host), Tamás Freund (The Brain Prize in 2011), Erwin Neher (Nobel Prize winner in 1991), Attila Gere (host winemaker), Edvard Moser (Nobel Prize winner in 2014), John O'Keefe (Nobel Prize winner in 2014), György Buzsáki (The Brain Prize in 2011), Veronika Solt. Crouching: István Katona, Zoltán Nusser, May-Britt Moser (Nobel Prize winner in 2014), Norbert Hájos. Lying: József Csicsvári. Villány, 29 January 2009.

Viktor Varga, research fellow of the Laboratory of Cerebral Cortex Research (left) showing his lab to Bert Sakmann (Nobel Prize, 1991), 8 April 2013.

Director Tamás Freund delivering his traditional Christmas Speech, 20 December 2014.

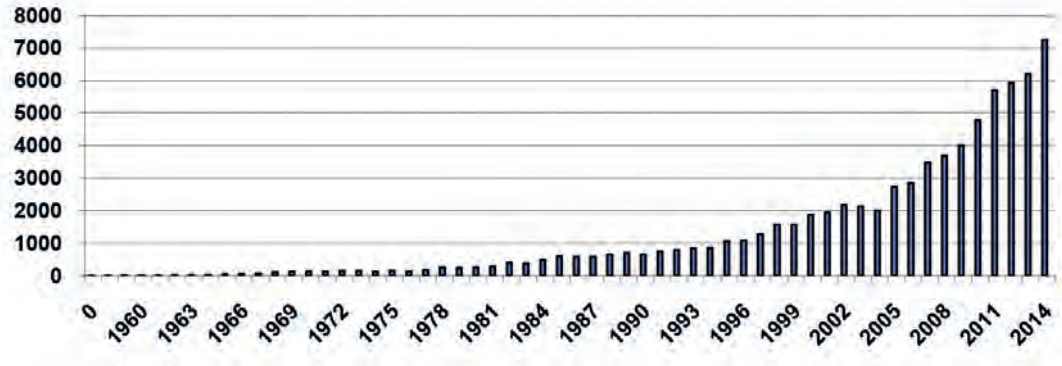
Inauguration of the busts of Santiago Ramón Y Cajal and János Szentágothai, created by Éva Freund, 20 December 2013. Standing in the middle from the left: E. Sylvester Vizi, Béla Halász, Tamás Freund.

Publications, scientometric data characterizing scientific activity of the Institute

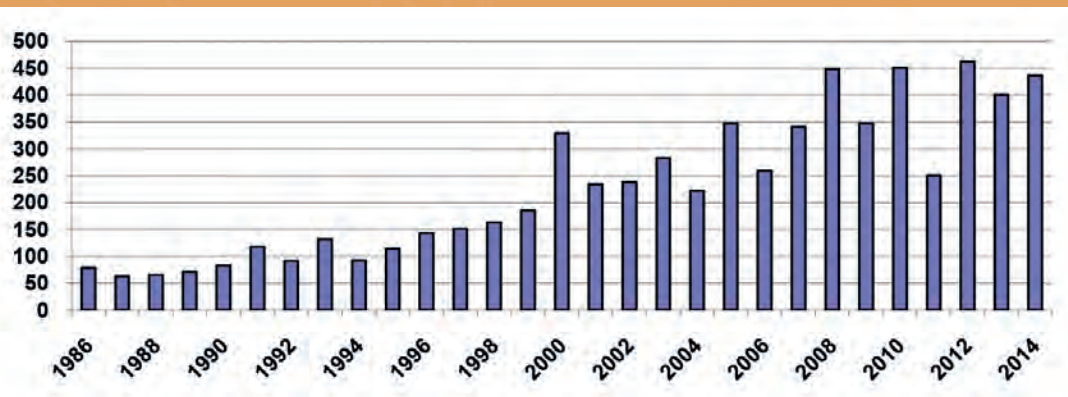
The IEM's most important "deliverables" are publications. In the past decade the Institute published about 60-80 peer-reviewed papers per year in leading international journals, with a cumulative impact factor of 330-460 per year.

The annual number of citations reached 7252 in 2014.

Number of citations received by the IEM:



Cumulative Impact factor - sum of impact factors for published journal articles:



The Brain Prize ceremony (from left to right) Tamás Freund, György Buzsáki and Péter Somogyi are receiving the award from Helga Nowotny, president of the European Research Council, and from Niels Axelsen, President of the Board of Trustees of The Brain Prize Foundation. Coppenhagen, 1 May 2011.

Basic facts and key figures (2014)

- Centre of neuroscience research in Hungary.
- Established in 1952, building built in 1962-64.
- Member of the Hungarian Academy of Sciences research network.

Budget (2014)

- 3 964 M Ft ~ 12,6 M €
- of which 1 676 M ~ 5,3 M € are revenues generated by the IEM:
  - 603 M Ft ~ 1,9 M € from Hungarian grants
  - 154 M Ft ~ 0,5 M € from foreign grants
  - 81 M Ft ~ 0,25 M € from industrial contracts

Investments in research infrastructure (2014):

- 604 M Ft ~ 1,9 M €

Personnel:

- 171 permanent employees, of which
  - 92 researchers (38 women, 39<35 years)

Organization & infrastructure

- 9000 m^2 area, more than 70 laboratories, seminar rooms and
- 15 research groups
- Core research facilities:
  - Animal farm /spf & conventional/,
  - Gene Technology Unit,
  - Hormone Lab and Isotope Unit,
  - Virus Technology Unit,
  - Nikon Microscopy Center at Institute of Experimental Medicine (NMC at IEM, from spring 2010),
  - Unit for Behavioral Studies (from autumn 2014),
  - FEI Unit (3 EMs, one with tomography from 2015),
  - Metabolic Phenotyping Unit (from winter 2014),
  - Cell Biology Unit (inclucing FACS laboratory),
  - 3D Printing Service.

- Service and Support Units
- Administration

Collaborations

- Common grants & publications with more than 70 laboratories from 15 Countries on 3 Continents
- 21 visiting scientists (6 from Hungarian Labs, 15 from Foreign Labs)
- Leading pharmaceutical companies
- spin-off companies

Scientific publications in 2014

- 436,518 IEM' cumulative impact factor
- 5,90 IF/publication
- 4,75 IF/researcher (employed full time)
- 7252 citations

Education – undergraduate and PhD training (2014)

- 69 undergraduate students
- 13 PhD students
- 28 training courses and summer schools

Editor's offices in IEM

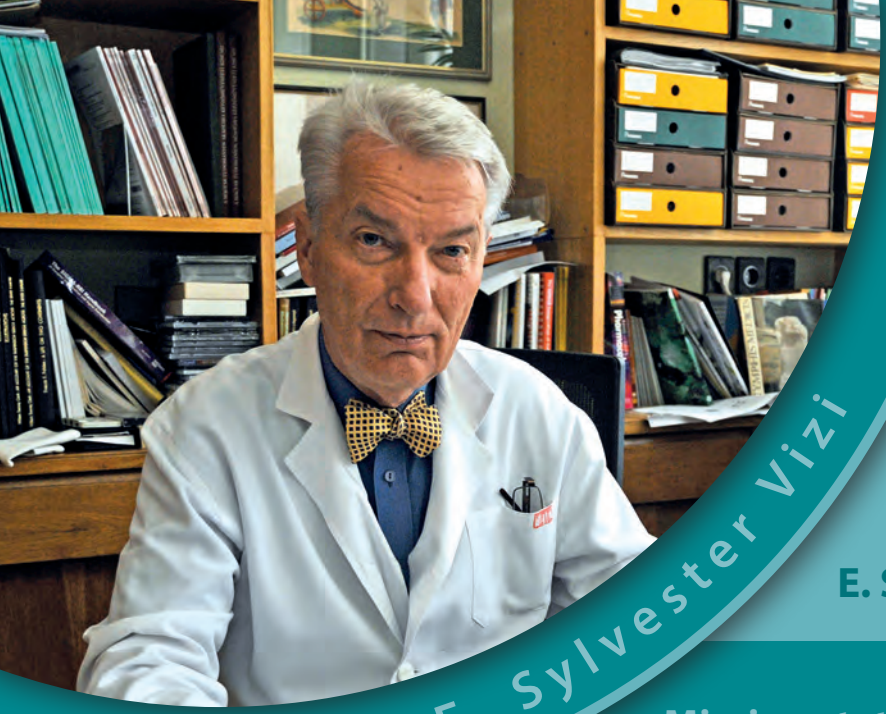
- Brain Research Bulletin (Section Editor E. S. Vizi)
- European Journal of Neuroscience (Section Editor L. Acsády)
- Hippocampus (Section Editor T. F. Freund)
- Science Advances (Associate Editor T. F. Freund)
- Frontiers in Behavioral Neurosciences (Associate Editor J. Haller)
- Inflammopharmacology (Section Editor B. Sperlágh)
- eNeuro (Receiving Editor Z. Nusser)

Brain Awareness

- Hungarian Brain Council membership
- Public programs, lectures, articles, interviews

Members of the IEM financial and service departments, including accounting, supplies, workshop, information technology unit, secretariat, animal facility, safety and transport. Sitting, from left: Anita Szabó, Erika Puschenreiter, Csilla Széles, Zsuzsa Györfi, Nikoletta Kohut-Veres, Sára Seregély, Renáta Antal Veres, Éva Nemes. Standing, first row: Erzsébet Tóthné Fekete, Diána Debreceni, Adrienne Rozs, Norbert Rozs, Judit Csek, János Fülöp, Andor Rácz, Sándor Török. Standing, second row: Miklós Bucsánszki, Attila Kovács, Dezső Szabó, Ferenc Oberfrank, Gabi Benkó, János Varga. Standing thirdrow: Nikolett Radó, József Poloniczki, Zoltán Sándor

Location map from bird's eye view.



Laboratory of Drug Research

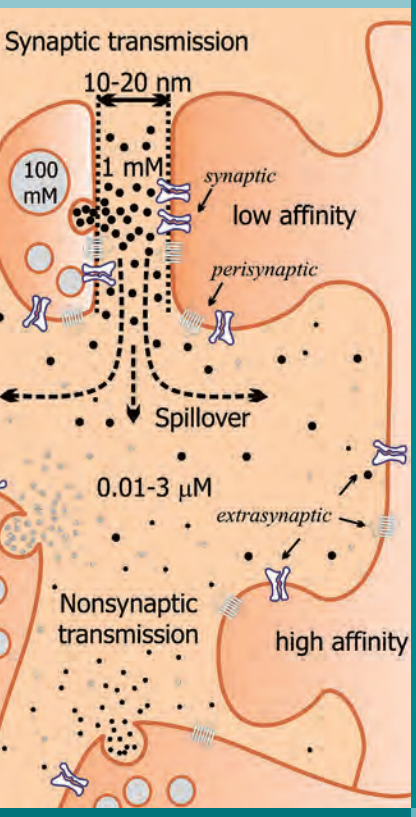
Department of Pharmacology

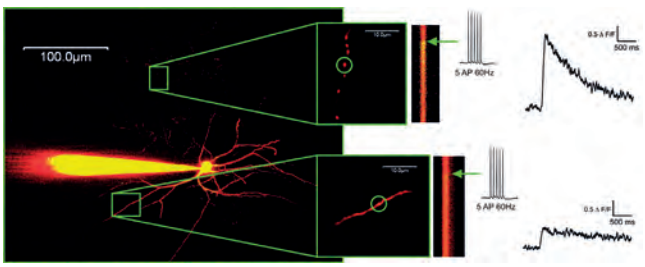
Head of Laboratory:
E. Sylvester Vizi MD, PhD

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-

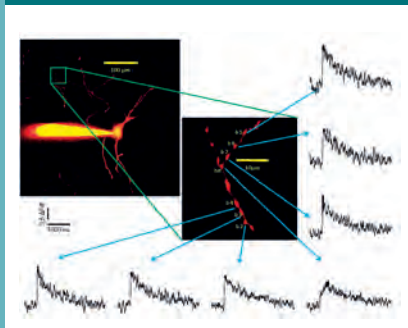


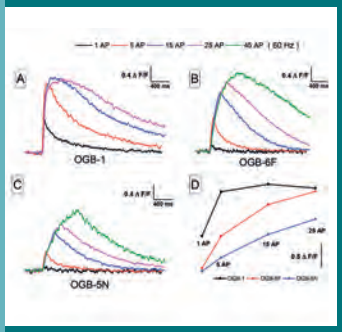


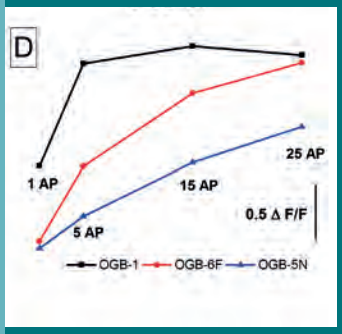
Senior scientists: Tibor Zelles MD, PhD, Gabriella Zsilla PhD
Ph.D. students: Máté Kisfali, Tibor Lőrincz , Viktória Humli , Gábor Polony MD, Zoltán Borbély
Graduate research assistant: Judit Szepesy
Undergraduate research assistants: Lakatos Marcell, student of Semmelweis University
Technicians: Anita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



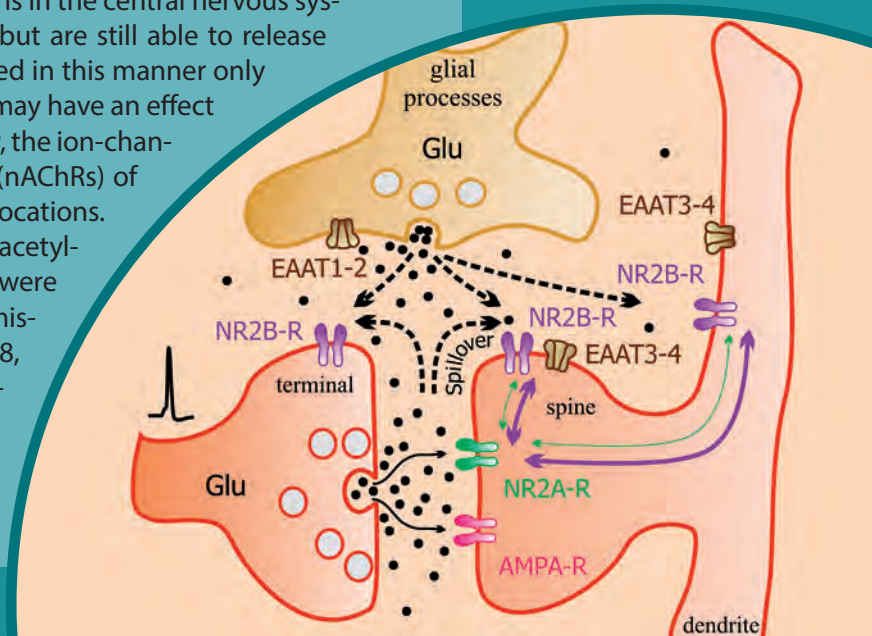


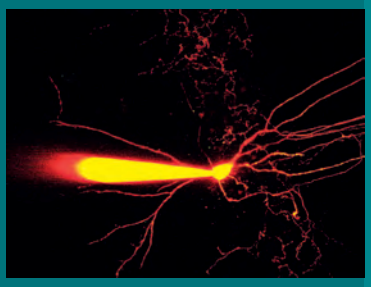


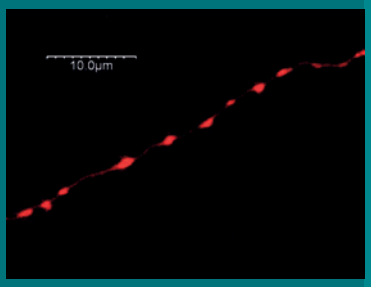
Nonsynaptic receptors and transporters as targets for drug treatment

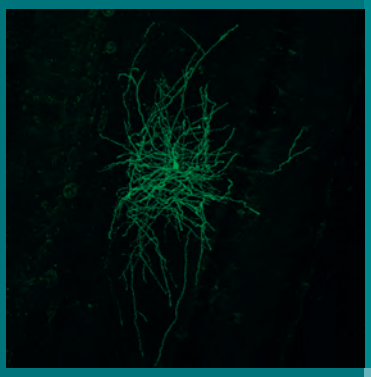
Presynaptic, preterminal axonal receptors are responsible for the local modulation of depolarisation-release coupling, including the propagation of action potentials (APs) and the modulation of release probability. Recently, strong evidence was obtained demonstrating that a high proportion (86%–93%) 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









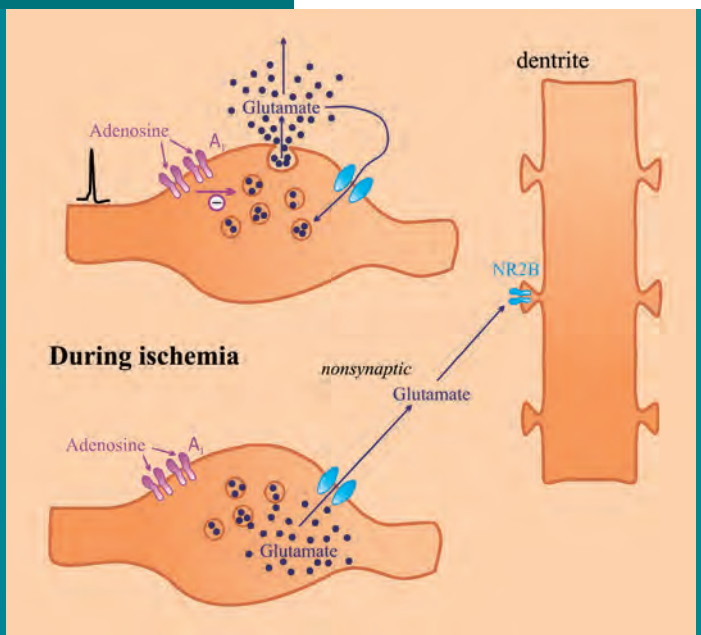
indicates that nAChRs expressed in the hippocampus are involved in synaptic plasticity and cognitive function, little is known about how this regulation occurs, particularly in brain regions known to be important for cognition. Stratum radiatum interneurons, unlike pyramidal cells, are rich in nAChRs. 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.

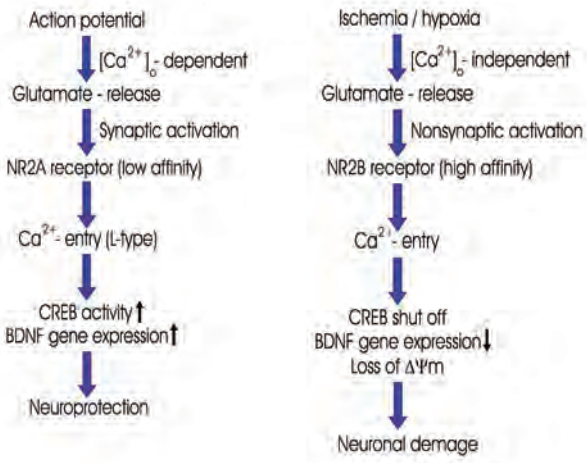
The NR2B glutamate receptor subtype is another nonsynaptic ionotropic receptor. Our laboratory intends to study the release of transmitters (e.g., glutamate) under ischaemic conditions. Extrasynaptic glutamate may activate nonsynaptic NR2B receptors, producing excitotoxicity. Using whole-cell patch-clamp 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





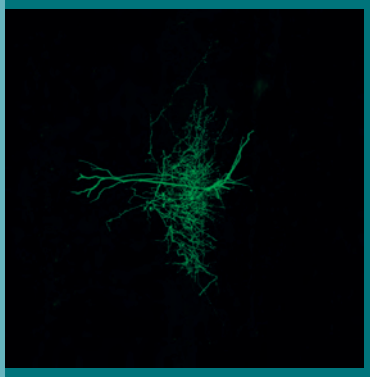
and ischaemic conditions result in $[Ca^{2+}]_o$-independent release of dopamine (Milusheva et al., Neuropharmacol. 58:816-825, 2010).

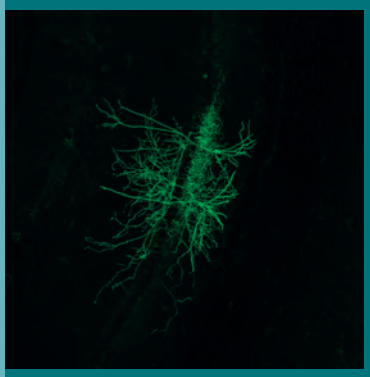
Mode of action of drugs of abuse

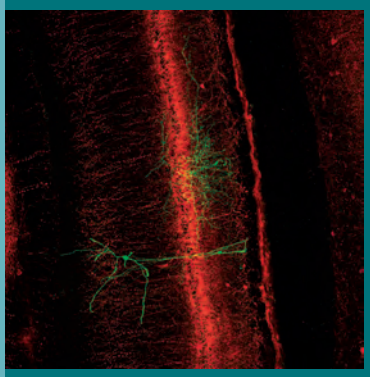
Recent investigations by the group have determined the mode of action of drugs of abuse. We provided neurochemical and pharmacological evidence that mephedrone, which is one of the most popular street drugs and functions in a similar manner to ecstasy (3,4-methylene-dioxymethamphetamine), triggers the 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).

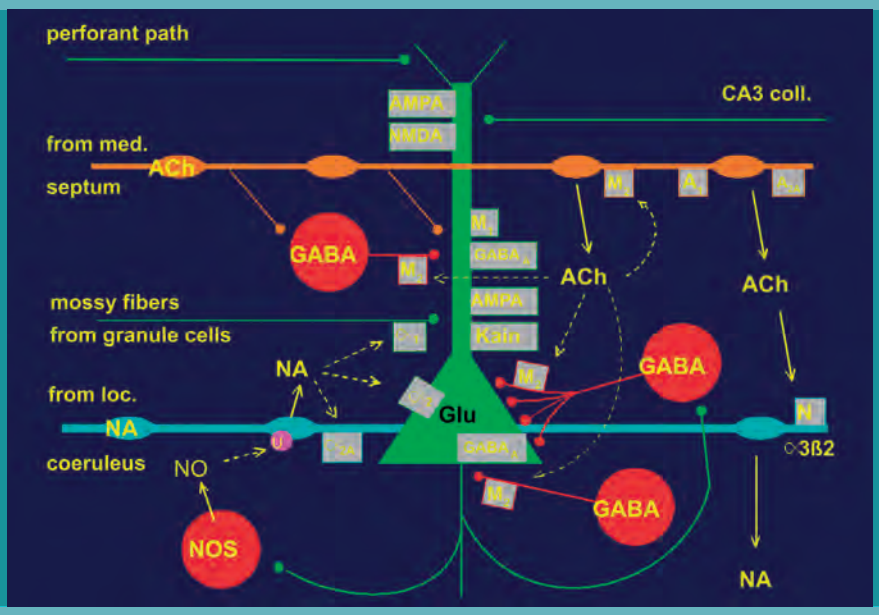
Study of Ca^{2+}-dynamics in hippocampal GABAergic varicosities. The role of presynaptic modulation and mitochondrial Ca^{2+} buffer systems

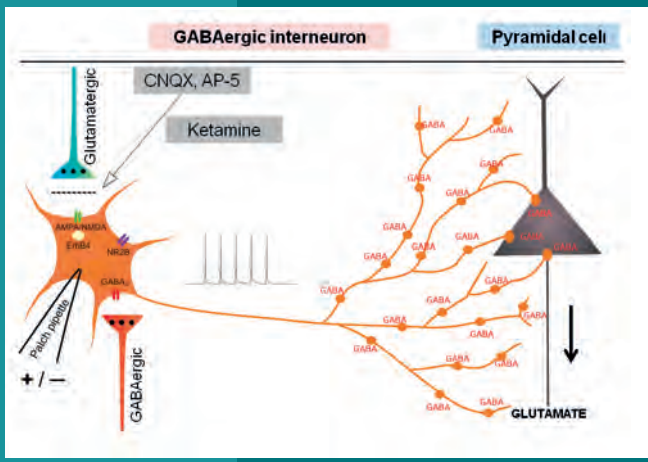
In the hippocampus, relatively uniform excitatory pyramidal cells are innervated by more than 21 different types of GABAergic interneuron. In contrast to electrophysiological studies, where the responses of the target principal cell to GABA released from the interneurons are recorded, we are able to 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-

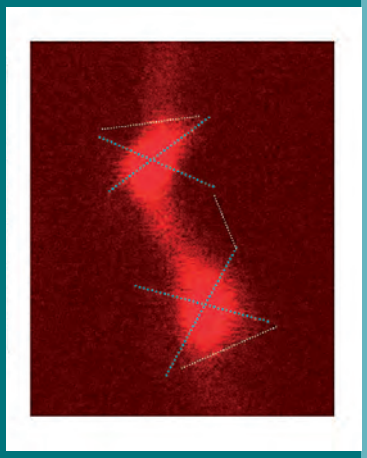


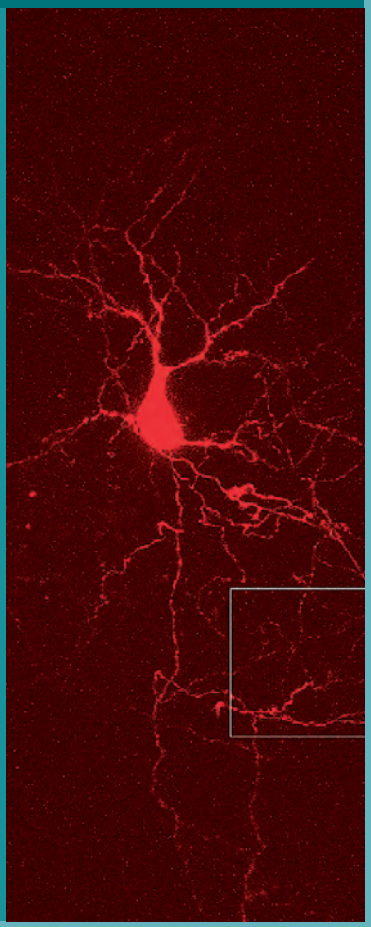












spiking (<50 Hz) and parvalbumin-positive fast spiking (>50 Hz) GABAergic interneurons present in the stratum radiatum and stratum oriens/pyramidale, respectively. No failures in Ca^{2+} transients were observed when the cell body was repeatedly stimulated, indicating reliable AP propagation from the 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

Laboratory of Molecular Pharmacology

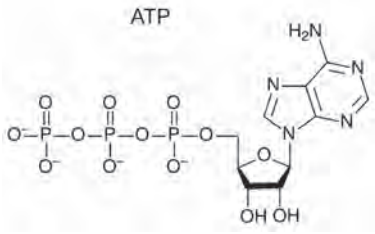
Department of Pharmacology

Head of Laboratory:
Beáta Sperlágh MD, PhD

Mission statement

Adenosine triphosphate (ATP) is one of the most versatile molecules in the living world. Besides its roles as an energy currency of cellular metabolism and as a building block of the genome, it is also an important extracellular signaling substance, acting on diverse families of P2X and P2Y receptors. The major research goal of the laboratory is to understand the role of ATP and other purines in the information processing of the normal and pathological nervous system, in order to identify target sites for therapeutic intervention in neuro-psychiatric diseases. In the past decades, the group has made substantial advances in the description of the release, extracellular metabolism and the presynaptic actions of purines, and in the identification of the receptors responsible. The group applies multidisciplinary approaches to study purinergic mechanisms, which include a wide variety of anatomical, molecular biological, neurochemical and pharmacological techniques and *in vivo* animal models of pain, neurodegenerative and psychiatric disorders. Their current research is aimed at the development of new purinergic drugs by identifying and validating new targets within this signalling system.

In addition to purinergic signalling, the lab has also substantially contributed to the research of the presynaptic modulation by other non-classical signalling systems, such as the cannabinergic system. They have described actions of exo- and endocannabinoids on the release of different neurotransmitters in the brain and identified the receptors and non-receptor mechanisms responsible. Their ongoing research includes the identification of presynaptic receptors influencing the neurotransmitter efflux from optogenetically-identified neuronal pathways.



The chemical structure of adenosine 5'-triphosphate

Simplified structure of P2X7 receptor upon single (upper panel) and prolonged (lower panel) agonist activation

Senior scientist: Ágnes Kittel PhD, Ed Beamer PhD
Research fellows: Rómeó D. Andó, Mária Baranyi
Ph.D. students: Katinka Bekő DVM, Gergely Horváth, Flóra Gölöncsér, Bence Koványi, Lilla Otrokocsi
Undergraduate research assistant: Szabina Kulcsár
Technician: Ilona Kéry
Secretary: Tünde Oroszi

Ongoing research support: Hungarian Research and Development Fund [NN107234]; Hungarian Office of Science and Technology [TÉT_10-1-2011-0050]; European Research Council Advanced Grant [294313-SERRACO]; Hungarian Brain Rese arch Program [KTIA_13_NAP-A-III/1].

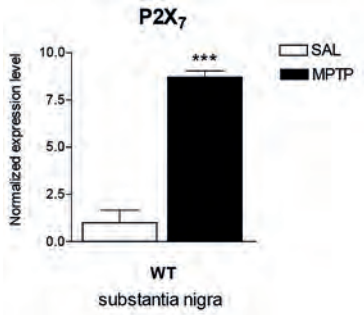
A third focus of the research is to study interactions between mitochondrial dysfunction, oxidative stress and dysregulated neurotransmitter release in the pathway leading to neurodegeneration, which characterizes many CNS diseases, including Parkinson's disease and ischemia-related neurodegeneration. Their current activity with external partners aims to develop new anti-Parkinsonian drugs with multiple sites of action.

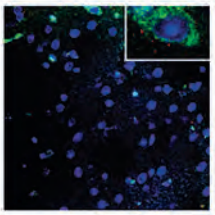
Purinergic signalling in the nervous system

The purine nucleotide ATP and its extracellular breakdown product adenosine are important neurotransmitters, neuromodulators and gliotransmitters in the nervous system. They are released from neurons and glial cells upon neuronal activity and pathological signals, and act on ionotropic P2X (P2X1-7), metabotropic P2Y ($P2Y_{1,2,4,6,11,12,13,14}$) and adenosine ($A_1$, A_{2A}, A_{2B}, A_3) receptors. Through these actions, purines participate not only in physiological information processing but also in the pathogenesis of neuro-psychiatric disorders and pain. The Sperlagh lab provided a major contribution to the knowledge on purinergic signalling, publishing more than 50 original papers in the past two decades in this field. Their achievements include the first demonstration of the presynaptic facilitation by P2 receptor activation, elucidation of the source and mechanisms of ATP release, identification, mapping and characterization of P2X and P2Y receptors involved in the regulation of transmitter release, and nucleotide catabolizing ectoenzymes (ectoATPase, adenylate kinase). Their technical repertoire also includes high resolution purine detection methods, e.g. a microelectrode biosensor technique and a variety of *in vivo* behavioral studies.

A particular focus of their interest is the ionotropic P2X7 receptor, which is a unique subtype of P2X receptors expressed on neuronal and non-neuronal cells. The group discovered that the activation of this receptor leads to increased glutamate and GABA release in the brain. Moreover, they showed that both the expression and functional responsiveness of P2X7 receptors in the brain are increased upon pathological stimuli. Based on these results they proposed the P2X7 receptor as a new target in various CNS diseases such as migraine, mood disorders and schizophrenia. The group works on the validation of this hypothesis, and on the identification of the mechanism of action of P2X7 receptor antagonists using various *in vivo* animal models and *in vitro* studies.

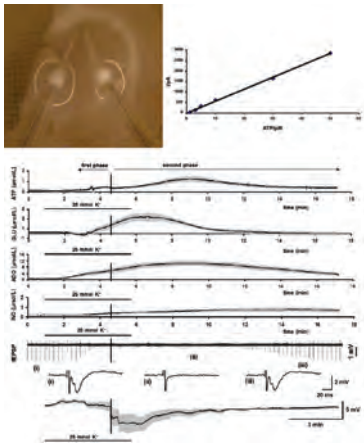
Another focus of the research is the metabotropic $P2Y_{12}$ receptor. Uniquely from P2X and P2Y receptor families, the $P2Y_{12}$ receptor is the molecular target of widely used antithrombotic drugs, such as clopidogrel or prasugrel. Accordingly, $P2Y_{12}$ receptors are highly expressed on platelets and their activation by ADP results in rapid thrombocyte aggregation. However, $P2Y_{12}$ receptors are also expressed in the central nervous system, i.e. on microglia, which raises the possibility of their utilization as a potential target in different pain modalities and neuroinflammatory diseases. The current results of the group indicate that $P2Y_{12}$ receptor inhibition leads to analgesic effects in inflammatory and neuropathic pain.

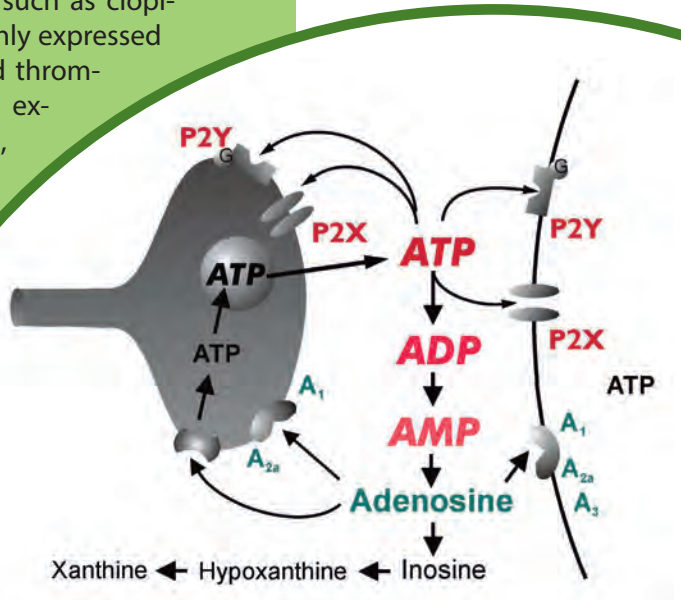




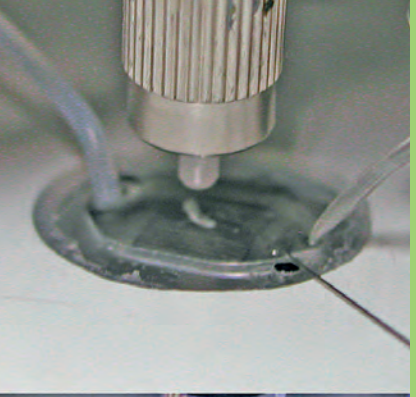
Upregulation of P2X7 receptor mRNA and protein expression in the substantia nigra (upper panel) and striatum (lower panel), respectively

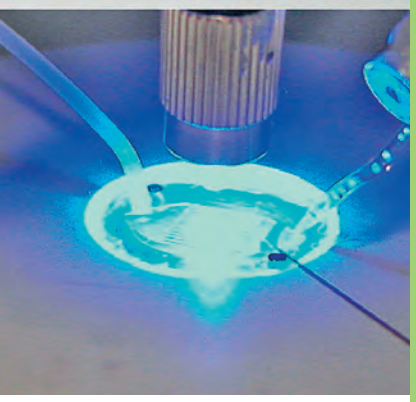
Parallel detection of the efflux ATP, adenosine, glutamate (GLU) by the microeletrode biosensor technique with simultaneous recording of field excitatory postsynaptic potentials from acute rat hippocampal slices





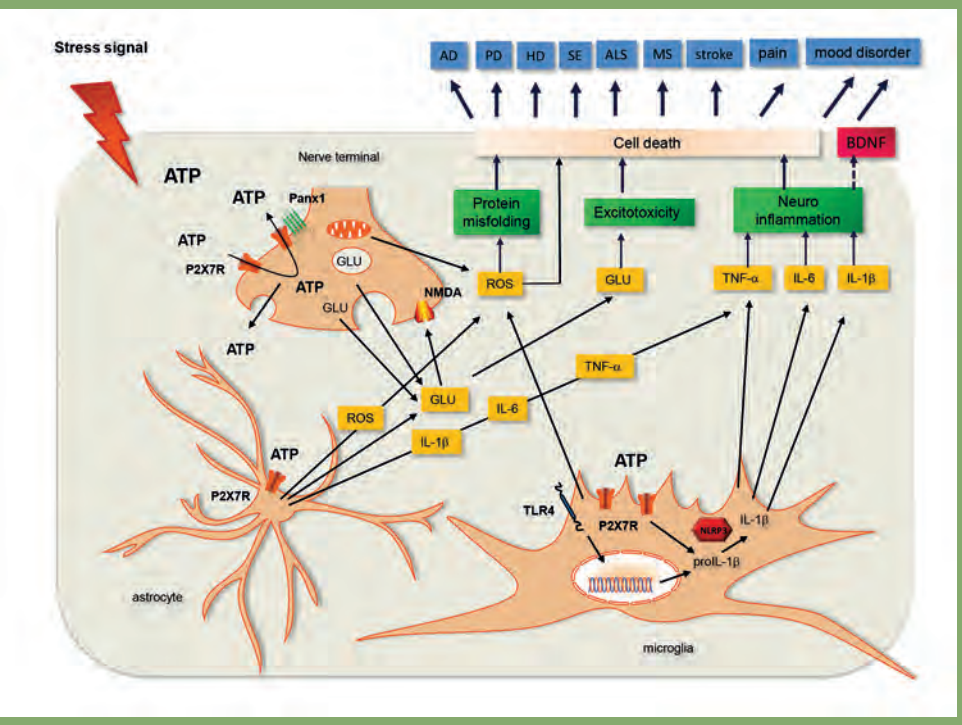
Purinergic signalling in the nervous system. The purinome consists of the enzymes and transporters responsible for the release, extracellular breakdown and uptake of nucleotides (ATP, ADP, AMP) and nucleosides (adenosine, inosine, hypoxanthine) as well as ionotropic (P2X) and metabotropic (P2Y, A_1, A_{2A}, A_3) receptors, respectively



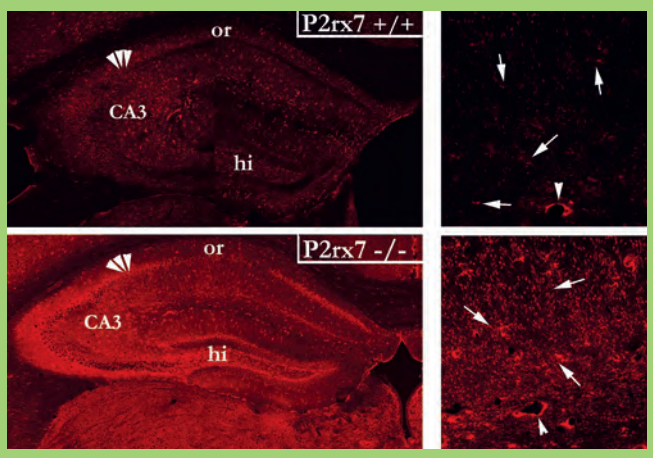


Receptor mediated modulation of neurotransmitter release

Although chemical signaling has a key role in all neural functions, measurements of transmitter concentrations and modulators directly in the extracellular space are rather rare. The main reason for this is that in the case of the traditional paradigm of synaptic transmission, transmitter release is proportional to the postsynaptic events and therefore could be efficiently monitored by electrophysiological recordings of postsynaptic events. However, several lines of recent evidence indicate that classical concepts of chemical neurotransmission represent oversimplified views. Even the fast-acting transmitters can act in a non-classical fashion, such as glutamate and GABA, which can "spill over" from synapses, or have quasi-paracrine actions maintaining tonic levels, or activating receptors by diffusion over large areas, leading to important functional consequences. These observations are even more valid for slow transmitters and non-conventional mediators, such as 5-HT and peptides. More than ever before, there is a need to perform direct measurements to answer



Common disease mechanism by P2X7R mediated pathways in CNS disorders of different etiology from Sperlágh and Illes TIPS 2014.

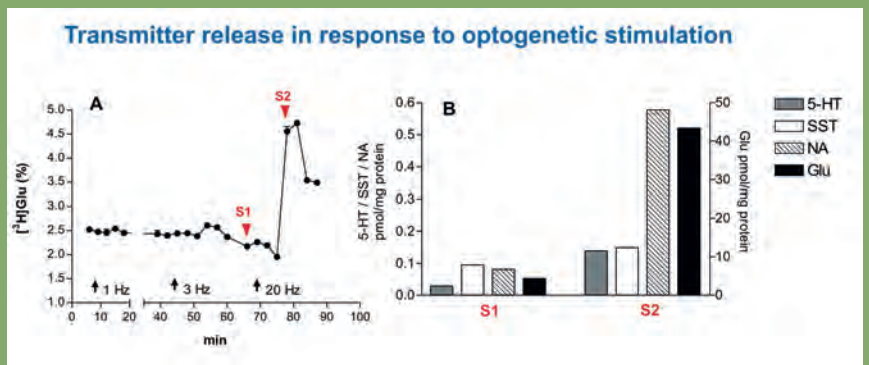


Upregulation of NR2B receptor protein in P2X7 receptor deficient mice (P2rx7-/-)

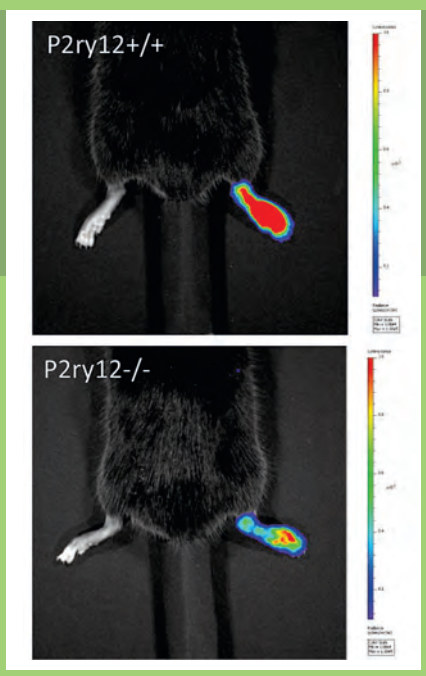
important questions on the timing, dynamics, quantity and spatial localization of the release of different transmitters. The overall aim of the current studies is to understand how the different (i.e. fast and slow) activity patterns of median raphe (MR) neurons are translated to different patterns of neurotransmitter release in one of its target areas, the hippocampus. Using optogenetic techniques, they examine how photostimulation affects glutamate and 5-HT release from the terminals of ChR2/eGFP/virus-labelled MR neurons in acute hippocampal slices. They assume that selective stimulation of ChR2-containing raphe-hippocampal fibres with different paradigms will result in qualitative and quantitative changes in the efflux of transmitters. Identifying the principles of these changes will help to understand the role of different transmitters, in particular glutamate and 5-HT, in the subcortical modulation of network activity and behaviour. In addition they explore modulatory mechanisms by M1, α_2, NMDA, AMPA $GABA_A$, $GABA_B$ and P2 receptors converging on the terminals of ChR2/eGFP/virus-labelled median raphe neurons.

Novel, multitarget drugs for neurodegenerative diseases

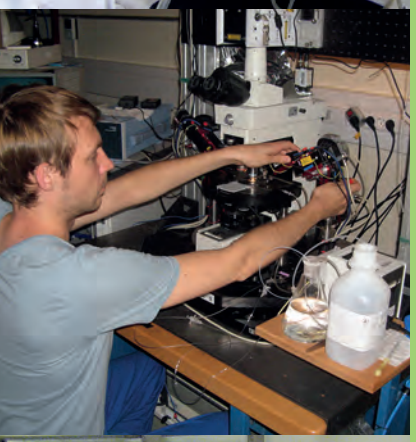
Neurodegenerative diseases (Parkinson disease, Alzheimer's disease, stroke, etc.) are characterized by the progressing loss of neurons and typically occur in the elderly population. Therefore, their incidence is continuously rising and they represent a considerable economic and social burden. Nevertheless, the treatment of neurodegenerative diseases is not yet resolved. The most likely reason for this failure is that the process leading to neurodegeneration is remarkably complex and involves numerous self-amplifying and complementary mechanisms at subcellular, cellular and systems levels. Therefore, compounds acting at single targets are unlikely to result in clinically beneficial action. Recently, a new concept of drug development has been elaborated in the lab, utilizing drugs with multiple modes of action. The group revealed



Photostimulation of the terminals of ChR2/eGFP /virus-labelled MR neurons elicits activity-dependent neurotransmitter efflux in rat hippocampal slices



In vivo imaging reveals decreased myeloperoxidase activity in the hind-paw of P2Y12 receptor deficient mice (p2ry12 -/-) after CFA treatment, when compared to wild-type mice (p2ry12+/+)



that mitochondrial dysfunction and oxidative stress have a supra-additive impact on the pathological, cytoplasmic accumulation of monoamines and their subsequent release in animal models of ischemia and Parkinson's disease. Moreover, they showed that this oxidative stress induced pathological monoamine release provides an additional source of highly reactive free radicals during their breakdown. Therefore, those drugs that simultaneously target mitochondrial dysfunction, oxidative stress and pathological dopamine release may have disease-modifying potential in addition to symptomatic improvement. Utilizing national (Department of Medicinal Chemistry, Semmelweis University, Budapest) and international (ICES, A*STAR, Singapore) collaboration with medicinal chemists, the lab works on the translation of this concept into anti-Parkinsonian drug design.

Selected publications from the last 10 years:

- Sperlágh B., Illes P. P2X7 receptor: an emerging target in central nervous system diseases. TRENDS PHARMACOL SCI. (2014) 35(10):537-547. doi: 10.1016/j.tips.2014.08.002
- Horváth G., Gölöncsér F., Csölle C., Király K., Andó R. D., Baranyi M., Koványi B., Máté Z., Hoffmann K., Algaier I., Baqi Y., Müller C. E., Von Kügelgen I., Sperlágh B., $P2Y_{12}$ receptor blockade alleviates inflammatory and neuropathic pain and cytokine production in rodents, NEUROBIOL DIS. (2014) 70:162-78. doi: 10.1016/j.nbd.2014.06.011.
- Ficker C., Rozmer K., Kató E., Andó R. D., Schumann L., Krügel U., Franke H., Sperlágh B., Riedel T. and Illes P. Astrocyte-neuron interaction in the substantia gelatinosa of the spinal cord dorsal horn via P2X7 receptor-mediated release of glutamate and reactive oxygen species. GLIA. (2014) 62:1671-86. doi: 10.1002/glia.22707.
- Gölöncsér F., Sperlágh B. Effect of genetic deletion and pharmacological antagonism of P2X7 receptors in a mouse animal model of migraine. J HEADACHE PAIN (2014) 15(1):24.
- Csölle C., Baranyi M., Zsilla G., Kittel Á., Gölöncsér F., Illes P., Papp E., Vizi E.S. and Sperlágh B., Neurochemical changes in the mouse hippocampus underlying the antidepressant effect of genetic deletion of P2X7 receptors. PLOS ONE (2013) 8:e66547.
- Csölle C., Andó R. D., Kittel A., Gölöncsér F., Baranyi M., Soproni K., Zelena D., Haller J., Németh T., Mócsai A., Sperlágh B. The absence of P2X7 receptors (P2rx7) on non-haematopoietic cells leads to selective al-

from left: Rómeó D. Andó, Ilona Kéry, Flóra Gölöncsér, Ágnes Kittel, Mária Baranyi, Bence Koványi, Katinka Bekő, Gergely Horváth, Lilla Otrokocsi, Ed Beamer. Sitting: Beáta Sperlágh

teration in mood-related behaviour with dysregulated gene expression and stress reactivity in mice. INT J NEUROPSYCHOPHARMACOL. (2013) 16:213-33.

- Timár C. I., Lorincz A. M., Csépányi-Kömi R., Vályi-Nagy A., Nagy G., Buzás E. I., Iványi Z., Kittel A., Powell D. W., McLeish K. R., Ligeti E. Antibacterial effect of microvesicles released from human neutrophilic granulocytes. BLOOD (2013) 121:510-8.
- Mátyus P., Huleatt P., Chai C. L. L., Sperlágh B., Ling K. M., Magyar K., Papp-Behr Á., Deme R., Túrós G., Gyires K., Novel (hetero)aryl propargylamines for the treatment of neurodegenerative disorders. PCT HU1300122/ W1300122 (2013)
- Dunkel P., Chai C. L., Sperlágh B., Huleatt P. B., Mátyus P. Clinical utility of neuroprotective agents in neurodegenerative diseases: current status of drug development for Alzheimers, Parkinsons and Huntingtons diseases, and amyotrophic lateral sclerosis. EXPERT OPIN INVESTIG DRUGS. (2012) 21:1267-308.
- Heinrich A., Andó R. D., Túri G., Rózsa B., Sperlágh B. K + depolarization evokes ATP, adenosine and glutamate release from glia in rat hippocampus: a microelectrode biosensor study. BR J PHARMACOL. (2012) 167:1003-20
- Hracskó Z., Baranyi M., Csölle C., Gölöncsér F., Madarász E., Kittel A., Sperlágh B. Lack of neuroprotection in the absence of P2X7 receptors in toxin-induced animal models of Parkinsons disease. MOL NEURODEGENER. (2011) 6:28
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- Sperlágh B., Vizi E. S., Wirkner K., Illes P. P2X7 receptors in the nervous system. PROG NEUROBIOL (2006) 78:327-346
- Köfalvi A., Rodrigues R. J., Ledent C., Mackie K., Vizi E. S., Cunha R. A., Sperlágh B. Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: A combined immunochemical and pharmacological analysis. J NEUROSCI (2005) 25: 2874-2884.
- Milusheva E., Baranyi M., Kittel Á., Sperlágh B., Vizi E. S. Increased sensitivity of striatal dopamine release to H_2O_2 upon chronic rotenone treatment. FREE RAD BIOL MED (2005) 39: 133-142

Laboratory of Cellular Neuropharmacology

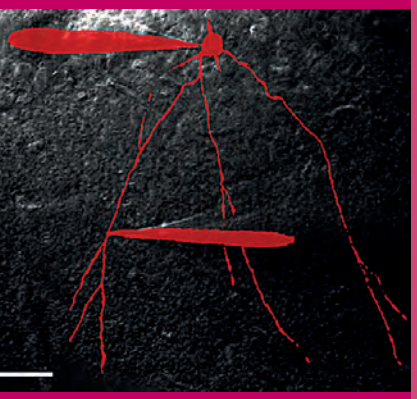
Department of Pharmacology

Head of Momentum-supported Laboratory: János Szabadics, PhD

Mission statement

The primary goal of the laboratory is to better understand the underlying neuronal circuitry of the hippocampus, in particular the cellular interface between the dentate gyrus and CA3 regions. The anatomical structure of the hippocampus is evolutionarily preserved among mammalian species, including human and rodents, and this cortical area plays crucial roles in certain learning and memory functions. The interaction of neuronal activities in the CA3 area of the hippocampus provided by mossy fibres (the axon of dentate gyrus granule cells) and auto-associative networks of local pyramidal cells is essential in the ability to distinguish novel situations from previously acquired memories. The mossy fibres introduce certain novel information about the environment in the CA3 network, and this incoming excitation is correlated with previously acquired memories that are represented by the interconnected synaptic network of local pyramidal cells. The research group focuses on the cellular mechanisms of this interaction using various techniques. The central methodology of the laboratory is *in vitro* patch clamp electrophysiology (including paired recordings of synaptically-coupled neurons, and direct dendritic and axonal recordings), which is combined with correlated anatomy and immunohistochemistry, calcium imaging, computational modelling and virus labelling. Combinations of these methods allow us to investigate how inputs are translated and processed into neuronal output within individual neurons, and what are the fundamental mechanisms of synaptic communications between individual neurons.

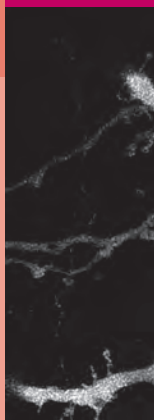
The laboratory started its operation on July 1st 2009 after a new young investigator position was created by the institute with the support of the Network of the European Neuroscience Institutes (ENI-Network). The current projects of the laboratory are funded by the Wellcome Trust, by the "Momentum" Young Investigator Program from the Hungarian Academy of Sciences and by the Hungarian Brain Research Program.



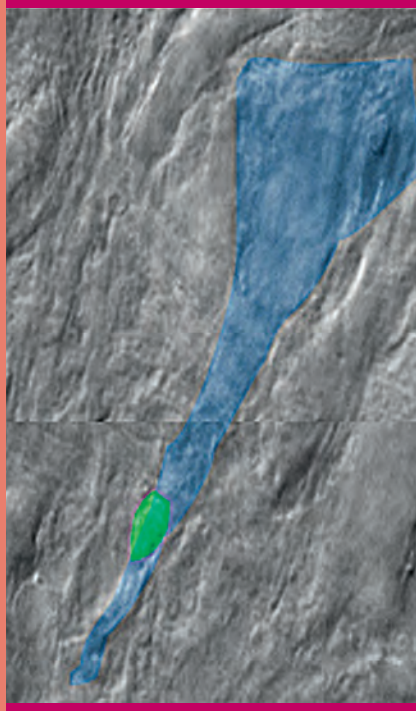
Dual somato-dendritic recording from a dentate gyrus granule cell.

Paired recording from a synaptically coupled mossy fibre axon and CA3 pyramidal cell.

Junior scientists: János Brunner, Máté Neubrandt
Undergraduate research assistant: Viktor Oláh
Technician: Dóra Kókay



The diversity of GABAergic neurons is manifested at several functional levels. One of these functionally defining properties is the cell-type specific innervations of GABAergic cells by excitatory pathways which enables pathway specific activation of distinct inhibitory circuits. We obtain simultaneous recordings from presynaptic mossy fibre terminals and synaptically-coupled CA3 neurons, which are identified with *post hoc* immunohistochemistry and morphological analysis to directly study monosynaptic connections between distant dentate gyrus granule cells and CA3 neurons, providing novel opportunities to better understand the neuronal circuitry of the hippocampus. Specifically, we are interested in how physiologically relevant activity patterns influence the neuronal output of individual hippocampal mossy fibres. Other major interests of the group are the mechanisms of the input integration in granule cells. To directly measure the propagation of signals along the dendritic arbors of granule cells we employ dendritic recordings, optical stimulation and calcium imaging, and verification of the obtained data by multicompartmental modelling. Similar research is pursued on the input integration of the CA3 GABAergic cells, which are the major targets of the mossy fibres, where we focus on the cell type-specific modulation of the input integration by potassium conductances. We also investigate the potential cellular consequences of the unique capability of the dentate gyrus to generate new neurons throughout the life of the animals by using a specific retroviral labelling method to birth-date adult-born granule cells. Altogether, these projects will reveal fundamental components of the cellular interface between the dentate gyrus and CA3 regions, and thus provide novel insights into the machinery of higher order neuronal functions.

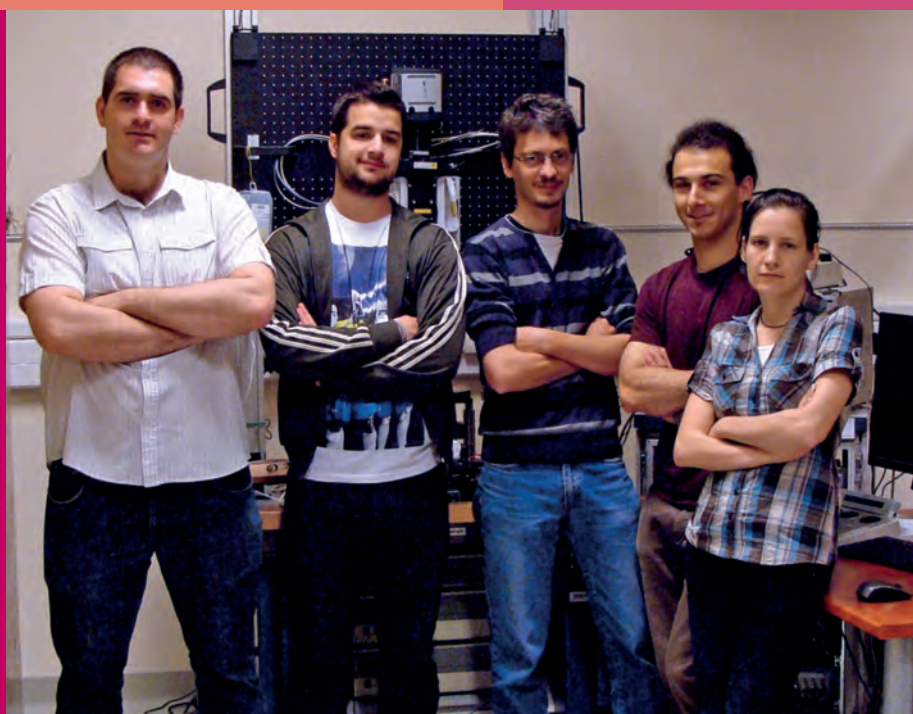


Nomarski image of the apical dendrite of a CA3 pyramidal cell (blue) and a mossy fibre terminal in acute slice.

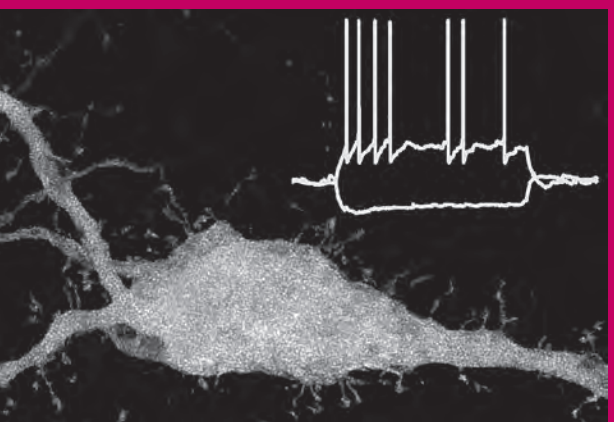
Selected publications from the last 10 years:

- Brunner J, Ster J, Van-Weert S, Andrási T, Neubrandt M, Corti C, Corsi M, Ferraguti F, Gerber U, Szabadics J. Selective Silencing of Individual Dendritic Branches by an mGlu2-Activated Potassium Conductance in Dentate Gyrus Granule Cells. J NEUROSCI 33: 7285-7298 (2013)
- Brunner J*, Neubrandt M*, Van-Weert S, Andrási T, Kleine Borgmann FB, Jessberger S, Szabadics J. Adult-Born Granule Cells Mature through Two Functionally Distinct States. eLIFE 3: e03104 (2014)

from left: János Brunner, Viktor Oláh, János Szabadics, Máté Neubrandt, Dóra Kókay



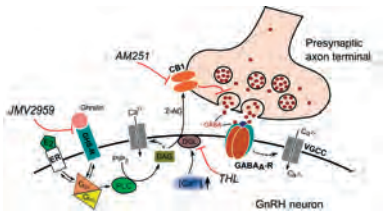
Spiny lucidum cell in the CA3 area.

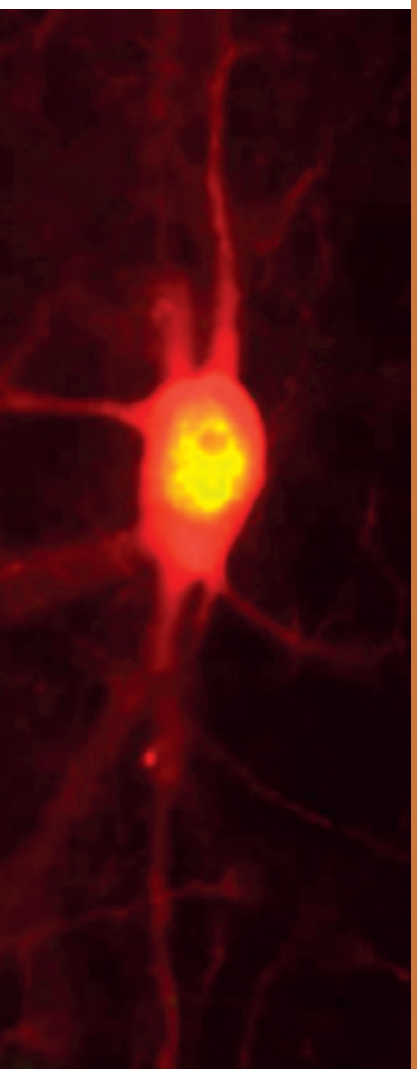


Laboratory of Endocrine Neurobiology

Department of Endocrine Neurobiology

Head of Laboratory:
Zsolt Liposits MD, PhD



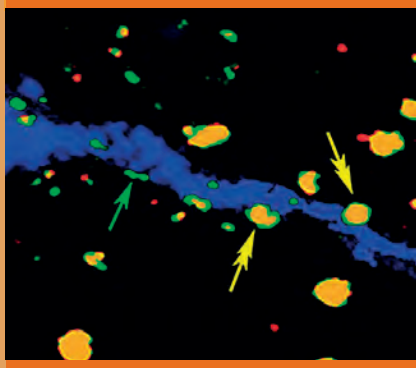


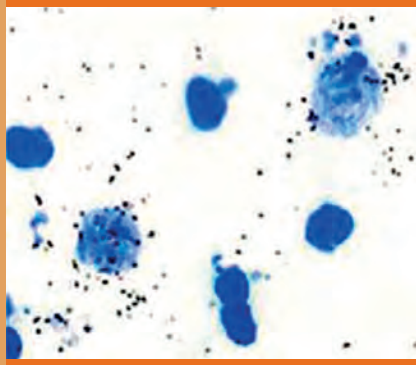
Mission statement

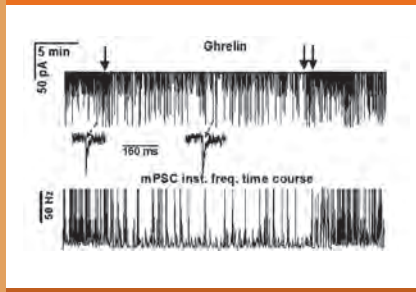
Reproduction, metabolism and adaptation are essential physiological functions of the human body that are regulated by the brain, mainly via the hypothalamus-pituitary-endocrine systems. Dysfunctions of the endocrine axes can result in severe illnesses including infertility, obesity and chronic stress. The prevention and proper medical treatment require the elucidation of central regulatory mechanisms that control the operation of the gonads, the thyroid and adrenal glands. The hypothalamus sends hormonal and neuronal messages to activate endocrine glands and peripheral organs. The communication is reciprocal. The secreted gonadal, thyroid and adrenal hormones report to the brain and the pituitary, in addition to exerting key effects on different peripheral organ systems. In the nervous system, hormone levels are sensed by specific receptors that relay the information to genomic and non-genomic cellular machineries. The hormonal regulation acting upon specific neuronal networks of the brain is manifested in the control of fertility, food intake, energy expenditure, water and salt consumption, adaptation to acute and chronic stress, mood, cognition, memory, sexual and aggressive behaviors. The Laboratory of Endocrine Neurobiology has been engaged to the exploration of the neuronal and hormonal mechanisms that take part in the physiological processes of reproduction, feeding and adaptation. State of the art methodologies are used to achieve these goals including recombinant DNA techniques, microarray, qRT-PCR, immunocytochemistry, *in situ* hybridization, electron microscopy, transgene technologies, fMRI and electrophysiology. The translation of the scientific results contributes to open new avenues in fertility, obesity and mood disorder research fields.

Senior scientists: Imre Farkas PhD, Erik Hrabovszky MD, PhD, Imre Kalló MD, PhD, Miklós Sárvári PhD
Postdoctoral fellow: Csaba Vastagh PhD
Ph.D. students: Zsuzsanna Bardóczi, Katalin Skrapits, Tamás Wilheim
Junior scientists: Csilla Maurnyi, Csilla Molnár
Technicians: Barna László, Hajnalka Bekó
Undergraduate students: Anna Csepregi, Veronika Csillag, Vivien Kanti, Zsófia Mauskopf
Secretary: Márta Turek

Hypothalamic regulatory mechanisms of reproduction





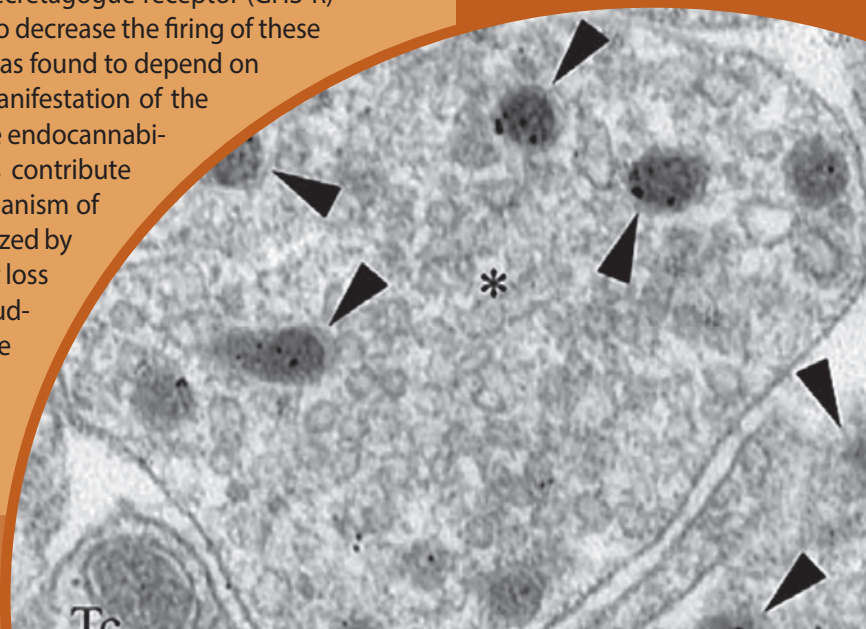


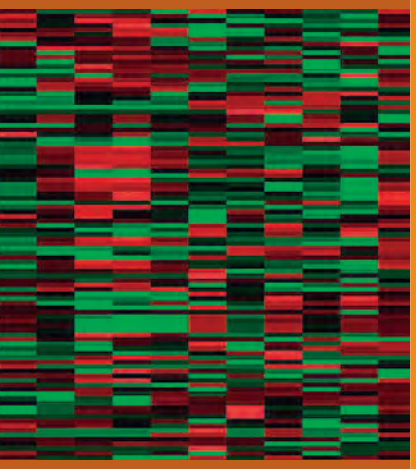
Gonadotropin-releasing hormone (GnRH)-synthesizing neurons represent the final output pathway of the hypothalamus in the neuroendocrine control of reproduction. Pulsatile GnRH secretion into the hypophysial portal circulation regulates the synthesis and release of the two adenohypophysial gonadotropins, LH and FSH, which in turn govern gonadal functions. Gonadal sex steroid hormones exert positive and negative feedback effects on the neurosecretory output of GnRH neurons via mechanisms that are poorly understood. A major research focus of the Laboratory of Endocrine Neurobiology has been on the neuronal and hormonal mechanisms that mediate the effects of 17β-estradiol on GnRH neuronal functions.

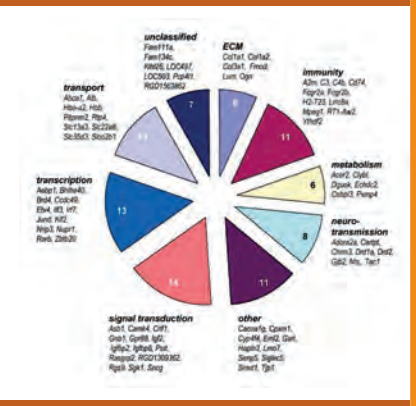
Members of the Laboratory provided the first neuromorphological evidence that, against a long-held view, GnRH neurons do possess receptors to sense circulating estrogen levels. The presence of the beta isoform of the estrogen receptor (ER-β) they observed in GnRH neurons of rats and humans was also found to characterize other hypothalamic systems that lack the classic estrogen receptor (ER-α), including vasopressin and oxytocin neurons of the magnocellular neurosecretory system. A recently revealed feature of GnRH neurons has been their glutamatergic character based on the expression of vesicular glutamate transporter-2 (vGLUT-2) mRNA and protein.

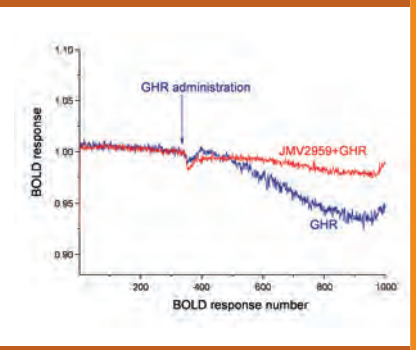
The Laboratory used light- and electron microscopic techniques on rodent and human tissues to reveal novel circuitries that may mediate sex steroid-, circadian-, metabolic- and stress signals to GnRH neurons. The newly-characterized input systems include histaminergic, cholinergic, peptidergic (AGRP/NPY), noradrenergic (DBH/NPY), GABA-ergic and glutamatergic afferents. Recent reports from the Laboratory on the distribution and connectivity to GnRH neurons of kisspeptin-, RF-amide related peptide- and neurokinin B-containing neurons have been of key importance to understand the regulation of the reproductive cycle in the human. It is noteworthy that human basal hypothalamic samples show a low level of overlap between kisspeptin, neurokinin B, and dynorphin immunoreactivities, contrasting and challenging the KNDy neuron concept in rodents. Furthermore, age and gender specific events characterize the expression of kisspeptin and neurokinin B in the human infundibular nucleus.

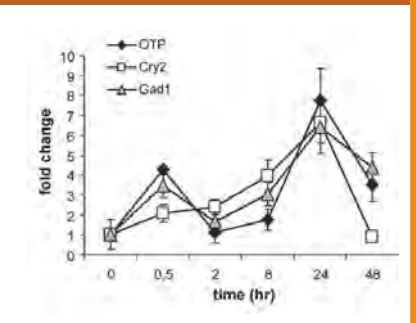
With combined electrophysiological and morphological approaches, the Laboratory has recently clarified a novel mechanism whereby endocannabinoids reduce the excitatory GABA-ergic afferent drive upon GnRH neurons. This may explain the known inhibitory actions of cannabinoids on reproduction. Studying the regulatory effects of the orexigenic hormone, ghrelin, we have revealed the expression of growth hormone secretagogue receptor (GHS-R) in GnRH neurons and the capability of ghrelin to decrease the firing of these neurons. The effectiveness of ghrelin's action was found to depend on the actual estradiol hormone milieu. In the manifestation of the effect of ghrelin, the principal role of retrograde endocannabinoid signaling was elucidated. These findings contribute to the better understanding of the pathomechanism of anorexia nervosa, the mental disease characterized by a high level of circulating ghrelin, severe weight loss and amenorrhea. Recent innovations and studies of the Laboratory have been aimed at the











expression profiling of GnRH neurons in order to discover novel regulatory mechanisms operating in the maintenance of GnRH neuron physiology.

Regulation of cortical functions by estradiol

The sex hormone 17β-estradiol (E2) is primarily synthesized in maturing ovarian follicles. Cyclic changes in serum E2 levels across the menstrual cycle exert profound effects on reproductive tissues in women. E2 also plays an important role in the maintenance of normal limbic and cortical functions. Around menopause, when E2 levels decline, the incidence of cognitive and mood disorders increases, which can be prevented with hormone replacement therapy. A major research interest of the Laboratory has been in the molecular mechanisms whereby E2 preserves good mood, capability of learning and processing memory via interactions with cortical and limbic structures. The classic actions of E2 are mediated by two estrogen receptor isoforms, ERα and ERβ. They are ligand-dependent transcription factors which regulate gene expression in the presence of E2. Prefrontal cortex (PFC) and the hippocampus are known targets of steroid hormone signaling. The Laboratory has studied the genomic responses of the prefrontal cortex and the hippocampus to E2 replacement and treatments with ERα and ERβ selective agonists. Estrogen receptor agonist-regulated genes were identified by microarray technology and selected changes were confirmed by quantitative real-time PCR. Several E2-regulated transcripts were also localized with high-resolution *in situ* hybridization and immunocytochemical techniques.
In the PFC, genomic alterations in response to E2 were partly related to dopaminergic neurotransmission, immune surveillance and transport processes. In the hippocampal formation, ovariectomy and subsequent treatment with estrogen receptor agonists powerfully tuned the innate immune system of middle-aged female rats. Analogous changes were observed in the hippocampus of post-menopausal women. The results shed light on the molecular mechanisms whereby estrogen replacement therapy preserves cortical and limbic functions.

Regulation of limbic functions via the reward system

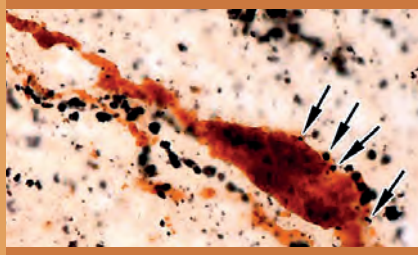
The reward system of the brain has a major contribution to the modulation of limbic functions including regulation of feeding, adaptation and reproduction. The ventral tegmental area (VTA) of the rostral mesencephalon has a pivotal role in triggering reward actions mainly via dopaminergic, mesolimbic and mesocortical projections. These neuron circuits are also regulated by gonadal steroids and metabolic signals. Recent studies of the Laboratory have made an attempt to elucidate the cooperation of neuronal and hormonal mechanisms that influences the initiation of the reward mechanism. Functional MRI (fMRI) measurements revealed the widespread action of the hunger signal, ghrelin, upon the neuronal networks of the reward system. The modulatory effects of ghrelin signaling have been demonstrated in cholinergic neurons of the latero-dorsal tegmentum and also in the basolateral amygdala.
The fMRI BOLD response evoked by amphetamine in the PFC is estrogen hormone dependent. Tract tracing studies revealed connections established by medial hypothalamic nuclei with the VTA. GABA and glutamate were con-

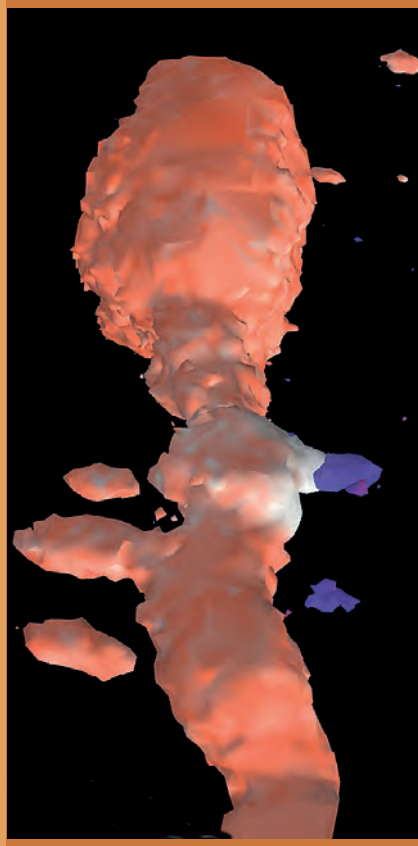
firmed as major neurotransmitters operating in the communication between the hypothalamus and VTA. The orexinergic input from the lateral hypothalamus to dopaminergic neurons of the VTA has been shown in the *post-mortem* human brain as well.

Funding: The research was supported by the Hungarian National Research Fund (OTKA K100722, K 83710, K101326), the National Development Agency of Hungary (NFU BONUS-HU08/2-2011-0006) and the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 245009.

Selected publications from the last 10 years:

- Hrabovszky E, Molnár CS, Borsay BA, Gergely P, Herczeg L, Liposits Z. Orexinergic input to dopaminergic neurons of the human ventral tegmental area. PLOS ONE 8: 12 (2013)
- Farkas I, Vastagh Cs, Sárvári M, Liposits, Z. Ghrelin decreases firing activity of gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid signaling dependent manner. PLOS ONE 8: 10 (2013)
- Sárvári M, Hrabovszky E, Kalló I, Solymosi N, Tóth K, Likó I, Szeles J, Mahó S, Molnár B, Liposits Z. Estrogens regulate neuroinflammatory genes via estrogen receptors alpha and beta in the frontal cortex of middle-aged female rats. J NEUROINFLAMM 8: 82 (2011)
- Sárvári M, Hrabovszky E, Kalló I, Galamb O, Solymosi N, Likó I, Molnár B, Tihanyi K, Szombathelyi Z, Liposits Z. Gene expression profiling identifies key estradiol targets in the frontal cortex of the rat. ENDOCRINOLOGY 151: 1161-1176 (2010)
- HrabovszkyE, Ciofi P, Vida B, Horváth MC, Keller É, Caraty A, Bloom GR, GhateiMA, Dhillo WS, Liposits Z, Kalló I. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. EUR J NEUROSCI 31:1984-1998 (2010)
- Farkas I, Kalló I, Deli L, Vida B, Hrabovszky E, Fekete C, Moenter SM, Watanabe M, Liposits Z. Retrograde endocannabinoid signaling reduces GABA-ergic synaptic transmission to gonadotropin-releasing hormone neurons. ENDOCRINOLOGY 151: 5818-5829 (2010)
- Vida B, Hrabovszky E, Kalamatianos T, Coen CW, Liposits Z, Kalló I. Oestrogen receptor alpha and beta immunoreactive cells in the suprachiasmatic nucleus of mice: distribution, sex differences and regulation by gonadal hormones. J NEUROENDOCRINOL 20: 1270-1277 (2008)





First row, from left: Barna László, Csaba Vastagh, Márta Turek, Miklós Sárvári
Second row: Csilla Molnár, Imre Farkas, Zsuzsanna Bardóczi, Imre Kalló, Flóra Bálint, Katalin Skrapits, Erik Hrabovszky, Tamás Wilheim, Zsolt Liposits

Laboratory of Molecular Neuroendocrinology

Department of Endocrine Neurobiology

Head of Laboratory:
Krisztina J. Kovács PhD

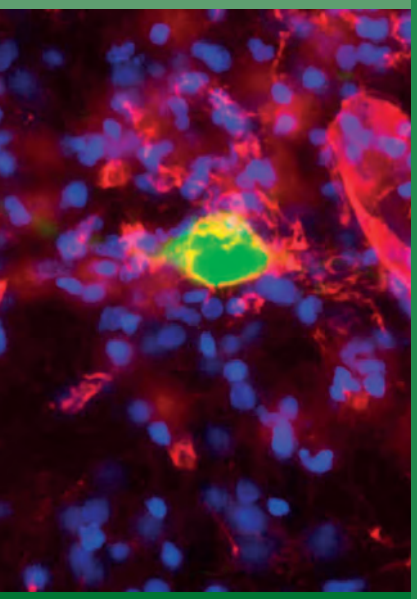
Mission statement

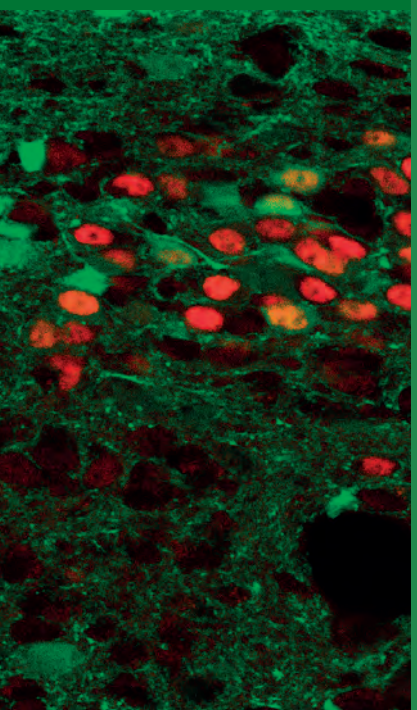
The brain orchestrates physiological responses to exogenous and endogenous stress challenges that serve adaptation. These responses are mediated by largely overlapping brain circuits of the limbic system, the hypothalamus and the brain stem. The final output is modulated according to the genetic background, current hormonal status and the overall physiological state of the organism, as well as on the epigenetic programming of early life events and previous stress experience.

The overall goal of our research is to reveal the pathways and mechanisms with which stress is perceived, processed and transduced into integrated neuroendocrine-, metabolic-, autonomic-, immune and behavioral responses. We aim to understand the assembly of neuronal circuits that are specifically recruited by physiological and psychological challenges, to identify signaling molecules that affect communication in these networks, and to study their regulation under stress. We are currently engaged in projects that aim to reveal interactions between immune-metabolic- and stress regulation. The research at the Laboratory of Molecular Neuroendocrinology involves combinations of various functional anatomical, molecular biological and physiological techniques from the molecular to the systems levels.

Understanding the neurobiology of stress by focusing on the brain circuits and genes that are associated with, or altered by, the stress response will provide important insights into the brain mechanisms by which stress affects psychological and physiological processes. Our current research focuses on the relationship between inflammation- stress- and neurological disease.

In addition to a strong research program, we are committed to translate this knowledge into the development of new treatment strategies for the prevention of stress-and inflammation-related disorders.





Senior Scientists: Ádám Dénes PhD, Szilamér Ferenczi PhD
PhD students: Rókus Kriszt, Ágnes Polyák and Zsuzsanna Winkler
Scientists Jr: Dániel Kuti and Bernadett Martinecz
Undergraduate students: Ágnes Gyöngy, Dóra Kővári and Edina Zelei

Functional Neuroanatomy of Stress-related Brain Circuits

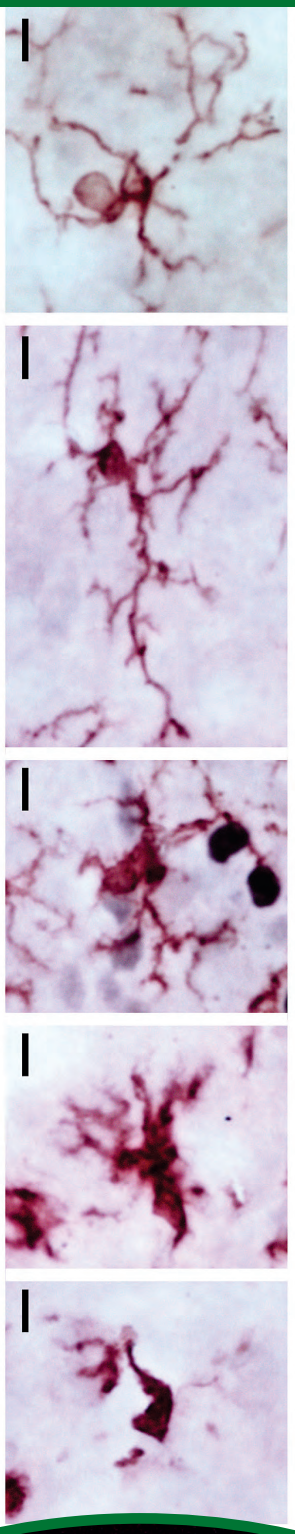
Using an immediate-early gene induction-based functional anatomical mapping strategy, previous work from the Laboratory of Molecular Neuroendocrinology has identified hypothalamic and extended brain circuits that are specifically recruited in response to physiological, psychological and immune stress challenges. They have established the spatial distribution and timing of transcriptional activation of stress-related neuropeptides, corticotropin-releasing hormone (CRH) and vasopressin, and identified the CRH-secreting parvocellular neurons as direct targets of corticosteroid negative feedback. The laboratory has provided ultrastructural and functional evidence for inhibitory GABAergic inputs that impinge upon stress-related CRH neurons and shown its functional plasticity in response to chronic variable stress.
The recent activity in this field involves projects that are aimed at understanding the neuroendocrine and immunological mechanisms which are responsible for chronic stress-induced depression. Our aim is to reveal how chronic stress exposure affects microglia function, neuroinflammation and reorganization of the limbic-hypothalamic neurocircuit in major depression. In addition to central circuits, there are ongoing research projects in the Laboratory that will establish the effects of chronic stress on the gut microbiome, mucosal immunity and gut-brain axis. In collaboration with biochemists, microbiologists and biotechnologists, the laboratory develops various psychosynbiotics.

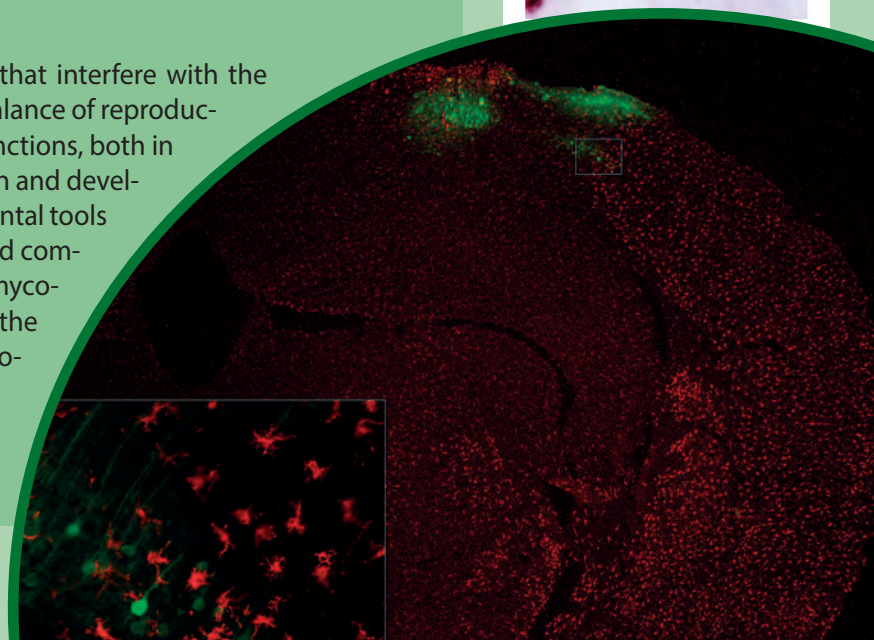
Psychoneurobiology of the Human Stress Response

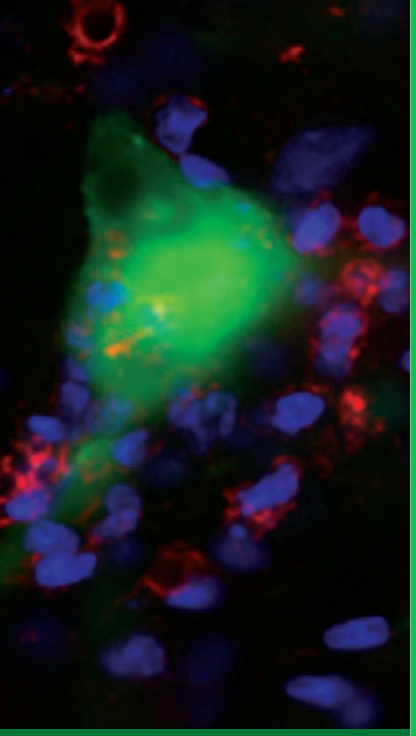
Understanding the neurobiology of stress in humans is far behind that of animal models. The Laboratory of Molecular Neuroendocrinology in collaboration with experts in genetics, psychology, psychiatry and medical technology is currently engaged in a multidisciplinary research program to analyze stress-related brain physiology and pathophysiology. With a combination of high resolution functional brain imaging techniques, autonomic recordings, behavioral analysis, and non-invasive collection of saliva samples for hormone measurement and DNA profiling, these tools are used to measure complex relationships between the recruitment of different brain areas and changes in hormonal, vegetative and behavioral responses, and to correlate these changes with genetic polymorphisms underlying individual responses to stress.

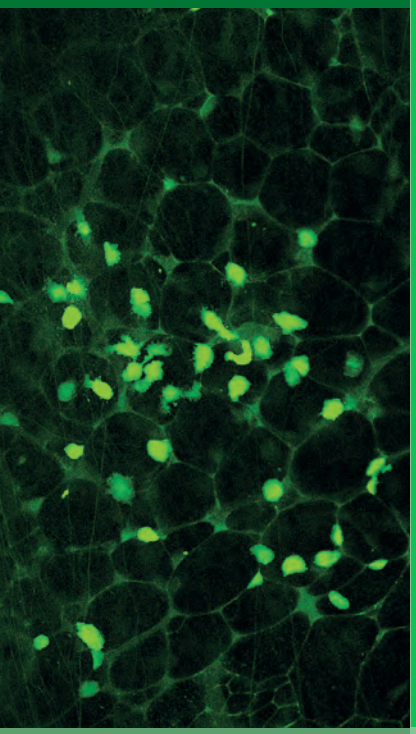
Endocrine Disruptors

Endocrine disruptors are various chemicals that interfere with the host's endocrine system and result in an imbalance of reproductive, metabolic, neurological and immune functions, both in humans and wildlife. In addition to the design and development - *in vivo* and *in vitro*- of new experimental tools with which to analyze the effects of single and complex exposures of the endocrine disruptor mycotoxin zearalenone and the pesticide atrazine, the Laboratory also aims to reveal the neuroendocrine mechanisms through which they act.









Inflammation and Obesity

Metabolic X syndrome is a serious metabolic condition characterized by abdominal obesity, glucose intolerance, insulin resistance, dyslipidemia and high blood pressure. Furthermore, in patients with MX, a low grade subclinical inflammation of the white adipose tissue is recognized. Diet-induced obesity and related peripheral and central inflammation are major risk factors for metabolic, neurological and psychiatric diseases. Recent work from the Laboratory revealed that the chemokine fractalkine (Cx3CL1) and its receptor Cx3CR1 play a pivotal role in recruitment, infiltration and proinflammatory polarization of leukocytes in the white adipose tissue of mice fed with a high fat diet.

Central and Peripheral Inflammation and Cerebrovascular Disease

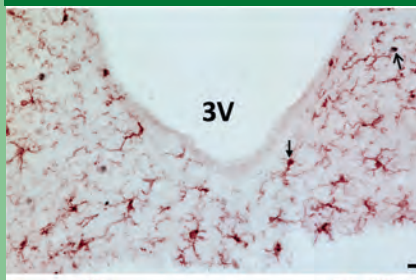
(Principal Investigator: Ádám Dénes PhD, from 2015 Head of the Laboratory of Neuroimmunology)

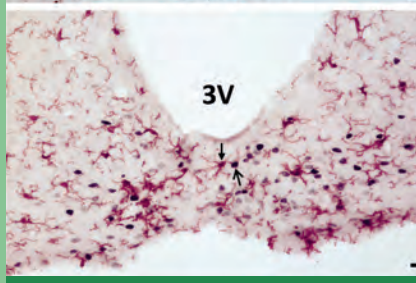
Inflammation is a key contributor to cerebrovascular disease. Recent data indicate that both central and systemic inflammatory processes are involved in the development of common brain diseases, such as stroke, schizophrenia, Alzheimer's- or Parkinson's disease and also contribute to worse clinical outcome. Our research aims at investigating mechanisms by which central and systemic inflammation mediate brain injury after stroke. Our data show that rodent models of systemic inflammation (induced by infection, obesity or atherosclerosis) show larger brain injury and worse neurological outcome after experimental stroke, and this can be prevented by blockade of inflammatory processes, such as actions mediated by the key proinflammatory cytokine interleukin 1 (IL-1). Blockade of IL-1 actions, inflammasome-mediated processes and other key proinflammatory mediators also reduces brain injury in mice without any systemic inflammatory burden after experimental stroke, indicating the potential therapeutic value of anti-inflammatory interventions. By using *in vivo* two photon imaging, SPECT/CT imaging, full transcriptome sequencing and transgenic animal models, we aim to understand how early inflamatory actions contribute to blood-brain barrier breakdown and excitotoxicity after acute brain injury. We also study the role of inflammation in the pathophysiology of neonatal asphyxia and central herpes virus infections.

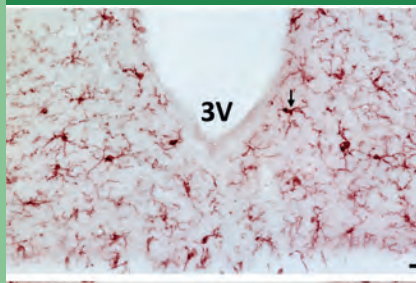
from left: Szilamér Ferenczi, Ádám Dénes, Ágnes Polyák, Krisztina Kovács, Nikolett Lénárt, Zsuzsanna Winkler, Dániel Kuti

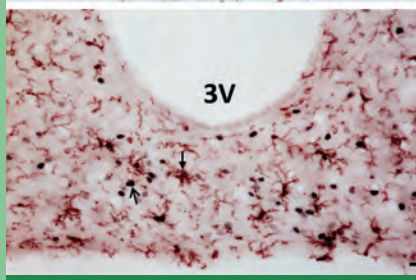
Selected publications from the last 10 years:

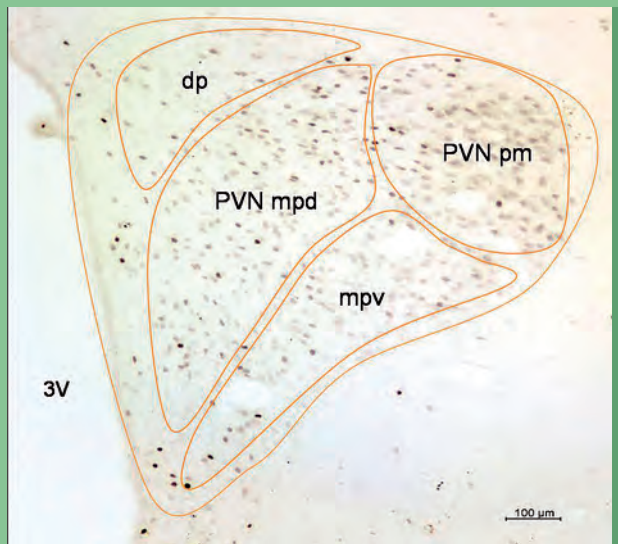
- Polyak A, Ferenczi S, Denes A, Winkler Z, Kriszt R, Pinter-Kubler B, Kovacs KJ The fractalkine/Cx3CR1 system is implicated in the development of metabolic visceral adipose tissue inflammation in obesity. BRAIN BEHAVIOR AND IMMUNITY (2014)
- Denes A, Pradillo JM, Drake C, Sharp A, Warn P, Murray KN, Rohit B, Dockrell D, Chamberlain J, Casbolt H, Francis S, Martinecz B, Nieswandt B, Rothwell N, Allan SM Streptococcus pneumoniae worsens cerebral ischaemia via IL-1 and platelet GPIbalpha ANNALS OF NEUROLOGY (2014)
- Kovacs KJ CRH: The link between hormonal-, metabolic- and behavioral responses to stress. JOURNAL OF CHEMICAL NEUROANATOMY 54: pp. 25-33. (2013)
- Smith CJ, Lawrence CB, Rodriguez-Grande B, Kovacs KJ, Pradillo JM, Denes A The Immune System in Stroke: Clinical Challenges and Their Translation to Experimental Research JOURNAL OF NEUROIMMUNE PHARMACOLOGY 8:(4) pp. 867-887. (2013)
- Pinter-Kubler B, Ferenczi S, Nunez C, Zelei E, Polyak A, Milanes MV, Kovacs KJ. Differential Changes in Expression of Stress- and Metabolic-Related Neuropeptides in the Rat Hypothalamus during Morphine Dependence and Withdrawal. PLOS ONE 8:(6) p. e67027. (2013)
- Miklós I, Kovács KJ Reorganization of Synaptic Inputs to the Hypothalamic Paraventricular Nucleus During Chronic Psychogenic Stress in Rats BIOLOGICAL PSYCHIATRY 71:(4) pp. 301-308. (2012)
- Kriszt R, Krifaton C, Szoboszlay S, Cserháti M, Kriszt B, Kukolya J, Czéh Á, Fehér-Tóth S, Török L, Szoke Z, Kovács KJ, Barna T, Ferenczi S A New Zearalenone Biodegradation Strategy Using Non-Pathogenic Rhodococcus pyridinivorans K408 Strain PLOS ONE 7:(9) Paper e43608. 9 p. (2012)
- Bajayo A, Bar A, Denes A, Bachar M, Kram V, Attar-Namdar M, Zallone A, Kovacs KJ, Yirmiya R, Bab I Skeletal parasympathetic innervation communicates central IL-1 signals regulating bone mass accrual PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 109:(38) pp. 15455-15460. (2012)
- Denes A, Ferenczi S, Kovacs KJ Systemic inflammatory challenges compromise survival after experimental stroke via augmenting brain inflammation, blood-brain barrier damage and brain oedema independently of infarct size JOURNAL OF NEUROINFLAMMATION 8: p. 164. (2011)
- Ferenczi S, Zelei E, Pinter B, Szoke Z, Kovacs KJ Differential regulation of hypothalamic neuropeptide y hnRNA and mRNA during psychological stress and insulin-induced hypoglycemia MOLECULAR AND CELLULAR ENDOCRINOLOGY 321:(2) pp. 138-145. (2010)

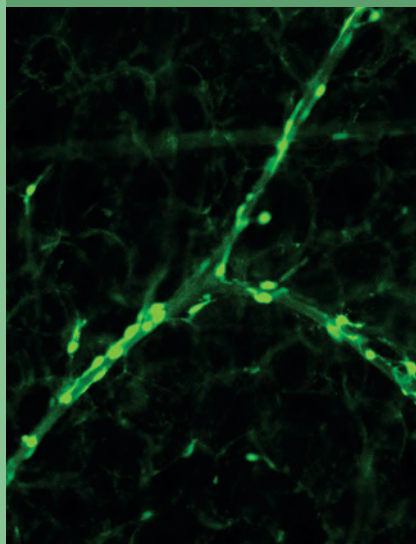












Laboratory of Integrative Neuroendocrinology

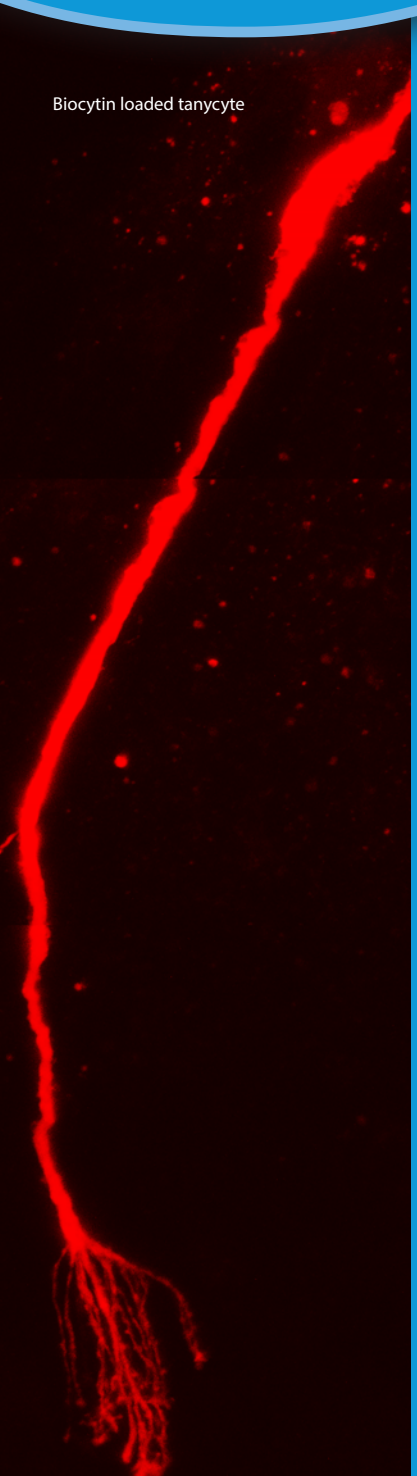
Department of Endocrine Neurobiology
Head of Momentum-supported Laboratory:
Csaba Fekete, MD, PhD

Mission statement

Obesity has risen to epidemic level in Europe. It causes devastating and costly health problems and reduces life expectancy. Despite the high impact of obesity on population health, reasonable medical therapy is currently unavailable due to the absence of proper drug targets. Therefore, understanding the mechanisms regulating the energy homeostasis has critical importance.

Complex interplay of peripheral organs and the central nervous system is critical for the regulation of energy homeostasis. The peripheral organs report the actual conditions of energy stores and the amount of consumed nutrients via peripheral sensory nerves and circulating hormones and metabolites to the energy homeostasis related circuits of the central nervous system. These circuits integrate the peripheral signals with inputs from other neuronal circuits, like the reward related neuronal networks, and regulate the energy homeostasis by controlling the hypothalamic-pituitary-endocrine axes, food intake, locomotor activity and the sympathetic and parasympathetic inputs of the peripheral organs.

The major goal of the Laboratory is to elucidate the anatomy and physiology of the neuronal networks involved in the central regulation of the energy homeostasis in rodents and humans. Special attention is paid to research focusing on the integration of the hypophysiotropic thyrotropin-releasing hormone-releasing hormone synthesizing neurons into neuronal networks regulating energy homeostasis. The laboratory described the anatomy and the physiological role of neuronal circuits involved in the regulation of the HPT axis during fasting. In their current studies they elucidate the role of the short and long term neuronal plasticity in this regulatory process. The laboratory also demonstrated



Biocytin loaded tanycyte

Postdoctoral fellows: Zoltán Péterfi PhD, Mónika Tóth PhD, Barbara Vida PhD
PhD students: Erzsébet Farkas, Andrea Kádár, Anett Szilvásy-Szabó, Györgyi Zséli
Junior scientists: Judit Szabon MSc, Edina Varga
Technician: Ágnes Simon

that the local increase of T3 (the active form of thyroid hormone) concentration in the hypothalamus that is caused by increased type 2 deiodinase activity of the special glial cells, the tanycytes, is responsible for the infection induced inhibition of the HPT axis. As a continuation of these studies, they further explore the role of tanycytes in the regulation of the HPT axis and the energy homeostasis. Another focus of the Laboratory is elucidation of the anatomy and physiology of novel satiety related neuronal networks. The methodologies used by the laboratory to achieve these goals include expression profiling, laser capture microdissection, immunohistochemistry, *in situ* hybridization, electron microscopy, transgene technologies, electrophysiology, optogenetics and metabolic profiling.

Ongoing Research Support:
Lendület program of the Hungarian Academy of Sciences, Hungarian Scientific Research Fund (OTKA K109710), National Brain Research Program, Seventh EU Research Framework Programme (Health-F2-2010-259772).

Selected publications from the last 10 years:

- Fekete C, Lechan RM. Central Regulation of Pituitary-Thyroid Axis Under Physiological and Pathophysiological Conditions. ENDOCRINE REVIEWS 35(2)pp. 159-194 (2014)
- Fonseca TL, Medina MC, Campos MPO, Wittmann G, Werneck-de-Castro JP, Arrojo e Drigo Rafael, Mora-Garzon ME, Ueta CB, Caicedo A, Fekete Csaba, Gereben B, Lechan RM, Bianco AC. Coordination of hypothalamic and pituitary T3 production regulates TSH expression. JOURNAL OF CLINICAL INVESTIGATION 123:(4) pp. 1492-1500. (2013)
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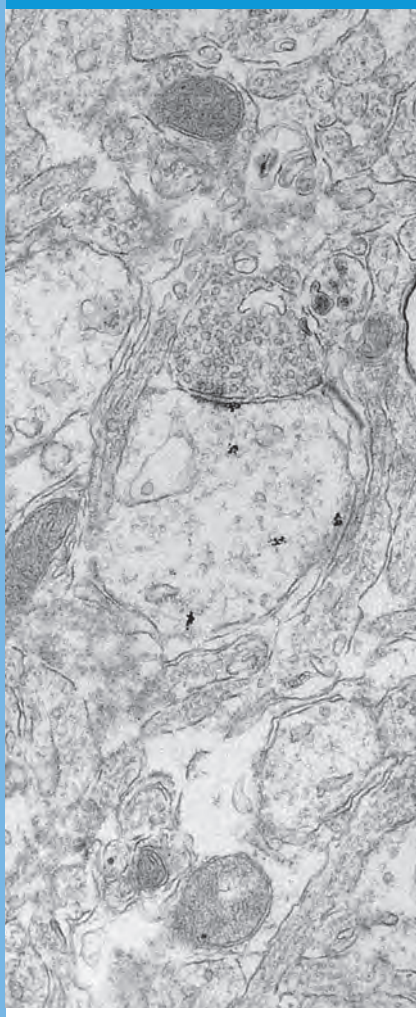
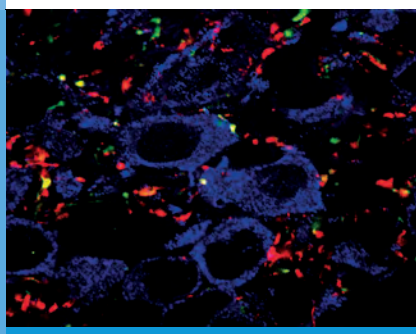
- Füzesi T, Wittmann G, Liposits Z, Lechan RM, Fekete C. Contribution of noradrenergic and adrenergic cell groups of the brainstem and agouti-related protein (AGRP)-synthesizing neurons of the arcuate nucleus to neuropeptide-Y innervation of corticotropin-releasing hormone neurons in hypothalamic paraventricular nucleus of the rat. ENDOCRINOLOGY 148: pp. 5442-5450. (2007)
- Fekete C, Gereben B, Doleschall M, Harney JW, Dora JM, Bianco AC, Sarkar S, Liposits Z, Rand W, Emerson C, Kacskovics I, Larsen PR, Lechan RM. Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: Implications for the nonthyroidal illness syndrome. ENDOCRINOLOGY 145: pp. 1649-1655. (2004)

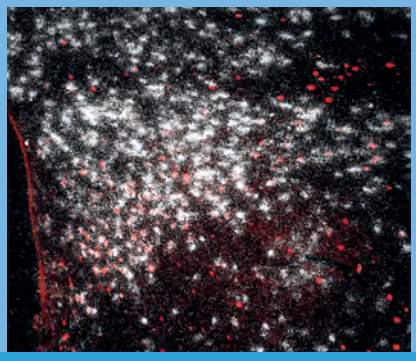
Ultrastructural localization of the neuronal nitric oxide synthase in the hypothalamic paraventricular nucleus



Noradrenergic and adrenergic innervation of CRH neurons in the hypothalamic paraventricular nucleus.



from left: Zsuzsa Beliczai, Erzsébet Farkas, Anett Szilvásy-Szabó, Csaba Fekete, Judit Szabon, Barbara Vida, Györgyi Zséli



Presence of VGLUT2 mRNA in the refeeding-activated neurons in the hypothalamic paraventricular nucleus.

Laboratory of Molecular Cell Metabolism

Department of Endocrine Neurobiology

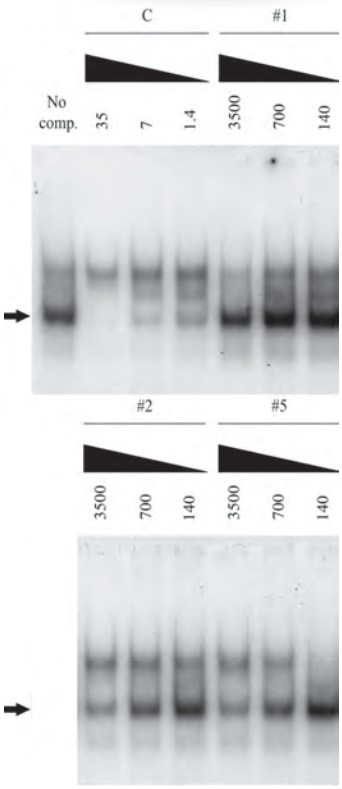
Head of Laboratory:
Balázs Gereben, DVM, PhD

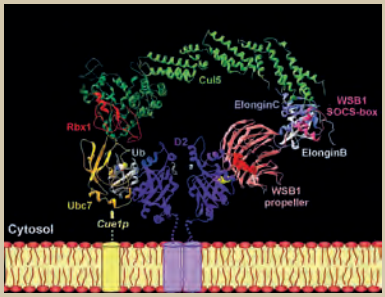
Mission statement

The major goal of the Laboratory is to understand how cell-type specific thyroid hormone (TH) regulation affects brain function and nervous system-controlled peripheral events under physiological and pathophysiological conditions, and to identify the underlying cellular and molecular pathways.

TH is a master regulator of cellular metabolism and proliferation, and exerts a fundamental impact on brain development and function. Despite its relatively stable plasma level, intracellular concentration of TH undergoes rapid and turbulent changes to meet the current needs of specific cellular conditions. The hypothalamo-hypophyseal-thyroid (HPT) axis dominates plasma TH levels via its stable prohormone, thyroxin (T4). Therefore, the axis is unable to perform quick and cell-type specific regulation of intracellular TH levels. This is achieved by cell-type specific TH metabolism catalyzed by deiodinase enzymes, allowing rapid activation and inactivation of TH. In the brain, the type 2 deiodinase (D2) selenoenzyme catalyzes TH activation in the glial compartment by activating T4 to T3, the compound that can effectively bind TH receptors. In contrast, type 3 deiodinase (D3) is responsible for T3 degradation in neurons.

The Laboratory contributed to the description of a complex molecular network allowing temporally and spatially controlled regulation of TH-dependent gene expression. They combine methods of cellular and molecular neurobiology with cell-type specific *in vivo* modulation of gene expression in transgenic mice. They aim to understand how neuro-glial TH economy mediates the function of hypothalamic hypophysiotropic neurons and cell proliferation in adult neurogenic brain niches.





Dissecting the molecular regulation of deiodination

The Laboratory studies molecular regulation of D2 and D3 at multiple regulatory levels including transcriptional and post-transcriptional

Ph.D. students: Péter Egri, Petra Mohácsik
Junior scientist: Zsuzsanna Kvarta-Papp
Technician: Andrea Juhász
Undergraduate student: Richárd Sinkó

Ongoing Research Support: Hungarian Scientific Research Fund (OTKA K109415), National Brain Research Program, EU FP7.

events. These efforts involve the identification of molecular elements and protein-protein interactions allowing the rapid regulation of T3 generation via ubiquitination using fluorescence resonance energy transfer and recombinant protein studies. They also investigate the regulation of the D2-encoding *dio*2 gene during hypothalamic response to inflammation, a phenomenon they described as a component of the nonthyroidal illness syndrome.

Understanding neuron-glial coupling in thyroid hormone metabolism

They study the mechanism and molecular components of neuron-glial coupling of TH metabolism and the biological impact of this process. They revealed a novel pathway regulating hypothalamic HT signaling and investigate how local TH affects the regulation of the HPT axis and the intracellular energy homeostasis of hypothalamic neurosecretory neurons. They also aim to identify TH dependent pathways in the regulation of cell proliferation and differentiation in adult neurogenesis.

In vivo assessment of thyroid hormone signaling in the brain

The Laboratory is involved in the generation of transgenic mouse models for cell-type specific modulation and assessment of TH signaling.

Selected publications from the last 10 years:

- Egri P., Gereben B. Minimal requirements for ubiquitination mediated regulation of thyroid hormone activation. J MOL ENDOCRINOL. 53(2):217-26 (2014)
- Kalló I., Mohácsik P., Vida B., Zeöld A., Bardóczi Z., Zavacki A. M., Farkas E., Kádár A., Hrabovszky E., Arrojo E Drigo R., Dong L., Barna L., Palkovits M., Borsay B. A., Herczeg L., Lechan R. M., Bianco A. C., Liposits Z., Fekete C. and Gereben B. A novel pathway regulates thyroid hormone availability in rat and human hypothalamic neurosecretory neurons. PLOS ONE. 7(6):e37860. doi: 10.1371/journal.pone.0037860 (2012)
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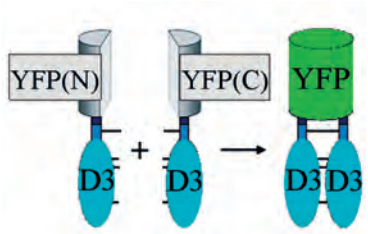
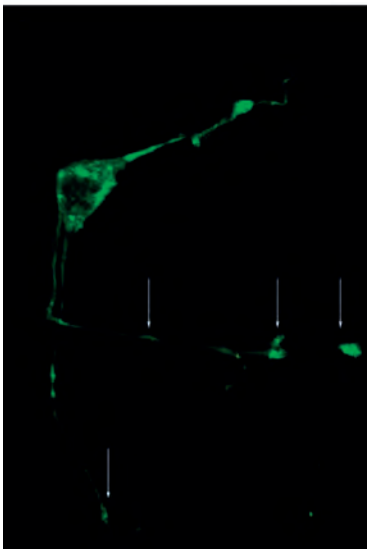
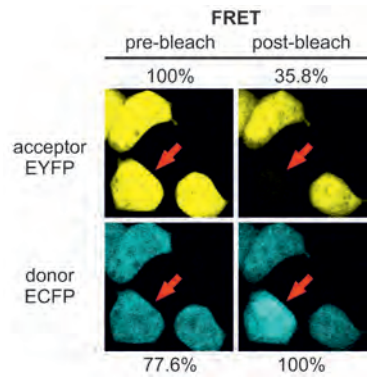
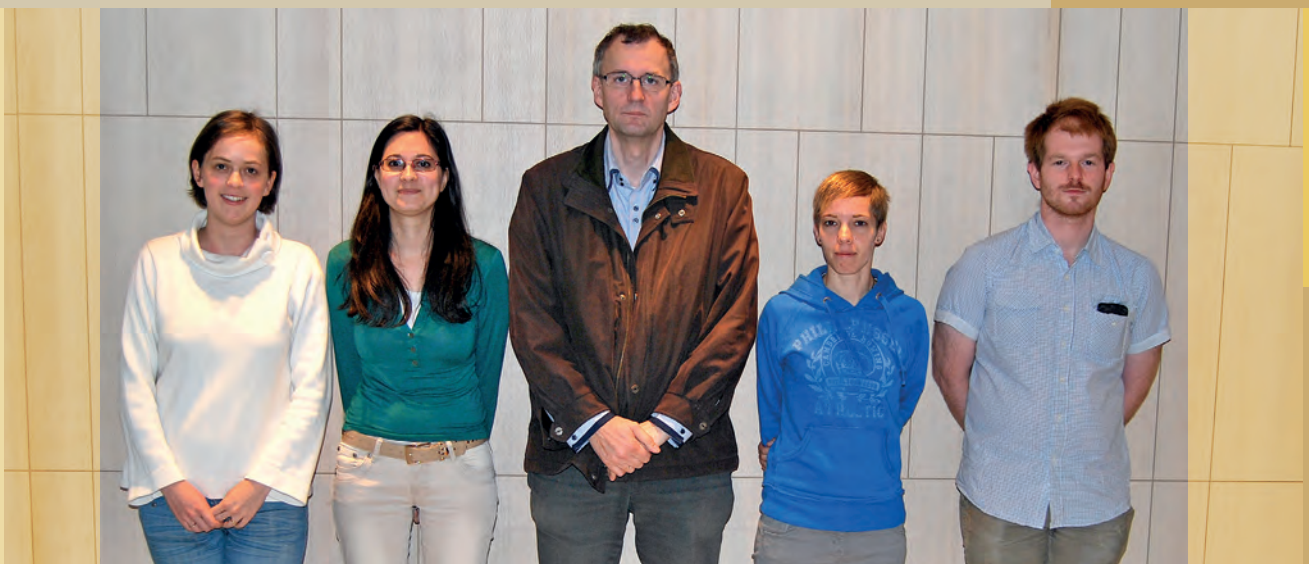








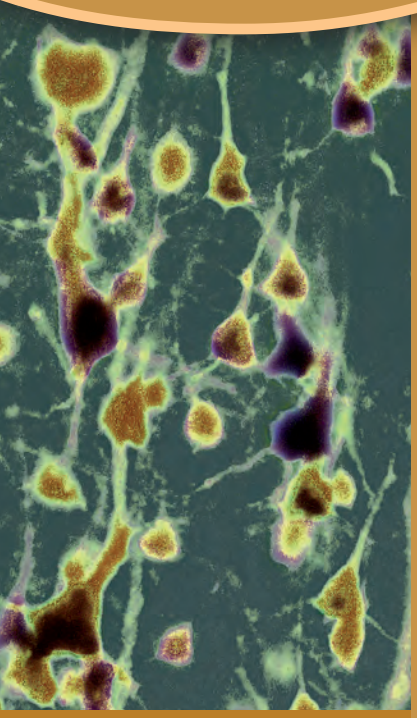
from left: Petra Mohácsik, Zsuzsanna Kvárta-Papp, Balázs Gereben, Andrea Juhász, Péter Egri

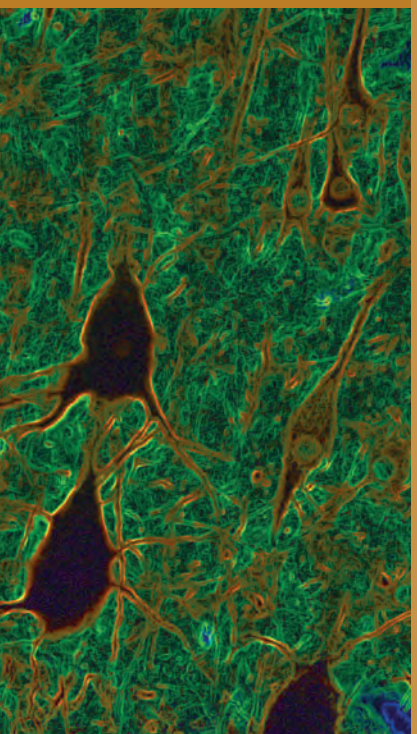
Tamás F. Freund

Laboratory of Cerebral Cortex Research

Department of Cellular and Network Neurobiology

Head of Laboratory: Tamás F. Freund, PhD

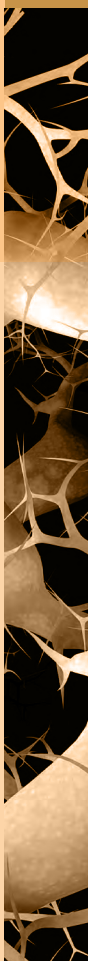




Mission statement

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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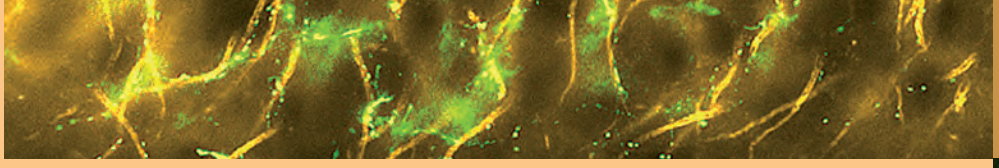
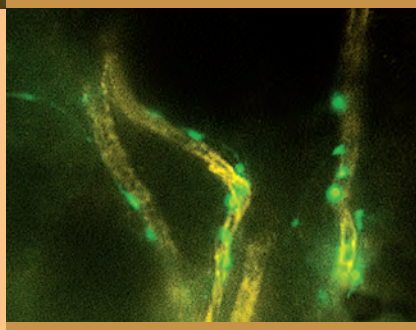
Ongoing Research Support: Hungarian National Research Foundation (OTKA K83251, OTKA NN102802, K109790), European Research Council Advanced Grant (ERC-2011-ADG-294313 SERRACO), European Union (FP7-ICT-2013-FET-F/ 604102, Human Brain Project).



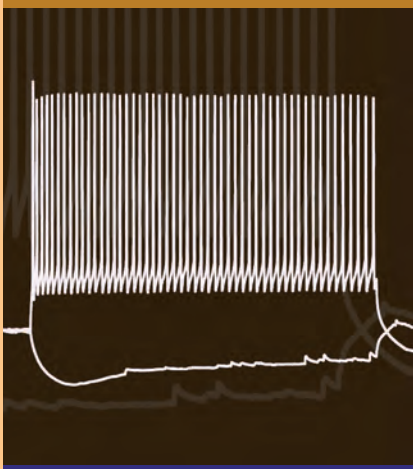
Chandelier cell terminals targeting axon initial segments.

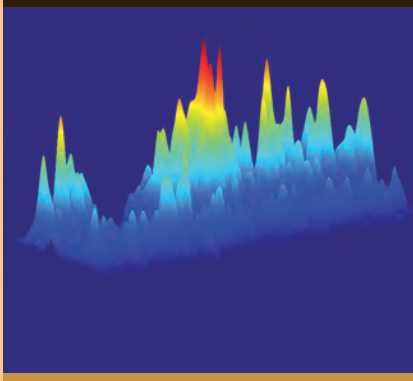


cidate the functional roles of inhibitory cell types in the control of population synchrony and synaptic plasticity in the hippocampus, their local as well as subcortical modulation via selective afferent pathways - including GABAergic and cholinergic septal, as well as serotonergic raphe input - and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

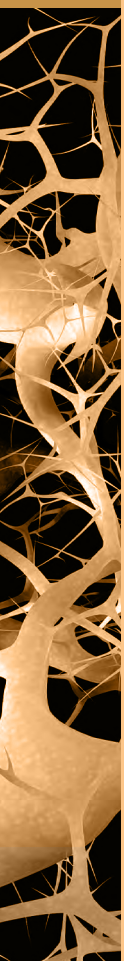


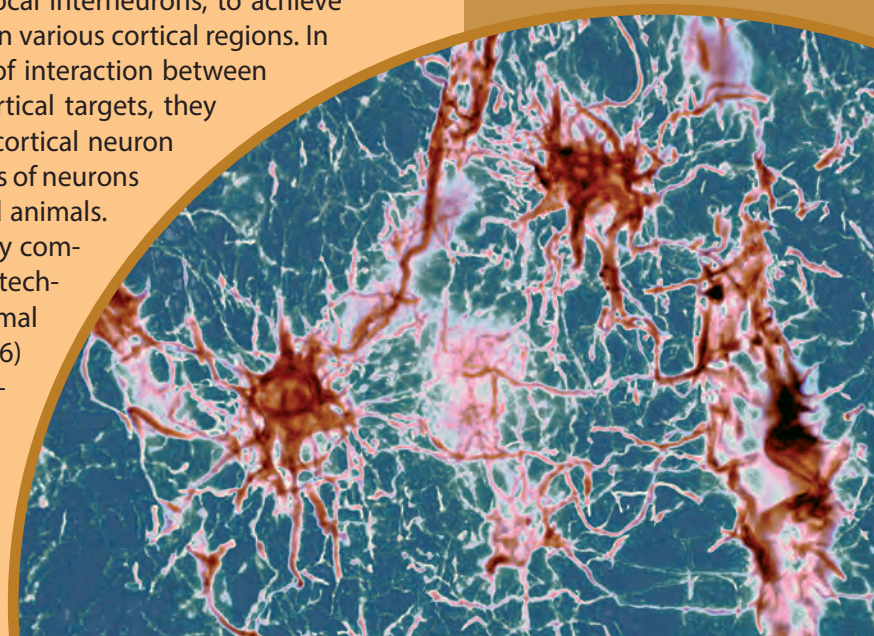


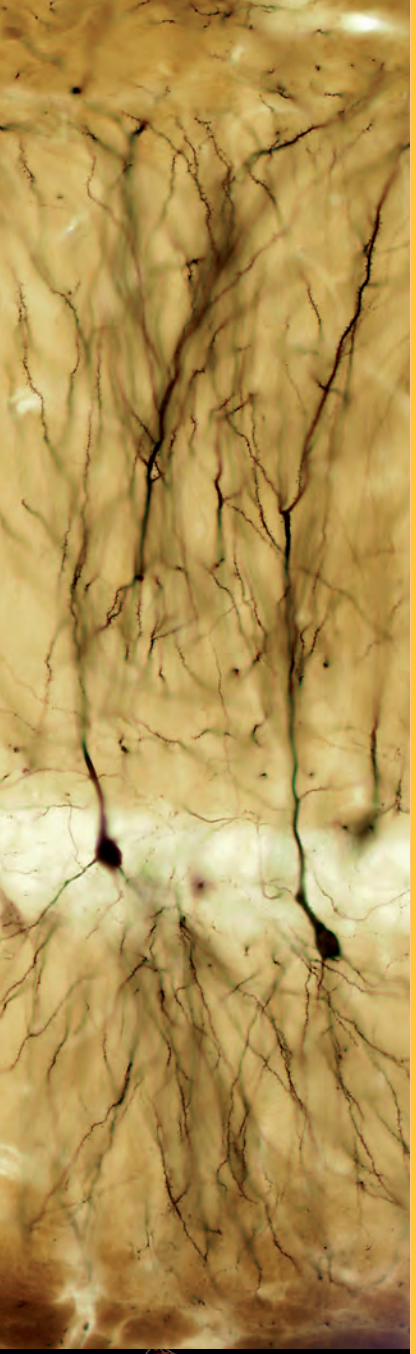
Glial cells in epileptic tissue

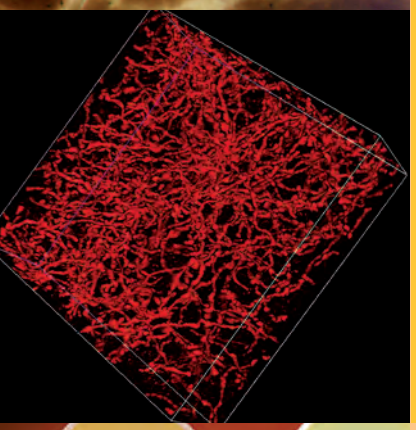
Subcortical control of hippocampal microcircuits

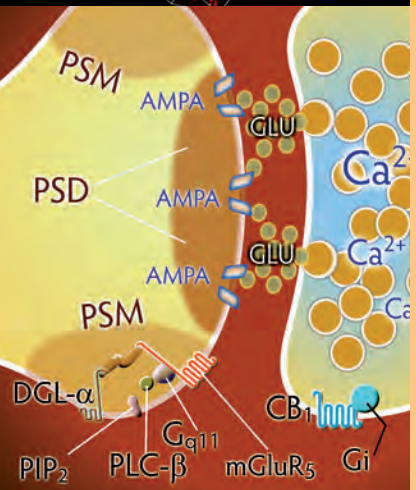
Since the early 1990's the Freund group made major discoveries related to the selective innervation of hippocampal interneurons by GABAergic pacemaker neurons in the medial septum, and the role of this connection in the formation of hippocampal theta oscillations. They showed that other subcortical pathways 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.











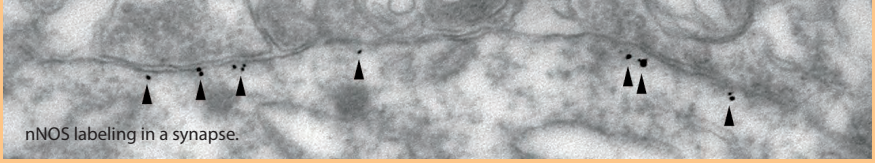
The group investigates the GABAergic feedback from the hippocampus to the medial septal pacemaker circuitry by selectively manipulating the somatostatin-expressing hippocampo-septal (HS) fibers with the optogenetic approach, and record the response of medial septal neurons both in urethane-anesthetized 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-

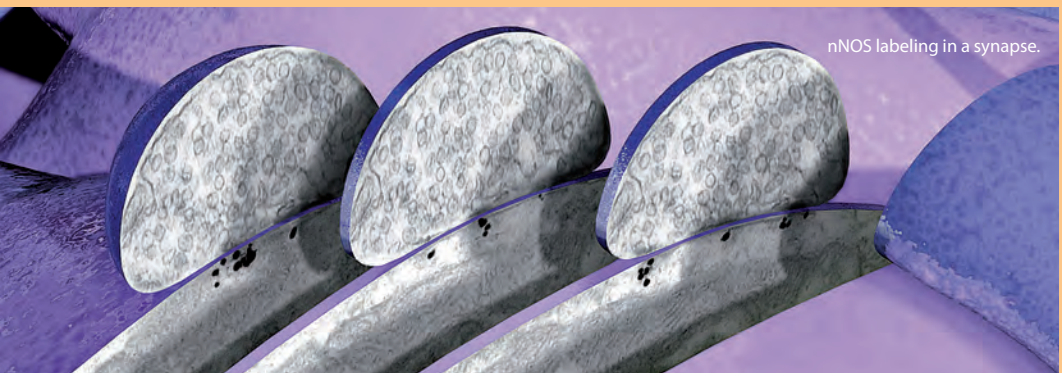


nNOS labeling in a synapse.

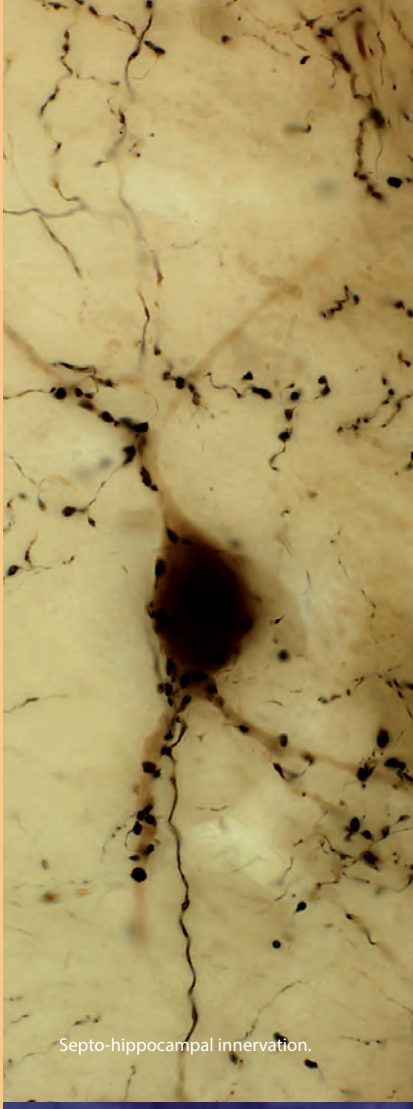
cordings that will take place at the Psychiatry Clinic of Semmelweis University Medical School. In addition, schizophrenia is investigated also in model animals. Polymorphisms at G72 genetic locus are considered to show one of the most robust associations with the disease. Transgenic mice that express 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

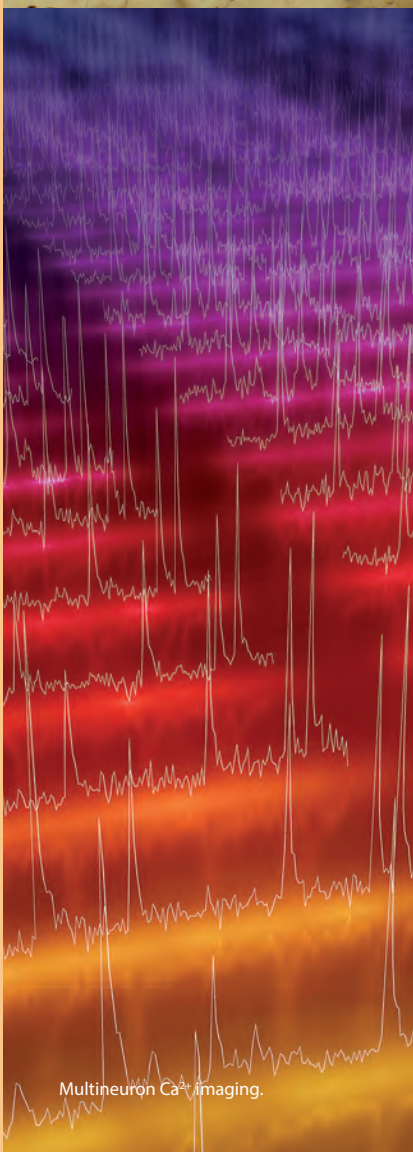
The Freund group described new signaling mechanisms at GABAergic synapses including nitric oxide and glutamate receptors. They challenged the classical views of retrograde endocannabinoid signaling by the demonstration of the dependence of this process on an intact nitric oxide pathway, and the selective 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



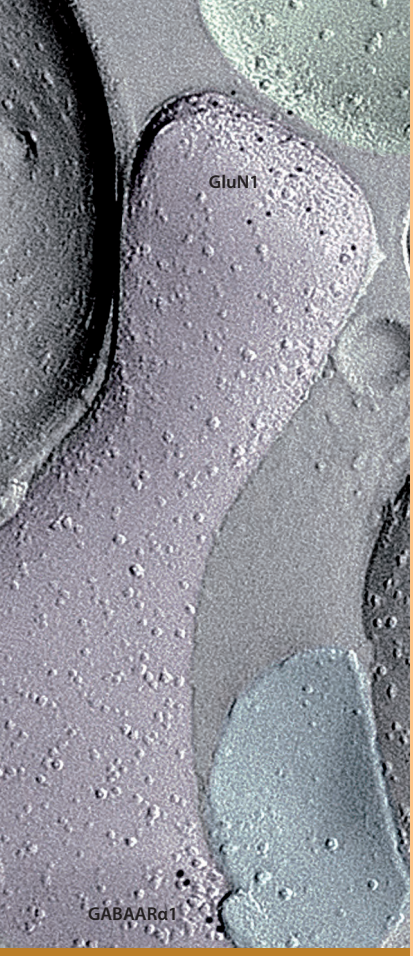
nNOS labeling in a synapse.



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)
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- Szabadits E., Cserép C., Szonyi A., Fukazawa Y., Shigemoto R., Watanabe M., Itohara S., Freund T. F., Nyiri G. NMDA receptors in hippocampal GABAergic synapses and their role in nitric oxide signaling. J NEUROSCI. 31:5893-904. (2011)
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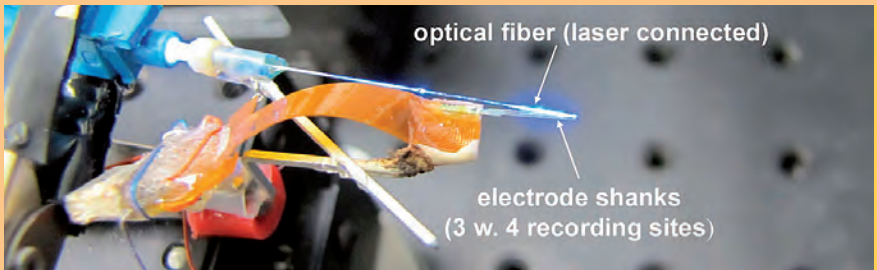
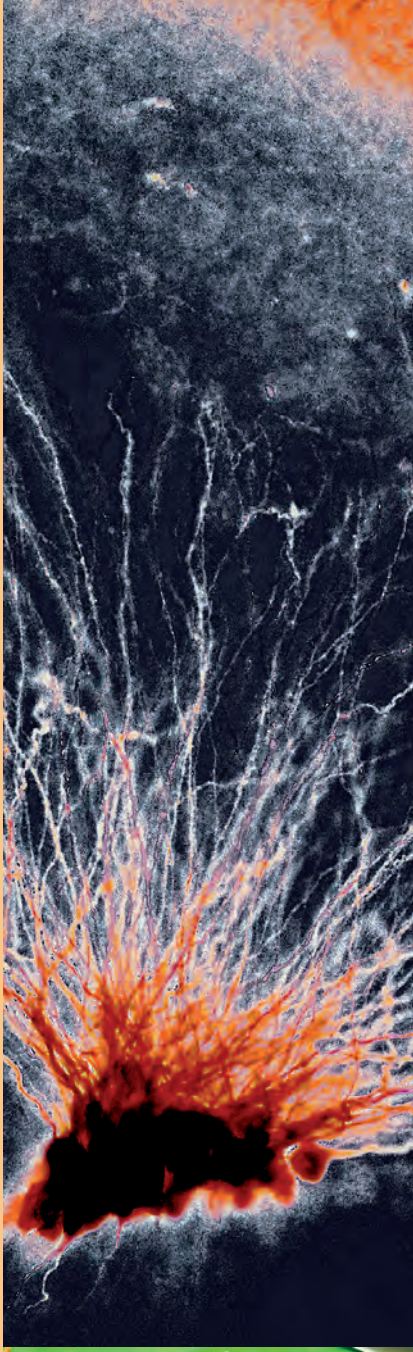
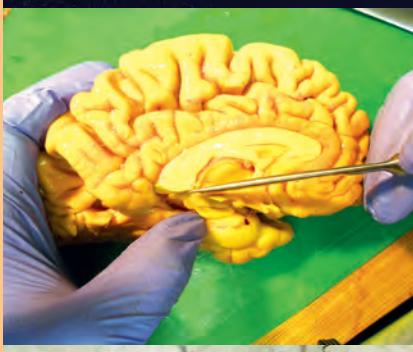
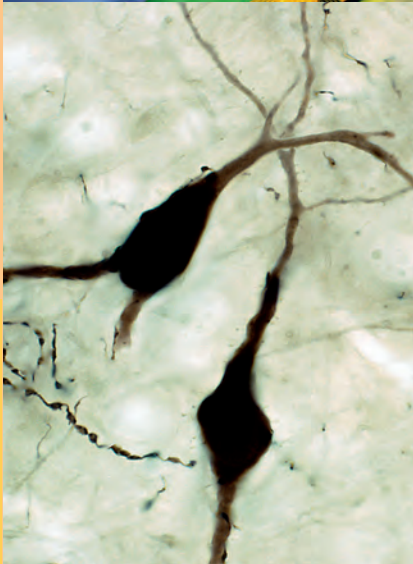






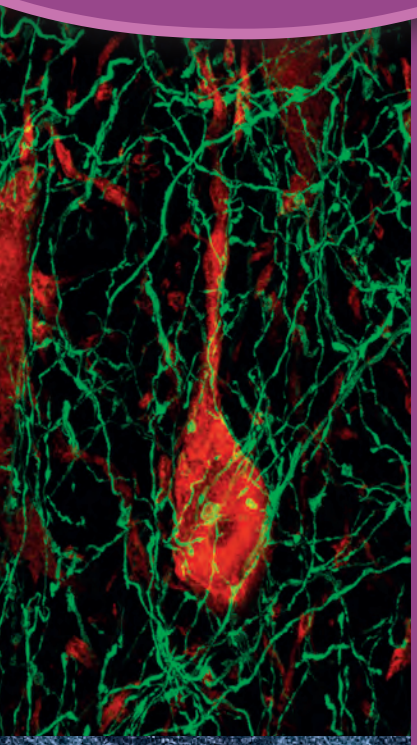


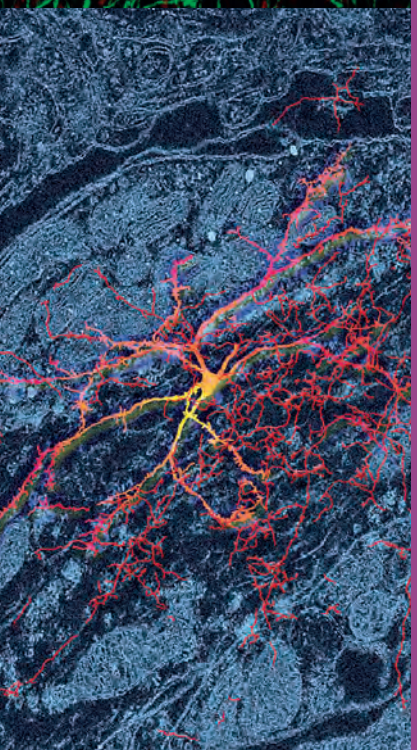
László Acsády

Laboratory of Thalamus Research

Department of Cellular and Network Neurobiology

Head of Laboratory:
László Acsády, PhD





Mission statement

All higher order brain operations require continuous interaction between thalamus and neocortex. Every cortical territory is in reciprocal connection with the thalamus and the lesion of any thalamic nucleus causes symptoms similar to that of the cortical area it is connected to. Thalamus and cortex develop together during the ontogenesis and evolve together during the phylogenesis. Normal communication between thalamus and cortex is disrupted in the major neurological and neuropsychiatric diseases. *These data indicate that thalamus and cortex form one functional unit and one cannot be understood without the other.* Still presently thalamus is mainly studied in isolation, we lack a coherent view on thalamocortical functions. We know especially little about the role of non-sensory thalamus which constitutes the majority of this structure.
Our research shows that the basic synaptic architecture of individual thalamic regions is highly variable. As a consequence the principles of information transfer in the thalamus display region specific features.

Therefore the mission of the Laboratory of Thalamus Research is:

- *to understand the generation and function of nucleus specific thalamocortical signals in normal and diseased states*
- *to understand the nature and significance of the perpetual two-way interaction between thalamus and cortex.*

To this end László Acsády's group utilizes a combined morphological and *in vivo* electrophysiological approach. The technological repertoire consists of light, electron, confocal, and superresolution microscopy, immunocytochemistry, virus mediated gene transfer, transgenic technology, juxta- and intracellular recording and labeling, optogenetics and the use of multishank, multisite silicon probes *in vivo.*

Senior scientists: Péter Barthó, Hajnalka Bokor, Csaba Dávid, Ferenc Mátyás
Postdoctoral fellows: Gergely Komlósi, Nóra Hádinger
Students: Zita Rovó, Viktor Plattner, Lejla Faradzs-Zade, Ákos Babitzky
Technician: Krisztina Faddi

MAJOR RESULTS AND RESEARCH DIRECTIONS

A novel inhibitory system in the thalamus

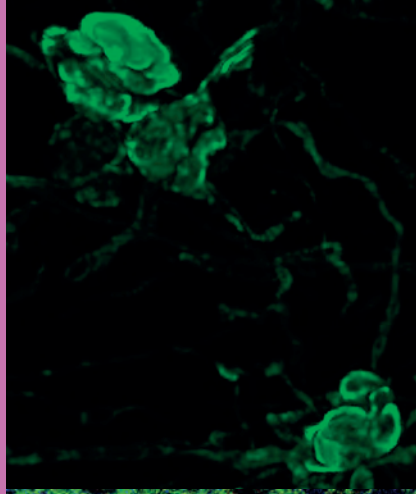
The major inhibitory input of the thalamus arises from the reticular thalamic nucleus, which provides GABAergic afferents to all thalamic nuclei. László Acsády's group revealed another inhibitory system which selectively innervates higher order thalamic relays (Barthó et al., 2002; Bokor *et al.,* 2005). In collaboration with Prof. Anita Lüthi's group (Univ. of Lausanne) they demonstrated that this system (called "extrareticular" or "extrathalamic" inhibition) is different from the reticular inhibition in synaptic organization, postsynaptic targets kinetics, short term plasticity connectivity and firing pattern (Wanaverbecq et *al.*, 2008). The novel system proved to be very powerful. The axons formed large terminals with multiple synapses, which exerted non-depressing inhibitory currents even at high presynaptic firing rates and effectively altered the firing pattern of the target cells. The organization of extrareticular afferents did not depend on the locus of origin (e.g. basal ganglia, or diencephalon,) or the species (rat or monkey) (Bodor *et al.*, 2008). Since its description, the role of extrareticular inhibition has been demonstrated in sensory transmission, central pain and epileptic activity.

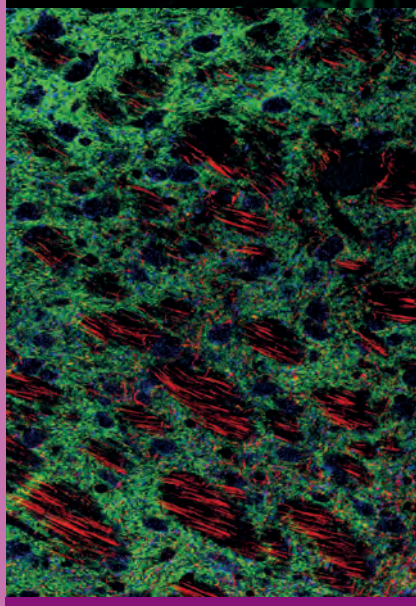
Sensory physiology – the whisker system

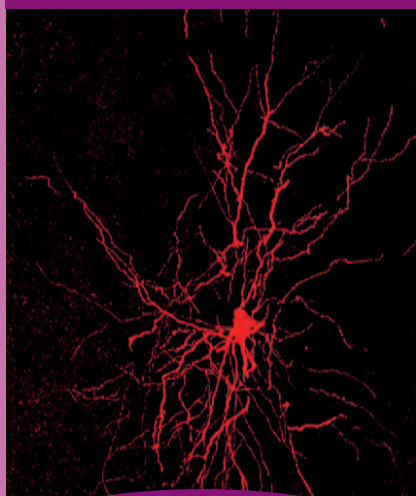
The rat whisker system is one of the best characterized sensory systems. László Acsády's group used the whisker system as a model to test specific predictions emerging from the organization of extrareticular inhibition. Together with Prof. Martin Deschenes's laboratory (Univ. of Lavalle), they demonstrated that extrareticular inhibition is able to block the peripheral sensory transmission in the thalamus via a feed-forward inhibitory mechanism (Lavallee *et al.*, 2005). They also demonstrated a novel principle of thalamic synaptic organization in the somatosensory nucleus of n. posterior (Groh, Bokor et al., 2013, see below). These results clearly show that certain thalamic nuclei are not designed for faithful transmission of sensory information, as earlier suspected, but their activity is regulated in a more complex manner probably using disinhibition and integration of signals of distinct origin.

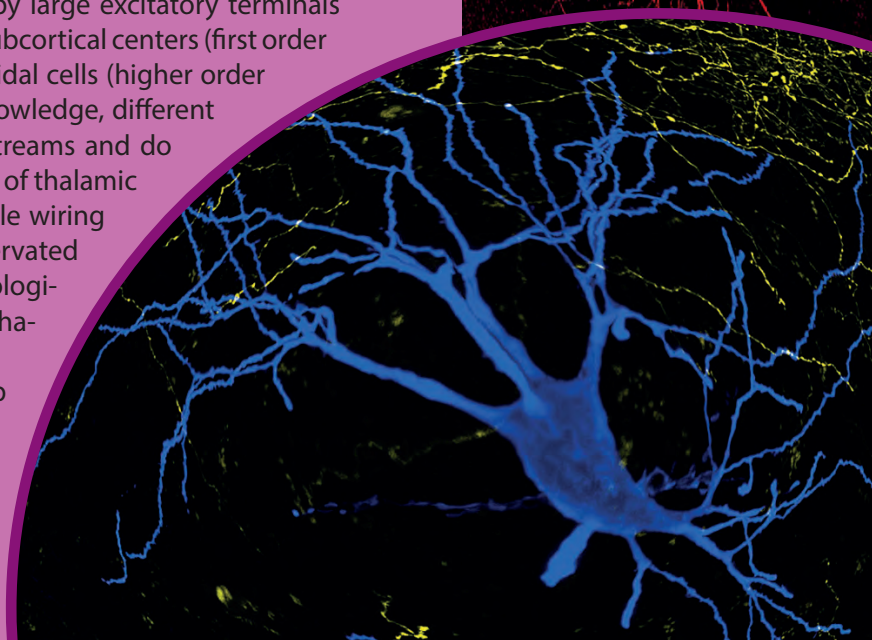
The thalamic computational unit

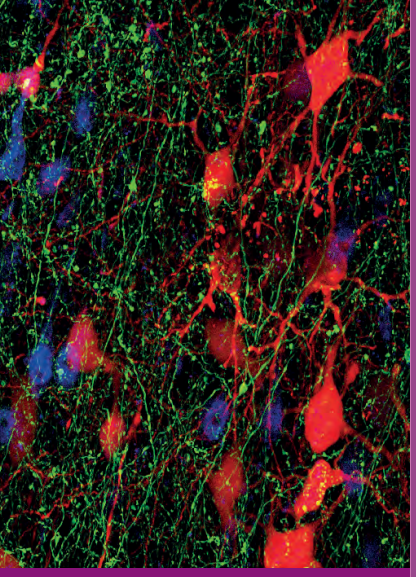
The activity of thalamic neurons is driven by large excitatory terminals (called "drivers"), which originates either in subcortical centers (first order thalamic nuclei) or in cortical layer 5 pyramidal cells (higher order thalamic nuclei). According to text book knowledge, different driver pathways are organized in parallel streams and do not interact at the thalamic level. Separation of thalamic information channels is achieved by a simple wiring principle, a single thalamic relay cell is innervated by a single type of driver input. This morphological unit was thought to underlie the basic thalamic function – the faithful relay.
In contrast to this, a recent study of the group (Groh, Bokor et al., 2013) demonstrated that drivers of different origin (cortex and brainstem) can converge on single thalamic neurons. The two drivers interacted syner-

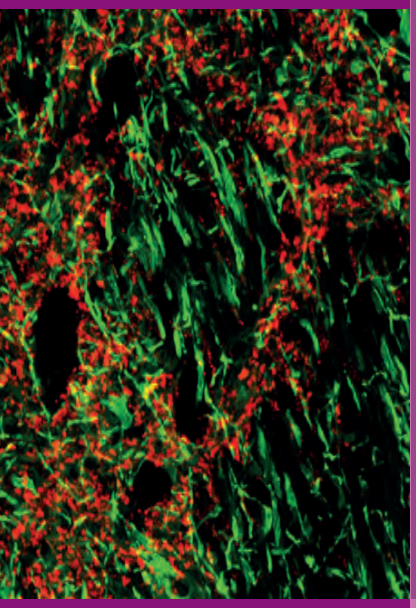


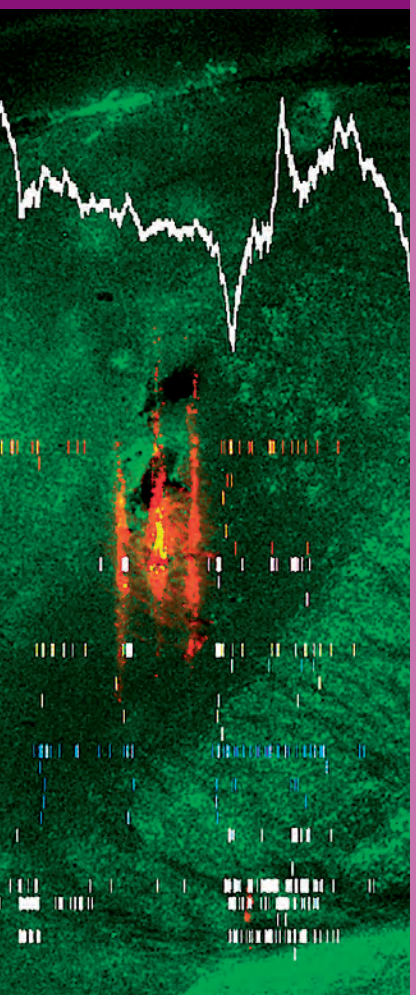












gistically in a time-dependent manner and when co-activated, supralinearly increased the output of thalamus. Convergence of different drivers in single thalamocortical cells was unexpected, demonstrated a novel type of computational unit in the thalamus and questioned the general validity of the relay concept. Together with the data about extrareticular inhibition the results indicated *that integration of different excitatory and inhibitory information channels* rather than simple relay is the main operational principle in several thalamic nuclei. Indeed, analyzing the morphology of drivers in the primate thalamus the group found that classical subcortical drivers are actually present in only 40% of the thalamus (Rovó *et al.*, 2012).

Motor thalamus and basal ganglia

Parkinson's, Huntington's and other major diseases of the basal ganglia cause debilitating syndromes. A recent discovery of the Thalamus Research Group is that the basal ganglia terminals in the motor thalamus, display identical morphological features to the extrareticular terminals described earlier in the sensory thalamus (Bodor *et al.*, 2008). This indicates that basal ganglia terminals are tailored for faithful inhibitory signal transmission even at high presynaptic firing rates, which can explain their effectiveness in controlling thalamocortical activity in normal and pathological states. Interestingly, rodent and primate basal ganglia terminals were identical in the thalamus demonstrating the evolutionary conserved nature of this pathway.

Thalamocortical oscillations

Oscillations in different frequency ranges bind the activity of neuronal populations in time. Using simultaneous thalamic and cortical recordings the Thalamus Research Group demonstrated that thalamic and cortical activity is timed to each other in a cycle-be-cycle manner during slow oscillation and cortical influence on thalamic activity is nucleus specific (Slézia *et al.*, 2011).
More recently, using viral mediated gene transfer and pharmacogenetic approaches they identified the role of extrasynaptic GABA-A receptors in thalamocortical oscillations (Rovó *et al.*, 2014). In another study, by simultaneously recording the somatic activity of excitatory thalamocortical cells together with axonal activity of reciprocally coupled inhibitory reticular thalamic cells they were able to show that during natural sleep a dynamically fluctuating thalamocortical network controls the duration of sleep spindles via the major inhibitory element of the circuits, the nRT (Barthó *et al.*, 2014).

Emerging technologies

The Thalamus Research Group recently implemented and successfully used the following state-of-the art techniques, which are now indispensible to study brain functions.

1) *Recording neuronal ensemble activity in the thalamocortical system* (Barthó et al., 2014).
2) *Control of neuronal activity in a spatially and temporally coordinated manner* (Rovó, Mátyás et al., 2014;, Barthó et al., 2014).
3) *Superresolution microscopy* (Rovó, Mátyás et al., 2014).

FUTURE DIRECTIONS

The Laboratory of Thalamus Research continues to study non-sensory functions of the thalamus by combining morphological and in vivo physiological approaches. In the near future the laboratory aims to include behavioral analysis into its repertoire. Ongoing/planned projects, briefly:

- synaptic organization and function of basal ganglia recipient thalamic nuclei
- the role of intralaminar nuclei in the coordination of large scale electrical activity and behavior
- the role of midline thalamus in arousal, appetitive and aversive behavior

Selected publications from the last 10 years:

- Barthó P., Slézia A., Mátyás F., Faradzs-Zade L., Ulbert I., Harris K. D., & Acsády L. (2014) Ongoing Network State Controls the Length of Sleep Spindles via Inhibitory Activity. *Neuron*, 82, 1367–1379.
- Rovó Z., Mátyás F., Barthó P., Slézia A., Lecci S., Pellegrini C., Astori S., Dávid C., Hangya B., Lüthi A., & Acsády L. (2014) Phasic, Nonsynaptic GABA-A Receptor-Mediated Inhibition Entrains Thalamocortical Oscillations. *J. Neurosci.*, 34, 7137–7147.
- Groh A., Bokor H., Mease R. A., Plattner V. M., Hangya B., Stroh A., Deschenes M., & Acsády L. (2013) Convergence of Cortical and Sensory Driver Inputs on Single Thalamocortical Cells. *Cereb. Cortex.* Epub ahead of print
- Rovó Z., Ulbert I., & Acsády L. (2012) Drivers of the primate thalamus. *J. Neurosci.*, 32, 17894–17908.
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- Bodor A. L., Giber K., Rovó Z., Ulbert I., & Acsády L. (2008) Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J. Neurosci.*, 28, 3090–3102.
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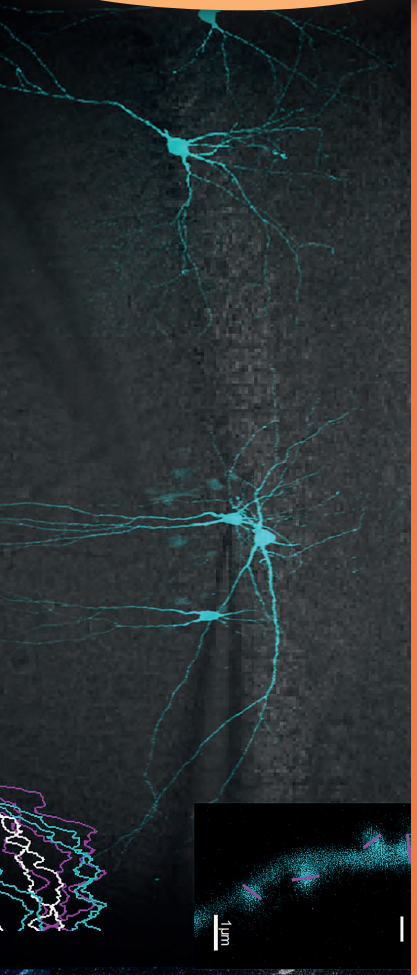
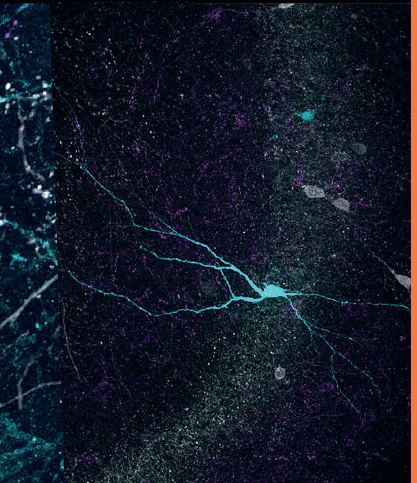
- Lavallee P., Urbain N., Dufresne C., Bokor H., Acsady L., & Deschenes M. (2005) Feedforward inhibitory control of sensory information in higher-order thalamic nuclei. *J. Neurosci*, 25, 7489–7498.

Standing, from left: Krisztina Faddi, Gergely Komlósi, Viktor Plattner, Ákos Babiczky, László Acsády, Ferenc Mátyás, Péter Barthó, Csaba Dávid
Sitting: Nóra Hádinger, Hajnalka Bokor, Lejla Faradzs-Zade

Laboratory of Cellular Neurophysiology

Department of Cellular and Network Neurobiology
Head of Momentum-supported Laboratory:
Zoltan Nusser, DVM, PhD





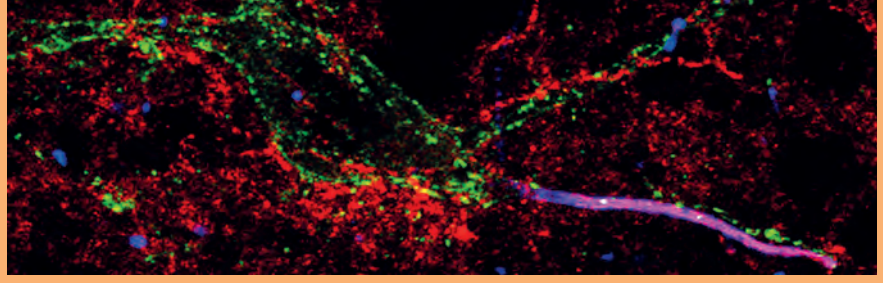
Mission statement

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

Senior scientists: Mark D. Eyre PhD, Noémi Holderith PhD, Nóra Lenkey MD, PhD, Andrea Lőrincz PhD, Máté Sümegi PhD
Ph.D. students: Tekla Kirizs MD, Tímea Éltes MD, Katalin Szigeti MD, Miklós Szoboszlay
Undergraduate research assistants: Borbála Bolonyai
Technicians: Éva Dobai, Bence Kókay, Dóra Rónaszéki

The research in the Laboratory of Cellular Neurophysiology is supported by a European Research Council Advanced Grant, a Project Grant from the Wellcome Trust, and a Hungarian Academy of Sciences 'Lendület' Grant.

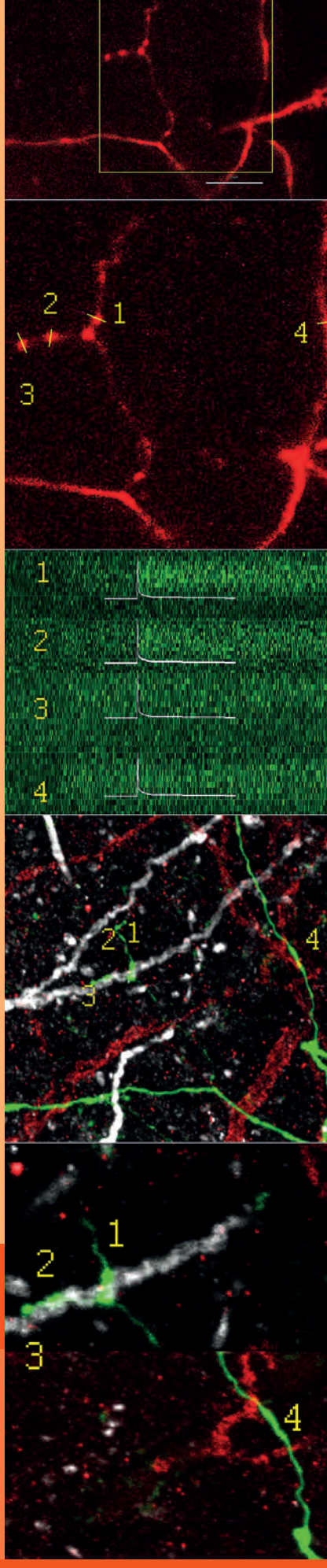


functionally testable predictions of the functional consequences of specialized ion channel distributions. *In vitro* electrophysiology and imaging approaches are used to test the functional predictions of our models.

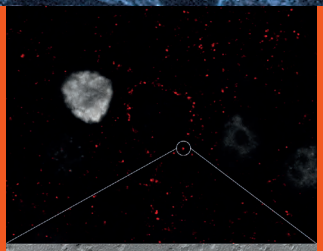
4. Provide a quantitative description of the microcircuit of the cerebellar cortex with special focus on electrically coupled GABAergic interneurons and their roles in network operations.

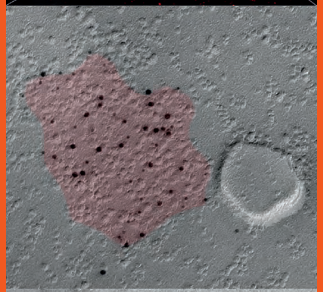
Understand the role of identified nerve cells in olfaction

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

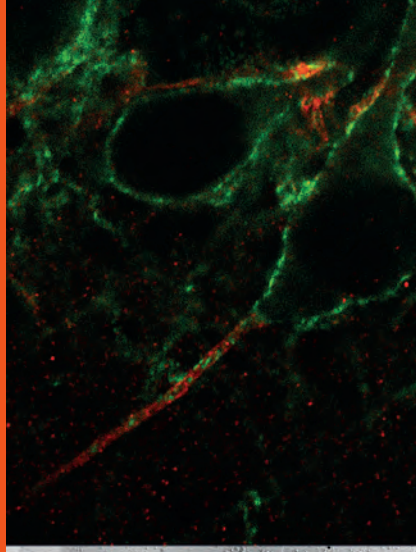
Understanding chemical synaptic neurotransmission in the CNS has been in the spotlight of neuroscience for many decades. A tremendous amount of information has been gathered regarding the molecular events leading to the release of neurotransmitter from synaptic vesicles, the diffusion of neurotransmitter 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.

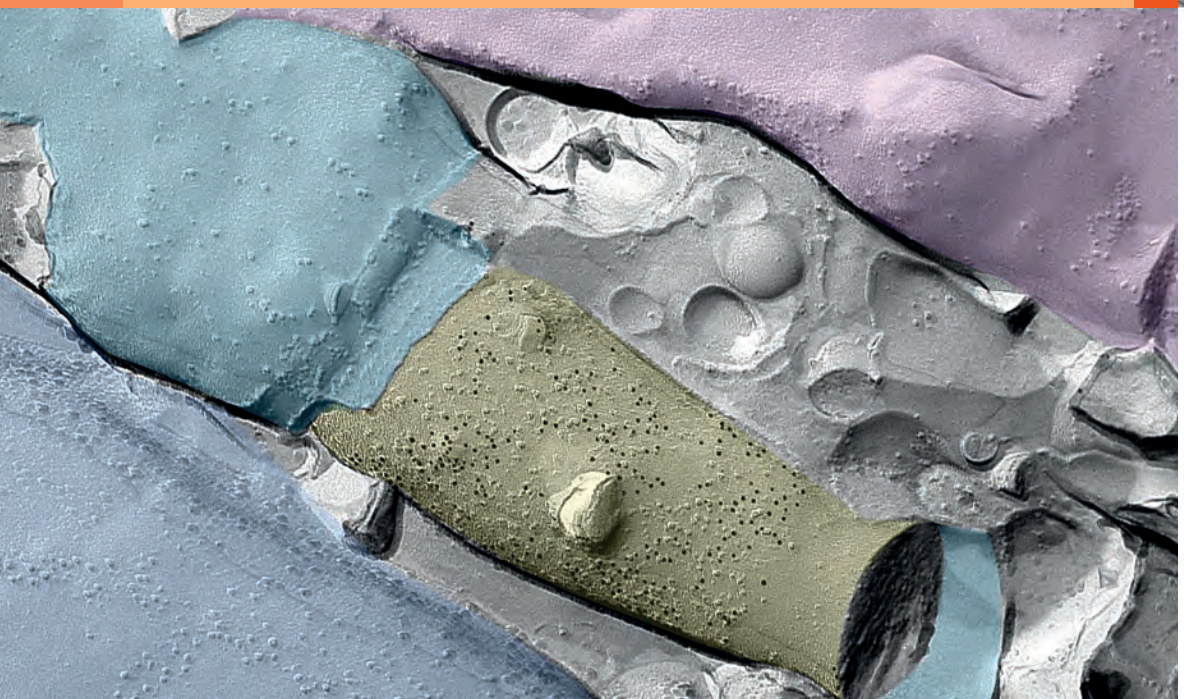
Creating a molecular map of the neuronal surface

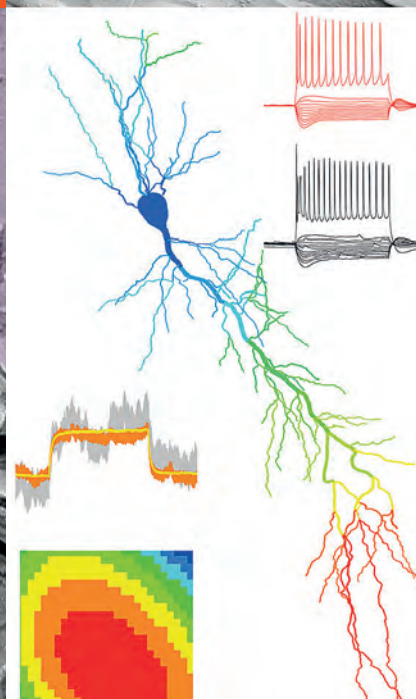
The most fundamental function of nerve cells is the integration of their synaptic inputs to generate action potentials (APs). It is a generally accepted view that APs are generated in the axon initial segment (AIS). However, input synapses are usually distributed over a large dendritic tree. Because 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

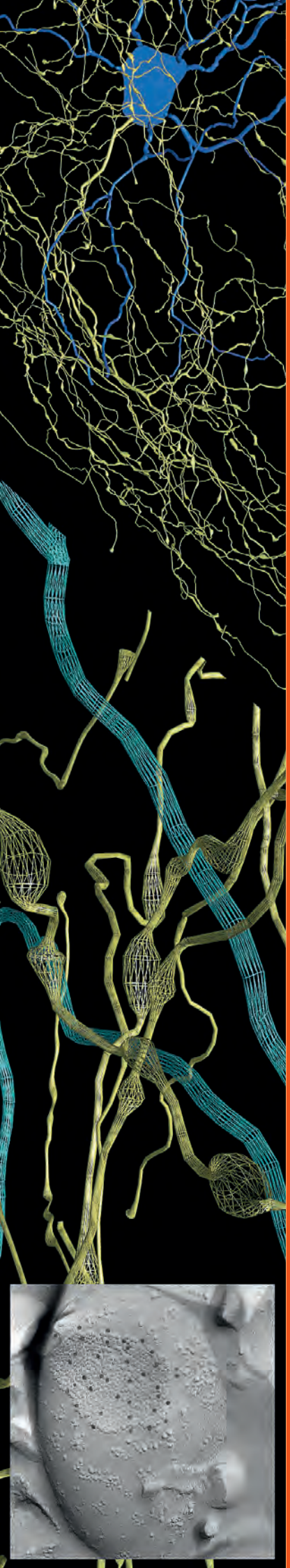
passive, the effect of a synapse on output generation would depend on its dendritic location. However, in the past decade, it has become apparent that dendrites of most nerve cells are not passive, but contain a large number of voltage-dependent conductances, which endow dendritic trees with an unanticipated 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.
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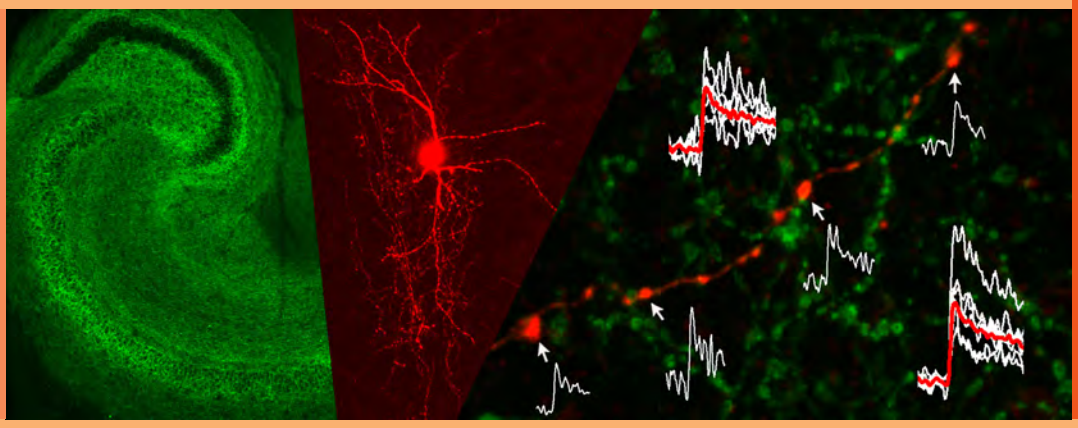
- Cathala L., Holderith N. B., Nusser Z., DiGregorio D. A. & Cull-Candy S. G. (2005). Changes in synaptic structure underlie the developmental speeding of AMPA receptor-mediated EPSCs. Nat Neurosci, 8, 1310-1318.
- Farrant M., & Nusser Z. (2005). Variations on an inhibitory theme: phasic and tonic activation 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.

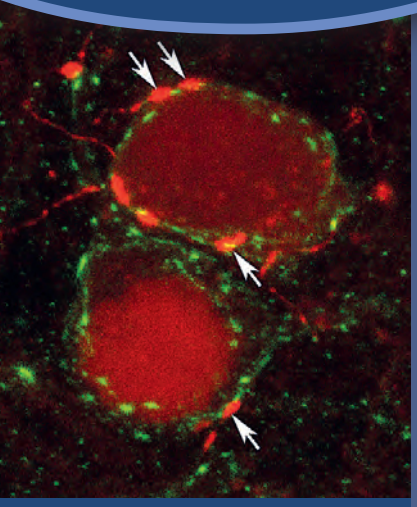


Labortory of Network Neurophysiology

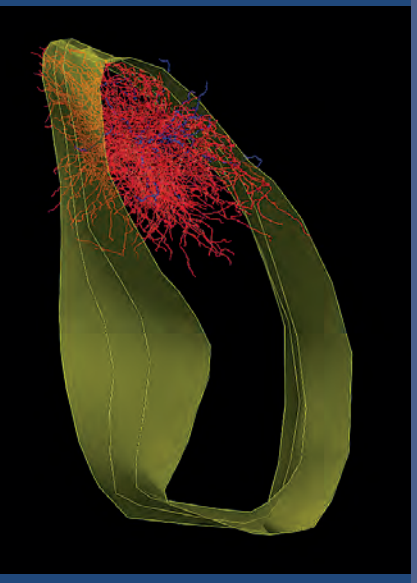
Department of Cellular and Network Neurobiology
Head of Momentum-supported Laboratory:
Norbert Hájos, PhD

Mission statement

Information flow in neuronal networks composed of different cell types is primarily determined by structural and functional properties of synaptic communication. Thus, on one hand, the actual number and the precise spatial location of synaptic junctions between pre- and post-synaptic neurons influence the efficacy of signal transfer. On the other hand, the probability of transmitter release and the number, types and distribution of transmitter receptors shape the synaptic transmission. On top of this complexity, plasticity of synaptic signaling at short and long time-scales controls the spread of the information within a microcircuit. *The mission of the Lendület Laboratory of Network Neurophysiology is to uncover the principles of information processing in cortical microcircuits at cellular and network levels.* Specifically, the group of Norbert Hájos aims to understand the logic of synaptic connectivity among distinct types of cortical neurons at both structural and functional levels; to uncover the synaptic mechanisms underlying the generation and propagation of synchronous neuronal activities in local cortical networks, and to elucidate the precise mechanisms of how synaptic communication among neurons is controlled under physiological and pathological conditions. In order to reach these goals, light and electron microscopy, electrophysiology, imaging techniques and optogenetic tools are combined.
A new research direction of the group is to elucidate the organization of inhibitory neuronal circuits in the amygdalar nuclei. The amygdala plays a significant role in emotional reactions and memory formation. This brain region is tightly controlled by subcortical afferents (carrying



Boutons of a parvalbumin-expressing basket cell (red) surround the somata of excitatory principal cells (green). Arrows indicate the contact sites.



3D reconstruction of a parvalbumin-expressing fast spiking basket cell in the basolateral amygdala. Dendrites in blue, axons in red, borders of the BLA in yellow.

Postdoctoral fellows: Boglárka Barsy, PhD; Orsolya Papp, PhD
Visiting scientist: Viktória Vereczki, MD PhD
Junior research fellow: László Végh
Ph.D. students: Tibor Andrási, Judit Veres
Undergraduate research assistants: Richárd Kozma, Gergő A. Nagy, Attila Vikór
Technicians: Erzsébet Gregori, Éva Krizsán

Ongoing Research Support: Fellowship of the Hungarian Academy of Sciences (Lendület, LP2012-23), National Office for Research and Technology (OMFB-01678/2009; KTIA_AIK_12-1-2013-0005), European Research Foundation (294313-SERRACO).

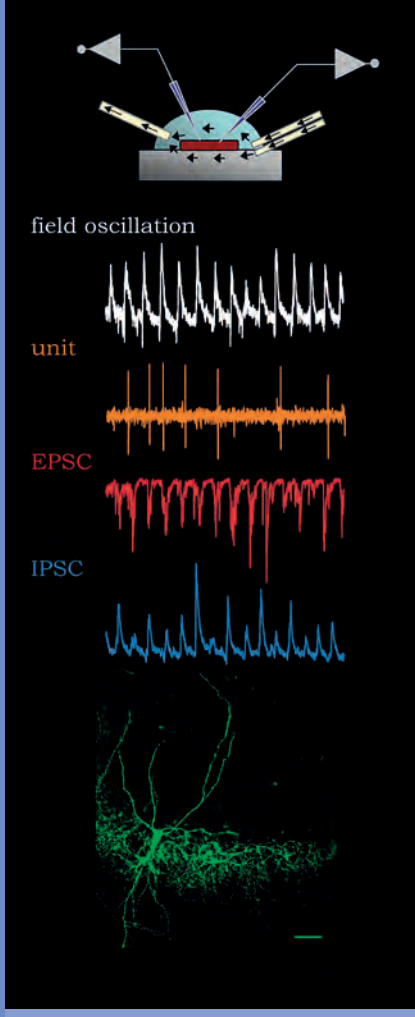
information about emotions, motivation and autonomic states) that likely impact the activity of principal neurons via local inhibitory cells. Uncovering the neuronal operation in amygdalar networks could help in understanding the cellular mechanisms that underlie psychiatric disorders including anxiety, depression or panic attack.

Synaptic mechanisms of hippocampal oscillations

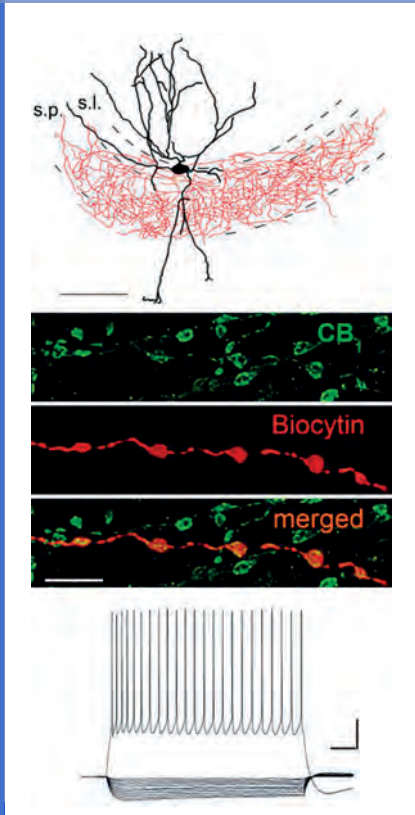
Oscillations in local field potentials are generated by synchronous neuronal activities. Rhythms with characteristic frequency bands and spatial outspread can be recorded during different brain states, suggesting that they emerge from the distinct behaviour-dependent operation of neuronal circuits. In order to reveal the cellular and synaptic mechanisms underlying the generation of distinct types of oscillations, *in vitro* models should be introduced. Norbert Hájos designed a novel type of recording chamber, which allows the maintenance of oscillatory activities in brain slice preparations in combination with high-resolution optical imaging. Using this new design, the group of Norbert Hájos revealed that the oscillations at gamma (30-50 Hz) frequencies emerging intrinsically in the CA3 region of the hippocampus propagate in the neighbouring hippocampal CA1 area by entraining local, inhibitory interneurons within CA1. Thus, in contrast to locally emerging gamma oscillations, where reciprocal recurrent feed-back mechanisms generate these fast network activities, the spread of gamma oscillations into other regions is mediated by rhythmic recruitment of feed-forward inhibition. In addition, Norbert Hájos teamed up with Attila Gulyás, working in the laboratory of Tamás Freund, to clarify the synaptic mechanisms underlying the generation of sharp wave-ripples in the CA3 area, synchronous events that are known to play a role in memory processes. They found that the sharp wave-ripples in CA3 are generated by a microcircuit composed of excitatory pyramidal cells and fast spiking basket cells, similarly to the generation of gamma oscillations. Thus, the same microcircuit is able to generate distinct synchronous network activities within the hippocampus, most likely depending on the different cellular and synaptic parameters.

Control of synaptic communication by endocannabinoids

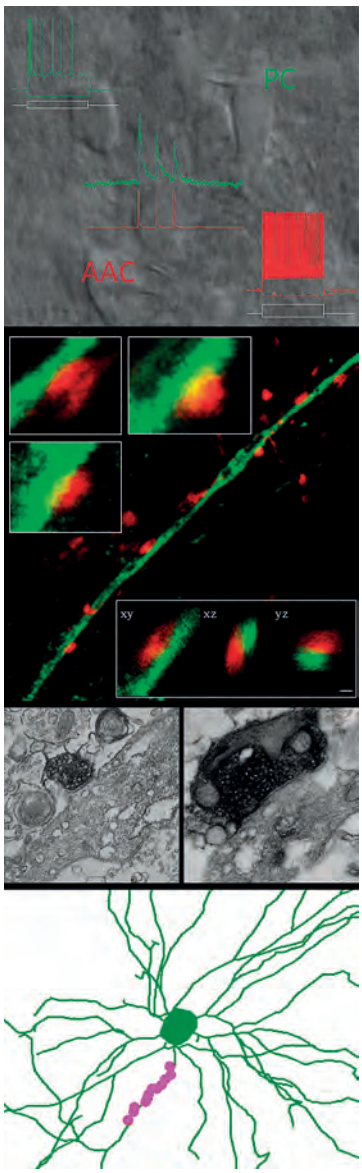
The majority of axon terminals in cortical structures are equipped with CB_1 cannabinoid receptors. Thus, the endogenous ligands of these receptors, the endocannabinoids are in the position to have a widespread impact on the synaptic communication in neuronal networks. The Hájos group revealed that CB_1 activation at excitatory synapses onto fast spiking basket cells by exogenous ligands was responsible for the suppression of network oscillations in the hippocampus. These data have elucidated the synaptic mechanisms of how administration of marihuana can impact the cognitive processes in animals and humans. Furthermore, they discovered together with István Katona's laboratory that, in addition to excitatory pyramidal cells, GABAergic interneurons in the hippocampus could also produce endocannabinoids and these retrograde signalling molecules mediated long-term depression of synaptic communication at excitatory inputs. In addition, teaming up with Zoltán Nusser's group, they clarified



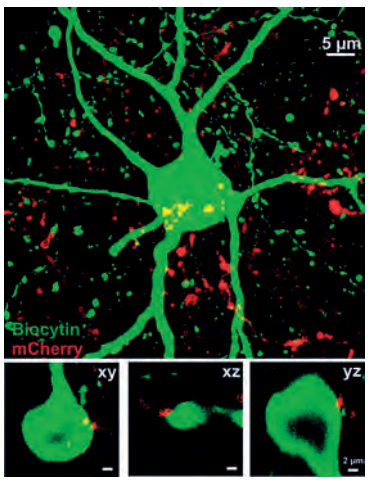
Schematic diagram of the flow in the dual-superfusion slice chamber. Simultaneous measurements of the oscillation and neuronal firing followed by monitoring the synaptic currents recorded in a fast-spiking basket cell in the CA3 region of the hippocampus (in green).



A basket cell in the hippocampus expresses CB_1 cannabinoid receptors on its axon terminals. Dendrites in black, axons in red. Below: voltage responses of the cell upon current injection.



An axo-axonic cell (red) specifically innervates the axon initial segment of a principal cell (green) in the basolateral amygdala.



GABAergic afferents from the basal forebrain (red) contact a GABAergic interneuron in the basolateral amygdala (green).

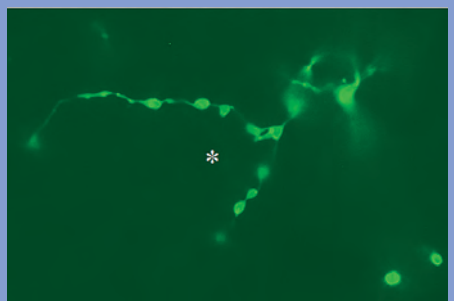
that activation of CB_1 receptors at inhibitory synapses reduced GABA release by suppressing Ca^{2+} entry into presynaptic axon terminals via N-type Ca^{2+} channels. Thus, the control of the intraboutonal Ca^{2+} concentration in GABAergic axon terminals by CB_1 function leads to the effective reduction of synaptic inhibition. These results substantially contribute to our understanding how endocannabinoid-mediated signalling regulates synaptic communication within cortical networks.

Microcircuits in the basolateral amygdala complex

The amygdala is a part of a complex system, the so-called emotional brain. Formed by several nuclei with specific functions this brain region is essential both in memory formation and the execution of relevant behavioural responses upon stress or emotional impact. The Hájos group has begun to define the principles of the synaptic organization within neuronal networks of the basolateral amygdala complex. This cortical structure is composed of excitatory principal cells and GABAergic interneurons, but the connectivity among neurons, which is pivotal to understanding the circuit operation, is unknown. Using light and electron microscopic techniques and *in vitro* electrophysiological recordings they examine the structural and functional properties of the synaptic output of inhibitory neurons innervating the principal neurons in the basolateral amygdala. For instance, the group clarified that a special cortical interneuron type, the axo-axonic cell (AAC) innervating the axon initial segments of principal cells, inhibits or delays the firing of their postsynaptic targets. Importantly, they found that single AACs preferentially innervate the portion of the AIS where action potentials are generated with the highest likelihood, regardless of the number of synapses forming a given connection. These results defined a fine organization of AAC innervation, maximizing their inhibitory efficacy by strategically positioning synapses along the AISs. In the future, the connectivity of other interneuron types will be uncovered in detail to build a functional wiring diagram of the inhibitory networks in the basolateral amygdala.

Subcortical modulation of amygdalar networks

Each cortical structure receives inputs from subcortical areas, which convey information about the external world and the internal state of the organism. The basal forebrain, one of the subcortical regions involved in attention, learning and memory processes, gives rise to both cholinergic and GABAergic innervations to its target cortical areas. The basolateral amygdala is innervated by cholinergic and GABAergic neurons located in a part of the basal forebrain, specifically within the ventral pallidum and substantia innominata. While cholinergic afferents influence both the excitability and the synaptic communication widespread within the basolateral amygdala, the GABAergic components of this subcortical input target preferentially, if not exclusively, local GABAergic interneurons. Using optogenetic tools combined with *in vitro* electrophysiology, the group investigates the cellular and network impact of this subcortical GABAergic input on amygdalar

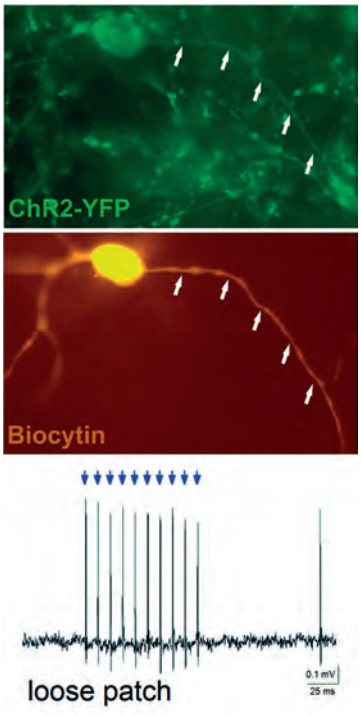


Channelrhodopsin 2-expressing GABAergic boutons from the basal forebrain (green) surround the soma of an interneuron (asterisk) in the basolateral amygdala.

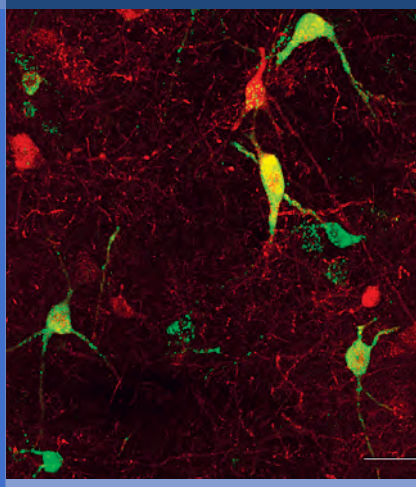
network operation. The overall aim is to clarify how basal forebrain inputs can gate the information processing driven by cortical and thalamic inputs at the amygdalar level.

Selected publications from the last 10 years:

- Szabó GG, Lenkey N, Holderith N, Andrási T, Nusser Z and Hájos N. Presynaptic calcium channel inhibition underlies CB_1 cannabinoid receptor-mediated suppression of GABA release. J Neurosci. 34:7958-63 (2014)
- Zemankovics R, Veres JM, Oren I and Hájos N. Feedforward inhibition underlies the propagation of cholinergically induced gamma oscillations from hippocampal CA3 to CA1. J Neurosci. 33:12337-51. (2013)
- Hájos N, Karlócai MR, Németh B, Ulbert I, Monyer H, Szabó G, Erdélyi F, Freund TF and Gulyás AI. Input-output features of anatomically identified CA3 neurons during hippocampal sharp wave/ripple oscillation in vitro. J Neurosci. 33:11677-91. (2013)
- Papp OI, Karlócai MR, Tóth IE, Freund TF and Hájos N. Different input and output properties characterize parvalbumin-positive basket and axo-axonic cells in the hippocampal CA3 subfield. Hippocampus 23:903-18. (2013)
- Nagy AG, Botond G, Borhegyi Z, Plummer NW, Freund TF and Hájos N. DAG-sensitive and Ca^{2+} permeable TRPC6 channels are expressed in dentate granule cells and interneurons in the hippocampal formation. Hippocampus 23:221-32. (2013)
- Péterfi Z, Urbán GM, Papp OI, Németh B, Monyer H, Szabó G, Erdélyi F, Mackie K, Freund TF, Hájos N, Katona I. Endocannabinoid-mediated long-term depression of afferent excitatory synapses in hippocampal pyramidal cells and GABAergic interneurons. J Neurosci. 32:14448-63 (2012)
- Holderith N, Németh B, Papp OI, Veres JM, Nagy GA, Hájos N. Cannabinoids attenuate hippocampal gamma oscillations by suppressing excitatory synaptic input onto CA3 pyramidal neurons and fast spiking basket cells. J Physiol. 589:4921-34 (2011)
- Hájos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O. Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. Eur J Neurosci. 29:319-27. (2009)
- Hájos N, Mody I Establishing a physiological environment for visualized in vitro brain slice recordings by increasing oxygen supply and modifying aCSF content. J Neurosci Meth. 183:107-13. (2009)



Blue light-induced firing in a channelrhodopsin 2 (ChR2)-expressing parvalbumin-containing neuron in the basal forebrain.



Neurons expressing parvalbumin (red) in the basal forebrain project into the basolateral amygdala indicated by the content of a retrograde tracer, Fluogold.

Standing, from left: Richárd Kozma, László Végh, Tibor Andrási, Norbert Hájos, Attila Vikor, Attila Gergő Nagy
Sitting: Erzsébet Gregori, Orsolya Papp, Éva Krizsán, Judit Veres

Laboratory of Neuronal Signaling

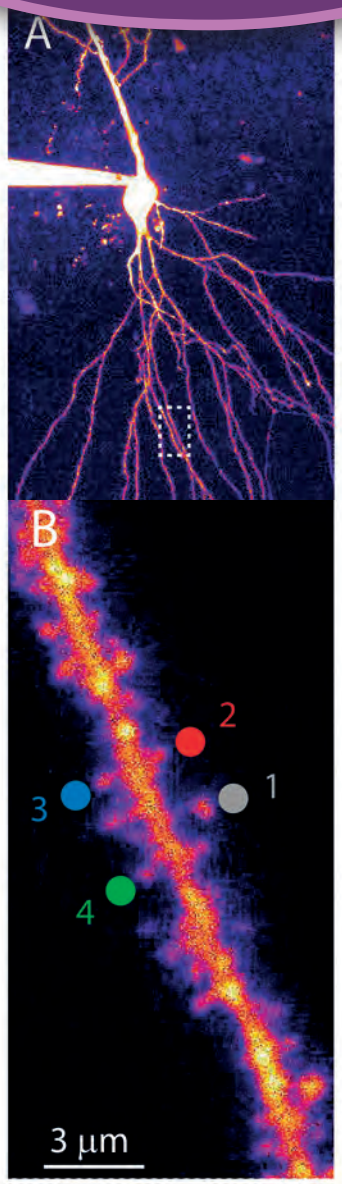
Department of Cellular and Network Neurobiology
Head of Momentum-supported Laboratory:
Judit Makara, MD, PhD

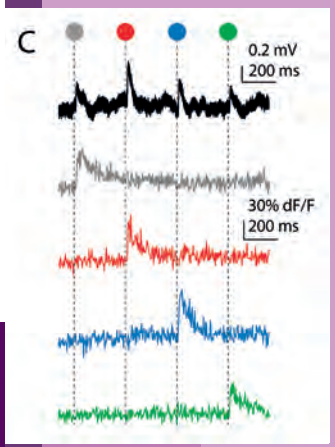
Mission statement

The Laboratory of Neuronal Signaling was established in 2011. The main focus of the lab is to understand how the active, voltage-dependent properties of dendrites and dendritic spines contribute to information processing on the single cell and network level during physiologically relevant learning-related behaviors.

Most mammalian neurons receive thousands of excitatory synaptic contacts that are located on their elaborate dendritic tree. The passive and active electrical properties of dendrites thus have profound influences on the integration of synaptic inputs. Recent *in vivo* studies confirmed that in certain cortical neuron types, active dendritic nonlinearities contribute to neuronal output. Understanding the molecular details of dendritic function is therefore essential to disentangle behaviorally relevant circuit computations.

Voltage dependent ion conductances in dendrites and spines may generate local nonlinear interactions of voltage and Ca^{2+} signals arising from multiple spatio-temporally coactive synaptic inputs, such as those provided by the correlated activity of a neuronal ensemble. These mechanisms can enable neurons to detect and transmit specific forms of information encoded in the activity of the neuronal network, as well as to potentially reorganize synaptic strength depending on the actual spatial and temporal parameters of incoming input patterns. Furthermore, dynamic regulation of voltage-dependent conductances provides a new layer of cellular plasticity mechanisms that may affect the precision, rate and timing of the output of neuronal populations receiving the same input patterns.





A) Z-stack of a CA1 pyramidal neuron loaded with the Ca^{2+} sensitive dye OGB-1. B) Dendrite in dashed box in A shown at high magnification. Four spines are selected for two-photon glutamate uncaging stimulation. C) Voltage (upper black trace) and Ca^{2+} (color coded lower traces) signals evoked in the spines.

Postdoctoral fellows: Bertalan Andrásfalvy MD PhD, Jens Weber PhD, Marina Polito PhD
Undergraduate research assistant: Ádám Magó
Technician: Adrienn Ráksai-Maár

Ongoing Research Support: Hungarian Academy of Sciences (Lendület LP2011-012), International Senior Research Fellowship from the Wellcome Trust (WT090915/Z/09/Z).

We use a combination of two-photon imaging, two-photon glutamate uncaging and electrophysiology to study these mechanisms in the rodent hippocampal circuitry *in vitro*. We also aim to investigate whether changes in dendritic excitability are associated with *in vivo* learning paradigms.

Effects of dendritic properties on synaptic signaling and plasticity

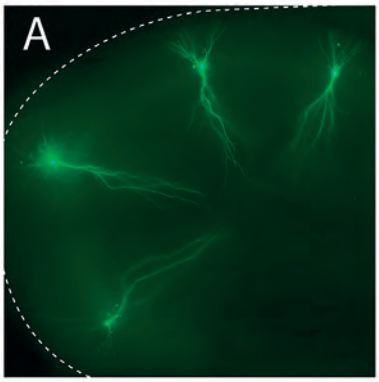
The dendritic tree is a heterogeneous structure which includes compartments of different morphological varieties (from the thick trunk to thin terminal branches), with branch-specific regulation of ion channel composition. These passive and active dendritic properties may shape synaptic input integration in a wide parameter range. Using CA1 pyramidal cells as a model neuron, we systematically investigate how the above factors influence the electrical and biochemical interactions between multiple spatially clustered excitatory synaptic inputs, depending on their location. We also study the impact of these dendritic properties on synaptic plasticity.

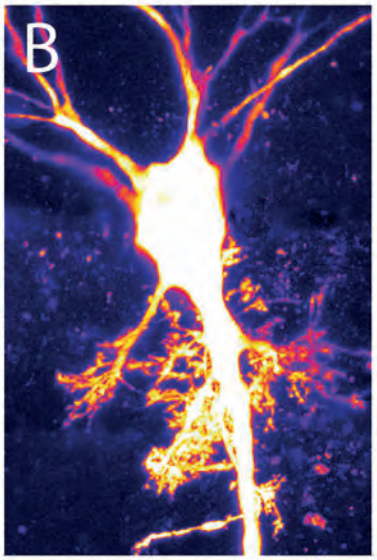
Dendritic integration and ensemble formation in hippocampal CA3 pyramidal neurons

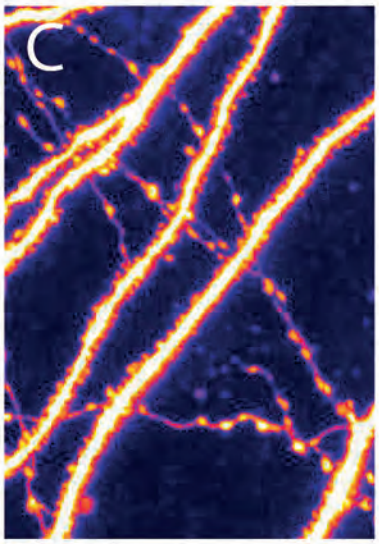
We have recently characterized active integrative properties of thin proximal dendrites (receiving recurrent innervation) of CA3 pyramidal neurons. We found a prominent role of NMDA receptor-mediated amplification of correlated synaptic inputs, which determined action potential output probability and reliability, and whose kinetics was powerfully regulated by K^+ currents. We are now extending our investigations to the active integration of different afferent pathways, as well as to the short- and long-term regulation of K^+ channel-mediated modulation of integration. We also aim to study whether changes in dendritic excitability and synaptic connectivity in the recurrent CA3 network are associated with *in vivo* experience as a potential correlate of memory formation.

Selected publications from the last 10 years:

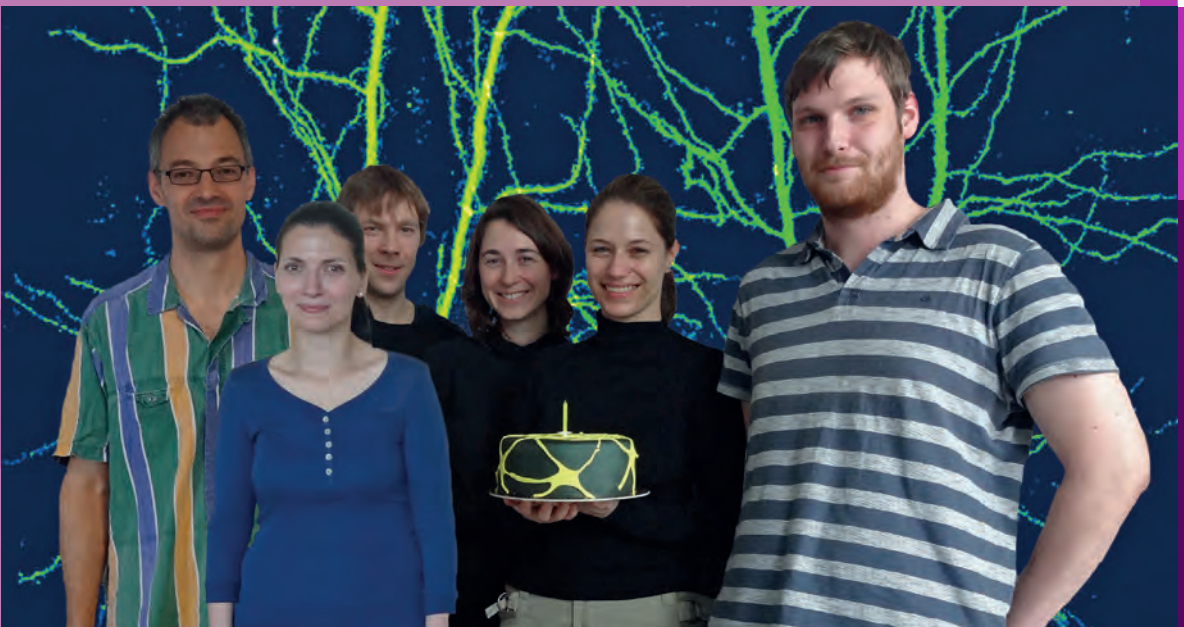
- Harnett MT*, Makara JK*, Spruston N, Kath WL, Magee JC (2012) Synaptic amplification by dendritic spines enhances input cooperativity. Nature 491:599-602. *equal co-authors
- Makara JK, Magee JC (2013) Variable dendritic integration in hippocampal CA3 pyramidal neurons.Neuron 80:1438-50.







Low magnification mon-tage of dye-loaded CA3 pyramidal neurons (A) and high magnification z-stacks showing perisomatic (B) and distal basal (C) dendritic structure of a CA3 pyramidal neuron.



from left: Bertalan Andrásfalvy, Adrienn Ráksai-Maár, JensWeber, Marina Polito, Judit Makara, Ádám Magó

Laboratory of Cellular and Developmental Neurobiology

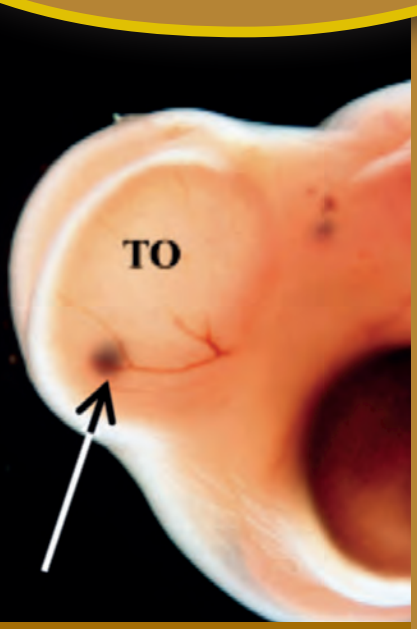
Department of Gene Technology and Developmental Neurobiology

Head of Laboratory:
Emilia Madarász, PhD

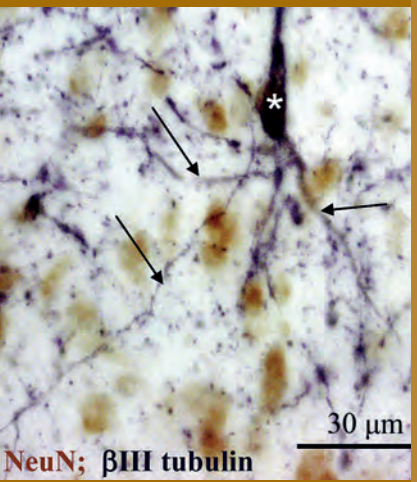
Mission statement

The activities of the Laboratory of Cellular and Developmental Neurobiology (CDNB) have been focused on events and mechanisms of neuron formation. Starting from one-cell derived clones of neural stem cells established from different stages of brain development and by using *in vitro* neurogenesis models, the roles of retinoid signalling, cell adhesion, metabolic and energy pathways, and the changes in ionic homeostasis have been addressed. Cell-fate commitment has been monitored by studying the expression of development-regulatory pre- and pro-neural genes, as well as position-specific genes determining cell identity along the neuraxis. Cellular phenotypes characteristic to different stages of *in vitro* differentiation have been identified by immunocytochemical methods. Primary brain cell cultures, purified microglia cultures and co-culture systems have been used to study cellular interactions and microglia reactions in response to damaging stimuli. Metabolic pathways of differentiating and damage-responding cells were monitored by measuring O_2-consumption in the presence of defined metabolic fuels (collaboration: Semmelweis Univ., Dept. Medical Biochem.). Label-free optical assays have been developed (collaboration: MicroVacuum Ltd) for studying the adhesive preferences of developing/regenerating cells. Fluorescent time-lapse microscopy was used for analysing the activation-dependence of motility of optogenetic neural stem/progenitor cells.

The CDNB gives regular theoretical and practical postgraduate training on "In vitro Cell Technology" and "Neural cell differentiation" and provides cell biological and culture facilities for other units of IEM-HAS.



NE-4C-derived mouse cells (blue; arrow) in the anterior brain vesicle of a 6-day old chick embryo grafted on the 3rd day of embryonic development (Demeter et al., 2004).



NE-4C stem cell-derived neuron (blue; * cell body, arrows: neurites) in the forebrain parenchyma of a 9-day old chick embryo, 6 days after implantation. Brown (NeuN) stainings shows the cell nuclei of host neurons (Demeter et al., 2004).

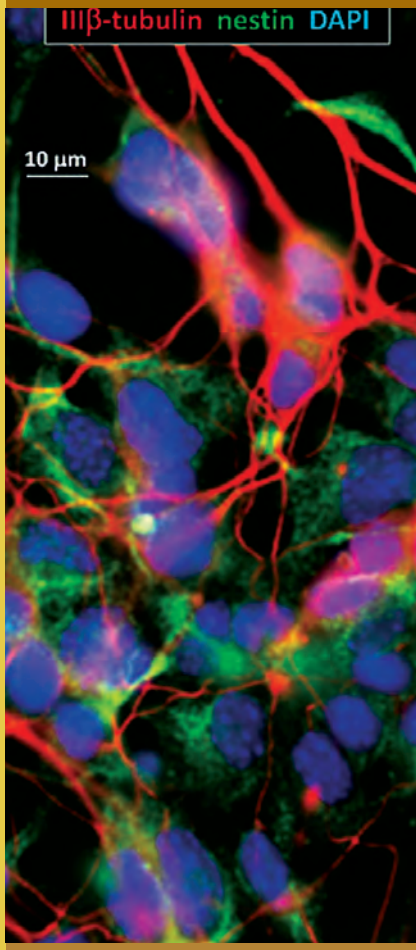
Senior scientists: Zsuzsanna Környei, PhD; Judit Szelényi, PhD, DSc
Marie-Curie fellow: Murali Kumarasamy
Ph.D. students: Attila Jády, Rebeka Fekete, Timea Kőhidi
Undergraduate research assistants: Noémi Papp, Judit Pomothy, Flóra Vásárhelyi-Nagy
Technician: Katalin Gaál

Isolation and characterization of neural stem cells

Pheno- and genotypically identical neuroectodermal stem/progenitor cells including NE-4C (ATCC CRL-2925), NE-7C2 and WNE clones have been established from the early embryonic (E 9-11.5) mouse neuroectoderm, and radial glia-like (RGl) stem/progenitor clones have been isolated from various regions of embryonic and adult wild-type or optogenetic mouse brains.

The embryonic neuroectodermal stem cells express various stem cell markers including Oct4 and develop into neurons, astrocytes and oligodendrocytes upon treatment with *all-trans* retinoic acid (RA). Non-induced stem cells do not express region-specific genes, while their differentiating progenies transcribe a series of positional genes which are not co-expressed *in vivo*. *In vitro*, different neuronal subtypes develop from the identical early embryonic neural stem cells. The formation of neurons precedes astroglia-genesis in all early clones.

Radial glia-like cells grow on AK-cyclo (RGDfC) coated surfaces in serum-free culture conditions, in the presence of EGF (Markó et al., 2011). Neuronal differentiation of RGl cells can not be initiated by RA-treatment, but by withdrawal of EGF, and astrocyte or oligodendroglia production needs different procedures. The selective adhesion to AK-cyclo (RGDfC) (Markó et al., 2008) allowed the separation of RGl cells from various regions of the adult mouse brain including SVZ and SGZ neurogenic zones and the cortical or midbrain parenchyma. While all RGl cells display radial glia characteristics (elongated shape, *pax6, emx2, blbp, glast* -but not *oct4, nanog* expression), the derived neurons show origin-dependent phenotypes.



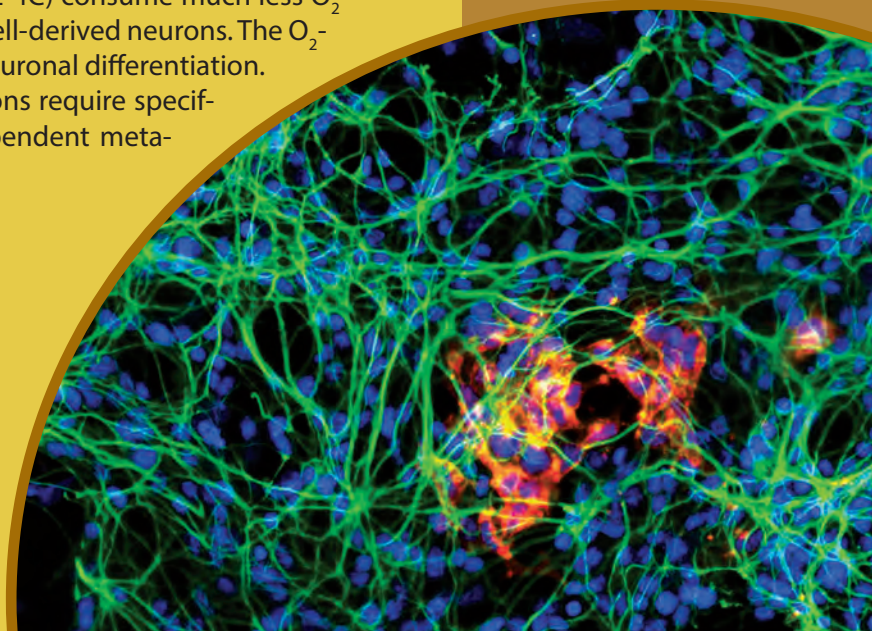
Progenitors (green) and neurons (red) developed from NE-4C embryonic neuroectodermal stem cells by the 6th day of induction with retinoic acid.

Metabolic characteristics and changes during neuron formation

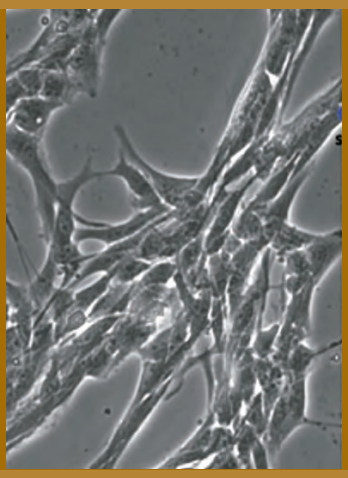
In comparison to early embryonic neuroectodermal stem cells, RGl cells showed different retinoid metabolism (Orsolits et al., 2013). In the early embryos, mesodermal tissues of the trunk are the main sources of retinoic acid, providing limited RA-availability for the sensitive anterior germinative zones. In the adult brain, meninges and choroid plexus produce RA resulting in high RA availability for the adult neurogenic zones; adult neural stem/progenitors, however, do not differentiate in response to RA exposure.

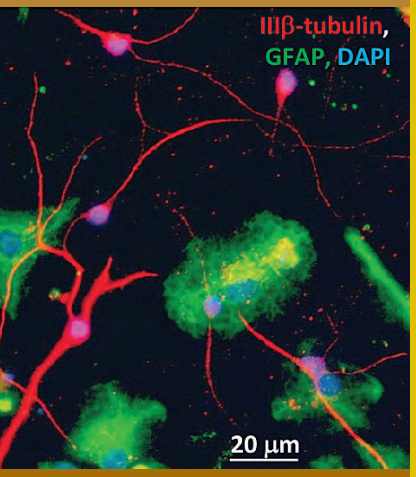
Non-committed neural stem cells survive and proliferate at low P_{O2}, but do not generate neurons (Zadori et al., 2011). Measuring O_2-consumption and extracellular acidification demonstrated that non-induced embryonic neuroectodermal stem cells (NE-4C) consume much less O_2 than the adult-derived RGl cells and stem cell-derived neurons. The O_2-consumption increases with the stage of neuronal differentiation.

Different neural stem/progenitor populations require specific, cell-type and developmental stage-dependent metabolic conditions for survival.

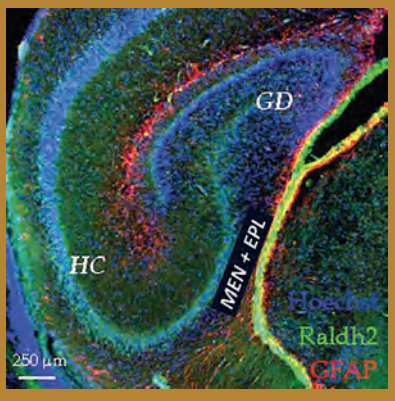


Groups of SSEA-1 immunreactive stem cells (red) persist in neuronal cutures differentiated from NE-4C embryonic neuroectodermel stem cells. Blu: Hoechst staining of cell nuclei; green: IIIβ-tubulin immunreactive neuronal processes. (Varga et al., 2008)





Adult SVZ-derived RGl cells (upper) and in vitro differentiating progenies on the 5th day after EGF withdrawal (lower).



Retinaldehyde-dehydrogenase 2 (Raldh2; green), a key enzyme of retinoic acid production is present in high amounts in the piamater (MEN) bordering the hippocampal formation (HC) and in the ependymal layer (EPL) lining the ventricles. GD: dentate gyrus.

Adhesive behaviour and motility of stem/progenitor cells

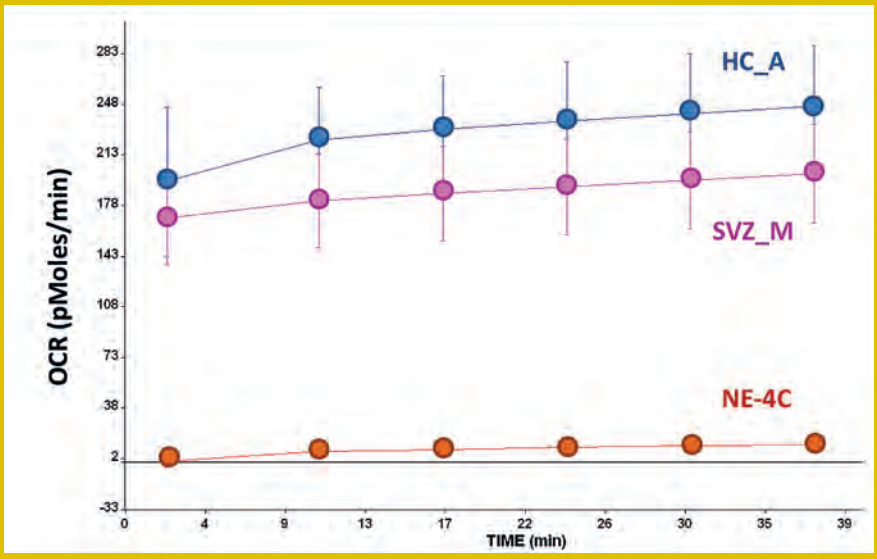
Optical waveguide light-mode spectroscopy (OWLS; www.owls-sensors.com), a sensitive, real time, label-free method, has been adapted for the quantification of cell adhesion and used in combination with live cell imaging, and cell adhesion-, dot-blot- and immunocytochemical assays. During initial attachment, alternating phases of ECM secretion and filopodial attachments were distinguished. The assays allowed differentiating active adhesion from passive settlement of cells on solid surfaces (manuscript in preparation). AK-cyclo(RGDfC) peptide-conjugate proved to anchor a number of non-differentiated cells including neural and embryonic stem (ES) cells.

Optogenetic neural stem cells (RGl^{ChR2+}) were established by transfecting RGl cells and by isolating RGl cells from optogenetic mouse embryos. Patch clamp assays demonstrated the light-gated cation channels in the cell membranes of RGl^{ChR2+} cells.

RGl^{ChR2+} and RGl^{ChR2-} cells were stimulated with light, and cell migration was recorded by time-lapse microscopy. The high motility of non-induced cells (migration distance ≥150 µm/24 h) decreased by the 2nd day of induction (40-80 µm/24 h), while intense intracellular nuclear migration appeared along the longitudinal cell axis. By the 5th day of induction, the majority of cells ceased moving and about 30% of the cells become process-bearing neurons. Illumination of light-sensitive cells on the 1st day did not cause motility changes, while a 24-hour stimulation on the 5th day resulted in markedly reduced motility and increased number of non-motile, process-bearing cells.

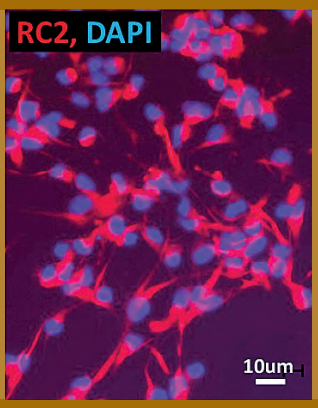
List of selected publications:

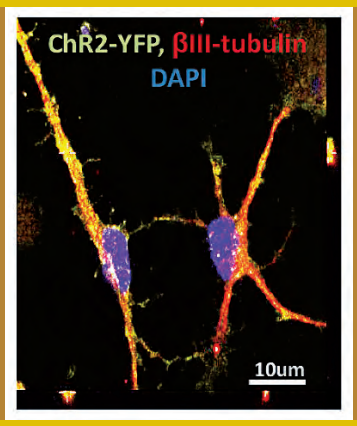
- Markó K., Kőhidi T., Hádinger N., Jelitai M., Mező G., Madarász E. Stem cells isolated by selective adhesion from distinct brain regions, PlosOne; | www.plosone.org 1 December 2011 | Volume 6 | Issue 12 | e28538
- Zádori A., Ágoston V. A., Demeter K., Hádinger N., Várady L., Kőhídi T., Gőbl A., Nagy Z., Madarász E. Survival and differentiation of neural stem cells at different oxygen levels. Exp. Neurology 2011, 227, (1), 136-148



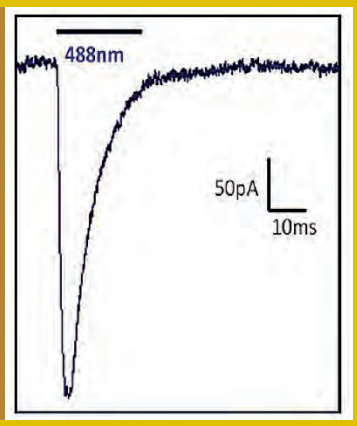
The rate of O_2-consumption (OCR) is significantly higher in adult-derived RGl stem cells than in the early embryonic (E9) NE-4C neuroectodermal stem cells.

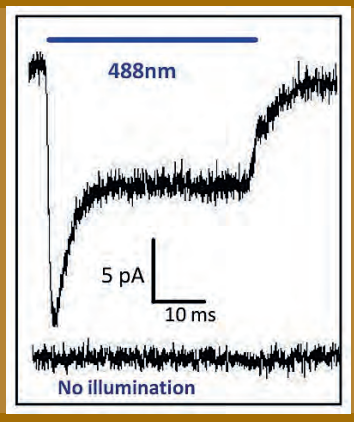
- Madarász E. (2013). Diversity of Neural Stem/Progenitor Populations: Varieties by Age, Regional Origin and Environment. In: Neural Stem Cells - New Perspectives, Dr. Luca Bonfanti (Ed.), ISBN: 978-953-51-1069-9, InTech
- Orsolits B., Borsy A., Madarász E., Mészáros Zs., Kőhidi T., Markó K., Jelitai M., Welker E., Környei Zs. Retinoid machinery of distinct neural stem cell populations with different retinoid responsiveness. Stem Cells Devl. 2013 Oct 15;22(20):2777-93.2013.
- Izak-Nau E., Kenesei K., Murali K., Voetz M., Eiden J., Duschl A., Puntes V., Madarász E. Interaction of differently functionalized fluorescent silica nanoparticles with neural stem- and tissue-type cells. Nanotoxicology 2013. DOI: 10.3109/17435390.2013.864427





Neurons derived from Chr2(H134)-EYFP expressing radial glia cells respond to illumination with inward cation current.





Chr2(H134)-EYFP construct expressing radial glia cells respond to illumination with inward cation current.

Standing: Judit Pomothy, Noémi Nagy, Rebeka Fekete, Flóra Vásárhelyi-Nagy, Katalin Gaál, Kumarasamy Murali, Attila Jády
Sitting: Emília Madarász, Zsuzsa Környei

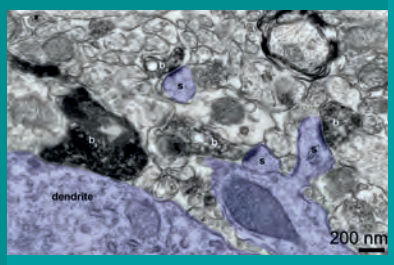
Laboratory of Molecular Neurobiology

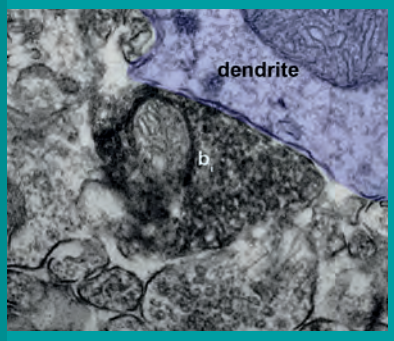
Department of Gene Technology and Developmental Neurobiology
Head of Momentum-supported Laboratory: István Katona, PhD

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-





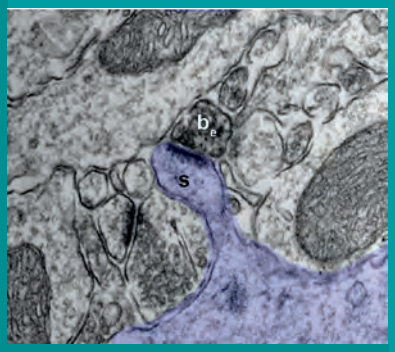


Figure 1.
These electron micrographs demonstrate the improved sensitivity for CB_1 receptors of a polyclonal antibody which was raised in FcRn overexpressing transgenic rabbits. Note the highly concentrated accumulation of the electron dense black DAB precipitate in both excitatory (b_e) and inhibitory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

Senior scientist: Zsolt Lele, PhD
Postdoctoral fellows: Marco Ledri, PhD; Steve Woodhams, PhD
Ph.D. students: Gyula Balla, Barna Dudok, Kata Kenesei, Zsófia László
Undergraduate students: Benjámin Barti, Emese Kovács, Vivien Miczán
Imaging specialist: László Barna
Lab Manager: Balázs Pintér
Technician: Erika Tischler

Research support:
1. European Research Council Starting Independent Research Grant 2009-2014
2. The Wellcome Trust International Senior Research Fellowship 2010-2016
3. Momentum Research Grant of the Hungarian Academy of Sciences 2013-2018

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

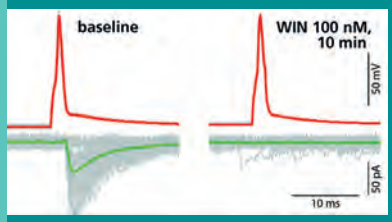
Our work during the last decade has revealed that presynaptic CB_1 receptors are ubiquitously distributed at most synapse types throughout the brain. These G-protein coupled receptors are among the most important presynaptic regulators of neurotransmitter release and mediate several forms of short-term 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.

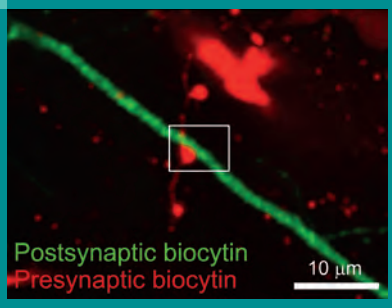
To facilitate quantitative studies on CB_1 receptors in brain circuits, we aim to improve the two most important tools of molecular neuroscience research, antibodies and microscopes. First, in collaboration with Prof. Imre Kacskovics (Immunogenes Kft), we employed the transgenic FcRn rabbit and mouse 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





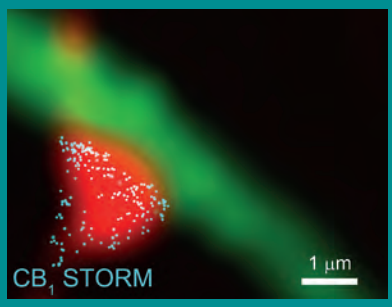


Figure 2.
The upper image shows that a CB_1 receptor agonist (WIN) readily inhibits action potential (red)–evoked inhibitory postsynaptic currents (green) in whole-cell patch-clamp paired recording. As seen in the middle image obtained by confocal microscopy, the axon (red) of the presynaptic GABAergic 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)*.

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- 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,

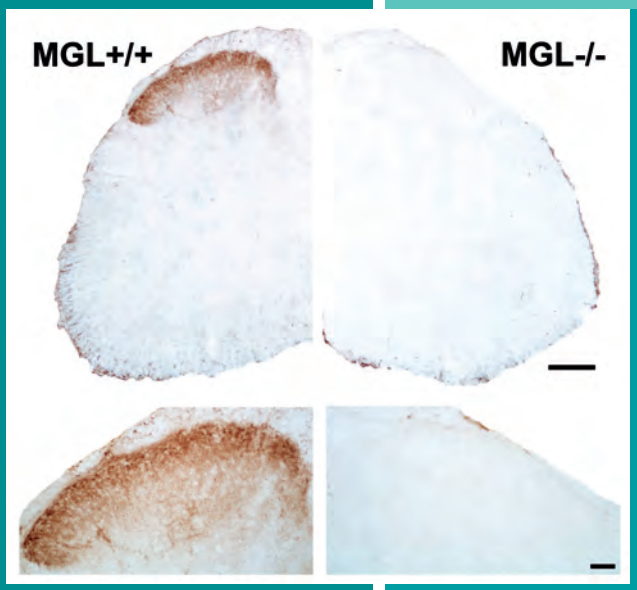

Figure 3.
Inhibition of monoacylglycerol lipase (MGL), a key regulator of endocannabinoid and prostaglandin signaling produces potent analgesia, but the anatomical location of its antinociceptive effects were unknown. These light micrographs demonstrate that MGL (brown DAB precipitate) is highly concentrated 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.

Vanegas H and Zeilhofer HU (2009) Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain senzitization. *Science*, 325:760-764.

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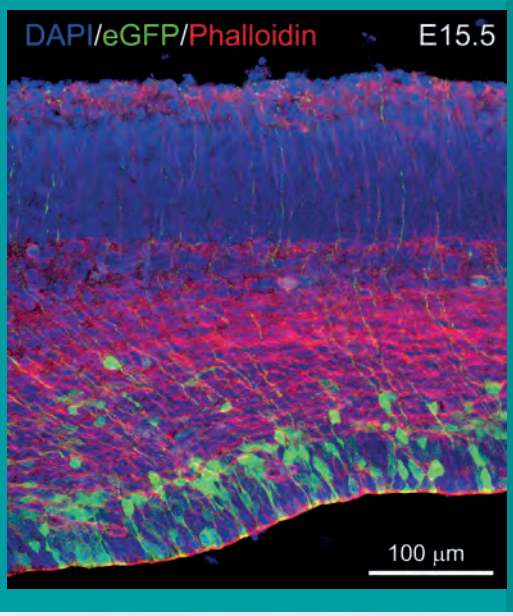


Figure 4.
Impaired migration of cortical cells (green) after *in utero* electroporation of a construct overexpressing an endocannabinoid-synthesizing enzyme reveals a new function of endocannabinoid signaling in the developing brain.

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

Laboratory of Behavioural and Stress Studies

Department of Behavioural Neurobiology

Head of Laboratory: József Haller, PhD

Mission statement

The group studies the neurobiological background of the stress response and emotional behavior. The approach is biomedical: laboratory models of human disorders are in focus. The main goal is to understand how the stress response develops, how it contributes to the development of behavioral dysfunctions, and how these are controlled by stress-induced changes in neural function. Furthermore, the group is interested in the identification of novel treatment opportunities for the behavioral dysfunctions studied. In order to mimic the clinical problems as closely as possible, several new behavioral models were developed. The main focus is on anxiety, depression and aggression. The goal of such studies is the identification of the complex interaction between the type of stressors and the type of the resulting behavioral dysfunctions. Research performed by the group is multidisciplinary. Endocrinological techniques are employed to characterize the hormonal consequences of stressors and their correlation with behavioral effects. Stress-induced changes in autonomic arousal and its correlation with behavioral dysfunctions is studied by *in vivo* biotelemetry. Brain mechanisms are studied by behavioral pharmacological, immunocytochemical, and recently, by optogenetic and epigenetic techniques. Finally, the possibilities of pharmacological intervention are explored by behavioral pharmacological studies that are based on the locally-discovered neural correlates and mechanisms that underlie stress-induced behavioral dysfunctions. The group is involved in drug development programs initiated locally or performed on request by pharmaceutical companies.

A mouse explores one of the compartments of the place-preference box, which is used to investigate the rewarding or aversive nature of drugs. Note that each compartment is marked differently to easy their recognition by experimental subjects.

Senior scientists: Manó Aliczki, PhD; Eszter Bodóné Sipos, PhD; Kornél Demeter, PhD; Éva Mikics, PhD; Máté Tóth, PhD; Dóra Zelena, MD, PhD
Ph.D. students: Diána Balázsfi, Anna Fodor, László Biró, Zoltán Balogh
Graduate research assistant: Lívia Farkas
Undergraduate research assistants: Bíborka Bruzsik, Péter Csikota, Áron Kerényi, Christina Miskolczi, Pál Pottyondy, Tamás Szabó, László Szente, Bibiána Török, Eszter Urbán
Technicians: Katalin Gyimesiné Pelczer, Beáta Barsvári
Research advisors: István Barna, Gábor Makara, MD, PhD

Studies on aggression

Studying aggression is one of the long-standing interests of the group. Its unique contribution to this field is the discovery that etiological factors of aggression-related human psychopathologies induce abnormal forms of aggression in laboratory rodents. The theoretical bases of this new approach in aggression research were laid down in cooperation with MR Kruk (University of Leiden, the Netherlands) (Haller and Kruk, 2006), and was recently amended in cooperation with scientists from Tufts University, and the University of Groeningen (Miczek, de Boer and Haller, 2013). Recently, the group together with scientists from the UK, Switzerland, and Germany proposed that such models may be used to study the impact of early-life stressors on the development of antisocial behavior (Haller et al., J Neuroendocrinol, 2014). Based on initial findings (Mikics et al., 2004) and the principles established later on, the group developed two models of abnormal aggression, one for psychopathologies characterized by instrumental/proactive and another for disorders differentiated by emotional/reactive aggression (e.g. antisocial personality disorder and intermittent explosive disorder, respectively).
The second discovery of the group is that the neural underpinnings of abnormal aggression are largely etiological factor- and emotionality-dependent (Tulogdi et al., 2010; Tóth et al., 2012), revealing that there are multiple neurobehavioral "roads" to abnormal aggression a finding to be considered when novel treatment options for aggression-related psychopathologies are evaluated. At present, the group studies the developmental mechanisms that lead to the emergence of etiological factor-dependent brain alterations and the details of aggression-related neural communication by epigenetic and optogenetic techniques, respectively.

Endocannabinoids, stress and behavior

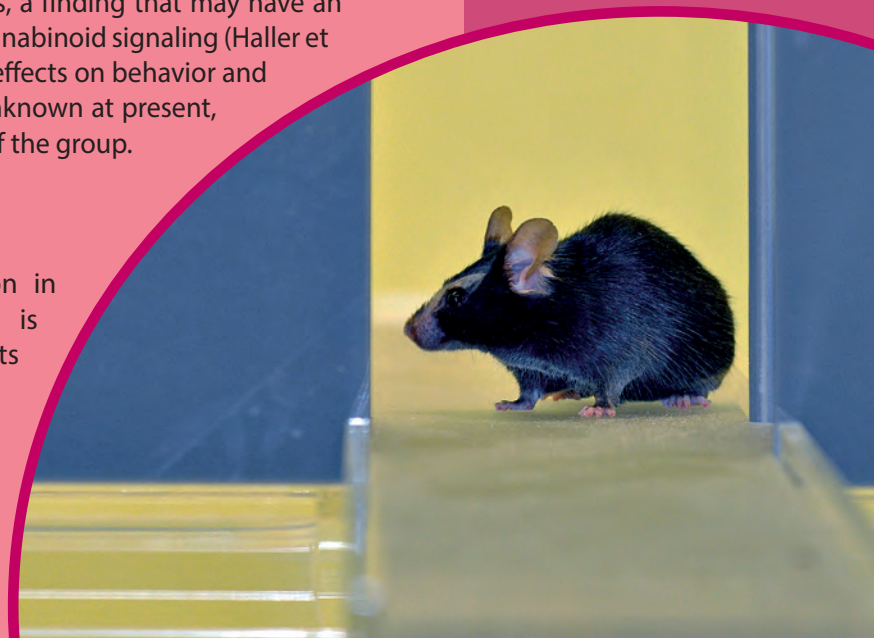
By using transgenic animals, this group was the first to establish a direct link between cannabinoid CB1 receptors and anxiety. Subsequent research involving the administration of stressors concomitantly with behavioral testing revealed that the role of cannabinoids is more complex than previously thought. The first publication on the issue demonstrated that the effects of cannabinoid agents largely depend on how aversive the testing environment is; moreover, cannabinoid effects are sometimes opposite depending on testing conditions (Haller et al., 2009). More recent findings of the group show that endocannabinoid signaling has a large impact on the way in which an individual copes with stress, a finding that may have an impact on the therapeutic implications of cannabinoid signaling (Haller et al., 2014). The relationship between specific effects on behavior and general effects on stress-coping is largely unknown at present, and constitutes one of the research targets of the group.

Vasopressin, stress and behavior

The role of vasopressin neurotransmission in stress responses and behavioral control is among the long-standing scientific interests

A mouse pokes his nose in one of the holes of the operant learning apparatus. Operant learning is frequently used to investigate the effects of environmental stressors, genetic influences and drugs on cognition, e.g. learning and memory as well as cognitive flexibility.

A mouse standing in the central area of the of the elevated plus-maze apparatus, which is frequently used to investigate anxiety states.



The three-chamber social interaction box is used to investigate social motivation, partner preference and social recognition. The subject is placed in the middle chamber; time spent in the two adjacent chambers, which may be left empty or may host familiar or unfamiliar individuals provides important information on the social behavior of subjects.

The recording of heart rates, body temperature, and locomotion by in vivo biotelemetry. Upper panell: on-line signals; lower panel: numeric values.

of the group. By studying the vasopressin-deficient Brattleboro rat, the group showed that vasopressin neurotransmission affects the development of the organism; this peptide is the primary secretagogue of the hypophyseal component of the stress-response in pups, and contributes to the development of anxiety and depression in newborn and adult rats. Recently, the group showed for the first time that vasopressin promotes maternal behavior in lactating dams, surprisingly in conjunction with contributing to the development of depression-like symptoms; the neural underpinnings of these roles were also investigated (Fodor et al., 2012). The group also revealed that in contrast to adults, the mineralocorticoid aldosterone is the main stress-responsive adrenocortical hormone in pups (Varga et al., 2013). Together with their earlier findings on age-related changes in central stress-controlling mechanisms, these findings show that the neuroendocrine stress response is specific in newborns. The developmental implications of this phenomenon are currently under study.

Emerging research areas

Within a consortium involving several groups of the Institute, the laboratory is engaged in optogenetic studies into the behavioral roles of the median raphe, a major source of cortical serotonergic innervation. In contrast to the dorsal raphe, neurons of this nucleus preferentially establish synaptic contacts with target neurons whose behavioral and physiological roles are largely unknown. They focus on anxiety and learning.
Prompted by earlier interest in conditioned fear (Mikics et al., 2008), the group initiated studies into the roles of glutamate receptor subtypes in mediating trauma-induced behavioral dysfunctions. By scrutinizing molecular details of glutamatergic neurotransmission, these studies have the potential of unraveling novel treatment options for post-traumatic stress disorder.

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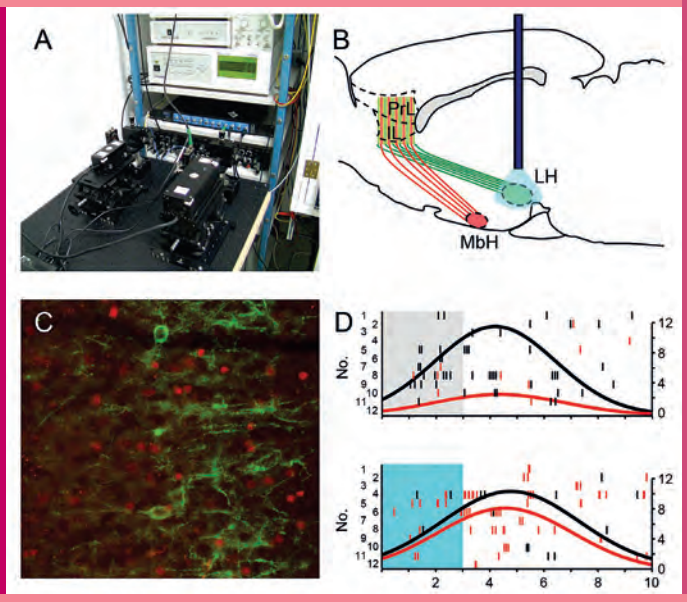
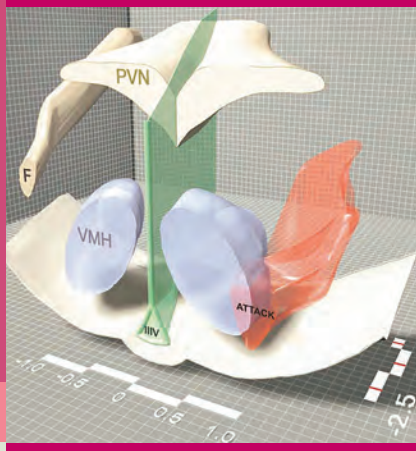
Maternal behavior is studied in conjunction with stress responses in the vasopressin-deficient Brattleboro rat.



Optogenetic experiments in a nutshell. *A*, The photostimulator producing laser light of specific wavelength; *B*, A graph showing the main features of an experimental design; *C*, Microscopic image showing the investigated neurons; *D*, Some of the results revealing the role of particular neural projections in the control of aggression.



A three-dimensional reconstruction of the hypothalamic attack area, a brain structure from where attacks on conspecifics can rapidly and reliably be elicited by electrical stimulation. The figure was published by the Behavioral Neurobiology department and by Dr. Menny R. Kruk from the Leiden University, The Netherlands. Abbreviations: attack; the hypothalamic attack area; F, fornix; IIIV, third ventricle; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus. The scales show distance from Bregma (mm).

Back row, from left: Kornél Demeter, László Bíró, Eszter Sipos, Dóra Zelena, János Varga, Máté Tóth, József Haller
Front row: Beáta Barsvári, Lívia Farkas, Diána Balázsfi, Anna Fodor, Éva Mikics, Manó Alicki, Katalin Gyimesiné Pelczer

László Barna

Nikon Microscopy Center at IEM HAS

Core facility unit

Head of Unit:
László Barna, MSc

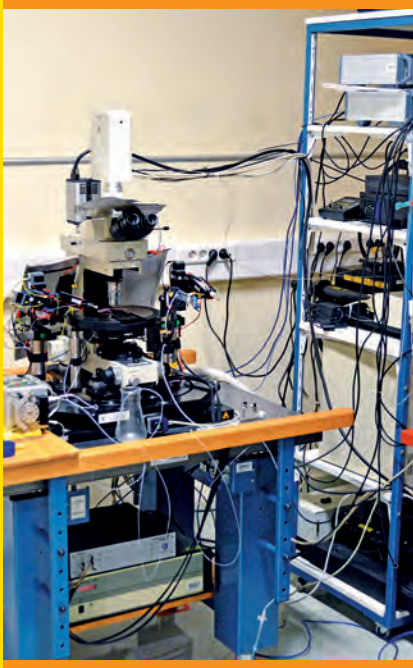
Mission statement

Successful mapping of the human genome at the beginning of the 21st century uncovered an unprecedented gold-mine of information about the blueprint of human biology. Postgenomic biology now turns its attention to decipher this code with the ambitious goal of a complete understanding of how exactly the ~21,000 protein-coding genes and an unexpected variety of other regulatory elements together orchestrate the beautiful complexity of life, from the level of cell physiology to behavioral phenomena. By additionally uncovering the molecular underpinnings of diseases, this enormous work will also help researchers to exploit the genome for a new generation of more effective and selective medicines.

These major objectives would not be feasible without a few other revolutionary developments in the life sciences. First of all, single molecules can now be tagged by genetically encoded fluorescent proteins and can be tracked to elucidate their dynamic behavior in living cells. To aid these investigations, new microscopic tools were developed which allow better spatial and temporal resolution, increase experimental flexibility by spectral detection, provide a more reliable environment for long-term live imaging protocols and, most importantly, support quantitative analysis of image data down to the molecular level. However, as new microscope systems become considerably more complex, research institutes must face the great challenge of combining all these components together, but still operate these complex systems in a functionally simple way for the end users.

To circumvent this problem, the Institute of Experimental Medicine (IEM), EM, Nikon Austria GmbH and its Hungarian distributor, Auro-Science Consulting Kft, established the Nikon Microscopy Center at the IEM in spring 2010. In terms of this collaboration, Nikon Austria has contributed with state-of-the-art microscope hardware and software for experimental use by IEM researchers, whereas Auro-Science provides an exclusive support service for their maintenance and application. The IEM constructed a new 126 m^2 microscopy room specially designed for imaging experiments.

The overall mission of the new Nikon Microscopy Center is to support IEM researchers in their leading edge research activities. By using these state-of-the-art microscopes and the specialized expertise of Nikon and Auro-Science staff, it provides a unique opportunity for IEM scientists to discover new molecular and cellular processes regulating brain cell activity, and also facilitates discovery of new pathophysiological mechanisms of brain disorders.

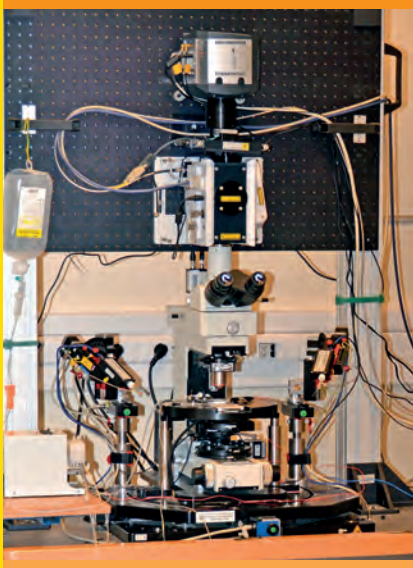


Microscopes:

Since the spring of 2014, there are 5 major microscope systems available for IEM HAS researchers.

1. A1R confocal system

The first of these microscopes is the flagship confocal system of Nikon placed on a fully automated Eclipse Ti-E inverted research microscope, which is equipped with 4 lasers, a Solent Scientific environmental chamber, the A1R resonant confocal head, 4 channel and spectral detector, and high-end Nikon objectives. This microscope is used for cell biological experiments, for example to study the signaling pathways in living neurons and glia that occur in or near their plasma membranes, and for neuroanatomical investigations.

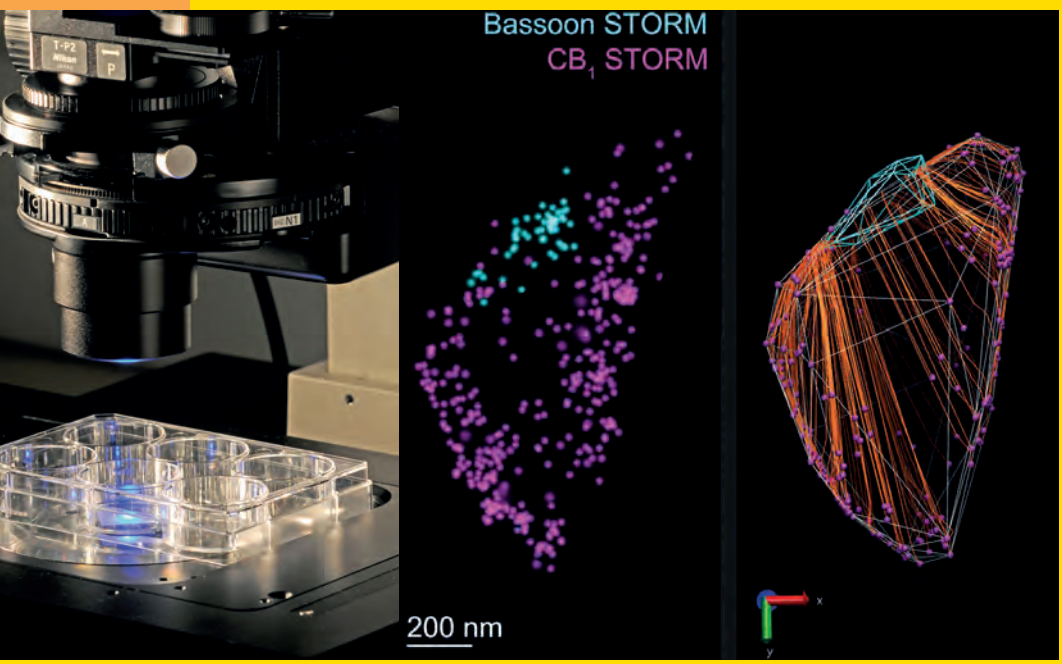


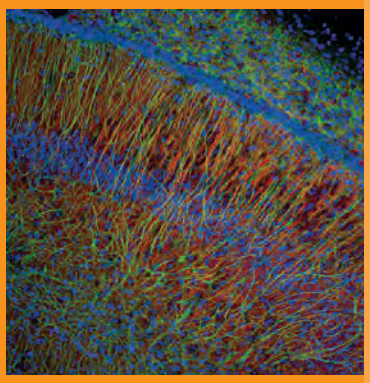
2. C2 confocal system

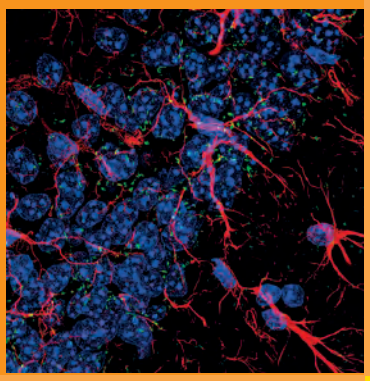
The second microscope is based on an Ni-E motorized upright body equipped with a C2 confocal head and 4 lasers. This microscope is predominantly used for anatomical experiments.

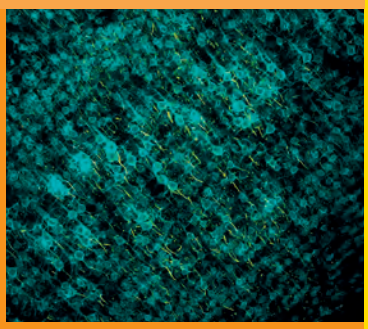
3. N-STORM super-resolution system

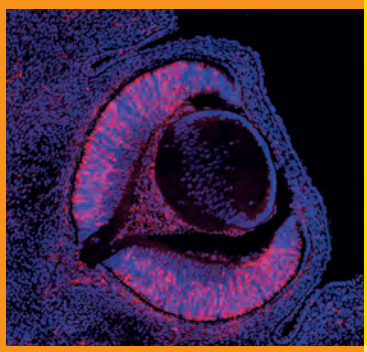
The third microscope is based on a Ti-E inverted body and it is equipped with Andor iXon camera, objectives and software to enable single mol-











ecule localization microscopy. This super-resolution system enables fluorophore localization precision of 10-20 nm (x-y) and 40-50 nm (z) in brain tissue, and is therefore an ideal solution for molecular imaging at the nanoscale level. This system is also equipped with a C2 confocal head which makes simultaneous confocal imaging possible, to provide a cellular context for the molecular data.

4. C1 patch-clamp setup

The fourth system is based on a fixed-stage Eclipse FN1 microscope equipped with a C1 confocal head, 3 lasers, 25x NA1.1 objective, IR DIC with sCMOS camera and is predominantly used to combine whole cell patch clamp electrophysiological experiments with high resolution confocal imaging of molecular dynamics in given subcellular compartments.

5. Spinning disk patch-clamp setup

The fifth microscope also stands on an FN1 microscope designed for patch-clamp electrophysiology, but it is equipped with an Andor Revolution spinning disk system and also with IR DIC imaging. This microscope is used to combine single neuron recording and monitoring of population activity by optical imaging.

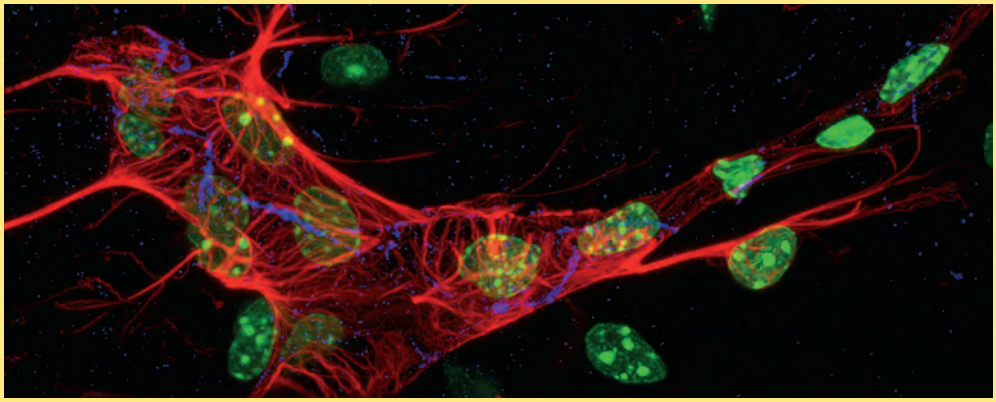
Achievements:

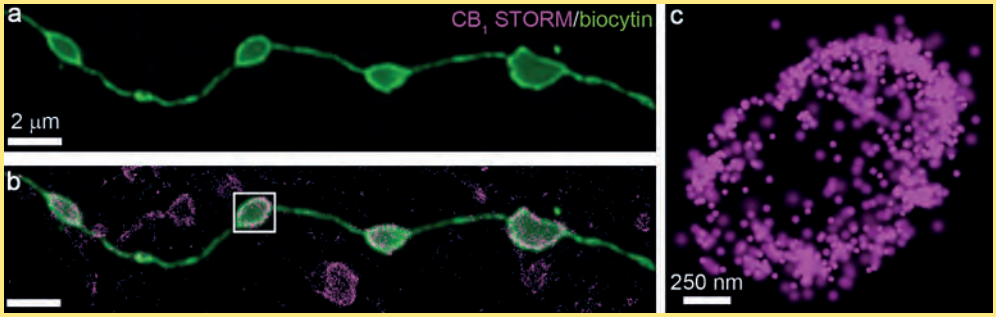
During the first 5 years of its activity, several major milestones have been achieved by the Nikon Microscopy Center:

- 15 out of 16 research groups have used the NMC
- More than 100 researchers or students worked with the equipments of the NMC
- Altogether 18,000 working hours have been spent at the NMC
- IEM HAS researchers used the NMC equipments in ~ 100 scientific projects
- So far, 40 studies were published in leading neuroscience journals.

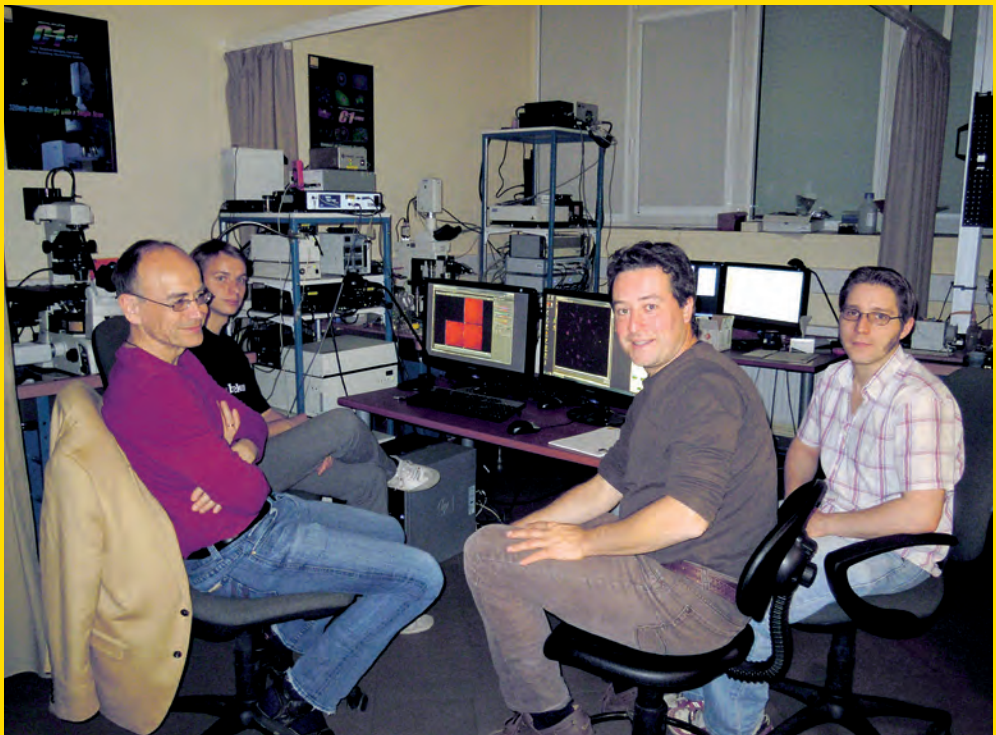
Most important recent publications:

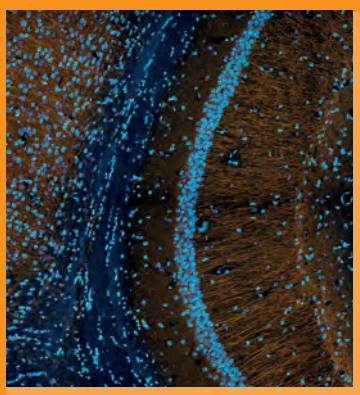
- Giber et al Nat Neurosci 2015
- Dudok et al Nat Neurosci 2015
- Denes et al PNAS 2015
- Murali et al Nanoscale 2015
- Szőnyi et al Brain Structure and Function 2014
- Veres et al J Neurosci 2014
- Szabó et al J Neurosci 2014
- Brunner et al eLIFE 2014
- Egri and Gereben, J Mol Endocrinol 2014
- Kisfali et al, J Physiol (Lond) 2013

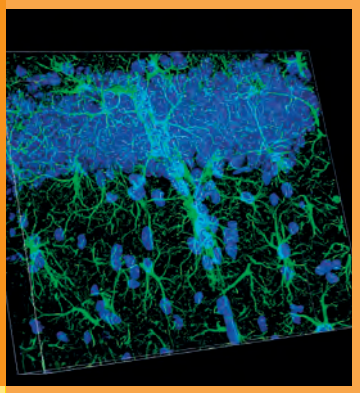


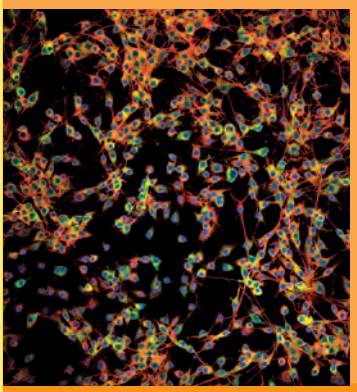


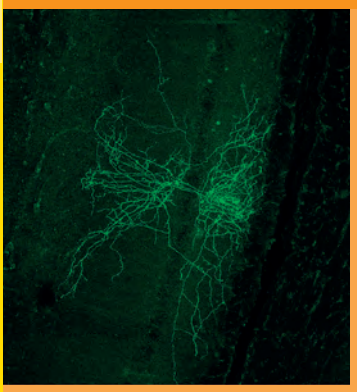
Tom Südhof (on the left, Nobel Prize in 2013) visiting the Nikon Microscopy Centre.

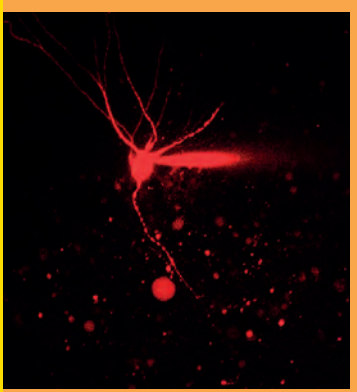












Gábor Szabó

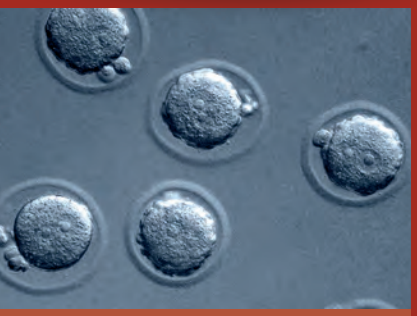
Medical Gene Technology Unit

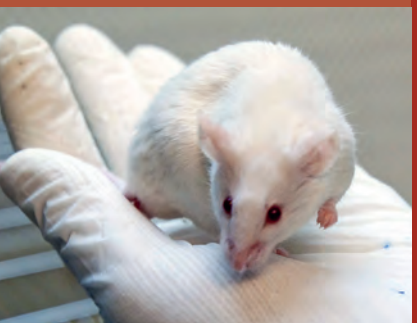
http://ogr.koki.hu

Head of Unit:
Gábor Szabó, MD, PhD

Manager of Unit:
Ferenc Erdélyi, MSc, Dr. Univ.

Mission statement and general description of the unit





The use of research animals, especially genetically modified mouse models have proved an indispensable tool to study basic biological processes and diseases at the molecular, cellular and system's levels *in vivo*, in the context of a whole live organism.

The **Medical Gene Technology Unit** (**MGTU**) of the institute was established a decade ago with the mission to maintain and breed existing rodent models as well as derive new genetically modified mouse models for biomedical research. The **MGTU** provides a variety of animal- and gene technology services to the whole scientific community including research groups of the institute, academia and the pharmaceutical industry.

This state-of-art facility comprises of two closely related **Animal- and Gene Technology Division** (**ATD & GTD**). The **ATD** operates in three separated areas with defined hygienic levels and different functions. The receiving-quarantine area operates as a conventional animal facility. The minimal disease area (**MD**), which is accessible to researchers houses rat and mouse colonies for experimental use with SPF hygienic status and in addition provides facilities for behavioral, pharmacological and surgical experiments to research groups. In the **fully restricted SPF area** breeding colonies of wild type and genetically modified mice are housed predominantly in individually ventilated cages. They currently maintain more than 100 different genetically modified mouse colonies and provide breeding genotyping and related services upon request amounting to over 20 000 GM mice annually. The Unit also provides animal breeding services for the recently established independent Research Units of the institute comprising *Behavioral Studies, Metabolic Phenotyping* and *Virus Technology*.

The **Genetic Testing Laboratory** of the **GTD** performs PCR-based genotyping of knockout and transgenic mice, supporting breeding and genetic crossings. The laboratory has developed tests for more then 100 mouse genes, modified alleles, and marker genes and annually performs more than 25,000 genomic PCR or Taqman-based qPCR reactions, respectively.

The **Microinjection & Embryo Laboratory** of the **GTD** operates behind the SPF barrier area. Over the past 5 years they have successfully re-derived more than 180 GM mouse lines through embryo transfer, and have cryopreserved over

Animal technologists: Katalin Bartosek, Mária Geszlerné Ecsédi, Mária Kaziné Szűcs, Adrienn Máthé, Miklós Ménessy, Csaba Pósa, Rozália Szafner and Miklós Tóth
Gene technologists: Ágnes Dobos MSc, Zsuzsanna Erdélyi MSc and Zoltán Máté MSc
Embyologist-transgenic technologist: Katalin Kovács
Senior scientist: Zoya Katarova PhD
Student research assistants: Anikó Fülöp and Dorina Glowacz

90 lines by embryo vitrification or sperm freezing, thus laying down the basis of a national mouse embryo bank. They also apply IVF technology for both re-derivation and embryo freezing.
The **GTD** routinely generates transgenic mice by pronuclear microinjection of both plasmid- and BAC-based transgenes and also utilizes transposon-based transgenesis. The Unit is also involved in managing complex transgenic projects from transgene design, engineering and microinjection to derivation and establishing and initial characterization of transgenic mouse lines. They have generated numerous transgenic mouse lines expressing fluorescent tags in the nervous system widely used in many neuroscience laboratories as well as a number of models for neurological diseases to support their own research or as a collaboration and/or as a service provided to other research groups and companies.

The virtual animal facility

The operation of **MGTU** is assisted by a custom-made **Facility Management software**, which allows tracking and archiving of all animals based on birth-dating and genotyping data, trace their ancestry, history of breeding as well as archiving data related to all activities of the Division. Researchers have a remote access to all data on their own colonies and can personally manage the breeding and genotyping electronically.

External service activity of MGTU

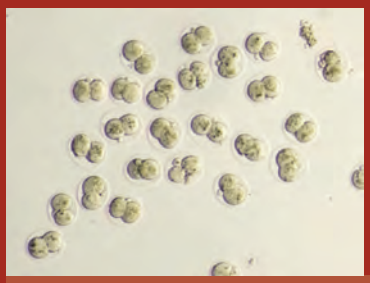
The major services that are currently offered by the facility to consumers include colony maintenance (breeding and genotyping) of GM mice, re-derivation, cryopreservation and recovery of mouse stocks and transgenic mouse production by pronuclear microinjection, including transgene design and engineering. The Unit has recently started to successfully implement the TALEN and CRISPR technology for generating KO and KI mouse models.

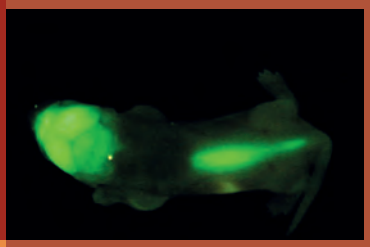
Development and research activity of MGTU

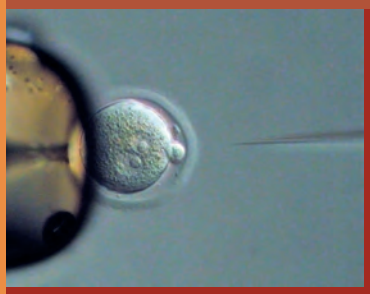
MGTU continuously adapts, develops and introduces the latest animal- and gene technologies to better serve the whole research community. The following advanced techniques have been added to the service repertoire in the recent years: *in vitro fertilization, sperm freezing, transposon-based transgenic technology, targeted nuclease-mediated knock-out and knock-in by using TALEN and CRISPR technologies.*

Research Unit of MGTU

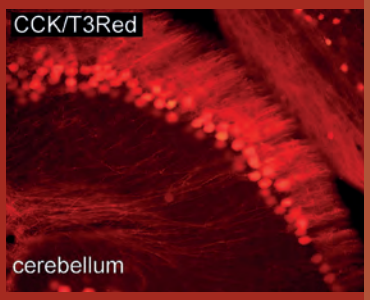
This unit is a recent addition of the former Laboratory of Molecular Biology and Genetics to **MGTU**. The research activity continues to focus on the role of GABA signaling, especially the function of different molecular forms of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD65 and GAD65 and embryonic forms) in complex brain functions, development and diseases. Due to the complexity of signals in the brain, they often use simpler model systems from the periphery such as neuronal and embryonic stem (mES) cells or the developing eye lens and peripheral olfactory system affording the utilization of cell biology and genetic manipulation approaches. Another line of research has been the generation of transgenic mice expressing fluorescent markers in different classes of neurons (both GABAergic and glutamatergic) or models with genetically altered GAD expression to study the GABA signaling during development and in severe neurological and psychiatric diseases.

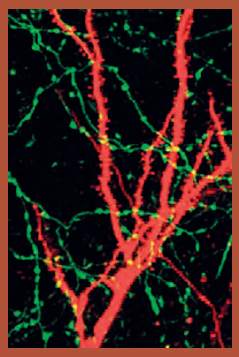


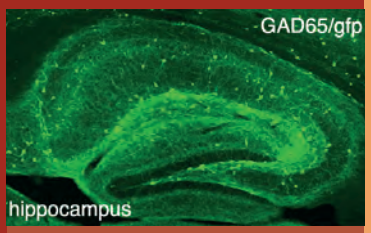


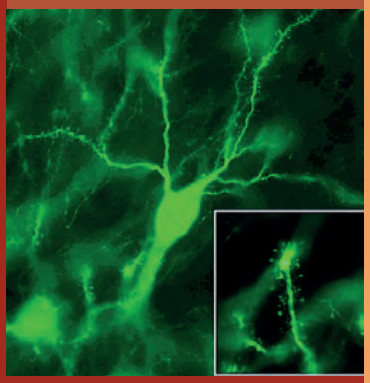


MGTU is the largest and most advanced research infrastructure of its kind in the country. Based on a national evaluation program, it was awarded the status of **Strategic Research Infrastructure** in 2010, renewed in 2014.









Major research areas and achievements

1. Studies on the developmental regulation of GABA signaling components

Using a variety of *in vivo* and *in vitro* systems including the brain, eye lens and stem cells they have observed a common temporal regulation of different GAD isoforms, notably the induction of GAD67 is preceded by the sequential and transient expression of two alternatively spliced embryonic GAD messages encoding the truncated GAD25 and GAD44, which were originally identified by this group. The switch from embryonic to adult GADs parallels differentiation suggesting a role of truncated GADs in this process. Furthermore, highly correlated expression was found between different GAD forms and downstream GABA signaling components. These studies contribute to our basic understanding of the ontogeny of GABA signaling and its link to downstream second messenger signaling systems that could explain its pleiotropic effect of GABA on cell proliferation, neuronal differentiation and migration and synaptic plasticity, which is critical to understanding the phenotypic alterations in diseases caused by deficient GABA signaling in both brain and/or periphery.

2. Characterization and role of GABA signaling in undifferentiated ES cells

We have found that in undifferentiated mES cells both $GABA_AR$ and $GABA_BR$ trigger synergistic rise of the intracellular Ca^{2+}, but modulate oppositely cell proliferation acting through different mechanisms and second-messenger pathways, which indicates that even at the earliest stage of development GABA can exert distinct effects mediated by different GABA receptors. Modulation of proliferation may be a major mechanism of GABA action also during neurogenesis and in some highly aggressive cancers.

3. Studies on the subcellular roles of different GADs using the eye lens as a genetic model

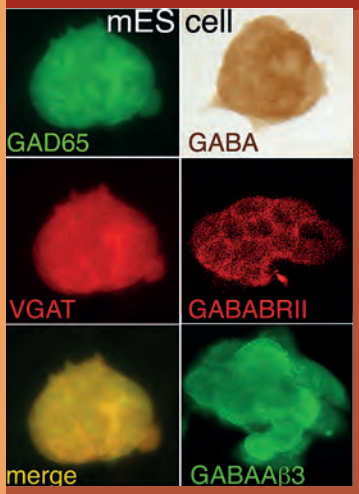
The ocular lens is an excellent *in vivo* model system of cellular differentiation, which was used to discern the specific roles of the GAD isoforms. They have found that GAD65 and GAD67 are spatially segregated in the primary vs. secondary lens fibers, respectively and are coordinately expressed with different downstream GABA signaling components during lens fiber differentiation. They have demonstrated for the first time that the developing lens expresses a fully functional GABA signaling, a novel finding that is expected to add new insights into the role of GABA in eye morphogenesis. The lab has also confirmed that activation of both GABA receptors triggers increase of $[Ca^{2+}]_i$ in lens epithelial and fiber cells. Overexpression of GAD in the lens of transgenic mice leads to specific ocular defects, affects proper vision and intracellular Ca^{2+} signaling allowing specific conclusions to be drawn concerning the specific subcellular functions of different GADs in the proliferative and elongation phases of lens fiber differentiation.

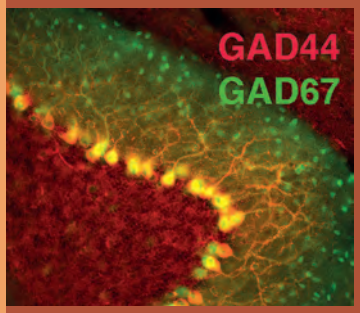
4. GAD forms in the developing olfactory system

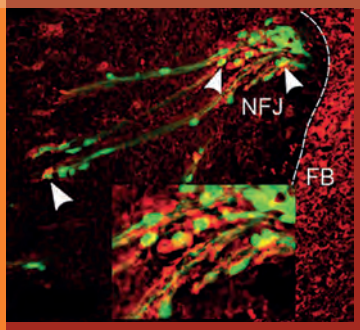
Similar to the developing lens they have found that GAD65 and GAD67 are expressed in a strictly segregated fashion in the developing olfactory system associated with profoundly different cellular functions. Despite segregated expression and function the two GADs play complementary roles in the regulation of GnRH migration. Their studies clearly indicate that GAD65 and GAD67 may exert different functions through distinct intracellular GABA pools activating distinct downstream effector systems.

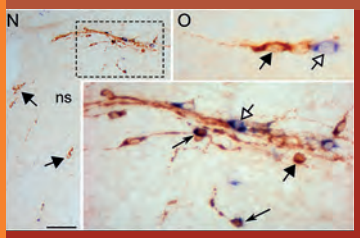
Most important publications from the last 10 years:

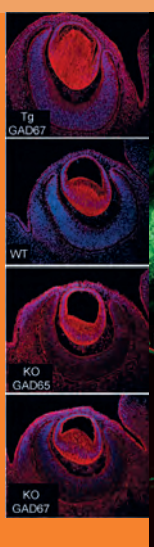
- López-Bendito G., Sturgess K., Erdélyi F., Szabó G., Molnár Z., Paulsen O. Preferential origin and layer destination of GAD65-GFP cortical interneurons. CEREB CORTEX 14: 1122-1133 (2004)
- Kwakowsky A., Schwirtlich M., Zhang Q., Eisenstat D. D., Erdélyi F., Baranyi M., Katarova Z. D., Szabó G. GAD isoforms exhibit distinct spatiotemporal expression patterns in the developing mouse lens: correlation with Dlx2 and Dlx5. DEV DYNAM 236: 3532-3544 (2007)
- Kwakowsky A., Schwirtlich M., Kooy F., Ábrahám I., Máté Z., Katarova Z., Szabó G. GABA neurotransmitter signaling in the developing mouse lens: Dynamic regulation of components and functionality. DEV DYNAM 237: 3830-3841 (2008)
- Betley J. N., Wright C. V., Kawaguchi Y., Erdelyi F., Szabo G., Jessell T. M., Kaltschmidt J. A. Stringent specificity in the construction of a gabaergic presynaptic inhibitory circuit. CELL 139: 161-174 (2009)
- Brill M. S., Ninkovic J., Winpenny E., Hodge R. D., Ozen I., Yang R., Lepier A., Gascon S., Erdelyi F., Szabo G., Parras C., Guillemot F., Frotscher M., Berninger B., Hevner R. F., Raineteau O., Gotz M. Adult generation of glutamatergic olfactory bulb interneurons. NATURE NEUROSCIENCE 12: 1524-1533 (2009)
- Schwirtlich M., Emri Z., Antal K., Mate Z., Katarova Z., Szabo G. GABAA and GABAB receptors of distinct properties affect oppositely the proliferation of mouse embryonic stem cells through synergistic elevation of intracellular $Ca2^{+}$. FASEB J 24: 1218-1228 (2010)
- Gulyas A. I., Szabo G. G., Ulbert I., Holderith N., Monyer H., Erdelyi F., Szabo G., Freund T. F., Hajos N. Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. JOURNAL OF NEUROSCIENCE 30:(45) pp. 15134-15145 (2010)
- Schwirtlich M., Kwakowsky A., Emri Z., Antal K., Lacza Z., Cselenyak A., Katarova Z., Szabo G. GABAergic signaling in primary lens epithelial and lentoid cells and its involvement in intracellular Ca(2+) modulation. CELL CALCIUM 50: 381-392 (2011)
- Vasudevan A., Won C., Li S., Erdelyi F., Szabo G., Kim K. S. Dopaminergic neurons modulate GABA neuron migration in the embryonic midbrain. DEVELOPMENT 139: 3136-3141 (2012)
- Chiovini B., Turi G. F., Katona G., Kaszás A., Pálfi D., Maák P., Szalay G., Szabó M. F., Szabó G., Szadai Z., Káli S., Rózsa B. Dendritic spikes induce ripples in parvalbumin interneurons during hippocampal sharp waves. NEURON 82: 908-24 (2014)

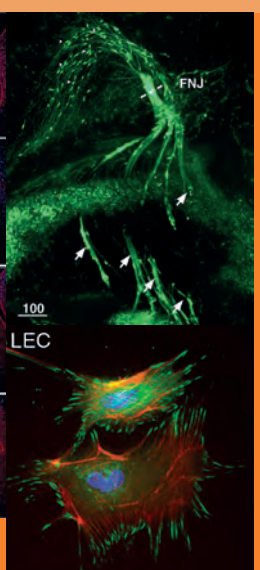












Unit for Behavioral Studies

Head of Unit:
József Haller, PhD

Mission statement

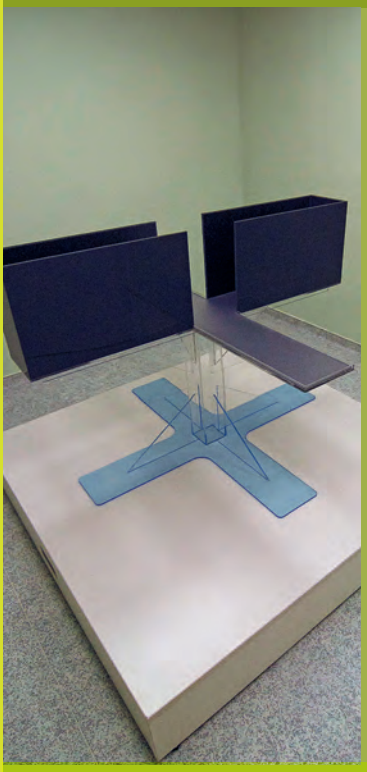
The Unit was created in 2014 to serve the behavioral research needs of the Institute and potentially to meet extra-institutional requests of this kind. The role of, and the demand for, behavioral studies is increasing in neuroscience (the main profile of the Institute), which prompted the initiative of constructing a specialized unit for such studies. The Unit works independently, but assists all interested parties to reach their scientific goals. The Unit consists of 5 animal maintenance rooms, which can host in total 240 mice, and 180 rat cages, a fully equipped surgery room and 5 research rooms where behavioral studies can be carried out, an anteroom, a main hall, and a corridor. Cooperation with users can take four forms: supplying users with space and equipment, providing technical help and scientific consultation, research cooperation, and performing commissioned research. Work is assisted by a computer-based reservation and utilization system, and links with the Intranet of the Institute.

Facilities for behavioral research

Behavioral categories that can be studied in the Unit include (note that the same equipment may be used for several purposes depending on use conditions):

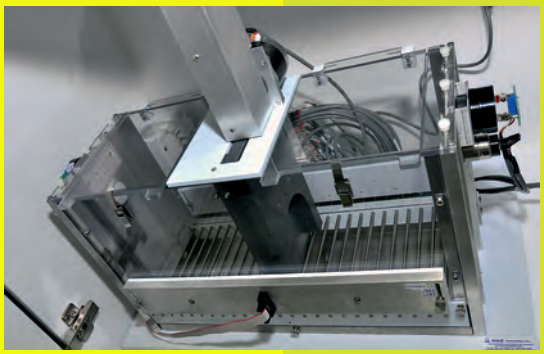
- *learning and memory* (available tests/equipment: open-field (habituation learning), object recognition, social recognition, familiarity recognition, shuttle-box (associative learning), operant conditioning, Morris water-maze, olfactory discrimination, 5 choice serial reaction, fear memory (cue-induced, contextual), Y-Maze (spontaneous alternation test), and radial maze);

Senior scientist: Áron Tulogdi, Ph.D
Technician: Nikolett Venczkóné Bakos



- *anxiety* (available tests/equipment: open-field (avoidance of central area), elevated plus-maze, social interaction, light-dark box, social avoidance, startle response, conditioned fear, ultrasonic vocalization, hole-board)
- *depression* (available tests/equipment: shuttle box (learned helplessness), forced swimming, tail suspension, prepulse inhibition, social defeat (defeat-induced depression)
- *schizophrenia* (available tests/equipment: PCP-induced social withdrawal, prepulse inhibition, amphetamine-induced hyper-locomotion);
- *social behaviors* (available tests/equipment: sociability (three-chambered box), partner preference, sexual preference, social interaction, resident/intruder test (aggression);
- *addiction* (available tests/equipment: place preference, drug self-administration, biotelemetry (the assessment of drug tolerance);
- *drug-induced responses and drug interaction* (available tests/equipment: place aversion/preference; drug discrimination, biotelemetry for heart rate, body temperature, locomotion, eating and drinking.
- *muscle strength, movement coordination, locomotor activity, and exploratory tendency* (available tests/equipment: balance beam; wire suspension, rotarod, open-field, hole-board).

Behavioral investigations are supported by a movement-tracking and analyzing system (EthoVision, Noldus, The Netherlands), a semi-automated behavioral analysis system (Observer, Noldus, The Netherlands), and by equipment-specific hardware and software in the case of complex tests. Distant behavioral recording is ensured by a web camera system (the automation of behavioral observations is in progress).

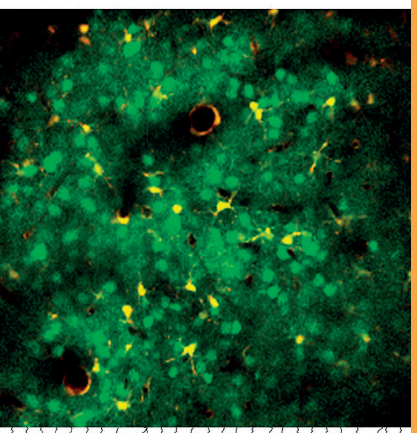


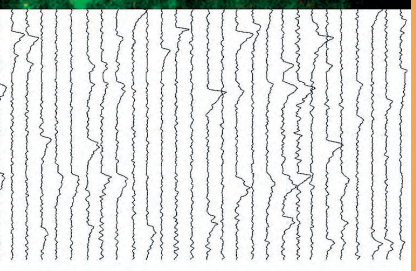
Two-Photon Imaging Unit

Head of Unit:
Balázs Rózsa, MD, PhD

Mission statement

To further understand the computational mechanism of the brain we need complex techniques to be able to measure from large population of neurons, but also with great details and subcellular resolution. The main goal of our group is to adopt and develop novel imaging techniques for scientific researches which make this quest attainable. With our novel developments it became possible to follow the activity of the entire dendritic tree of a neuron in three dimensions or to record hundreds of cell simultaneously in an intact neuronal network (Katona et al. 2012 Nature Methods, Katona et al. 2011 PNAS). Having these techniques in hand, we turn to various biological questions that could be only addressed with these novel tools. We are interested in better understanding the role of the integration on a single neuron interacting with a large network. For example we are studying the dendritic integration on a single neuron during sharp wave ripple oscillation (Chiovini et al. 2014 Neuron). A larger scale project is to find out the role of a single neuron within different network ensembles during learning tasks performed by the animal. Specifically weare investigating of the neuronal level coding of the visual cortex during different visual stimulation and behavioral paradigms.The Two-Photon Imaging Center is closely collaborating with research at the PázmányPéter Catholic University and also with the multidisciplinary research team of Femtonics Ltd.



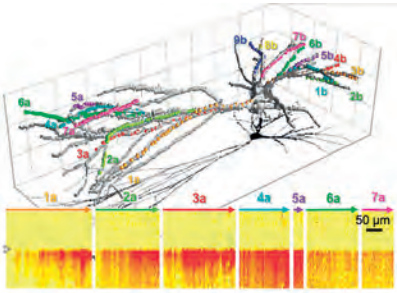


3D two-photon imaging

To understand the fast computational mechanisms of the brain, one needs to be able to perform rapid measurements at several sites along a single neuron as well as to image large populations of neurons. Traditional two-dimensional measurements are severely limited for such kinds of endeavors since neurons are located in three dimensions. To overcome this problem, we have developed new solutions to perform three-dimensional functional imaging with large scanning ranges along the z direction (Katona et al. 2012 Nature Methods, Katona et al. 2011 PNAS). With our three-dimensional microscopes we are able to maintain point or trajectory scanning which in combination with the ~800 µm penetration depth of two-photon technology makes our methodology very convenient for *in vivo* measurements of neuronal populations, too.

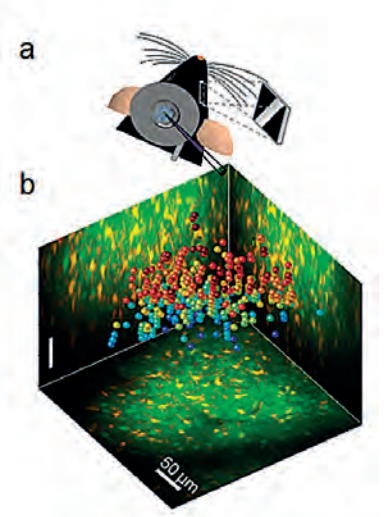
In vivo measurement of network activity

Population activity has long been studied in the visual cortex. We conduct *in vivo* two-photon imaging of cell assemblies in the V1 area by using bolus loading and genetic approaches. Neuronal network responses are recorded during different visual stimuli. In addition, active cells are selected based on the previously recorded somatic activity and their dendritic responses are followed along with the network activity in three dimensions by using whole-cell patch clamp techniques.



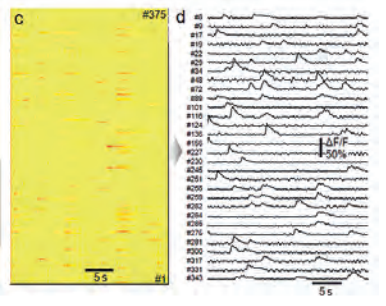
Two-photon uncaging

Two-photon uncaging takes advantage of the high spatial and temporal resolution of two-photon excitation to study dendritic integration, a post-synaptic mechanism. Used in combination with two-photon imaging, two-photon uncaging provides an opportunity to study the long-term structural and functional consequences of stimulation of structures such as dendritic spikes and dendritic spines. Besides performing experiments we develop new caging compounds and use these for our new measurements.



In vitro measurement of spontaneous neuronal network activity

It is thought that sharp wave-ripples (SPW-R) activity is involved in the process of memory consolidation. The properties of SPW-R events are similar to what was found in vivo. We investigate spontaneous single cell (pyramidal cell and interneuron) neuronal activities during SPW-R in the hippocampus CA1 and CA3 region under in vitro conditions. Fast spiking, PV+ basket cells as the clockworks for neuronal oscillations are important elements of hippocampal neuronal networks. Thus, beside the pyramidal cells we focus on PV+ interneurons to reveal the dendritic calcium dynamics during SPW-R (Chiovini et al. 2014, Neuron).



Selected publications:

- B. Chiovini, G. F. Turi, G. Katona, A. Kaszas, D. Palfi, P. Maak, G. Szalay, M. F. Szabo, Z. Szadai, Sz. Kali and B. Rozsa. Dendritic spikes induce ripples in parvalbumin interneurons during hippocampal sharp waves. Neuron (2014)
- Gergely Katona, Gergely Szalay, Pál Maák, Attila Kaszás, Máté Veress, Dániel Hillier, Balázs Chiovini, E. Sylvester Vizi, Botond Roska & Balázs Rózsa. Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes, Nature Methods (2012)
- G. Katona, A. Kaszás, G. F. Turi, N. Hájos, G. Tamás, E. S. Vizi, B. Rózsa. Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons, Proc. Natl. Acad. Sci. USA,Volume 108, No. 5 (2011), Page 2148-2153
- B. Chiovini, G. F. Turi, G. Katona, A. Kaszás, F. Erdélyi, G. Szabó, H. Monyer, A. Csákányi, E. S. Vizi, B. Rózsa (2010). Enhanced Dendritic Action Potential Backpropagation in Parvalbumin-positive Basket Cells During Sharp Wave Activity, Neurochemical Research Volume 35, Number 12, 2086-2095

Metabolic Phenotyping Unit

Head of Unit:
Csaba Fekete, MD, PhD

The Unit, established in December 2014, is equipped with an EchoMRI-700 - Whole Body Magnetic Resonance Analyzer and with a TSE PhenoMaster Metabolism Unit for 8 Mice and 8 rats to facilitate research focusing on the regulation of energy homeostasis and metabolic phenotyping of novel transgenic mice.

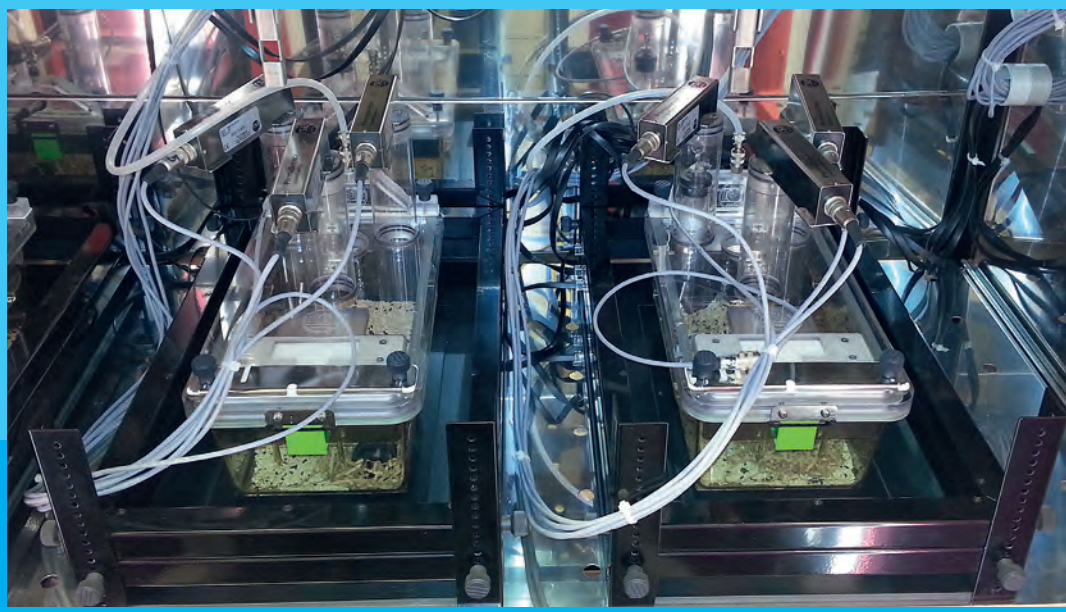
EchoMRI-700 - Whole Body Magnetic Resonance Analyzer allows very quick and precise analyses of body composition without the need of anesthesia. The equipment can be used for mice but also for large rats up to 700 g. The system determines the total body fat content, the lean body mass and the free and total water content of the animals.

These measurements are necessary to understand the nature of the bodyweight change caused by pharmacological or genetic manipulations, but also for normalization of indirect calorimetry data with lean body mass.

The TSE PhenoMaster Metabolism Unit can be used to monitor the energy expenditure, substrate utilization, food and liquid intake, body weight and locomotor activity of rats or mice. The climate chamber housing the metabolic cages allows performing the experiments in a wide temperature range including termoneutral conditions. With the help of the Stellar Telemetry system, the indirect calorimetry measurements can be combined with monitoring of the core body temperature and heart rate.

Combination of metabolic phenotyping with optogenetics or central drug administration can help to understand the physiological role of neuronal pathways discovered by *ex vivo* and *in vitro* methods.

The facility can be used by researchers of the Institute, but it is also open for collaborative projects with researchers working in other academic institutes or in universities. A researcher, Mónika Tóth PhD can provide help for the users of the laboratory.



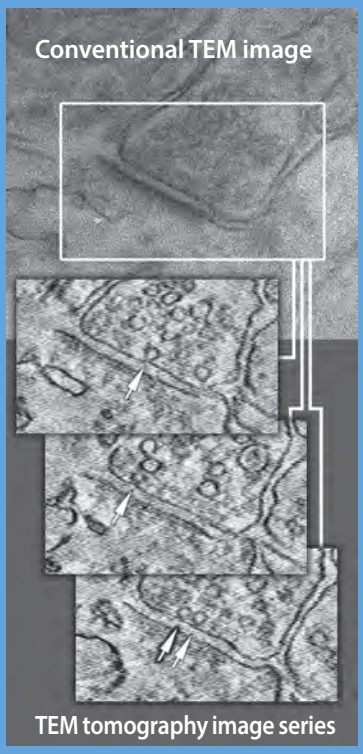
FEI Unit

Head of Unit:
Gábor Nyíri, PhD

The FEI TEM Unit opened its doors in early 2015. Its main equipment is a FEI Tecnai T12 G2 Biotwin transmission electron microscope (TEM) optimised for electron tomography. The microscope is equipped with a high quality long-life LaB_6 cathode, a sophisticated vacuum system, a computer-controlled stage (CompuStage) optimised for tomography, and a high bit-depth 16 megapixel bottom mounted Eagle CCD-camera. The exceptionally precise CompuStage enables the use of the automatic montage function and stage tracking with saving of multiple stage positions. The system is capable of collecting tilt-series from sections up to 250 nm thickness, through a range of -70 – +70 degrees, and batch tomography on serial sections is also feasible.

Further instruments of the Unit are the Leica UC6 and UC7 ultracut machines for the preparation of ultrathin electron microscopic samples, a light microscope for checking sections and blocks at different stages of the cutting process, and a Leica EM ACE200 carbon evaporator, that is able to deposit thin carbon layers on the surface of electron microscopic sections with a precision of 0.1 nm. The thin carbon coating on the section improves the mechanical stability of the sample, and helps to dissipate the heat and charge during imaging.

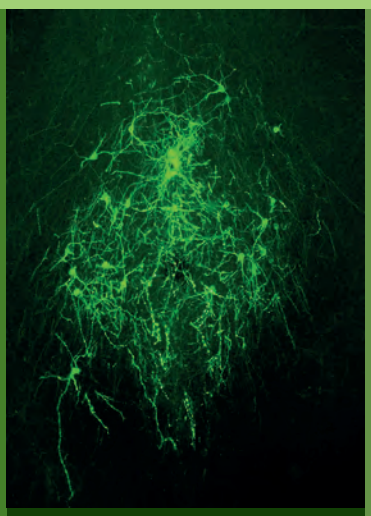
Transmission electron microscopy is the highest resolution imaging application for neuroanatomical research. A modern TEM can achieve a resolution of 0.2 nm, and its images contain all the ultrastructural features, besides the specifically labelled molecules. In the past few decades large steps were taken towards volume-electron microscopy, i.e. to extend the TEM's superb X-Y resolution into the Z-direction as well. One very successful approach is TEM-tomography, during which the relatively thicker (100-200 nm) – compared to the 40-80 nm thickness used in conventional TEM – plastic embedded tissue samples are imaged through a range of different tilt angles (usually between -65 - +65 degrees with 1-2 deg. increment steps) and the real 3D volume is reconstructed via software calculations from the acquired tilt-series to achieve sub-nanometer resolution in all 3 dimensions.



TEM tomography image series

Virus Technology Unit

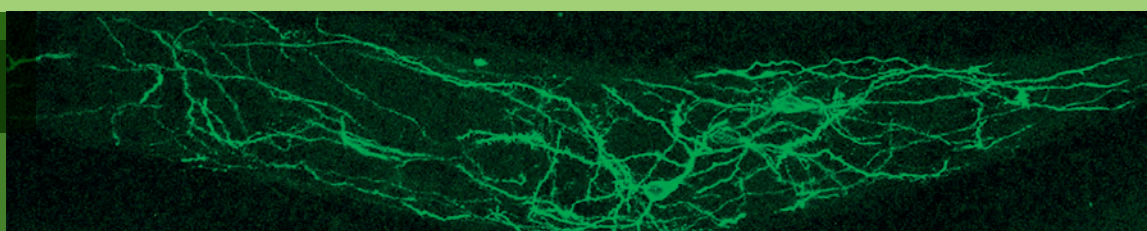
Head of Unit:
Viktor Varga, PhD



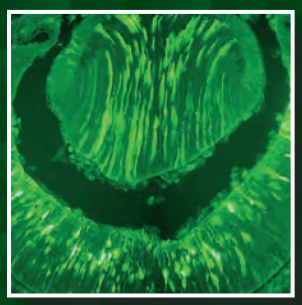
The molecular biology revolution of past decades enabled the introduction of genes from evolutionarily distant species into mammalian cells and as such the interrogation of biological phenomena at unprecedented specificity in living animals. The central components of gene transfer into target cells are recombinant viruses deprived of the machinery for normal viral operation rendering them incapable of replication in the host cell. The resulting viral particles carry the transgenes to be introduced into targets. By viral gene transfer modifications can be cell type, brain area and developmental time-specifically targeted. The dawn of optogenetics and the spread of high throughput approaches in various areas of neuroscience research e.g. connectomics, recording of neuronal activity by high density electrodes or by advanced imaging techniques resulted in the dramatic expansion of the viral gene transfer technique. Viruses have been incorporated into the methodological repertoire and now are routinely used by large number of labs around the world as basic and indispensable research tools.

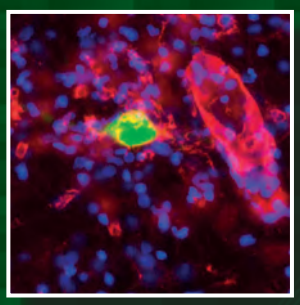
Viral gene transfer was gradually implemented in the IEM during the mid-2000s. The growing demand especially after the implementation of optogenetic manipulation motivated the allocation of a dedicated virus injection facility. Currently, two virus labs operate in the Institute capable of satisfying the demands of all current and future groups aiming to deploy viral gene transfer by providing biosafety level 2 (BSL-2) conditions. The Institute's groups utilize viral gene transfer for a multitude of purposes such as introducing microbial opsin genes for the manipulation or various neuron types in optogenetic experiments, suppressing or overactivating genes or labeling neurons for determining their connectivity. The injected viral agents belong to adeno-associated viruses or lentiviruses.

The virus injection facilities were planned in order to accommodate enough space for all phases of the viral gene transfer procedure from the preparation of animals, through the injection process to the post-injection storage of the animals in ideal conditions during the incubation period and decontamination. Hence, the facilities have dressing rooms, injection labs with multiple injection setups, deep freezers and laminar boxes, animal rooms and decontamination rooms. The facilities' ventilation systems feed particle-filtered, cooled and humidified air to provide stable temperature and humidity level and remove contaminated air through high-efficiency particulate arrestance (HEPA) and small molecule adsorbing filters. Pressure gradients generated and maintained by ventilation prevent the outflow of contaminated air toward common areas outside the facilities. The injection setups are optimized for the targeted delivery of small amounts of virus solution. Injected animals are stored in animal rooms capable of holding up to 300 mice and 100 rats with programmable lighting under regular surveillance by an animal technician. Currently, more than 20 users representing more than 10 groups carry out viral gene transfer experiments. The virus injection facilities are supervised by the Institute's Biosafety Committee.



Brain photo: [11C]-MADAM imaging of the SERT (serotonin transporter) in the human brain with HRRT PET. Courtesy of Dr. Andrea Varrone, Karolinska Institutet
Design: Geopositive Consulting Ltd.





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