Laboratory of Molecular Pharmacology

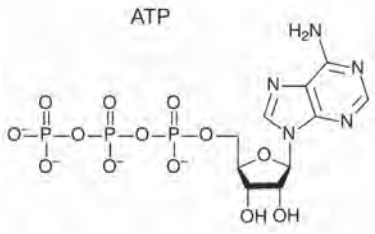
Department of Pharmacology

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].

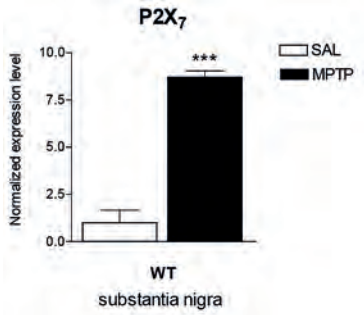
A third focus of the research is to study interactions between mitochondrial dysfunction, oxidative stress and dysregulated neurotransmitter release in the pathway leading to neurodegeneration, which characterizes many CNS diseases, including Parkinson's disease and ischemia-related neurodegeneration.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

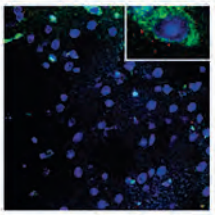
Purinergic signalling in the nervous system

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.

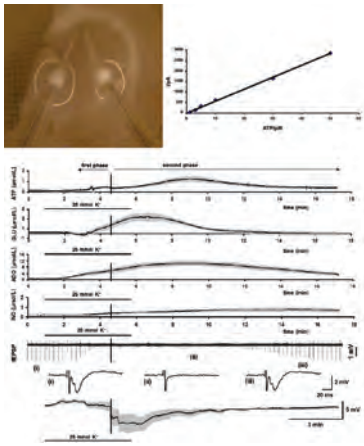
Another focus of the research is the metabotropic $P2Y_{12}$ receptor. Uniquely from P2X and P2Y receptor families, the $P2Y_{12}$ receptor is the molecular target of widely used antithrombotic drugs, such as clopidogrel or prasugrel. Accordingly, $P2Y_{12}$ receptors are highly expressed on platelets 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.

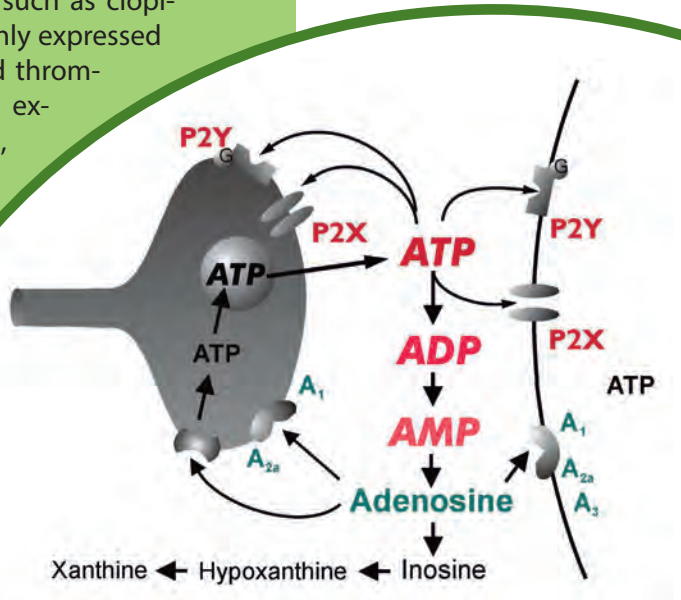




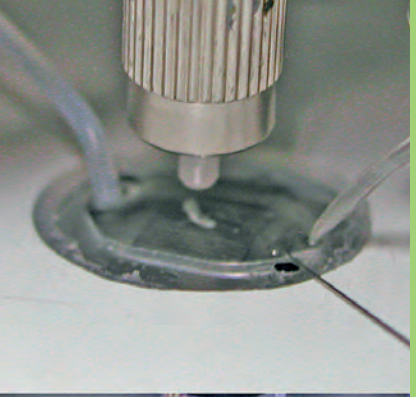
Upregulation of P2X7 receptor mRNA and protein expression in the substantia nigra (upper panel) and striatum (lower panel), respectively

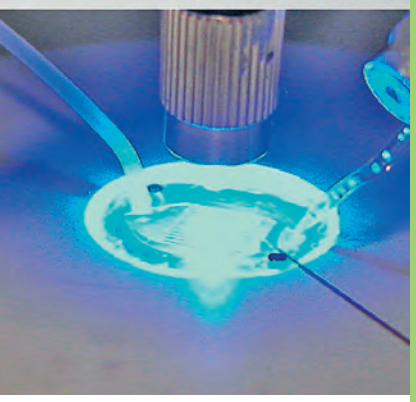
Parallel detection of the efflux ATP, adenosine, glutamate (GLU) by the microeletrode biosensor technique with simultaneous recording of field excitatory postsynaptic potentials from acute rat hippocampal slices





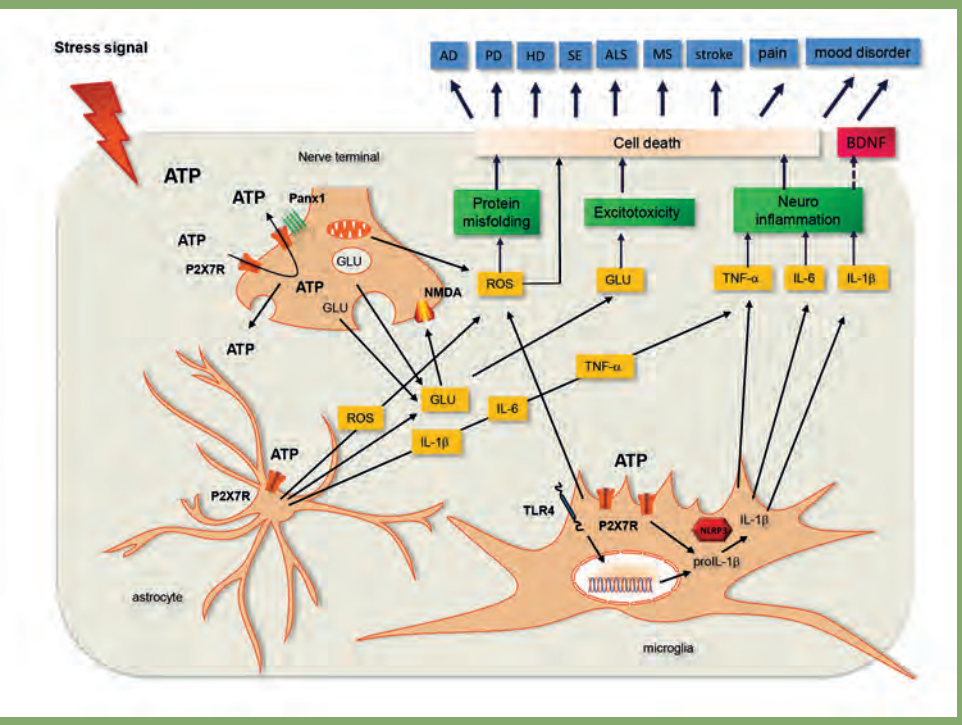
Purinergic signalling in the nervous system. The purinome consists of the enzymes and transporters responsible for the release, extracellular breakdown and uptake of nucleotides (ATP, ADP, AMP) and nucleosides (adenosine, inosine, hypoxanthine) as well as ionotropic (P2X) and metabotropic (P2Y, A_1,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



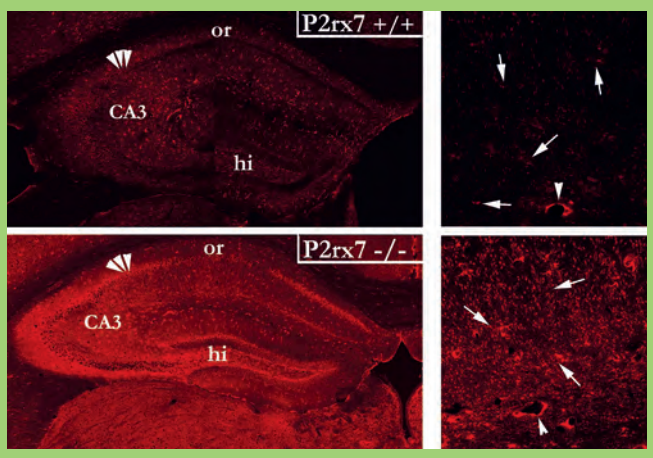


Receptor mediated modulation of neurotransmitter release

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.

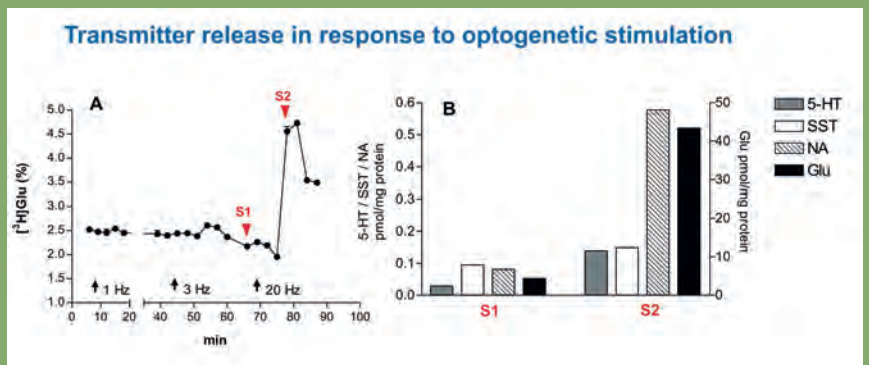


Upregulation of NR2B receptor protein in P2X7 receptor deficient mice (P2rx7-/-)

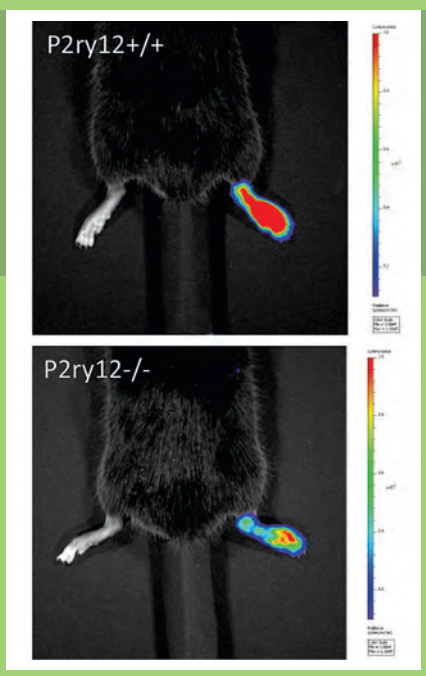
important questions on the timing, dynamics, quantity and spatial localization of the release of different transmitters. The overall aim of the current studies is to understand how the different (i.e. fast and slow) activity patterns of median raphe (MR) neurons are translated to different patterns of 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

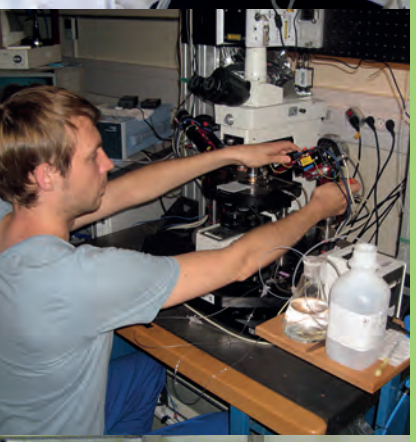
Neurodegenerative diseases (Parkinson disease, Alzheimer's disease, stroke, etc.) are characterized by the progressing loss of neurons and typically occur in the elderly population. Therefore, their incidence is continuously rising and they represent a considerable economic and social burden. Nevertheless, 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
- Andó R., Méhész B., Gyires K., Illes P., Sperlágh B. A comparative analysis of the activity of ligands acting at P2X and P2Y receptor subtypes in models of neuropathic, acute and inflammatory pain. BR J PHARMACOL. (2010) 159:1106-17.
- 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