Characteristics of main research directions investigated at the institute and the achievements 2010−2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

The Institute of Experimental Medicine, Czech Academy of Sciences, is the leading institution in the Czech Republic for biomedical research, particularly in the fields of **neuroscience**, **regenerative medicine and stem cells** as well as **molecular biology of cancer, genetic ecotoxicology, pharmacology and teratology**. The Institute is an internationally recognized center in these fields, and as such it was selected as an **EU Center of Excellence (MEDIPRA).** The institute's already-established position is documented by the extensive collaboration that exists between Institute scientists and their colleagues in Europe, North America, Asia and Australia. Recently, a number of foreign PhD students, postdoctoral fellows, as well as senior scientists have been working in our departments, financed from our institutional budget as well as from a number of projects of the European Commission. The Institute regularly organizes a number of international workshops, symposia and conferences as well as summer schools for young researchers.

We are proud to collaborate with leading Czech institutions, namely with Charles University's First, Second and Third Medical Faculties and Faculty of Science, the Institute for Clinical and Experimental Medicine (IKEM) of the Ministry of Health, the Institute of Macromolecular Chemistry CAS, the Institute of Physics CAS, the Institute of Physiology CAS, and the Institute of Organic Chemistry and Biochemistry CAS. Scientists of the Institute are involved in both the pregraduate and postgraduate training of students. During the period 2010-2014 the Institute cooperated as part of several Research Centers, namely the Neuroscience Centre (2005-2011), Center for Cell Therapy and Tissue Repair (2005-2011), Center for New Antivirals and Antineoplastics (2005-2011), Research Centre for Cell Therapy and Tissue Repair (2011-2013), Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (2012-2015), Project of Excellence in the field of neuroscience (2012-2018), Center for Studies on Toxicity of Nanoparticles (2012-2018), Center for Development of Original Drugs (2012-2018), and Center of Orofacial Development and Regeneration (2014-2018).

The Institute of Experimental Medicine (IEM) has collaborated for several years on a joint project of six institutes of the Academy of Sciences of the Czech Republic and two faculties of Charles University in Prague aimed at establishing a European Centre of Excellence in Biomedicine and Biotechnology (BIOCEV), in Vestec near Prague. The financing of the building's construction as well as equipping it with the necessary research set-up was mostly provided by European structural funds. The Centre will open at the end of 2015. As a part of BIOCEV, the IEM has established a program focused on Functional Genomics, a group focused on investigating the Auditory Function in Mutant Mice (group leader J. Popelář, PhD.) that collaborates closely with the IEM Department of Auditory Neuroscience. Another group that has been formed in the frame of the BIOCEV program concerning Biomaterials and Tissue Engineering is a group specifically focused on the Application of Stem Cells and Biomaterials in Cell Therapy (group leader Assoc. Prof. P. Jendelová). This group closely collaborates with the IEM Department of Neuroscience. The IEM is represented on the Board of BIOCEV by Prof. Josef Syka.

The IEM has since 2013, also participated in the establishment of a new institute in the frame of the Czech Technical University, i.e. Czech Institute of Informatics, Robotics and Cybernetics (CIIRC) situated in Prague 6 – Dejvice. The Institute is involved in the organization of a group on Neuroinformatics of Normal and Pathological Hearing Function (group leader D. Šuta, PhD.) in the Department of Cognitive Systems and Neuroscience. This group collaborates closely with the IEM Department of Auditory Neuroscience. The planned opening of the Institute is in 2016.

The publication activity of the Institute is stable, most of its publications appear in journals with high impact factors, such as Physiological Reviews, Nature, Cell, Trends in Neuroscience, Trends in Pharmacology, The Journal of Cell Biology, NeuroImage, Journal of Physiology, Journal of Cell Science, Molecular Pharmacology, Biophysical Journal, Carcinogenesis, Glia, Stem Cells, Journal of Cerebral Blood Flow and Metabolism, Journal of Dental Research, Hippocampus, Leukemia, and Journal of Leukocyte Biology. From 2010 to 2014 the employees of the Institute published **414 publications with a total IF of 1628,128.** The number of publications and the impact factor is therefore considerably larger than that published during the previous evaluated period, from 2005 to 2009 (353 publications with a total IF of 1340,991).

At present, the Institute of Experimental Medicine belongs to the biomedical group of research institutes of the Czech Academy of Sciences and is the only institute in the Czech Republic engaged in a comprehensive medical research program encompassing a number of diverse fields. Within the last five years scientists in the Institute obtained 117 Czech and 21 foreign grants, predominantly from the EU. The Institute also fosters **research from bench to bedside.** The newly established **Innovation Biomedical Center** provides researchers in the Institute, as well as offering outside scientists the possibility to join its Business Incubator, to establish spin-off companies and to profit from the Center's advisory and practical services.

Department of Cellular Neurophysiology (part of team number 1, "Cellular and Molecular Neurophysiology")

The research of the Department of Cellular Neurophysiology, headed by Miroslava Anděrová, is focused on the characterization of intercellular signaling pathways within neuronal-glial circuits and intracellular signaling mechanisms in glia cells under physiological and pathological conditions. In addition, the role of glutamergic and purinergic pathways is studied in neuronal-glial signaling in the cortex and hippocampus with a specific emphasis on the astroglial NMDA and P2X receptors. Neuronal-glial circuits under physiological and pathophysiological conditions are imaged in situ/in vivo in order to investigate calcium signaling cascades in Alzheimer's disease; the morphology and physiology of glia during normal brain ageing; cellular, molecular and morphological changes in glial cells during pathological states; the role of ion channels and transporters in astrocytic regulatory volume processes during oxygen-glucose deprivation or hypoosmotic stress; the membrane properties of reactive glia and ongoing glia proliferation, cell death and glial scar formation; three-dimensional reconstruction of glial cells and their morphometric analysis; glial expression of TRP channels under physiological/pathological conditions. Ion channels and calcium signaling in neural stem or progenitor cells at different stages of differentiation and their functional properties are studied after transplantation into injured CNS in order to study the role of morphogenes on the differentiation potential of neonatal neural stem or progenitor cells with the main focus on Wnt- and Shh-signaling pathways, differentiation of region-specific neural stem/progenitor cells after their transplantation

into the ischemic brain and the role of glial cells in CNS regeneration. By employing a complex of electrophysiological, video-imaging and molecular biological techniques, the main receptors responsible for calcium signaling pathways are identified and the intracellular signaling cascades localized. The research is also focused on elucidating the role of glial cells in the pathophysiology of brain ischemia and mechanical brain injury using electrophysiological and immunohistochemical methods, Ca2+ imaging and 3D confocal morphometry. Further, the research also focuses on the regeneration of nervous tissue, namely on the functional properties of differentiated neural stem/progenitor cells transplanted into the site of injury or the differentiation potential of polydendrocytes following CNS disorders, such as ischemia or Alzheimer disease.

From 2010 to 2014, the Department published a total of 37 papers, IF=141,837.

Department of Molecular Neurophysiology (part of team number 1, "Cellular and Molecular Neurophysiology")

The research of the Department of Molecular Neurophysiology, headed by Govindan Dayanithi, is focused on the molecular signaling mechanisms in excitable and non-excitable cells, physiology of calcium signaling and calcium homeostasis in magnocellular neurons and terminals, molecular mechanisms involved in the activation of plasma membrane calcium entry pathways with the modulatory effect of intracellular calcium release, physiology of vasopressin and oxytocin in the central and peripheral nervous systems using newly developed transgenic rat models to visualize fluorescent vasopressin and oxytocin (vasopressin-eGFP; oxytocin-eCFP and oxytocin-mRFP1), physiology of vasopressin and oxytocin signaling in dorsal root ganglia neuron-glia interactions, nociception, pregnancy and lactation and the physiopathology of calcium signaling in stem cells and neurodegeneration. The results show that the physiological significance of the complexity of Ca2+ homeostatic mechanisms in the somatodendritic region of supraoptic neurones and their terminals can be multifaceted, attributable, in a major way to their specialized electrical activity and Ca2+-dependent neurohormone release. The intracellular Ca2+ measurements resulting from dorsal root ganglia neurons revealed that these larger neurons responded to arginine-vasopressin, oxytocin, high K+ and capsaicin, indicating a novel physiological effect of arginine-vasopressin and oxytocin in pain and a possible physiological consequence of oxytocin in dorsal root ganglia neurons during pregnancy and lactation. In addition, the physiopathological responses and pharmacological profiles of human embryonic stem cell-derived neural precursors is analyzed in terms of their intracellular Ca2+ responses to high K+, ATP, glutamate and intracellular Ca2+ releasing agents at different passages (P1 through P10) during the course of cell differentiation. Results show that these cells respond to different physiological stimuli by an increase in intracellular Ca2+ that varies during the course of cell differentiation. Also the number of cells responding to the above agents is higher in passage 7 (P7) NPs when compared to other passages. The cells from P7 express functional glutamate receptors, purinoreceptors, voltage-dependent Ca2+ channels and show spontaneous Ca2+ oscillations as typically observed in neuronal/endocrine cells. Preliminary results show that pre-differentiation of neural precursors derived from adipose and bone marrow mesenchymal stem cells leads to an activation of Ca2+ signaling cascades and enhances the functional activity of the stem cells. While pre-differentiated mesenchymal stem cells express functional voltage gated Ca2+ channels, P2X and P2Y purinergic receptors, glutamate receptors, OT and AVP receptors, the release of Ca2+ from the endoplasmic reticulum via ryanodine receptors and also exhibited spontaneous intracellular Ca2+ oscillations, undifferentiated mesenchymal stem cells express only purinergic receptors. Understanding the

functional properties of stem cells may enable the better control of their regenerative potential and help to improve strategies for their use in transplantation and treatment.

From 2010 to 2014, the Department published a total of 15 papers, IF=54,836.

Department of Neuroscience (team number 2, "Neurosciences")

Neurological disorders and their consequences represent a significant economic burden on Czech society not only because of the high costs connected with their direct treatment, but also because of the associated long-term and expensive after-care and rehabilitation, not to mention the decreased working capacity of the patients. Rapid diagnosis and properly targeted treatment of neurological diseases may therefore significantly decrease the economic expenses of our healthcare system, especially since the incidence of pathologies such as stroke, Alzheimer´s and Parkinson´s diseases and brain tumors is increasing with changes in life style and the increasing age of the population. However, to be able to provide a targeted cure instead of mostly palliative treatment, we need to understand the pathogenic mechanisms in detail.

The Department, headed by Prof. Eva Syková, focuses on research from bench to bedside, particularly on the mechanisms and treatment of brain and spinal cord injury, on brain homeostatic mechanisms and extrasynaptic transmission, and their roles in a number of physiological and pathological states. The members of this Department are leaders in the field of brain homeostasis, the role of glia and the extracellular matrix in pathophysiology as well stem cell therapy and tissue engineering in the Czech Republic. Within the period of 2010-2014 the Department has collaborated with many European and US research centers in a number of EU projects, namely DiMI, ENI-NET, STEMS, RegMedTeach, AXREGEN, EdU-GLIA, SCI_MOL_BIOL, and others.

The focus of the **Laboratory of Tissue Culture and Stem Cells** is on the characterization, cultivation, phenotyping and differentiation of adult, embryonic, fetal and induced pluripotent stem cells. Various types of iron-oxide nanoparticles have been used and developed for the labeling of stem cells and their imaging in vivo by MRI. Mesenchymal stem cells (MSCs), neural precursor cells derived from immortalized fetal cells line (SPCs) or neural precursors from induced pluripotent stem cells (iPSCs) are studied in animal models of stroke, spinal cord injury (SCI), Alzheimer´s disease and Amyotrophic lateral sclerosis (ALS). Stem cells in these animal models resulted in morphological changes, in behavioral improvement as well as in prolonged survival of the animals. MSCs are also natural anti-inflammatory agents, decrease apoptosis, promote angiogenesis, produce cytokines and growth factors and have the potential to assist the regeneration of nerve tissue. The effect is found after systemic implantation as well as direct application to the injury site. Neural precursors from SPCs and iPSCs differentiate into neurons, astrocytes and oligodendrocytes. In SCI macroporous polymeric hydrogels are used as a suitable carrier for cell growth in both in vitro cultures and in vivo after implantation. These scaffolds seeded with stem cells in order to bridge the defects and promote regeneration of injured tissue. The goal of this cell therapy is to repair, replace or enhance the biological function of damaged nerve tissue and to treat neurodegenerative diseases. Two clinical studies for SCI and ALS are being conducted based on this research.

Research in the **Laboratory of Diffusion Studies and Imaging Methods** focuses on the origin, mechanisms and pathophysiological significance of ionic and volume changes in the extracellular space (ECS). Diffusion of substances through the ECS is the underlying mechanism of extrasynaptic (volume) transmission. Changes in ECS diffusion parameters which occur during

physiological and pathological states, e.g. lactation, aging, ischemia, brain edema, hydrocephalus, Alzheimer's disease, tumors, and epilepsy, are studied in animal models. For diffusion studies the real-time iontophoretic method using ion-selective microelectrodes as well as diffusion-weighted MRI are used to study ECS volume and geometry – factors affecting the function of the CNS in health and disease. The primary research aims are to improve therapy and diagnostic methods for CNS diseases and the prevention of CNS damage.

The **Laboratory of Biomaterials and Biophysical Methods** develops advanced natural and synthetic biomaterials, namely decelullarized CNS matrix, hydrogels and nanofiber scaffolds for regenerative medicine and tissue engineering, and evaluates their performance using biological models of stroke and spinal cord injury. In collaboration with the Institute of Physics CAS, detailed research of the effects of magnetic field and low-temperature atmospheric plasma on biological systems as well as the development of novel devices for medical applications are performed.

From 2010 to 2014, the Department published a total of 94 papers, IF=351,957; 5 patents have resulted from departmental research. Research of the department has resulted in 4 ongoing clinical studies. Bioinova, Ltd., is a spin-off company of the Department (for details see Appendix 3.3).

Department of Molecular Biology of Cancer (part of team number 3, "Molecular Biology of Cancer and Teratology")

The most important area of research in the Department of Molecular Biology of Cancer, headed by Pavel Vodička, is focused on investigating the molecular characteristics of cancer, predominantly colorectal cancer. In molecular-epidemiologic designed studies, molecular markers are studied as they reflect increases in predisposition to cancer, early diagnosis, individual responses to anti-cancer treatment, and a patient's long-term prognosis. The main interest is in the role of DNA repair processes under both physiological and pathological conditions. This cell's function is covered by at least six pathways, it is an important defense mechanism against DNA instability, and therefore plays a crucial role in the transformation of malignant cells. On the other hand, activity of DNA repair is also involved in the response of cancer cells to treatment with chemotherapeutics. Such treatment is targeted at DNA damage induction, ultimately triggering cell death. High activity of DNA repair processes may therefore influence the resistance of cancer cells to this treatment. The research is conducted on different kinds of human biological material, such as tumor tissue, peripheral blood cells, or plasma.

From 2010 to 2014, the Department published a total of 61 papers, IF=262,676.

Department of Teratology (part of team number 3, "Molecular Biology of Cancer and Teratology")

The Department of Teratology, headed by Miroslav Peterka, focuses on studying the developmental abnormalities in humans as well as experimental models. The causes and mechanisms of inborn defect formation are studied using two experimental models (developing chick embryos and mouse tooth germs), and using the clinical-epidemiological approach. The main target is to contribute to the knowledge about normal and abnormal development, pathogenesis of inborn defects and possibilities of their prevention.

The **Laboratory of Embryogenesis** concentrates on testing harmful chemical and physical factors and generating estimations of minimum embryotoxic doses using a chick embryotoxicity screening test; understanding the mechanisms of cleft beak development in chick embryos and its possible prevention and reparation; clinical/epidemiological studies searching for the causes

underlying the origin of orofacial clefts in humans, based on a critical analysis of case- and family-history data; monitoring of the newborn sex ratio as a tool for detecting ecological accidents.

The **Laboratory of Odontogenesis** is focused on the studies of tooth development under normal, pathological and experimental conditions. It was found that rudimentary structures are involved significantly during odontogenesis. Although the rudiments stop their growth and/or regress under normal conditions, their abnormal development can be implicated in the formation of inborn defects of the dentition. Knowledge of the development and role of rudimentary structures during odontogenesis can contribute not only to elucidating the evolution of dentitions, but also to understanding pathogenesis of distinct dental anomalies. If the rudiments continue developing instead of regressing, they can give rise to supernumerary teeth. From this point of view, the regressing/revitalizing rudiments are the natural model for studying inhibition/stimulation mechanisms of tooth development and for testing possibilities of tooth regeneration.

From 2010 to 2014, the Department published a total of 19 papers, IF=60,316.

Department of Transplantation Immunology (part of team number 4, "Transplantation immunology, Tissue Engineering and Pharmacology")

The Department of Transplantation Immunology, headed by Vladimír Holáň, is primarily focused on the study of cellular and molecular mechanisms specific to transplantation immunity and to use this knowledge in the regulation of immune responses. The aim is to improve the survival of genetically diverse cells and tissue transplants. The basic model proposed involves the use of cultured and specifically differentiated stem cells (mesenchymal, limbal cells), which are transplanted with different kinds of nanofiber scaffolds for the purpose of repairing severe damage to the skin and ocular surface as well as replenishing limbal stem cell deficiencies. It uses a wide range of methods of cell and molecular biology, such as cell cultures, differentiation of stem cells, the study of gene regulation (PCR, real time PCR), the production and detection of cytokines using assays (ELISA, ELISPOT), flow cytometry, MACS, Western blotting, experimental models of transplantation of skin, cornea, limbus, and stem cell transfers. The main objective is to test the knowledge gained in preclinical models and their potential use in clinical practice (particularly in patients with severe ocular surface wounds). Using a model of immunological reaction to histocompatibility antigens, the research is focused on studying the activation and function of regulatory T cells in transplantation immunity and tolerance. Since successful treatment of the severely damaged ocular surface requires the transfer of limbal stem cells, the isolation, growth and characterization of stem cells was recently started. For the transfer of stem cells, various types of nanofibre scaffolds were used, which represent optimal 3D matrices for stem cell growth. Well-established methods for monitoring the immune response enabled the study of the cytokine response in various experimental models of immunoregulation. The ultimate goal of the research is to acquire insights into the mechanisms of the specific immune response, isolate and transplant stem cells and propose novel strategies for targeted immunoregulation. Recent experiments have also been focused on the targeted differentiation of mesenchymal stem cells in vitro and their use for ocular surface reconstruction. This is one of the models for testing the possibilities of using stem cell transplantation in regenerative and reparative medicine.

The **Laboratory of Eye Histochemistry and Pharmacology** examines the causes of non-healing lesions in the anterior eye segment in various eye injuries or illnesses and the regeneration of tissues of the anterior segment of the eye, particularly the cornea, with the restoration of visual

function. For the healing of anterior eye segment lesions, special attention is paid to the nanofibers as carriers of stem cells and dosage forms.

From 2010 to 2014, the Department published a total of 19 papers, IF=49,05.

Department of Tissue Engineering (part of team number 4, "Transplantation immunology, Tissue Engineering and Pharmacology")

The Department of Tissue Engineering, headed by Prof. Evžen Amler, was established in 2005 after the research team moved from the Institute of Physiology AS CR. Currently, three main research topics are investigated in the laboratory: tissue engineering, controlled drug delivery and protein engineering. The laboratory closely collaborates with the Department of Biophysics, Charles University in Prague, the 2nd Faculty of Medicine and the Department of Nonwovens, Faculty of Textile Engineering, Technical University of Liberec. The research is concentrated on the development of novel three-dimensional scaffolds utilizing biodegradable materials. Textiles, both woven and non-woven, as well as composite scaffolds are generated mainly employing a nanofiber-based approach and applied separately or in combination with an isotropic gel. Grafts based on autologous chondrocytes and mesenchymal stem cells are used for tissue defect regeneration (namely of cartilage and bone). A special technique for the rapid evaluation of biomechanical properties in miniature tissue pieces has been developed.

From 2010 to 2014, the Department published a total of 24 papers, IF=68,055; 3 patents have resulted from departmental research.

Department of Pharmacology (part of team number 4, "Transplantation immunology, Tissue Engineering and Pharmacology")

Activities of the Department of Pharmacology, headed by Zdeněk Zídek, are governed by the scientific aims of the "Human Health" program. The ultimate goal is the research and development of original low-molecular weight drugs, notably those targeting immune-related diseases. The hitherto obtained results have demonstrated immunosuppressive properties of newly synthesized derivatives of pyrimidine, and also immunobiological activities of some compounds of natural origin. More advanced studies are focused on the analysis of rational chemical structures and synthesis of compounds. These studies should facilitate the transfer of experimental data to preclinical and clinical phases of research, and ultimately to commercial practice. Optimization of the structure is ensured by a tight and immediate backward communication between chemical and biological organizational compartments of the project. An indispensable part of the study is the determination of safety and understanding the action mechanism of the drugs. The therapeutic potential of promising drug candidates is determined using experimental models of autoimmune and inflammatory human diseases.

From 2010 to 2014, the Department published a total of 24 papers, IF=46,687; 1 patent has resulted from the departmental research.

Department of Auditory Neuroscience (team number 5, "Auditory Neuroscience")

The Department of Auditory Neuroscience, headed by Prof. Syka, has existed since the foundation of the Institute of Experimental Medicine in 1975. The main research aims of the department are oriented towards investigations of the structure and function of the auditory system in animals and humans under normal and pathological conditions and during ontogeny and ageing.

In the **Laboratory of Auditory Physiology and Pathology**, recordings of neuronal activity in individual auditory centers using multielectrodes have revealed the basic principles of

the neuronal processing of simple tones as well as complex sounds such as artificially generated rippled noise or animal vocalizations. The development of the hearing organ during ontogeny and changes in the expression of calcium-binding proteins and other neuroactive substances are studied with immunostaining methods and confocal microscopy analysis. Behavioral conditioning tests associated with permanent or pharmacologically-induced reversible lesioning of cortical structures are used to study the lateralization of auditory functions in the rat auditory cortex. Pathologies of the peripheral and central parts of the auditory system, appearing as a consequence of noise exposure or in conjunction with aging, are investigated in experimental animals and in human subjects. Among the methods used in the laboratory for this purpose are the recording of extracellular single neuron activity and auditory evoked responses, the assessment of hearing thresholds, measurements of psychoacoustic functions, startle reactions, recording of different types of otoacoustic emissions, measurement of calcium currents with two photon confocal microscopy, as well as immunohistochemical and western blotting techniques used for evaluating changes in the expression of neuroactive proteins in the peripheral and central parts of the auditory system in experimental animals. Age-related changes of hearing function are investigated in special strains of rodents with accelerated aging (C57 mice or Fischer 344 rats). Special attention is given to the GABA inhibitory system in the central auditory pathway, since it is known that this system is vulnerable when animals are exposed to noise and during aging. Collaboration with ENT clinics and with the MRI unit at the IKEM is oriented towards investigations of hearing function at different ages, the characterization of changes in the brain due to presbycusis and the genetic background of inherited deafness. Possible methods for the prevention or treatment of inner ear diseases by the application of biologically active drugs or genes to the cochlea were experimentally tested using nanoparticles as a targeted transporting tool in the frame of the European project NANOEAR. Since 2014, the department has participated in the Marie-Curie project TARGEAR and in the COST project TINNET, i.e. the specific project oriented towards the investigation of tinnitus.

In the **Laboratory of Synaptic Physiology** the mechanisms underlying the plasticity of excitatory and inhibitory synaptic transmission are studied in rodent brain slices using electrophysiological and immunohistochemical techniques. The Calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) is mostly used as a model of the central type of synapse due to its large size enabling direct examination by the patch-clamp technique. Recent projects in the lab are aimed at revealing the physiological roles of inhibitory transmitters, their receptors and uptake systems in the MNTB neurons. Experimental work has provided evidence of the novel excitatory nature of the classical inhibitory transmitters GABA and glycine. The results show that chloride-permeable glycine receptors, G-protein coupled GABA-B receptors, N-type Ca2+ channels and calcium-activated potassium conductances work in concert to support the extremely high reliability of glutamatergic synaptic transmission at MNTB neurons. All activities of the laboratory run in close collaboration with the Department of Biomedicine, University of Basel.

From 2010 to 2014, the Department published a total of 36 papers, IF=192,005.

Department of Genetic Ecotoxicology (team number 6, "Genetic Ecotoxicology")

The Department of Genetic Ecotoxicology, headed by Radim Šrám, was formed from the Laboratory of Genetic Ecotoxicology, which in turn was founded in 1991 as a joint venture of the Institute of Experimental Medicine CAS and the Regional Institute of Health of Central Bohemia with the aim of coordinating the international Teplice Program (1991–1999). This program, which

studied the effect of air pollution on the health of the population living in the coal basin of Northern Bohemia, was carried out in collaboration with the U. S. Environmental Protection Agency and was supported by the EC program PHARE. This international collaboration helped to establish molecular epidemiology methods and to use them to assess the risk of exposure to air pollution. The major findings included the fact that carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) in the ambient air are responsible for most of the genotoxicity of complex mixtures and that exposure to c-PAHs in the early stages of pregnancy significantly increases intrauterine growth retardation (IUGR). Furthermore, in polluted regions the relationship between c-PAHs exposure and DNA adduct levels, as well as the effect of genetic polymorphisms on DNA adducts, were also studied. The department participates in other international collaborative projects (EC, US EPA, HEI); among these, the EC project EXPAH (Effects of PAHs in environmental pollution on exogenous and endogenous DNA damage, QLK4-CT-2000-00091) has been the most important. Research in the department concentrates mostly on the effects of air pollution on genetic material, on the mechanisms of changes induced by environmental factors as well as modeling the relationships between individual factors (e. g. air pollution vs. life style), and the genetic damage caused by genotoxic and carcinogenic compounds, including polycyclic aromatic hydrocarbons, and other xenobiotics. The research is organized in several levels: model studies on human cell cultures; molecular-epidemiological studies on model populations using biomarkers of exposure, effect and susceptibility; reproductive epidemiology – the effect of the environment on pregnancy outcomes (the involvement of genetic material, genetic polymorphisms, gene expression, and oxidative stress); the effect of air pollution on upper respiratory diseases in children and the modulatory effects of genetic polymorphisms and gene expression on childhood morbidity.

The **Laboratory of Molecular Epidemiology** conducts molecular epidemiological studies, including the risk assessment of mutagen and carcinogen exposure, using biomarkers of exposure, effect and susceptibility (DNA adducts, chromosomal aberrations, micronuclei, oxidative damage to DNA, proteins and lipids) analyses of genetic polymorphisms and RNA expression profiles, studies of the effect of the environment on pregnancy outcomes, and the effect of the environment on children's health.

The **Laboratory of Genetic Toxicology** concentrates on the mechanisms of toxic effects of complex mixtures, ambient air particles and combustion generated and engineered nanoparticles in human cell cultures (human diploid embryonic fibroblasts (HEL12469), A549 and BEAS-2B lung cells). Studies include genotoxic (DNA adducts, DNA strand breaks, oxidative damage to DNA, formation of micronuclei) as well as epigenetic effects (intercellular communication, dioxin–like activity). The laboratory is also involved in computational modelling of toxic effects of engineered nanoparticles.

The **Laboratory of Genomics** studies gene expression profiles both in populations exposed to air pollution, tobacco smoke and other factors and *in vitro* human cell lines (HEL12469, A549, BEAS-2B) treated with model compounds and organic extracts from air pollutants. The laboratory further concentrates on the analysis of single nucleotide polymorphisms affecting the metabolism of xenobiotics, DNA repair, immune responses and other biological processes and conducts, sequencing, and DNA methylation analyses of samples exposed to environmental pollutants.

From 2010 to 2014, the Department published a total of 83 papers, IF=300,086.

Department of Technology Transfer

The Department of Technology Transfer of the IEM operates the Innovative Biomedical Centre (IBC), which consists of:

- Specialized Business Incubator (office area, lab space, GMP-certified clean rooms and support facilities) for early stage spin-off companies in biomedicine;
- Centre of support for competitiveness in Biomedicine, offering consultations and advisory services for early stage companies in biomedicine (legal support, web presentation, accounting, information systems, marketing, management and strategic planning of startup companies);
- Centre of Applied Research in biomedicine (laboratories for applied research and scale-up technologies focused on regenerative medicine, cell therapy, the development of biomaterials and pharmaceuticals as well as the design of clinical studies)

The IBC enables applied research in the field of advanced human medical products in the Czech Republic by providing a unique business incubator equipped with clean rooms for aseptic manipulation of cells and a robust quality assurance system.

Special services are provided for companies at reduced prices in these incubator projects. The incubator of the IBC is entirely operated based on private investments and on in-house income. The candidate companies are not automatically entitled to reduced prices; they must go through an entrance process in which the committee of the IBC incubator will make a final decision, based in particular on the quality of the submitted business plan, commercial potential and to what extent the focus of the company corresponds with the strategic objectives of the IEM.

The pre-incubator is a short-term program (about 6 months) that is dedicated to candidates with an interest in setting up spin-off companies. Consulting focuses on external (product from demand and a market point of view, profitability, customs and market segmentation, distribution possibilities, substitution products and competition, value, market communication, etc.) and internal analyses (production aspects, such as costs and production capacity, as well as company resources, including finances, personnel, material and instrumentation, etc.). With the assistance of the IBC, the candidate composes a business plan based on these external and internal analyses.

The business incubator is primarily dedicated to spin-off companies established in association with the scientific outcomes of the IEM AS CR. Currently, there are five companies in the IBC, Bioinova, s.r.o., EponaCell s.r.o., STUDENT SCIENCE s.r.o., MRSUPPORT s.r.o., and CELLNOVA s.r.o. The virtual incubator is dedicated to companies that are located elsewhere for any reason, but which are interested in the training programs of the IBC, consulting and other services. These companies can transfer their official address as well as their address in the trade register to the IBC. The IBC will provide these companies with administrative facilities and presentations on web sites.

The Department has prepared a system of commercialization of scientific outputs, which was awarded a grant (under the program GAMA of the Technology Agency of the Czech Republic) to fund projects such as proof-of concept in the amount of 17 mil. CZK (approx. 618,500 EUR).

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Cellular and Molecular Neurophysiology

The scientific team consists of two departments: Department of Cellular Neurophysiology (head Miroslava Anděrová) and Department of Molecular Neurophysiology (head Govindan Dayanithi).

Department of Cellular Neurophysiology

Increased expression/function of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in reactive astrocytes following ischemia.

Astrocytes respond to ischemic brain injury by proliferating, increasing the expression of intermediate filaments, hypertrophy, resulting in glial scar formation, and altering the expression of ion channels, receptors and transporters that maintain ionic/neurotransmitter homeostasis. Here, we showed how the increased expression of Hcn1-4 genes encoding hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in reactive astrocytes following focal cerebral ischemia (FCI) or global cerebral ischemia (GCI) and we also characterized their functional properties. Using single-cell RT-qPCR we demonstrated that 2 weeks after ischemia reactive astrocytes express high levels of Hcn1-4 transcripts and immunohistochemical analyses confirmed the presence of HCN1-3 channels in reactive astrocytes 5 weeks after ischemia. Electrophysiological recordings revealed that post-ischemic astrocytes are significantly depolarized and compared to astrocytes from non-injured brains they display large hyperpolarization activated inward currents, the density of which increased 2-3-fold in response to ischemia. Their activation was facilitated by cAMP and their amplitudes were decreased by HCN channel inhibitors or low extracellular Na+ concentration. Until now, these channels were described only in neurons. Since HCN channels are mainly permeable for sodium and potassium ions, their increased expression in reactive astrocytes indicates that they may markedly influence the basic astrocytic functions in the central nervous system, and consequently, the extent of nervous tissue damage following ischemia. Astrocytic HCN channels could therefore be an important therapeutic target in post-stroke therapy. The team of the Department of Cellular Neurophysiology designed the study, performed immunohistology, prepared cells for single-cell PCR, conducted electrophysiology, data interpretation and the writing of the paper.

Honsa P., Pivonkova H., Harantova L., Butenko O., Kriska J., Dzamba D., Rusnakova V., Valihrach L., Kubista M. and Anderova M. (2014): Increased expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in reactive astrocytes following ischemia. Glia 62 (12), 2004–2021. IF 5.466

Cell death/proliferation and alterations in glial morphology contribute to changes in diffusivity in the rat hippocampus after hypoxia-ischemia.

To understand the structural alterations that underlie early and late changes in hippocampal diffusivity after hypoxia/ischemia, the changes in the apparent diffusion coefficient of water (ADCW) were studied in 8-week-old rats after hypoxia/ischemia using DW-MRI. In the hippocampal CA1 region ADCW analyses were carried out during 6 months of reperfusion and compared with alterations in cell number/cell type composition, glial morphology and ECS diffusion parameters obtained using the real-time iontophoretic method. In the early phases of reperfusion (1-3 days) neuronal cell death, glial proliferation and developing gliosis were accompanied by an ADCW decrease and tortuosity increase. Interestingly, ECS volume fraction was decreased only on the first day after H/I. In the late phases of reperfusion (starting 1 month after H/I), when the CA1 region consisted mainly of microglia, astrocytes and NG2-glia with markedly altered morphology, ADCW, ECS volume fraction and tortuosity were increased. Three-dimensional confocal morphometry revealed enlarged astrocytes and shrunken NG2-glia, and in both the contribution of cell soma/processes to total cell volume was markedly increased/decreased. In summary, the ADCW increase in the CA1 region, underlain by altered cellular composition and glial morphology, suggests that considerable changes in extracellular signal transmission might occur in the late phases of reperfusion after hypoxia/ischemia. The team of the Department of Cellular Neurophysiology designed the study, performed immunohistology, immunohistochemistry quantification, proliferation/apoptosis quantification, surgeries, 3D morphometry, data interpretation, and the writing of the paper.

Anderova M, Vorisek I, Pivonkova H, Benesova J, Vargova L, Cicanic M, Chvatal A, Sykova E. (2011): Cell death/proliferation and alterations in glial morphology contribute to changes in diffusivity in the rat hippocampus after hypoxia/ischemia. JCBFM 31, 894-907, IF 5.008

Impact of Global Cerebral Ischemia on K+ Channel Expression and Membrane Properties of Glial Cells in the Rat Hippocampus.

Astrocytes and NG2 glia respond to CNS injury by the formation of a glial scar. Since the changes in K^+ currents in astrocytes and NG2 glia that accompany glial scar formation might influence tissue outcome by altering K^+ ion homeostasis, we aimed to characterize the changes in K^+ currents in hippocampal astrocytes and NG2 glia during an extended time window of reperfusion after ischemic injury. Global cerebral ischemia was induced in adult rats by bilateral, 15-minute common carotid artery occlusion combined with low-pressure oxygen ventilation. Using the patch-clamp technique, we investigated the membrane properties of hippocampal astrocytes and NG2 glia *in situ* 2 hours, 6 hours, 1 day, 3 days, 7 days and 5 weeks after ischemia. Astrocytes in the CA1 region of the hippocampus progressively depolarized starting 3 days after ischemia, which coincided with the decreased Kir4.1 protein expression in gliotic tissue. Other K^+ channels described previously in astrocytes, such as Kir2.1, Kir5.1, TREK2 and TWIK1 did not show any changes in their expression in the hippocampus after ischemia. However, marked changes in TREK1 expression were detected. NG2 glia displayed increased input resistance, decreased membrane capacitance and increased delayed outwardly rectifying and A-type K^+ currents 3 days after ischemia, accompanied by their proliferation. Our results show that the membrane properties of astrocytes after ischemia undergo complex alterations, which might profoundly influence the maintenance of K^+ homeostasis in damaged tissue, while NG2 glia display membrane currents typical of proliferating cells. The team of the Department of Cellular

Neurophysiology designed the study, performed immunohistology, immunohistochemistry quantification, proliferation quantification, surgeries, electrophysiology, Western blots, data interpretation, and the writing of the paper.
Pivonkova H, Benesova J, Butenko O, Chvatal A, Anderova M.(2010): Impact of Global Cerebral Ischemia on K+ Channel Expression and Membrane Properties of Glial Cells in the Rat Hippocampus. *Neurochemistry International* 57(7):783-94, IF 3.541

Distinct expression/function of potassium and chloride channels contributes to the diverse volume regulation in cortical astrocytes of GFAP/EGFP mice.

Recently, we have identified two astrocytic subpopulations in the cortex of GFAP-EGFP mice, in which the astrocytes are visualized by the enhanced green–fluorescent protein (EGFP) under the control of the human glial fibrillary acidic protein (GFAP) promotor. These astrocytic subpopulations, termed high response- (HR-) and low response- (LR-) astrocytes, differed in the extent of their swelling during oxygen-glucose deprivation (OGD). In the present study we focused on identifying the ion channels or transporters that might underlie the different capabilities of these two astrocytic subpopulations to regulate their volume during OGD. Using three-dimensional confocal morphometry, which enables the quantification of the total astrocytic volume, the effects of selected inhibitors of K^+ and Cl^- channels/transporters or glutamate transporters on astrocyte volume changes were determined during 20 minute-OGD *in situ*. The inhibition of volume regulated anion channels (VRACs) and two-pore domain potassium channels (K_{2P}) highlighted their distinct contributions to volume regulation in HR-/LR-astrocytes. While the inhibition of VRACs or K_{2P} channels revealed their contribution to the swelling of HR-astrocytes, in LR-astrocytes they were both involved in anion/K^+ effluxes. Additionally, the inhibition of Na^+-K^+-Cl^- co-transporters in HR-astrocytes led to a reduction of cell swelling, but it had no effect on LR-astrocyte volume. Moreover, employing real-time single-cell quantitative polymerase chain reaction (PCR), we characterized the expression profiles of EGFP-positive astrocytes with a focus on those ion channels and transporters participating in astrocyte swelling and volume regulation. The PCR data revealed the existence of two astrocytic subpopulations markedly differing in their gene expression levels for inwardly rectifying K^+ channels (Kir4.1), K_{2P} channels (TREK-1 and TWIK-1) and Cl^- channels (ClC2). Thus, we propose that the diverse volume changes displayed by cortical astrocytes during OGD mainly result from their distinct expression patterns of ClC2 and K_{2P} channels. The team of the Department of Cellular Neurophysiology designed the study, performed immunohistochemistry, 3D morphometry and pharmacological studies, cell collection for single cell PCR, data interpretation, and the writing of the paper.
Benesova, J., Rusnakova, V., Honsa, P., Dzamba, D., Kubista, M. Anderova, M. (2012): Distinct expression/function of potassium and chloride channels contributes to the diverse volume regulation in cortical astrocytes of GFAP/EGFP mice. PLoS ONE 7(1): e29725. doi:10.1371/journal.pone.0029725 IF 4.004

The increased activity of TRPV4 channel in the astrocytes of the adult rat hippocampus after cerebral hypoxia/ischemia.

The polymodal transient receptor potential vanilloid 4 (TRPV4) channel, a member of the TRP channel family, is a calcium-permeable cationic channel that is gated by various stimuli such as cell swelling, low pH and high temperature. Therefore, TRPV4-mediated calcium entry may be involved in neuronal and glia pathophysiology associated with various disorders of the central nervous system, such as ischemia. The TRPV4 channel has been recently found in adult rat cortical

and hippocampal astrocytes; however, its role in astrocyte pathophysiology is still not defined. In the present study, we examined the impact of cerebral hypoxia/ischemia (H/I) on the functional expression of astrocytic TRPV4 channels in the adult rat hippocampal CA1 region employing immunohistochemical analyses, the patch-clamp technique and microfluorimetric intracellular calcium imaging on astrocytes in slices as well as on those isolated from sham-operated or ischemic hippocampi. Hypoxia/ischemia was induced by a bilateral 15-minute occlusion of the common carotids combined with hypoxic conditions. Our immunohistochemical analyses revealed that 7 days after H/I, the expression of TRPV4 is markedly enhanced in hippocampal astrocytes of the CA1 region and that the increasing TRPV4 expression coincides with the development of astrogliosis. Additionally, adult hippocampal astrocytes in slices or cultured hippocampal astrocytes respond to the TRPV4 activator 4-alpha-phorbol-12,-13-didecanoate (4αPDD) by an increase in intracellular calcium and the activation of a cationic current, both of which are abolished by the removal of extracellular calcium or exposure to TRP antagonists, such as Ruthenium Red or RN1734. Following hypoxic/ischemic injury, responses of astrocytes to 4αPDD agonist are significantly augmented. Collectively, we show that TRPV4 channels are involved in ischemia-induced calcium entry in reactive astrocytes and might be involved in the pathogenic mechanisms of astroglial reactivity following ischemic insult. The team of the Department of Cellular Neurophysiology designed the study, performed immunohistochemistry surgeries, electrophysiology, Western blots, calcium imaging, cell culture preparation, data interpretation, and the writing of the paper.

Butenko, O., Dzamba, D., Benesova, J., Honsa, P., Benfenati, V., Rusnakova, V., Ferroni, S., and Anderova, M. (2012): The increased activity of TRPV4 channel in the astrocytes of the adult rat hippocampus after cerebral hypoxia/ischemia. PloS ONE 7(6): e39959. doi:10.1371/journal.pone.0039959. IF 4.004

The inhibitor of volume regulated anion channels DCPIB activates TREK potassium channels in cultured astrocytes.

The ethacrynic acid derivative, 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid (DCPIB) is considered to be the most specific and potent inhibitor of volume-regulated anion channels (VRACs). In the central nervous system DCPIB has been shown to be neuroprotective through mechanisms that are principally associated with its action on VRACs. We hypothesized that DCPIB could also regulate the activity of other astroglial channels involved in cell volume homeostasis. Functional and molecular experiments have been performed in rat cortical astrocytes in primary culture and in hippocampal astrocytes *in situ*. The effect of DCPIB has been determined by patch-clamp electrophysiology, immunoblotting and immunocytochemical techniques. The results have been verified through comparative analysis with recombinant channels expressed in COS-7 cells. In primary cultured rat cortical astrocytes, DCPIB promoted the activation of a K^+ conductance mediated by open rectifier two-pore-domain K^+ (K_{2P}) channels. The DCPIB effect occluded that of arachidonic acid which activates K_{2P} channels K_{2P} 2.1 (TREK-1) and K_{2P} 10.1 (TREK-2) in cultured astrocytes. Immunocytochemical and western blot analyses confirmed that cultured astrocytes express K_{2P} 2.1 and K_{2P} 10.1 proteins. Moreover, DCPIB opened recombinant K_{2P} 2.1 and K_{2P} 10.1 expressed in heterologous system. In brain slices DCPIB did not augment the large background K^+ conductance in hippocampal astrocytes but caused an increment in the basal K^+ current in neurons. Our results indicate that the neuroprotective effect of DCPIB could be due, at least in part, to the activation of TREK channels. They also suggest that DCPIB could be used as a template to build new pharmacological tools able

to increase background K^+ conductance in astroglia and neuronal cells. The team of the Department of Cellular Neurophysiology carried out electrophysiology, data interpretation, and the partial writing of the paper.
Minieri L., Pivonkova H., Harantova L., Anderova M. and Ferroni S. (2015): Intracellular Na+ inhibits volume regulated anion channel in rat cortical astrocytes J Neurochem , 132 (3), pp. 286-300. IF 3.973.

The role of Astrocytic potassium channels in CNS disorders.

Astrocytes are particularly complex cells responding to a large number of stimuli, such as extra/intracellular ion concentration shifts, pH shifts or increased neurotransmitter and hormone levels. Under physiological conditions, one of their key functions is to maintain ionic homeostasis in the brain during neuronal activity by a process called potassium spatial buffering. Several mechanisms are believed to underlie the maintenance of K^+ homeostasis, among which astrocytic K^+ conductance plays an important role. Potassium spatial buffering is based on the expression of weakly inwardly rectifying K^+ (Kir) channels, predominantly Kir4.1, which enable the high K^+ permeability of the plasma membrane and establish a strongly negative resting membrane potential in astrocytes. Additionally, two-pore domain K^+ channels, namely TREK1,2 and TWIK1, which are also highly expressed in astrocytes, might play a significant role in K^+ spatial buffering due to their constitutive open state allowing "passive" K^+ ion fluxes. Recent data indicate that the expression and functioning of astrocytic K^+ channels are seriously affected under many acute and chronic pathological conditions. In particular, a decrease in the inward K^+ current together with a reduced Kir4.1 channel expression or Kir4.1 channel mislocation have been shown in both the retina and brain after insult. These changes are accompanied by astrocytic depolarization, ultimately leading to impaired K^+ buffering, and also to a decreased ability of astrocytes to clear excess extracellular glutamate, both of which contribute to ongoing damage of the nervous tissue. In this review we focus on summarizing current knowledge about the expression and functioning of K^+ channels in astrocytes during the acute and chronic stages of CNS disorders, such as ischemia or epilepsy. The role of other proteins that might functionally interact with K^+ channels and influence K^+ buffering during CNS disorders, namely aquaporins and connexins, are also discussed.
Anderova M. and Pivonkova H., Chapter:. Astrocytic Potassium Channels in CNS Disorders. pp17-36, 2012 Chapter in the book: Astrocytes: Structure, Functions and Role in Disease Editors: Oscar González-Pérez, Publisher: NovaPublisher

Distinct effects of Sonic hedgehog and Wnt-7a on differentiation of neonatal neural stem/progenitor cells in vitro.

Sonic hedgehog (Shh) and Wnt-7a are morphogens involved in embryonic as well as ongoing adult neurogenesis. Their effects on differentiation and membrane properties of neonatal neural stem/progenitor cells (NS/PCs) were studied in vitro using NS/PCs transduced with either Shh or Wnt-7a. Eight days after the onset of in vitro differentiation the cells were analyzed for the expression of neuronal and glial markers using immunocytochemical and Western blot analysis, and their membrane properties were characterized using the patch-clamp technique. Our results showed that both Shh and Wnt-7a increased the numbers of cells expressing neuronal markers; however, quantitative immunocytochemical analysis showed that only Wnt-7a enhanced the outgrowth and development of processes in these cells. In addition, Wnt-7a markedly suppressed

gliogenesis. Electrophysiological analysis revealed that Wnt-7a increased, while Shh decreased the incidence of cells displaying a neuron-like current pattern, represented by outwardly rectifying K+ currents and tetrodotoxin-sensitive Na+ currents. Moreover, Shh promoted proliferation during the entire course of differentiation. Thus we can conclude that Shh and Wnt-7a interfere differently with the process of neuronal differentiation and that they promote distinct stages of neuronal differentiation in neonatal NS/PCs.
Prajerova, I., Honsa, P., Chvatal, A., Anderova, M. (2010): Distinct effects of Sonic hedgehog and Wnt-7a on differentiation of neonatal neural stem/progenitor cells in vitro. *Neuroscience*, 15;171(3): 693-711, IF 3.2

Neural Stem/Progenitor Cell Proliferation and Differentiation: Role of Sonic Hedgehog and Wingless/Int-1 Proteins.

The Sonic hedgehog and Wingless-Int protein (Wnt) signaling pathways have proven to be essential at various stages of neural development, but also in the ongoing neurogenesis of the adult hippocampus and subventricular zone under physiological conditions as well as in pathological states, such as traumatic brain injury, ischemia or neurodegenerative diseases. Here we review key findings demonstrating the role of Sonic hedgehog and Wingless-Int proteins (Wnts) in modulating the proliferation of neural stem/progenitor cells and affecting the fate decision of neural stem/progenitor cells during embryonic development. Moreover, we also review current findings elucidating the role of these morphogens during neonatal and adult neural stem cell differentiation and their possible role in adult neurogenesis induced by neurodegenerative disorders of the CNS.
Anderova M. and Honsa P. Chapter: Neural Stem/Progenitor Cell Proliferation and Differentiation: Role of Sonic Hedgehog and Wingless/Int-1 Proteins, in the book: Stem Cells and Cancer Stem Cells Volume 4, 2012, pp 3-18 Editor: Hayat M.A. Publisher: Springer

RT-qPCR work-flow for single-cell data analysis.

Individual cells represent the basic unit in tissues and organisms and are in many aspects unique in their properties. The introduction of new and sensitive techniques to study single-cells opens up new avenues to understand fundamental biological processes. Well established statistical tools and recommendations exist for gene expression data based on traditional cell population measurements. However, these workflows are not suitable, and some steps are even inappropriate for application on single-cell data. Here, we present a simple and practical workflow for the preprocessing of single-cell data generated by reverse transcription quantitative real-time PCR. The approach is demonstrated on a data set based on profiling of 41 genes in 303 single-cells. For some pre-processing steps we present options and also recommendations. In particular, we demonstrate and discuss different strategies for handling missing data and scaling data for downstream multivariate analysis. The aim of this workflow is to provide a guide to the rapidly growing community studying single-cells by means of reverse transcription quantitative real-time PCR profiling. The team of the Department of Cellular Neurophysiology carried out cell isolation, FACS sorting, induction of ischemia, and the partial writing of the paper.
Ståhlberg A, Rusnakova V, Forootan A, Anderova M, Kubista M (2012): RT-qPCR work-flow for single-cell data analysis. Methods 59 (2013) 80–88 IF 4.482

Deletion of alpha-syntrophin in perivascular astrocytes affects their ability to regulate their volume.

Brain edema accompanying ischemic or traumatic brain injuries, originates from a disruption of ionic/neurotransmitter homeostasis that leads to the accumulation of K+ and glutamate in the extracellular space. Their increased uptake, predominantly provided by astrocytes, is associated with water influx via aquaporin-4 (AQP4). As the removal of perivascular AQP4 via the deletion of α-syntrophin was shown to delay edema formation and K+ clearance, we aimed to elucidate the impact of α-syntrophin knockout on volume changes in individual astrocytes in situ evoked by pathological stimuli using three dimensional confocal morphometry and changes in the extracellular space volume fraction (α) in situ and in vivo in the mouse cortex employing the real-time iontophoretic method. RT-qPCR profiling was used to reveal possible differences in the expression of ion channels/transporters that participate in maintaining ionic/neurotransmitter homeostasis. To visualize individual astrocytes in mice lacking α-syntrophin we crossbred GFAP/EGFP mice, in which the astrocytes are labeled by the enhanced green fluorescent protein under the human glial fibrillary acidic protein promoter, with α-syntrophin knockout mice. Three-dimensional confocal morphometry revealed that α-syntrophin deletion results in significantly smaller astrocyte swelling when induced by severe hypo-osmotic stress, oxygen glucose deprivation (OGD) or 50 mM K+. As for the mild stimuli, such as mild hypo-osmotic or hyperosmotic stress or 10 mM K+, α-syntrophin deletion had no effect on astrocyte swelling. Similarly, evaluation of relative α changes showed a significantly smaller decrease in α-syntrophin knockout mice only during severe pathological conditions, but not during mild stimuli. In summary, the deletion of α-syntrophin markedly alters astrocyte swelling during severe hypo-osmotic stress, OGD or high K+. RT-qPCR analyses of a large number of cortical astrocytes revealed no changes in the expression of genes associated with K+/glutamate uptake or cell volume regulation. The team of the Department of Cellular Neurophysiology carried out the creation of transgenic animals with fluorescently labeled astrocytes, PCR, Western blotting, immunohistochemistry, 3D confocal microscopy, immunohistochemistry, quantification of GFAP staining, data interpretation, and the partial writing of the paper.

Dmytrenko L., Cicanic M.1, Anderova M., Vorisek I., Ottersen O.P., Sykova E. M., Vargova L. (2013): The impact of alpha-syntrophin deletion on the changes in tissue structure and extracellular diffusion associated with cell swelling under physiological and pathological conditions. PloS ONE, IF 3.5

Anderova M., Benesova J., Mikesova M., Dzamba D., Honsa P., Kriska J., Butenko O.' Novosadova V., Valihrach L., Kubista M., Dmytrenko L., Cicanic M. and Vargova L**. (**2014): Altered astrocytic swelling in the cortex of α-syntrophin-negative GFAP/EGFP mice. *PloS ONE,* IF 3.5

Cerebral ischemia expands the differentiation potential of polydendrocytes.

We have elucidated the role of adult polydendrocytes (also known as NG2 glial cells), which constitute a fourth major glial cell type in the adult mammalian central nervous system (CNS), in brain regeneration after ischemic injury. Although much evidence suggests that these cells are multipotent in vitro, their differentiation potential in vivo under physiological or pathophysiological conditions is still controversial. We have defined the differentiation potential of cortical polydendrocytes in vivo after middle cerebral artery occlusion (MCAO) with a main focus on their proliferation, differentiation and the electrophysiological properties of newly derived cells using a transgenic mouse line in which green fluorescent protein (GFP) has been knocked into the Cspg4 gene. We have also defined their differentiation potential in vitro after

their isolation from the site of an ischemic injury, with a main focus on their ability to form neurospheres and to differentiate into oligodendrocytes, astrocytes and neurons.
Honsa, P., Pivonkova, H., Dzamba, D., Filipova, M., Anderova, M. (2012): Polydendrocytes Display Large Lineage Plasticity following Focal Cerebral Ischemia. *PloS ONE 7 (5): e36816, doi: 10.1371/journal.pone.0036816.* IF 3.5

Focal cerebral ischemia induces the neurogenic potential of mouse Dach1-expressing cells in the dorsal part of the lateral ventricles.

The mDach1 gene, involved in the development of the neocortex and the hippocampus, is expressed by neural stem cells (NSCs) during early neurogenesis, and its expression also continues in a subpopulation of mDach1-expressing cells in the dorsal part of the lateral ventricles (LV) of the adult mouse brain. In this study we aimed to elucidate the role of mDach1-expressing cells in adult neurogenesis/gliogenesis under physiological as well as post-ischemic conditions, employing transgenic mice in which the expression of green fluorescent protein (GFP) is controlled by the D6 promotor of the mDach1 gene. The GFP^+ cells isolated from the dorsal part of the LV of controls formed neurospheres and differentiated into a glial phenotype, while those isolated after MCAo also differentiated into cells with the properties of neuronal precursors. In situ analyses revealed that GFP^+ cells expressed the phenotype of adult NSCs or neuroblasts in controls and following ischemia. Moreover, after MCAo we found a significantly increased number of GFP^+ cells expressing doublecortin and the number of GFP^+ cells migrating through the rostral migratory stream into the olfactory bulb, where they probably differentiated into $calretinin^+$ interneurons. Collectively, our results reveal the involvement of the mDach1 gene in adult neurogenesis. Cells expressing this gene exhibit the properties of adult NSCs or neuroblasts and respond to MCAo by enhanced neurogenesis.
Honsa, P., Pivonkova, H., Anderova, M (2013): Focal cerebral ischemia induces the neurogenic potential of mouse Dach1-expressing cells in the dorsal part of the lateral ventricles. Neuroscience 240, 39-53. IF 3.38

The inhibitor of volume regulated anion channels DCPIB activates TREK potassium channels in cultured astrocytes.

The ethacrynic acid derivative, 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid (DCPIB) is considered to be a specific and potent inhibitor of volume-regulated anion channels (VRACs). In the central nervous system, DCPIB was shown to be neuroprotective through mechanisms principally associated to its action on VRACs. We hypothesized that DCPIB could also regulate the activity of other astroglial channels involved in cell volume homeostasis. In cultured astrocytes DCPIB promoted the activation of a K^+ conductance mediated by two-pore-domain K^+ (K_{2P}) channels. The DCPIB effect occluded that of arachidonic acid which activates K_{2P} channels K_{2P} 2.1 (TREK-1) and K_{2P} 10.1 (TREK-2) in cultured astrocytes. Immunocytochemical analysis suggested that cultured astrocytes express K_{2P} 2.1 and K_{2P} 10.1 proteins. Moreover, DCPIB opened recombinant K_{2P} 2.1 and K_{2P} 10.1 expressed in an heterologous system. In brain slices DCPIB did not augment the large background K^+ conductance in hippocampal astrocytes but caused an increment in the basal K^+ current of neurons. Our results indicate that the neuroprotective effect of DCPIB could be due, at least in part, to the activation of TREK channels. DCPIB could be used as a template to build new pharmacological tools able to increase background K^+ conductance in astroglia and neuronal cells. The team of the Department

of Cellular Neurophysiology carried out immunohistochemistry, as well as some of the electrophysiology in vitro, in situ experiments, data interpretation, and the writing of the paper.
Minieri L, Pivonkova H, Caprini M, Harantova L, Anderova M, and Ferroni S. (2012): The inhibitor of volume regulated anion channels DCPIB activates TREK potassium channels in cultured astrocytes. *British Journal of Pharmacology 168(5):1240-54.* IF 5.03

Heterogeneity of Astrocytes: From Development to Injury – Single Cell Gene Expression.

Astrocytes perform control and regulatory functions in the central nervous system; heterogeneity among them is still a matter of debate due to limited knowledge of their gene expression profiles and functional diversity. To unravel astrocyte heterogeneity during postnatal development and after focal cerebral ischemia, we employed single-cell gene expression profiling in acutely isolated cortical GFAP/EGFP-positive cells. Using a microfluidic qPCR platform, we profiled 47 genes encoding glial markers and ion channels/transporters/receptors participating in maintaining K^+ and glutamate homeostasis per cell. Self-organizing maps and principal component analyses revealed three subpopulations within 10-50 days of postnatal development (P10-P50). The first subpopulation, mainly immature glia from P10, was characterized by the high transcriptional activity of all the studied genes, including polydendrocytic markers. The second subpopulation (mostly from P20) was characterized by low gene transcript levels, while the third subpopulation encompassed mature astrocytes (mainly from P30, P50). Within 14 days after ischemia (D3, D7, D14) additional astrocytic subpopulations were identified: resting glia (mostly from P50 and D3), transcriptionally active early reactive glia (mainly from D7) and permanent reactive glia (solely from D14). Following focal cerebral ischemia, reactive astrocytes underwent pronounced changes in the expression of aquaporins, nonspecific cationic and potassium channels, glutamate receptors and reactive astrocyte markers. The team of the Department of Cellular Neurophysiology carried out immunohistochemistry, cell isolation, cell sorting, ischemia induction, data interpretation, and the partial writing of the paper.
Rusnakova V., Honsa P., Dzamba D., Ståhlberg A., Kubista M. and Anderova M. (2013) Heterogeneity of Astrocytes: From Development to Injury – Single Cell Gene Expression. PloS ONE, IF 3.5

NMDA receptors in glial cells: pending questions.

Glutamate receptors of the N-methyl-D-aspartate (NMDA) type are involved in many cognitive processes, including behavior, learning and synaptic plasticity. For a long time NMDA receptors were thought to be the privileged domain of neurons; however, discoveries of the last 25 years have demonstrated the active role of glial cells as well. Despite the large number of studies in the field, there are many unresolved questions connected with NMDA receptors in glia that are still a matter of debate. The main objective of this review was to shed light on these controversies by summarizing results from all relevant works concerning astrocytes, oligodendrocytes and polydendrocytes (also known as NG2 glial cells) in experimental animals, further extended by studies performed on human glia. The results are divided according to the study approach to enable a better comparison of how findings obtained at the mRNA level correspond with protein expression or functionality. Furthermore, special attention is focused on the NMDA receptor subunits present in the particular glial cell types, which give them special characteristics different from those of neurons – for example, the absence of Mg^{2+} block and decreased Ca^{2+} permeability. Since glial cells are implicated in important physiological and pathophysiological roles in the

central nervous system (CNS), the last part of this review provides an overview of glial NMDA receptors with respect to ischemic brain injury.
Dzamba D., Honsa P. and Anderova M. (2013): NMDA receptors in glial cells: pending questions. Current Neuropharmacology, Curr Neuropharmacol. 2013 May;11(3):250-62.. IF 2.847

Analysis of in vitro and in vivo characteristics of human embryonic stem cell-derived neural precursors.

During the last decade, much progress has been made in developing protocols for the differentiation of human embryonic stem cells (hESCs) into a neural phenotype. The appropriate agent for cell therapy is neural precursors (NPs). Here, we demonstrate the derivation of highly enriched and expandable populations of proliferating NPs from the CCTL14 line of hESCs. These NPs could differentiate in vitro into functionally active neurons, as confirmed by immunohistochemical staining and electrophysiological analysis. Neural cells differentiated in vitro from hESCs and exhibit broad cellular heterogeneity with respect to developmental stage and lineage specification. To analyze the population of the derived NPs, we used fluorescence-activated cell sorting (FACS) and characterized the expression of several pluripotent and neural markers, such as Nanog, SSEA-4, SSEA-1, TRA-1-60, CD24, CD133, CD56 (NCAM), beta-III-tubulin, NF70, nestin, CD271 (NGFR), CD29, CD73, and CD105 during long-term propagation. The analyzed cells were used for transplantation into the injured rodent brain; the tumorigenicity of the transplanted cells was apparently eliminated following long-term culture. These results complete the characterization of the CCTL14 line of hESCs and provide a framework for developing cell selection strategies for neural cell-based therapies. The team of the Department of Cellular Neurophysiology carried out Immunohistochemical identification, electrophysiology, partially writing the paper.
Kozubenko N., Turnovcova K., Kapcalova M., Butenko O., Anderova M., Rusnakova V., Kubista M., Hampl A., Jendelova P., Sykova E. (2010): Analysis of in vitro and in vivo characteristics of human embryonic stem cell-derived neural precursors. *Cell transplantation*, 19(4):471-86. IF 5,251

Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex.

Astrocytes control tissue equilibrium and hence define the homoeostasis and function of the CNS (central nervous system). Being principal homoeostatic cells, astroglia are fundamental for various forms of neuropathology, including AD (Alzheimer's disease). AD is a progressive neurodegenerative disorder characterized by the loss of cognitive functions due to specific lesions in mnesic-associated regions, including the mPFC (medial prefrontal cortex). We therefore analyzed the expression of GS (glutamine synthetase) and GLT-1 (glutamate transporter-1) in astrocytes in the mPFC during the progression of AD in a triple-transgenic mouse model (3xTg-AD). GS is an astrocyte-specific enzyme, responsible for the intracellular conversion of glutamate into glutamine, whereas the removal of glutamate from the extracellular space is accomplished mainly by astroglia-specific GLT-1. We found a significant decrease in the numerical density (Nv, cells/mm3) of GS-positive astrocytes from early to middle age (1–9 months; at the age of 1 month by 17%, 6 months by 27% and 9 months by 27%when compared with control animals) in parallel with a reduced expression of GS (determined by Western blots), which started at the age of 6 months and was sustained up to 12 months of age. We did not, however, find any changes in the expression of GLT-1, which implies an intact glutamate uptake mechanism. Our results indicate

that the decrease in GS expression may underlie a gradual decline in the vital astrocyte-dependent glutamate–glutamine conversion pathway, which in turn may compromise glutamate homoeostasis, leading towards failures in synaptic connectivity with deficient cognition and memory. The team of the Department of Cellular Neurophysiology performed morphological analyses of neural tissue in transgenic and control mice using three-dimensional confocal morphometry developed at the Department, and was involved in the preparation of the manuscript. Kulijewicz-Nawrot M, Syková E, Chvátal A, Verkhratsky A, Rodríguez JJ. (2013) Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. ASN Neuro. 5(4):273-82. IF 3.638.

Department of Molecular Neurophysiology

Physiology of vasopressin and oxytocin.

In this project, we focused on the physiology and signaling mechanisms of vasopressin (AVP) and oxytocin (OT) neurons and their terminals in the central and peripheral (dorsal root ganglia) nervous system. In collaboration with our Japanese colleagues, a transgenic rats model was, for the first time, developed in order to visualize fluorescent vasopressin (AVP-eGFP) and oxytocin (OT-eCFP; OT-mRFP) neurons in the magnocellular nuclei and terminals, which successfully demonstrated distinct Ca^{2+} signaling cascades within neurons and terminals. This project resulted in several important publications listed below. The team of the Department of Cellular Neurophysiology carried out neuron and terminal isolation, confocal imaging, fluorescence microscopy, immunohistochemistry, data interpretation, and the partial writing of the paper.

Katoh A, Fujihara H, Ohbuchi T, Onaka T, Young WS, Dayanithi G, Yamasaki Y, Kawata M, Suzuki H, Otsubo H, Suzuki H, Murphy D, Ueta Y. (2010): Specific expression of an oxytocin-enhanced cyan fluorescent protein fusion transgene in the rat hypothalamus and posterior pituitary. Journal of Endocrinology 204: 275-285. IF 3.743

Todoroki M, H. Fujihara H, Otsubo H, Shibata M, Sakamoto H, Kawata M, Dayanithi G, Murphy D, Hiro H, Nagata S and Ueta Y. (2010): Induction of the arginine vasopressin-enhanced green fluorescent protein fusion gene in the locus coeruleus in these transgenic rats. Stress 13 (4):281-291. IF 3.463

Viero C, Shibuya I, Kitamura N, Fujihara H, Verkhratsky A, Katoh A, Ueta Y, Zingg HH, Chvatal A, Sykova E, and Dayanithi G. (2010) Oxytocin: crossing the bridge between basic science and pharmacotherapy. CNS Neuroscience & Therapeutics 16:e138-e156. IF 3.874

Dayanithi G, Forostyak O, Ueta Y, Verkhratsky A & Toescu EC (2012). Segregation of calcium signalling mechanisms in magnocellular neurones and terminals. Cell Calcium 51: 293-299. IF 4.21

Calcium signaling in human embryonic stem cells.

This research project was focused on the study of the physiological significance of Ca^{2+} signaling mechanisms in undifferentiated human embryonic stem cells (hESC) and during differentiation to neural cells (hESC-NPs: human embryonic stem cell-derived neural precursors). The functional properties of ion channels and receptors as well as the differentiation of stem cells (SCs) were analyzed and then compared to the Ca^{2+} signaling mechanisms of various types of SC

models such as: i) human immortalized neural SCs; ii) human spinal cord SCs and finally iii) rats mesenchymal SCs. They found that the pre-differentiation of embryonic SCs leads to an activation of Ca^{2+} signaling cascades and enhanced the functional activity of the cells. The Ca^{2+} signaling mechanisms and the physiological properties of hESC-derived neural precursors changed during maintenance *in vitro.* It is suggested that in future studies, the evaluation of homeostatic signaling mechanisms could be considered to be a key element in determining the 'quality of stem cells' before using them for transplantation and repair studies. In the study, the use of conditionally immortalized neural stem cell lines from human embryonic spinal cord tissue demonstrated that these clonal lines: i) retain a clear transcriptional signature of ventral spinal cord progenitors and a normal karyotype after extensive propagation in vitro, ii) differentiate into relevant ventral neuronal subtypes with functional Ca^{2+} channels and exhibit spontaneous Ca^{2+} oscillations, and iii) stably engrafte into lesioned rat spinal cord without tumorogenicity. The team of the Department of Cellular Neurophysiology carried out $Ca2^{+}$ signaling analysis and behavioral studies, data interpretation and the partial writing of the paper.

Forostyak O, Romanyuk N, Verkhratsky A, Sykova E, and Dayanithi G. (2013). Plasticity of calcium signaling cascades in human embryonic stem cell-derived neural precursors. Stem Cells and Development 22, 1506-1521.

Cocks G, Romanyuk N, Amemori T, Jendelova P, Forostyak O, Jeffries AR, Perfect L, Thuret S, Dayanithi G, Sykova E, et al. (2013). Conditionally immortalized stem cell lines from human spinal cord retain regional identity and generate functional V2a interneurons and motorneurons. Stem Cell Research & Therapy 7; 4(3):69.

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Neuroscience

The Department of Neuroscience, headed by Prof. Eva Syková, focuses on research from bench to bedside, particularly on the mechanisms and treatment of brain and spinal cord injury, brain homeostatic mechanisms and extrasynaptic transmission, as well as their roles in a number of physiological and pathological states. Bioinova, Ltd., is a spin-off company of the Department (for details see Appendix 3.3).

The use of magnetic nanoparticles in regenerative medicine.

During 2010-2014 we developed new nanoparticles based on either iron oxides (namely maghemite) with different polymer coatings, perovskites and gadolinium linked to Titanium oxide. Such particles showed a higher labeling efficiency compared with commercial products. Therefore, we patented our new method of particle surface modification. All the prepared nanoparticles were tested from the point of view of genotoxicity, cell viability, proliferation, as well as ability to differentiate using mesenchymal stem cells (MSCs) and spinal neural precursors (SPCs). The nanoparticles did not affect cell viability and proliferation, but their presence inside the cells caused oxidative damage to DNA, lipids and proteins. Nevertheless, in the presence of nanoparticles MSCs can differentiate into osteoblasts, adipocytes and chondrocytes. In the case of neural differentiation we studied SPC cells, which were transplanted into rats with a spinal lesion. In vivo, the labeled cells differentiated mainly into astrocytes, with only a portion differentiating into motoneurons. During the in vivo differentiation, some nanoparticles were driven out of the cells. The biological part of this research was performed at the Department, nanoparticles were prepared at the Institute of Macromolecular Chemistry CAS or the Faculty of Natural Science.

Chekina N, Horak D, Jendelova P, Trchova M, Benes MJ, Hruby M, Herynek V, Turnovcova K, Sykova E. Fluorescent magnetic nanoparticles for biomedical applications. JMaterChem. 2011; 21:7630-39.

Sponarova D, Horak D, Trchova M, Jendelova P, Herynek V, Mitina N, Zaichenko A, Stoika R, Lesny P, Sykova E. The use of oligoperoxide-coated magnetic nanoparticles to label stem cells. Journal of biomedical nanotechnology. 2011; 7(3):384-94.

Pollert E, Kaman O, Veverka P, Veverka M, Marysko M, Zaveta K, Kacenka M, Lukes I, Jendelova P, Kaspar P, Burian M, Herynek V. Core-shell La(1-x)Sr(x)MnO3 nanoparticles as colloidal mediators for magnetic fluid hyperthermia. Philosophical transactions. 2010; 368(1927):4389-405.

Kotkova Z, Kotek J, Jirak D, Jendelova P, Herynek V, Berkova Z, Hermann P, Lukes I. Cyclodextrin-based bimodal fluorescence/MRI contrast agents: an efficient approach to cellular imaging. Chemistry (Weinheim an der Bergstrasse, Germany). 2010; 16(33):10094-102.

Rehor I, Vilimova V, Jendelova P, Kubicek V, Jirak D, Herynek V, Kapcalova M, Kotek J, Cerny J, Hermann P, Lukes I. Phosphonate-titanium dioxide assemblies: platform for multimodal diagnostic-therapeutic nanoprobes. Journal of medicinal chemistry. 2011; 54(14):5185-94.

Novotna B, Jendelova P, Kapcalova M, Rossner P, Jr., Turnovcova K, Bagryantseva Y, Babic M, Horak D, Sykova E. Oxidative damage to biological macromolecules in human bone marrow mesenchymal stromal cells labeled with various types of iron oxide nanoparticles. Toxicology letters. 2012; 210(1):53-63.
Amemori T, Romanyuk N, Jendelova P, Herynek V, Turnovcova K, Prochazka P, Kapcalova M, Cocks G, Price J, Sykova E. Human conditionally immortalized neural stem cells improve locomotor function after spinal cord injury in the rat. Stem cell research & therapy. 2013; 4(3):68.
Babic M, Horak D, Jendelova P, Herynek V, Proks V, Vanecek V, Lesny P, Sykova E. The use of dopamine-hyaluronate associate-coated maghemite nanoparticles to label cells. International journal of nanomedicine. 2012;**7**:1461-74.

Poly(L-lysin)-coated particles were utilized for in vivo detection and quantification of labeled metastatic brain cells injected into experimental animals via intracardial injection. This research was performed in collaboration with our Norwegian partners; the Department's team was responsible for the development of cell labeling method and labeling efficiency.
Sundstrom T, Daphu I, Wendelbo I, Hodneland E, Lundervold A, Immervoll H, Skaftnesmo KO, Babic M, Jendelova P, Sykova E, Lund-Johansen M, Bjerkvig R, Thorsen F. Automated tracking of nanoparticle-labeled melanoma cells improves the predictive power of a brain metastasis model. Cancer research. 2013; 73(8):2445-56.

We also evaluated the targeting of mesenchymal stem cells labeled with PLL-coated SPIONs to the site of a spinal cord injury by means of a magnetic implant. We monitored both the distribution and the number of cells in the lesion site after their intrathecal administration and the effect of the magnetic implant on cell transplantation. The cell distribution correlated with the calculated distribution of magnetic forces exerted on the transplanted cells in the subarachnoid space and the lesion site. Our results showed that targeting efficiency can be increased by using magnets that produce spatially modulated stray fields. All experiments were designed and performed at the Department, the Institute of Physics CAS performed the theoretical calculations of the magnetic forces.
Vanecek V, Zablotskii V, Forostyak S, Ruzicka J, Herynek V, Babic M, Jendelova P, Kubinova S, Dejneka A, Sykova E. Highly efficient magnetic targeting of mesenchymal stem cells in spinal cord injury. International journal of nanomedicine. 2012; 7:3719-30.

Effects of high-gradient magnetic fields on MSC growth and differentiation.

Mechanical or magneto-mechanical stress applied to the cell membrane, cytoskeleton, or organelles plays a very important role in crucial intracellular processes. In this project, we experiment using micro-magnets and living cells to reveal the dramatic impact of a high magnetic field gradient on the spatial organization and growth of stem cells. We also proposed an approach of driving the MSC differentiation pathway through a spatially modulated low frequency high-gradient magnetic field (HGMF) generated by magnet arrays. We showed that oscillating HGMF and mechanical vibration affect adipogenic differentiation of MSCs via the transmission of mechanical stress to the cell cytoskeleton, resulting in F-actin remodeling and subsequent down-regulation of adipogenic genes adiponectin, PPARc, and AP2. Our findings offer insight into the regulation of cellular nanomechanics, and provide a basis for better controlled down-regulation of stem cell adipogenesis by HGMF, which may facilitate the development of challenging therapeutic strategies suitable for the remote control of biological systems. All biological experiments were

performed and designed at the Department, magnets and oscillating magnetic fields were provided by the Institute of Physics CAS.

Zablotskii V, Dejneka A, Kubinova S, Le-Roy D, Dumas-Bouchiat F, Givord D, Dempsey NM, Sykova E. Life on Magnets: Stem Cell Networking on Micro-Magnet Arrays. Plos One. 2013; 8(8).

Zablotskii V, Lunov O, Novotna B, Churpita O, Trosan P, Holan V, Sykova E, Dejneka A, Kubinova S. Down-regulation of adipogenesis of mesenchymal stem cells by oscillating high-gradient magnetic fields and mechanical vibration. Appl Phys Lett. 2014; 105(10).

The use of stem cells in spinal cord injury repair.

A comparison of stem cell therapies in a preclinical model is needed to understand the underlying mechanism behind behavioral recovery, how the implantation of stem cells effects the physiological and genetic functions concerning growth factor expression, immune response or glial scar modulation. In our investigation, we studied GMP manufactured human bone marrow mesenchymal stem cells (hMSCs) and two types of neural precursors (NPs), including human conditional fetal spinal line (SPC-01) and human induced pluripotent derived neural progenitors (iPS-NPs) in the treatment of a clinically relevant SCI model of balloon-induced spinal cord compression in rats. One week after lesion induction, injured rats received either intrathecal application of hMSCs, saline, or were implanted intraspinally at the level of the SCI with SPC-01**,** NP-iPS or saline. The route of delivery was chosen according to the most appropriate application for SCI. Rats were behaviorally evaluated using different tests, assessing their basic and advanced locomotor skills (BBB, flat beam test, rotarod). Sensory responses were measured using the plantar test. Tissue analyses of white/gray spared matter, axonal sprouting and glial scar modulation (GFAP staining) were performed. qPCR and Luminex multiplex cytokines (MIP-1α, IL-4, IL-1β, IL-2, IL-6, IL-12p70, TNF-α, and RANTES) were performed at 10, 14, 28 and 60 days after SCI, respectively, to detect host tissue responses to stem cell therapy. All stem cell grafted rats showed increased locomotor recovery when compared to controls. However, the NP-iPS treated group also scored significantly better in the advanced locomotor tests. In all stem cell treated groups white matter sparing was observed, while gray matter was preserved only in the NP-iPS treated group. Both NPs significantly increased the number of GAP43+ axons and slowly matured within the period of 4 months after transplantation. SPC cells differentiated more into glia, though 25% of cells displayed markers for motoneurons, while iPS-NPs showed different neuronal phenotype. hMSCs, unlike both NPs, decreased in levels of inflammatory cytokines within 10 days after stem cell application. These findings correlate with the span of survival of hMSCs for a short period of time (2 weeks), NPs survived longer (2 months) and matured slowly. From our results, we deduce that iPS-NPs, were the most suitable candidate for treatment of SCI due to their robust survival, tissue sparing, reduction of glial scarring, and increased axonal sprouting. The major advantage of hMSC use is its less invasive route of administration coupled with a strong anti-inflammatory effect, with repeated applications potentially overcoming its transiency.

Administration of growth factors leading to the mobilization of bone marrow cells or application of other cells which are not of neuronal origin (mesenchymal stromal cells from bone marrow and adipose tissue, olfactory glial cells) into rats with spinal cord lesions in a subacute phase has only a supporting and neuroprotective effect, leading to an increase in the volume of white matter and improvements in motor function. Differentiation of MSCs in vitro into neural phenotype does not affect the further improvement of motor function. Furthermore, intraspinal application of mesenchymal bone marrow stromal cells and olfactory ensheathing glia does not

have a synergistic effect on the improvement of motor functions. All studies were performed at the Department, except for the detection of cytokines (New York Medical College). SPC-01 cells were provided by King's College London and iPS cells were provided by INSERM.

Amemori T, Romanyuk N, Jendelova P, Herynek V, Turnovcova K, Prochazka P, Kapcalova M, Cocks G, Price J, Sykova E. Human conditionally immortalized neural stem cells improve locomotor function after spinal cord injury in the rat. Stem cell research & therapy. 2013; 4(3):68.
Urdzíková LM, Růžička J, LaBagnara M, Kárová K, Kubinová Š, Jiráková K, Murali R, Syková E, Jhanwar-Uniyal M, Jendelová P. Human mesenchymal stem cells modulate inflammatory cytokines after spinal cord injury in rat. Int J Mol Sci. 2014 Jun25; 15(7):11275-93.
Cocks G, Romanyuk N, Amemori T, Jendelova P, Forostyak O, Jeffries AR, Perfect L, Thuret S, Dayanithi G, Sykova E, Price J. Conditionally immortalized stem cell lines from human spinal cord retain regional identity and generate functional V2a interneurons and motorneurons. Stem cell research & therapy. 2013; 4(3):69
Romanyuk N, Amemori T, Turnovcova K, Prochazka P, Onteniente B, Sykova E, Jendelova P. Beneficial effect of human induced pluripotent stem cell-derived neural precursors in spinal cord injury repair. Cell transplantation. 2014.
Urdzikova L, Likavcanova-Masinova K, Vanecek V, Ruzicka J, Sedy J, Sykova E, Jendelova P. Flt3 ligand synergizes with granulocyte-colony-stimulating factor in bone marrow mobilization to improve functional outcome after spinal cord injury in the rat. Cytotherapy. 2011; 13(9):1090-104.
Amemori T, Jendelova P, Ruzickova K, Arboleda D, Sykova E. Co-transplantation of olfactory ensheathing glia and mesenchymal stromal cells does not have synergistic effects after spinal cord injury in the rat. Cytotherapy. 2010; 12(2):212-25.
Arboleda D, Forostyak S, Jendelova P, Marekova D, Amemori T, Pivonkova H, Masinova K, Sykova E. Transplantation of predifferentiated adipose-derived stromal cells for the treatment of spinal cord injury. Cellular and molecular neurobiology. 2011; 31(7):1113-22.

The use of stem cells in stroke model.

Administration of human neural precursors derived from induced pluripotent cells in an experimental model of stroke has a dual effect. In the first weeks after transplantation, the transplanted cells have a support function and neuroprotective effect and their administration reduces degeneration of *Substantia nigra*. In the long term, transplantation leads to maturation and differentiation of transplanted cells into striatal neurons that communicate with the structures of the recipient. Application of NPs from human pluripotent stem cells (iPS or ES) is safe, but it is necessary to respect their in vitro characteristics and transplant the cells in the correct stage of maturation and development. All experiments were performed at the Department; results from the stroke model were combined with results obtained at INSERM and published in 2 papers. Publications were based on Collaboration within STEMS consortium (EU research project). Patch clamp studies were done by the Department of Cellular Neurophysiology, Institute of Experimental Medicine CAS.

Polentes J, Jendelova P, Cailleret M, Braun H, Romanyuk N, Tropel P, Brenot M, Itier V, Seminatore C, Baldauf K, Turnovcova K, Jirak D, Teletin M, Come J, Tournois J, Reymann K, Sykova E, Viville S, Onteniente B. Human induced pluripotent stem cells improve stroke outcome and reduce secondary degeneration in the recipient brain. Cell transplantation. 2012; 21(12):2587-602

Kozubenko N, Turnovcova K, Kapcalova M, Butenko O, Anderova M, Rusnakova V, Kubista M, Hampl
A, Jendelova P, Sykova E. Analysis of in vitro and in vivo characteristics of human embryonic stem cells derived neural precursors. Cell transplantation. 2010; 19(4):471-86.
Seminatore C, Polentes J, Ellman D, Kozubenko N, Itier V, Tine S, Tritschler L, Brenot M, Guidou E, Blondeau J, Lhuillier M, Bugi A, Aubry L, Jendelova P, Sykova E, Perrier AL, Finsen B, Onteniente B. The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. Stroke; a journal of cerebral circulation. 2010; 41(1):153-9.

Spinal cord injury repair using hydrogels.

We have developed and tested biomodified poly(2-hydroxethyl methacrylate)-based (PHEMA) hydrogel scaffolds with oriented channels that can bridge a spinal cord lesion, support functional axonal re-growth and may serve simultaneously as stem cell carriers. The project was developed in collaboration with a team at the Institute of Macromolecular Chemistry CAS, which developed the hydrogel scaffolds and their modifications. Several new cell adhesive modifications of PHEMA hydrogels were successfully developed for tissue engineering application.

The role of the SIKVAV-modified hydrogel for guiding channels in the spinal cord repairing processes was determined in combination with mesenchymal stem cells (MSC) in a complete spinal cord injury. The results showed that this type of hydrogel scaffold with oriented guiding channels can successfully bridge a complete spinal cord transection and promote the aligned axonal ingrowth into the parallel pores; however, no additional effect of the MSCs on the ingrowth of tissue elements into the hydrogel scaffold was observed.

We also evaluated the surface modifications of HEMA and HPMA hydrogels by RGD. MSCs were seeded onto four different hydrogels: hydroxypropylmethacrylate-RGD prepared by heterophase separation (HPMA-HS-RGD) and 3 other hydrogels polymerized in the presence of a solid porogen: HPMA-SP, HPMA-SP-RGD and hydroxy ethyl methacrylate [2-(methacryloyloxy)ethyl] trimethylammonium chloride (HEMA-MOETACl). Adhesion capability and cell survival were evaluated 1, 7 and 14 days after the seeding of MSCs onto the hydrogel scaffolds. The cell-polymer scaffolds were then implanted into a hemisected rat spinal cord, and MSC survival in vivo and the ingrowth of endogenous tissue elements were evaluated 1 month after implantation. Our results showed that the adhesive molecule RGD supports angiogenesis, while hydrogels based on HEMA improve adhesiveness of MSCs, compared to hydrogels based on HPMA. Hydrogels based on hydroxypropylmethacrylamid (HPMA) support the ingrowth of axons compared to hydrogels based on 2-hydroxyethylmethacrylate (HEMA); the ingrowth of new axons is further supported by the inner structure of the hydrogel resembling a spider web, compared to a structure based on globular microparticles. However, after in vivo implantation HPMA-HS-RGD polymer in combination with MSCs can improve the behavioral outcome in rats with chronic spinal cord lesion.

We evaluated the use of a cell polymer construct based on a combination of the conditionally immortalized spinal progenitor cell line SPC-01_GFP3, derived from human fetal spinal cord tissue, with a serotonin-modified poly(2-hydroxyethyl methacrylate) hydrogel (pHEMA-5HT). We compared the effects of treatment with a pHEMA-5HT hydrogel seeded with SPC-01_GFP3 cells, a pHEMA-5HT hydrogel only and no treatment on the functional outcome and tissue reconstruction in hemisected rats. Prior to transplantation the cell-polymer construct displayed a high potential to support the growth, proliferation and differentiation of SPC-01 cells

in vitro. One month after surgery, the combined hydrogel-cell treatment reduced astrogliosis and tissue atrophy and increased axonal and blood vessel ingrowth into the implant; however, two months later only the ingrowth of blood vessels remained increased. SPC-01_GFP3 cells survived well in vivo and expressed advanced markers of neuronal differentiation. However, a majority of the transplanted cells migrated out of the lesion and only rarely remained in the hydrogel. No differences among the groups in motor or sensory recovery were observed. Despite the support of the hydrogel as a cell carrier *in vitro*, and good results *in vivo* one month postsurgery, there was only a small effect on long term recovery, mainly due to the limited ability of the hydrogels to support the *in vivo* growth and differentiation of cells within the implant.

All the hydrogels were developed in collaboration with the Institute of Macromolecular Chemistry CAS, where the polymer matrices were prepared. All in vitro and in vivo experiments were performed at the Department.

Kubinova S, Sykova E. Biomaterials combined with cell therapy for treatment of spinal cord injury. Regenerative medicine. 2012;**7**(2):207-24.

Pego AP, Kubinova S, Cizkova D, Vanicky I, Mar FM, Sousa MM, Sykova E. Regenerative medicine for the treatment of spinal cord injury: more than just promises? Journal of cellular and molecular medicine. 2012; 16(11):2564-82.

Kubinova S, Horak D, Hejcl A, Plichta Z, Kotek J, Sykova E. Highly superporous cholesterol-modified poly(2-hydroxyethyl methacrylate) scaffolds for spinal cord injury repair. Journal of biomedical materials research. 2011; 99:618-29.

Kubinova S, Horak D, Kozubenko N, Vanecek V, Proks V, Price J, Cocks G, Sykova E. The use of superporous Ac-CGGASIKVAVS-OH-modified PHEMA scaffolds to promote cell adhesion and the differentiation of human fetal neural precursors. Biomaterials. 2010; 31(23):5966-75.

Kubinova S, Horak D, Vanecek V, Plichta Z, Proks V, Sykova E. The use of new surface-modified poly(2-hydroxyethyl methacrylate) hydrogels in tissue engineering: treatment of the surface with fibronectin subunits versus Ac-CGGASIKVAVS-OH, cysteine, and 2-mercaptoethanol modification. Journal of biomedical materials research. 2014; 102(7):2315-23.

Kubinova S, Horak D, Hejcl A, Plichta Z, Kotek J, Proks V, Forostyak S, Sykova E. SIKVAV-modified highly superporous PHEMA scaffolds with oriented pores for spinal cord injury repair. J Tissue Eng Regen Med. 2013.

Hejcl A, Ruzicka J, Kapcalova M, Turnovcova K, Krumbholcova E, Pradny M, Michalek J, Cihlar J, Jendelova P, Sykova E. Adjusting the chemical and physical properties of hydrogels leads to improved stem cell survival and tissue ingrowth in spinal cord injury reconstruction: a comparative study of four methacrylate hydrogels. Stem cells and development. 2013; 22(20):2794-805.

Hejcl A, Sedy J, Kapcalova M, Toro DA, Amemori T, Lesny P, Likavcanova-Masinova K, Krumbholcova E, Pradny M, Michalek J, Burian M, Hajek M, Jendelova P, Sykova E. HPMA-RGD hydrogels seeded with mesenchymal stem cells improve functional outcome in chronic spinal cord injury. Stem cells and development. 2010; 19(10):1535-46.

Ruzicka J, Romanyuk N, Hejcl A, Vetrik M, Hruby M, Cocks G, Cihlar J, Pradny M, Price J, Sykova E, Jendelova P. Treating spinal cord injury in rats with a combination of human fetal neural stem cells and hydrogels modified with serotonin. Acta neurobiologiae experimentalis. 2013; 73(1):102-15.

Stem cells in the treatment of amyotrophic lateral sclerosis (ALS).

We found that the transplantation of rat GFP+ mesenchymal stem cells (MSC) in vitro and in situ or human MSCs applied intrathecally led to the prolongation of life span in ALS animals, improved survival of motoneurons and decreased apoptosis in the spinal cord. We also analyzed perineuronal nets, which were preserved in animals transplanted with MSCs. Based on preclinical data obtained in animal models, we have prepared a clinical trial in patients with ALS, intrathecally transplanting autologous bone marrow mesenchymal stromal cells, which was approved by the regulatory authority SÚKL. All patients underwent a single intrathecal administration of autologous MSC suspension (15-20x106 cells) by lumbar puncture and were subsequently followed up for 18 months to record all possible side effects of the therapy. During the follow-up visits, the effect of MSCs on the progression of the disease was also assessed – in all patients a functional evaluation of the clinical status was performed using spirometry, the ALS-functional rating scale, the Norris bulbar and spinal scale. All experiments we designed and performed at the Department. The clinical trial is running in collaboration with BioInova, a spinoff company of the Institue, and Motol Hospital in Prague.

Forostyak S, Homola A, Turnovcova K, Svitil P, Jendelova P, Sykova E. Intrathecal delivery of mesenchymal stromal cells protects the structure of altered perineuronal nets in SOD1 rats and amends the course of ALS. Stem cells. 2014; 32(12):3163-72.

Forostyak S, Jendelova P, Kapcalova M, Arboleda D, Sykova E. Mesenchymal stromal cells prolong the lifespan in a rat model of amyotrophic lateral sclerosis. Cytotherapy. 2011; 13(9):1036-46.

Study of the properties of the human adipose tissue-derived stromal cells (ASCs) from the diabetic and non-diabetic patients.

The aim of this study was to evaluate the properties of ASCs from diabetic patients and to compare them to the non-diabetic counterparts. ASCs were isolated from the adipose tissue of distal limbs of diabetic patients with critical ischemia. As a non-diabetic hAT-MSC, cells isolated from fat tissue obtained during orthopedic operations were used. Flow cytometry analysis confirmed mesenchymal phenotypes in both diabetic and non-diabetic ASCs with positive expression for CD29, CD73, CD 90, HLA-ABC and negative for CD31, CD45, CD235a, CD271 and HLA-DR+DP markers. Nevertheless, 40% of diabetic and 20% of non-diabetic ASC samples displayed high expressions of fibroblast marker, which inversely correlated with the expression of CD105. Moreover, significantly decreased expression of VEGFA and chemokine receptor CXCR4 in fibroblast-positive ASCs was found in diabetic patients, compared with their fibroblast-negative counterparts. Reduced osteogenic differentiation and the down-regulation of chemokine CXCL12 were found in fibroblast-negative diabetic ASCs. According to this study, adipose tissue from distal limbs of diabetic patients is not satisfactory as an autologous ASC source. All experiments were designed and performed at the Department, and fat tissue was collected at the IKEM.

Koci Z, Turnovcova K, Dubsky M, Baranovicova L, Holan V, Chudickova M, Sykova E, Kubinova S. Characterization of human adipose tissue-derived stromal cells isolated from diabetic patient's distal limbs with critical ischemia. Cell Biochem Funct. 2014; 32(7):597-604.

Diffusion studies during pathological states.

During the last 5-year period, the role of quantitative and qualitative remodeling of the extracellular matrix (ECM) and/or alteration in the astrocytic structural/functional properties in changes of the brain diffusivity was studied in tumorous and dysplastic human cortex, after the induction of transient hypoxia/ischemia in rats and on genetically modified mice lacking either Bral1 or α-syntrophin proteins. The effect of the changes in the ECS properties, including its volume and ion composition, on fine cellular mechanisms like long-term potentiation (LTP) and neuronal plasticity, underlying the formation of memory trails in hippocampus, was studied in connexin knock-out animals. The current state of knowledge concerning the role of the extracellular matrix and astrocytes in changes of the ECS diffusion parameters and signal transmission including the results were summarized in a high-impacted review, designed and written by the Department's team members.

Vargova L, Sykova E. Astrocytes and extracellular matrix in extrasynaptic volume transmission. Philos Trans R Soc Lond B Biol Sci 2014; 369(1654):20130608.

Focal cortical dysplasias (FCDs) of the brain are recognized as a frequent cause of intractable epilepsy. The correlation of the immunohistochemical analysis of human FCD tissue samples with results of diffusion measurements showed that overexpression of certain ECM molecules (tenascin R, tenascin C, and versican) and structural changes of fine astrocytic processes lead to increased amounts of diffusion barriers in the ECS. Hindered brain diffusion not only increases a local concentration of the neuroactive substances and contributes thus to the generation or spread of epileptic seizures, but also slowed down the movement of drugs and reduced the effect of the therapy. Diffusion measurement and study design were performed at the Department, human tissue was provided by Motol Hospital, pathological evaluation of the tissue was performed at 2nd Medical Faculty, Charles University, Prague..

Vargova L, Homola A, Cicanic M, Kuncova K, Krsek P, Marusic P, Sykova E, Zamecnik J. The diffusion parameters of the extracellular space are altered in focal cortical dysplasias. Neuroscience Letters. 2011; 499(1):19-23.

Zamecnik J, Homola A, Cicanic M, Kuncova K, Marusic P, Krsek P, Sykova E, Vargova L. The extracellular matrix and diffusion barriers in focal cortical dysplasias. The European journal of neuroscience. 2012; 36(1):2017-24.

Early and late changes in tissue diffusivity and the structural alteration which underlie them were studied in 8-week-old rats after transient global cerebral ischemia (GCI). In the early phases of reperfusion (1 to 3 days) neuronal cell death, glial proliferation and developing gliosis were accompanied by an apparent diffusion coefficient of water (ADC_W), an extracellular volume fraction (α) decrease and tortuosity (λ) increase. In the late phases of reperfusion (starting 1 month after H/I), when the CA1 region consisted mainly of microglia, astrocytes, and NG2-glia with markedly altered morphology, all of the studied diffusion parameters had increased. The changes of the ADC_W, determined by diffusion-weighted magnetic resonance imaging (DW-MRI), can thus be associated with either changes in the extracellular volume (acute phase) or in tortuosity (chronic phase), reflecting the alterations in the amount of the diffusion barriers. Correlation of the α and λ values with ADC_W changes in states involving cell volume increase, cell death or proliferation, glia morphology and ECM composition can bring important findings for improving the quality of diagnosis and prognosis in patients with ictus. Experiments were performed and

designed at the Neuroscience Department together with the Department of Cellular Neurophysiology (responsible for patch clamp studies and immunohistochemistry).
Anderova M, Vorisek I, Pivonkova H, Benesova J, Vargova L, Cicanic M, Chvatal A, Sykova E. Cell death/proliferation and alterations in glial morphology contribute to changes in diffusivity in the rat hippocampus after hypoxia-ischemia. J Cereb Blood Flow Metab. 2011; 31(3):894-907.

Bral1 is a brain-specific hyaluronan-binding link protein, which stabilizes the ECM and nodes of Ranvier in the myelinated white matter. In Bral1-/- mice, the hyaluronan-associated ECM no longer showed a typical nodal pattern, diffusion hindrances were reduced and CNS nerve conduction was markedly decreased even though there were no differences between wild-type and mutant mice in the clustering or transition of ion channels at the nodes or in the tissue morphology around them. The results indicate that the ECM contributes to the accumulation of sodium ions in the proximity of the nodes of Ranvier, probably by binding cations to negatively charged groups, but also by forming diffusion barriers, thus creating a pool of sodium ions. The IEM team performed measurements of the extracellular space diffusion parameters and apparent diffusion coefficient of water using the real-time iontophoretic method and diffusion weighted MRI, respectively. The team participated in the experiment planning, evaluation of the results and writing of the manuscript.
Bekku Y, Vargova L, Goto Y, Vorisek I, Dmytrenko L, Narasaki M, Ohtsuka A, Fassler R, Ninomiya Y, Sykova E, Oohashi T. Bral1: its role in diffusion barrier formation and conduction velocity in the CNS. J Neurosci. 2010; 30(8):3113-23.
Cicanic M, Sykova E, Vargova L. Bral1: "Superglue" for the extracellular matrix in the brain white matter. The international journal of biochemistry & cell biology. 2012; 44(4):596-9.

Modification or deletion of aquaporin-4 (AQP4) channels or their anchoring proteins, α-syntrophins (α-syn), have a protective effect e.g. during ischemia or epileptic activity, presumably via the reduction of astrocytic swelling, however, the detailed mechanisms have not yet been fully elucidated. Two comprehensive studies using a spectrum of methods and experimental models of physiological and pathological cell swelling were performed to detect changes in the brain diffusivity, volume of the ECS and individual astrocytes and correlated them with alterations in the tissue structure and protein expression. Comparison of the results acquired in α-syn-/- and wild type mice showed that water transport across AQP4 channels enhances and accelerates astrocyte swelling as well as recovery, mainly during severe hypotonic stress, increased extracellular potassium or ischemia/anoxia, but not during milder stimuli. α-syn-/- thus represents a promising target for new therapeutic approaches in pathological states associated with brain cell swelling, such as ictus or heart attack. The members of the Department were responsible for all experimental results (except from immunohistochemistry and image analysis), critical evaluation of the data, statistical analysis and preparation of the manuscript.
Anderova M, Benesova J, Mikesova M, Dzamba D, Honsa P, Kriska J, Butenko O, Novosadova V, Valihrach L, Kubista M, Dmytrenko L, Cicanic M, Vargova L. Altered astrocytic swelling in the cortex of alpha-syntrophin-negative GFAP/EGFP mice. PLoS One. 2014; 9(11):e113444.
Dmytrenko L, Cicanic M, Anderova M, Vorisek I, Ottersen OP, Sykova E, Vargova L. The impact of alpha-syntrophin deletion on the changes in tissue structure and extracellular diffusion associated with cell swelling under physiological and pathological conditions. PLoS One. 2013; 8(7):e68044.

The role of astrocytic networks and mutual neuroglial interaction was studied in mice deficient for connexins 30 and 43 (Cx30-/-Cx43-/-). Electrophysiological experiments showed that gap junctional communication mediated by astroglial connexins limit neuronal excitability, release probability and insertion of postsynaptic AMPA receptors, silencing synapses which result in a decrease of neuronal activity and synaptic transmission in pyramidal neurons of the CA1 region. Knock-outs revealed disorders in ion, glutamate and volume homeostasis maintained by astrocytes. A larger decrease in ECS volume as well as the delayed deswelling of astrocytes in Cx30-/-Cx43-/- mice may further increase extracellular concentrations of ions and neurotransmitters will enhancing even more synaptic activity and prolonging neuronal activation, resulting in higher susceptibility to epileptic seizures and spontaneous epileptogenic activity. Dr Vargova performed the measurements and evaluation of the relative changes of the extracellular volume in the hippocampus of mice deficient for connexins 30 and 43 and in their wild type controls. Dr Vargova and prof. Sykova participated also in the planning of the experimental design and writing of the manuscript.
Pannasch U, Vargova L, Reingruber J, Ezan P, Holcman D, Giaume C, Sykova E, Rouach N. Astroglial networks scale synaptic activity and plasticity. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(20):8467-72

The research of the Department resulted in four clinical studies, which were operated by Bioinova, Ltd., a subsidiary of the IEM, whose main objective is the transfer of the IEM's intellectual property into practice:

1. AMSC-ALS-001 (2011-000362-35): "A prospective, non-randomized, open label study to assess the safety and the efficacy of autologous multipotent mesenchymal stem cells in the treatment of amyotrophic lateral sclerosis".
2. AMSC-BDT-001 (2012-005599-33): "Utilization of autologous multipotent mesenchymal stem cells in the management of the large skeletal defects during revision total hip arthroplasty".
3. AMSC-DSD-001 (2010-024665-52): "Utilization of autologous mesenchymal cells in posterolateral spinal fusion in degenerative spine disease".
4. AMSC-RC-001 (2010-024664-17): "Utilization of autologous mesenchymal cells to enhance rotator cuff repair".

During the period of 2010-2014, Bioinova, Ltd. attracted approximately 462 000 Euro from non-public sources in order to realize these clinical studies.

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Molecular Biology of Cancer and Teratology

The scientific team consists of two departments: Department of Molecular Biology of Cancer (head Pavel Vodička) and Department of Teratology (head Miroslav Peterka).

Department of Molecular Biology of Cancer

Molecular and genetic characteristics of sporadic colorectal cancer in the Czech Republic.

Colorectal cancer (CRC), a common neoplasia, poses serious health problems in the Czech Republic. In its sporadic forms low penetrance genes are a prerequisite for individual susceptibility to CRC, the paradigm of "common disease-common variant" is applicable in this case. We have therefore focused on associations between DNA repair polymorphisms, cell cycle and DNA damage recognition genetic polymorphisms and CRC. A novel aspect of our study has been the investigation of the role of DNA MMR in sporadic CRC and simultaneous haplotype analyses of genetic polymorphisms of the previously mentioned genes. In our studies we have analyzed the link between gene variants (genes regulating several important pathways like DNA repair, folate metabolisms, DNA methylation, insulin resistance, obesity, cell cycles and ABC transporters) and the risk of sporadic CRC. We have investigated the role microRNA binding sites and the role of gene coding selenoproteins in CRC carcinogenesis. Since we are partners in the European consortium COGENT, these studies are clearly collaborative, however we substantially contributed to the setting the hypothesis, design and conducting the experiment.

Tomlinson IP, Dunlop M, Campbell H, Zanke B, Gallinger S, Hudson T, Koessler T, Pharoah PD, Niittymaeki I, Tuupanen S, Aaltonen LA, Hemminki K, Lindblom A, Foersti A, Sieber O, Lipton L, van Wezel T, Morreau H, Wijnen JT, Devilee P, Matsuda K, Nakamura Y, Castellvi-Bel S, Ruiz-Ponte C, Castells A, Carracedo A, Ho JW, Sham P, Hofstra RM, Vodicka P, Brenner H, Hampe J, Schafmayer C, Tepel J, Schreiber S, Voelzke H, Lerch MM, Schmidt CA, Buch S, Moreno V, Villanueva CM, Peterlongo P, Radice P, Echeverry MM, Velez A, Carvajal-Carmona L, Scott R, Penegar S, Broderick P, Tenesa A, Houlston RS. COGENT (COlorectal cancer GENeTics): an international consortium to study the role of polymorphic variation on the risk of colorectal cancer. Br. J. Cancer 2010; 102: 447-454. IF: 4.8

Jiraskova A, Novotny J, Novotny L, Vodicka P, Pardini B, Naccarati A, Schwertner HA, Hubacek JA, Puncocharova L, Smerhovsky Z, Vitek L. Association of serum bilirubin and promoter variations in HMOX1 and UGT1A1 genes with sporadic colorectal cancer. Int J Cancer 2012; 131:1549–1555. IF: 5.0

Landi D, Gemignani F, Pardini B, Naccarati A, Garritano S, Vodicka P, Vodickova L, Canzian F, Novotny J, Barale R, Landi S. Identification of candidate genes carrying polymorphisms associated with the risk of colorectal cancer by analyzing the colorectal mutome and microRNAome. Cancer 2012, 118:4670-80. IF: 5.1

Molecular characteristics of DNA repair in tumor tissue of colon and rectum carcinoma.

We developed and applied functional tests of DNA repair in tumor tissue and healthy mucosa in patients with colon and rectum tumors in order to characterize the main repair pathway (mismatch repair or NER and BER). We have also investigated the relationship between DNA repair capacity (functional test) and expression profiles of DNA repair genes in sporadic forms of colorectal cancer (CRC) cases as well as in controls. Several studies have been dedicated to the influence of DNA repair markers towards the effectiveness of chemotherapy. The impact of the DNA repair on the outcome of chemotherapy has been awarded by the chairman of the Czech Science Foundation with appropriate reflections in the media. We have been the main investigators in these studies with some participation of our German and Norwegian colleagues, we developed the functional assay, designed the experiments, interpreted the data and wrote the manuscripts. It should be noted that our Italian colleagues Dr. Alessio Naccarati and Dr. Barbara Pardini have been constituent members of our team for a decade (until 2012) and they still continue to work part time with us.

Slyšková J, Korenková V, Collins AR, Procházka P, Vodičková L, Švec J, Lipská L, Levý M, Schneiderová M, Liška V, Holubec L, Kumar R, Souček P, Naccarati A, Vodička P. Functional, genetic and epigenetic aspects of base and nucleotide excision repair in colorectal carcinogenesis. Clin Cancer Res 2012;18:5878-5887. IF: 8.193

Pardini B, Rosa F, Di Gaetano C, Slyšková J, Novotný J, Levý M, Landi S, Vodička P, Naccarati A. Variation within 3´-untranslated region of base excision repair genes and response to therapy in colorectal cancer patients: a potential modulation by microRNAs binding. Clin Cancer Res 2013;19(21):6044-6056. IF: 8.193

Slyskova J., Lorenzo Y., Karlsen A., Carlsen M.H., Novosadova V., Blomhoff R., Vodicka P., Collins A.R. Both genetic and dietary factors underlie individual differences in DNA damage levels and DNA repair capacity. DNA Repair (Amst). 2014 Apr;16:66-73. IF: 3.362

Epigenetic gene silencing in colorectal cancer- a potential biomarker for early diagnosis and efficacy of chemotherapy.

Our primary aim was to demonstrate the assumption that methylation of a gene promoter leads to gene silencing (transcriptional repression). We explored possible correlations between the silencing of genes in the target (tumor) and surrogate tissues (healthy mucosa, blood). It has been an important finding to prove genetic/epigenetic alterations in basic protective mechanisms (DNA mismatch repair genes) are present not only in the target tumor tissue, but also in a surrogate tissue, such as the peripheral blood leukocytes (PBL). We reported that some of these alterations may be shared between the normal intestinal mucosa and PBL of cancer patients. Such markers may become traits characterizing the carcinogenic process. The study was designed and executed mainly in our Department.

Vymetalkova Polakova V, Slyskova J, Korenkova V, Bielik L, Langerova L, Prochazka P, Rejhova A, Schwarzova L, Pardini B, Naccarati A, Vodicka P. Molecular characteristics of mismatch repair genes in sporadic colorectal tumors in Czech patients. BMC Medical Genetics 2014. IF: 2.450

Analysis of the role of genetic factors in pancreatic cancer risk and its prognosis.

The project examined new gene variants in the multigenic etiology of pancreatic cancer. Similarly as in colorectal cancer (CRC), we aimed to map DNA repair polymorphisms, cell cycle and DNA damage recognition gene polymorphisms and the risk of the disease. We have participated on studies concerning the importance of the KRAS signaling pathway in development

as well as the prognosis of pancreatic cancer. The main aim was to identify new targets for biological agents directed against oncogenic action of the KRAS track and to define markers for eventual use in individualized treatment of this disease. This project was a multicentric collaborative study, to which we proportionally participated with ideas, design and execution; however our role was not the major one.

Campa D, Rizzato C, Bauer A, Werner J, Capurso G, Costello E, Talar-Wojnarowska R, Jamroziak K, Pezzilli R, Gazouli M, Khaw KT, Key TJ, Bambi F, Mohelnikova-Duchonova B, Heller A, Landi S, Vodickova L, Theodoropoulos G, Bugert P, Vodicka P, Hoheisel JD, Neoptolemos JP, Soucek P, Büchler MW, Giese N, Canzian F. Lack of replication of seven pancreatic cancer susceptibility loci identified in two Asian populations. Cancer Epidemiology, Biomarkers & Prevention, 2013 Feb;22(2):320-3. IF: 4.324

Wolpin BM, Rizzato C, Kraft P, Kooperberg C, Petersen GM, Wang Z, Arslan AA, Beane-Freeman L, Bracci PM, Buring J, Canzian F, Duell EJ, Gallinger S, Giles GG, Goodman GE, Goodman PJ, Jacobs EJ, Kamineni A, Klein AP, Kolonel LN, Kulke MH, Li D, Malats N, Olson SH, Risch HA, Sesso HD, Visvanathan K, White E, Zheng W, Abnet CC, Albanes D, Andriotti G, Austin MA, Barfield R, Basso D, Berndt SI, Boutron-Ruault MC, Brotzman M, Buchler MW, Bas Bueno-de-Mesquita H, Bugert P, Burdette L, Campa D, Caporaso NE, Capurso G, Chung C, Cotterchio M, Costello E, Elena J, Funel N, Gaziano M, Giese N, Giovannucci EL, Goggins M, Gorman MJ, Gross M, Haiman C, Hassan M, Helzlsouer K, Henderson BE, Holly EA, Hu N, Hunter DJ, Innocenti F, jenab M, Kaaks R, Key TJ, Khaw KT, Klein EA, Kogevinas M, Kupcinskas J, Kurtz RC, LaCroix A, Landi MT, Landi S, Le Marchand L, Mambrini A, Mannisto S, Milne RL, Nakamura Y, Oberg AL, Owzar K, Panico S, Patel AV, Peeters PH, Peters U, Piepoli A, Porta M, Real FX, Riboli E, Rothman N, Scarpa A, Shu X, Silverman DT, Soucek P, Sund M, Talar-Wojnarowska R, Taylor PR, Theodoropoulos GE, Thornquist M, Tjoenneland A, Tobias GS, Trichopoulos D, Vodicka P, Wactawski-Wende J, Wentzensen N, Wu C, Yu H, Yu K, Zeleniuch-Jacquotte A, Hoover R, Hartge P, Fuchs C, Channock S, Stolzenberg-Solomon RS, Amundadottir L. Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. Nature Genet. 2014 Sep.;46(9):994-1000. IF: 29.648

Molecular biological and histopathological characteristics of lymphocytes infiltrating tumor tissue as a tool for prediction of risk of early recurrence of colorectal cancer.

The main aim of the project was to look for differences in gene expression related to the behavior of lymphocytes regarding the tumor tissue of patients with colorectal cancer (CRC) with a good prognosis after radical surgery as well as in early recurrent patients. We characterized the disease not only by classical clinico-pathological factors (radicality of resection, TNM, stage, grade, lymfangioinvasion), but also by molecular factors known to interact with the prognosis of patients (microsatellite instability - MSI, mutations of KRAS and BRAF oncogenes and CpG islets methylation - CIMP). We also identified a prospective molecular biological, histopathological and immunohistochemical profile of early recurrent CRC. Another objective was to retrospectively validate the molecular biological, histopathological and immunohistochemical profiles identified in the prospective part, with an aim to find an association with the tendency for early recurrence of colorectal cancer. We have been partners on this project and participated on the design of the study and on the execution of the experiments.

Vymetalkova V, Pardini B, Rosa F, Di Gaetano C, Novotny J, Levy M, Buchler T, Slyskova J, Vodickova L, Naccarati A, Vodicka P. Variations in mismatch repair genes and colorectal cancer risk and clinical outcome. Mutagenesis 2014 Apr 22;29(4):259–265. IF: 3.495

Identification of new genetic variants (SNPs and CNVs) and the determination of their impact on DNA repair function and susceptibility to CRC.

An additional area of research that was explored within the department was the identification of genetic variants and miRNA-binding sites which affect the survival and toxicity of 5-FU treatment. We also focused on the epigenetic analysis of genes involved in 5-FU treatment response as well as on the analysis of relevant variants in certain subgroups of colorectal cancer (CRC) patients. Furthermore, an identification of new genes and genetic variants which predispose to CRC in families with unknown genetic background as well as the analysis of genotype-environment interaction (patient's lifestyle etc.) were integral parts of the project. This project was designed mainly in our Department with important participation of our Italian members, Alessio Naccarati and Barbara Pardini and colleagues from the German Cancer Research Center, Heidelberg, Germany. The study was, however, executed mainly in our laboratory.

Pardini B, Kumar R, Naccarati A, Novotny J, Prasad RB, Forsti A, Hemminki K, Vodicka P, Bermejo JL. 5Fluorouracil-based chemotherapy for colorectal cancer and MTHFR/MTRR genotypes. Br J Clin Pharmacol. 2011 Jul; 72(1):162-3. IF: 3.578

Pardini B, Rosa F, Di Gaetano C, Slyšková J, Novotný J, Levý M, Landi S, Vodička P, Naccarati A. Variation within 3´-untranslated region of base excision repair genes and response to therapy in colorectal cancer patients: a potential modulation by microRNAs binding. Clin Cancer Res 2013;19(21):6044-6056. IF: 8.193

Pardini B, Bermejo JL, Naccarati A, Di Gaetano C, Rosa F, Legrand C, Novotny J, Vodička P, Kumar R. Inherited variability in a master regulator polymorphism (rs4846 associates with survival in 5-FU treated colorectal cancer patients. Mutat. Res. 2014 766-767:7-13. IF: 4.440

Study of long non-coding RNA, miR137 and its protein and mucin gene expression as a guide in the early diagnosis of CRC. Noninvasive examination of molecular markers in colorectal cancer (CRC) enable effective surgery thanks to early diagnosis of the disease or its recurrence. To take the most appropriate diagnostic and therapeutic strategy, we addressed the molecular characteristics of the tumor, as well as its clinical and histological behavior and searched for new non-invasive biomarkers. Long non-coding RNAs, microRNAs (miRNAs) or mucins were delineated as potential biomarkers that could help the early detection of CRC or its recurrence. The studies were designed at our Department in collaboration with our colleagues, currently working mainly in Italy. The execution of the experiments, data interpretation and writing of the manuscripts were led by our department. We also relayed in close collaboration with clinical departments (Surgical department, Thomayer hospital, Prague).

Svoboda M, Slyskova J, Schneiderova M, Makovicky P, Bielik L, Levy M, Lipska L, Hemmelova B, Kala Z, Protivankova M, Vycital O, Liska V, Schwarzova L, Vodickova L, Vodicka P. HOTAIR long non-coding RNA is a negative prognostic factor not only in primary tumors, but also in the blood of colorectal cancer patients. Carcinogenesis. 2014 Jul;35(7):1510-5. IF: 5.226

Genetics and immunity in early stages of colorectal adenocarcinoma: inflammatory environment in conventional vs germ-free animal models and in human samples.

We intended to characterize the genetic (MSI), epigenetic (methylation) and immunological changes in order to identify pathways in cancer induction in rats and early markers of carcinogenesis. Additional aims included: the selection of candidate genes of DNA repair, cell cycle, apoptosis and inflammation to create a profile of expressions and to validate animal models

appropriate for humans. We executed the analyses and participated in the design of the study, being partners on the project.
Polakova Vymetalkova V, Vannucci L, Korenkova V, Prochazka P, Slyskova J, Vodickova L, Rusnakova V, Bielik L, Burocziova M, Rossmann P, Vodicka P. Evaluation of tumor suppressor gene expressions and aberrant methylation in the colon of cancer-induced rats: a pilot study. Mol Biol Rep. 2013 Oct;40(10):5921-9.

Department of Teratology

A new view on molar development in the mouse model of odontogenesis.

We presented completely new aspects of molar development in mice. During rodent evolution, teeth progressively extinguished between incisor and molars and were replaced by a toothless gap - diastema. Our previous studies have reported transitory rudimentary primordia of the suppressed teeth. However, the primordia of suppressed teeth are transiently so big that they are generally considered to be the first functional molar (M1). We have proved, using in situ hybridization, DiI microinjection and time lapse in vitro, histology and 3D reconstructions, that the developmentally most advanced tooth primordium in the early mouse mandible is indeed a rudimentary structure and not the M1. Our results propose completely new insight into the regulation of early tooth development and on the existing molecular data achieved in the mouse model. The Department's team led and designed this study, performed the histological tests, prepared data for the computer made 3D reconstructions, performed Shh in situ hybridisation, DiI microinjection and time-laps experiments, the corresponding data analyses and interpretation, and was responsible for writing the paper and making documentations.
Prochazka, J. – Pantalacci, S. - Churava, S. - Rothova, M. - Lambert, A - Lesot, H. - Klein, O. - Peterka, M. - Laudet, V. - Peterkova, R.: Patterning by heritage in mouse molar row development. Proc. Natl. Acad. Sci. USA. 107 (2010) 15497 – 15502. IF 9.432

Tooth number can be regulated by activity of a single signal transduction pathway.

A series of mice carrying mutations in the Sprouty genes were studied, the protein products of which are antagonists of receptor-tyrosine kinase signaling. In Sprouty, loss-of-function mutants, splitting of gene expression domains and reduced apoptosis was associated with subdivision of the incisor primordium and a multiplication of its stem cell-containing regions. Interestingly, changes in Sprouty dosage led to a graded change in incisor number, with progressive decreases in Sprouty dosage leading to increasing numbers of teeth. The independent development of two incisors in mutants with large decreases in Sprouty dosage mimicked the likely condition of rodent ancestors. The Department's team performed the morphological analyses of developing incisors in mutant and control mice using histology and 3D reconstructions, and was involved in the preparation of corresponding documentation and paper writing.
Charles C, Hovorakova M, Ahn Y, Lyons DB, Marangoni P, Churava S, Biehs B, Jheon A, Lesot H, Balooch G, Krumlauf R, Viriot L, Peterkova R, Klein OD. Regulation of tooth number by fine-tuning levels of receptor-tyrosine kinase signaling. Development. 2011 Sep;138(18):4063-73. IF 6.898.

A new view on incisor development in the mouse model of odontogenesis.

We presented completely new aspects concerning incisor development in mice. Knowledge about the normal development of teeth is essential for the proper interpretation of developmental anomalies in mutant mice. A single Shh expression domain is generally correlated with lower incisor development in mice. In contrast, we identified that instead of the single domain, two spatially distinct regions of Shh expression appear in an anterior-posterior sequence. The initial anterior one represented a rudimentary incisor, whereas only the later posterior region corresponded to the prospective functional incisor. This implies that, as with Shh expression, other molecular data that have been ascribed to the progressive development of the mouse functional incisor at early stages, in fact, correspond to a rudimentary incisor whose development is aborted. The Department's team designed the study, performed the histological tests, prepared data for the computer made 3D reconstructions, performed Shh in situ hybridization, corresponding data analyses and interpretation, and was responsible for writing the paper and making documentation. Hovorakova M, Prochazka J, Lesot H, Smrckova L, Churava S, Boran T, Kozmik Z, Klein O, Peterkova R, Peterka M. Shh expression in a rudimentary tooth offers a new insight into the mouse lower functional incisor development. J Exp Zool B Mol Dev Evol. 316 B (5), pp. 347-358, 2011. IF 2.373.

Fate-map of the dental mesenchyme documented a dynamic development of the dental papilla and follicle.

A tooth is a hard product of a mature tooth germ, which results from the developmental interactions between the dental epithelium and mesenchyme. Dental epithelium is generally assumed to actively grow and wrap around the adjacent mesenchyme, which becomes a dental papilla. We fate mapped the dental mesenchyme, using in vitro tissue culture combined with vital cell labelling and tissue grafting, and showed that the dental mesenchyme is a much more dynamic population than previously suggested. The Department's team designed the paper, performed the experiments, collected and analyzed data, and were responsible for the interpretation of the results and the writing of the paper.

Rothova M, Peterkova R, Tucker AS. Fate map of the dental mesenchyme: dynamic development of the dental papilla and follicle. Dev Biol. 2012; 366(2):244-254. IF 3.637

Shape and size of the upper jaw in the patients after operation of unilateral cleft lip and palate.

This malformation is the most severe among orofacial clefts. Geometric morphometrics was used to determine the regions of size and shape differences in the patients when compared to a control group. These differences resulted from a combination of congenital hypoplasia and postoperative changes of the upper jaw in the patients. These results will be used in designing following orthodontic treatment. One member of the Department (M. Peterka) was involved in designing the study, analysis of data, interpretation of results and paper writing.

Ruskova H, Bejdova S, Peterka M, Krajicek V, Veleminska J. 3-D shape analysis of palatal surface in patients with unilateral complete cleft lip and palate. J Craniomaxillofac Surg. 2014 Jul;42(5):e140-7. IF2.597

Shape and size of the upper jaw in the patients after operation of the bilateral cleft lip and palate.

Bilateral complete cleft lip and palate is the most severe among orofacial clefts. Geometric morphometrics was used to determine the differences in the regions of size and shape in patients when compared to a control group. These differences resulted from a combination of congenital hypoplasia and postoperative changes of the upper jaw in patients. The results will be used in designing orthodontic treatment. One member of the Department (M. Peterka) was involved in designing the study, analysis of data, interpretation of results and paper writing.

Bejdova S, Krajicek V, Peterka M, Trefny P, Veleminska J. Variability in palatal shape and size in patients with bilateral complete cleft lip and palate assessed using dense surface model construction and 3D geometric morphometrics. J Craniomaxillofac Surg. 2012 Apr;40(3):201-8. IF 2.597.

Update of the knowledge on dental anomalies.

The anomalies addressed represent common congenital malformations that occur either as isolated findings (abnormalities in tooth size, shape, and form), as part of a syndrome (in e.g. ectodermal dysplasias), or are associated with cleft lip and palate. The paper focuses on genetic causes of abnormal tooth development in human and mouse models, and the implications of these abnormalities for clinical care. R. Peterková was the co-leader of this paper together with the first author O. Klein (San Francisco, USA). Members of our team contributed to the sections concerning the mouse odontogenesis model, normal tooth development in humans, and tooth anomalies in the patients with orofacial clefts.

Klein OD, Oberoi S, Huysseune A, Hovorakova M, Peterka M, Peterkova R. Am J Med Genet C Semin Med Genet. 2013 Nov;163C(4):318-32. doi: 10.1002/ajmg.c.31382. Epub 2013 Oct 4. Review. IF 4.440.

Discovery of the correlation between early Shh expression and rudimentary upper incisor development in the mouse.

We proved that the first developing structure and its molecular signaling in the mouse upper incisor region does not belong to a functional incisor, as generally assumed, but to a rudimentary, so called milk incisor, that is aborted. This study has completed our systematic revision of classical data on mouse odontogenesis: The primordia of functional teeth appear as late as embryonic day 13, while only rudiments develop prior to this. The Departmental team designed the study, performed the histological tests, prepared data for computer made 3D reconstructions, performed Shh in situ hybridisation, morphological analyses, data interpretation, and writing the paper.

Hovorakova M, Smrckova L, Lesot H, Lochovska K, Peterka M, Peterkova R. Sequential Shh expression in the development of the mouse upper functional incisor. J Exp Zool B Mol Dev Evol. 2013, 320:455-464. IF 2.123

Three-dimensional analysis of the early development of dentition.

We summarized our results provided during our 20 years-worth of research concerning odontogenesis in the laboratory mouse and in humans. Our results disprove the generally accepted concept of dentition development in both, mice and humans. We offer a new base for the interpretation of results of studies on interactions between dental epithelium and mesenchyme, and on the molecular control of tooth development in the mouse model. Such knowledge is important for development of future methods aimed at biological tooth replacement, when a tooth implant

resulting from controlled differentiation of living cells will be anchored into the jaw. The Departmental team led the designing of the experimental structure, contributed extensive documentation, and wrote the majority of the text. The paper summarizes results of our collaboration with the team of Dr. Lesot (Strasbourg, France) providing 57 common papers and 85 conference presentations.
Peterkova R, Hovorakova M, Peterka M, Lesot H. Three-dimensional analysis of the early development of the dentition. Aust Dent J. 2014, 59 Suppl 1:55-80. IF: 1.482.

Three-dimensional analysis of later phases of tooth development in the mouse model.

Five phenomena characterize the later period of tooth development: growth of the tooth germ; development of the cervical loop; histogenesis of the enamel organ; folding of the epithelial-mesenchymal junction associated with cusp formation; and change in cellular heterogeneity in the mesenchyme. All these processes are controlled by epithelial-mesenchymal interactions. These complex histo-morphogenetic events have been documented using histological sections and 3D reconstructions. When combined with functional tests in vitro, this approach enabled the search of possible relationships between simultaneous changes occurring in both the epithelial and ecto-mesenchymal compartments. The Department's team was involved in paper writing and preparation of documentation.
Lesot H, Hovorakova M, Peterka M, Peterkova R. Three-dimensional analysis of molar development in the mouse from the cap to bell stage. Aust Dent J. 2014, 59 Suppl 1:81-100. IF: 1.482.

Supernumerary incisor development in mice with an excess NF-κB activity.

A small, supernumerary upper incisor was found in mice with excess NF-κB activity and documented a revival of the developmental program that is suppressed under physiological conditions in mice, but stimulated in K5-Ikkβ mutants. A similar small incisor is physiologically present in the mouse-related group – lagomorphs. The Departmental team performed morphological analyses of developing incisors in the mutant and control mice using histology and 3D reconstructions, and was involved in the preparation of corresponding documentation and paper writing.
Blackburn J, Kawasaki K, Porntaveetus T, Kawasaki M, Otsuka-Tanaka Y, Miake Y, Ota MS, Watanabe M, Hishinuma M, Nomoto T, Oommen S, Ghafoor S, Harada F, Nozawa-Inoue K, Maeda T, Peterková R, Lesot H, Inoue J, Akiyama T, Schmidt-Ullrich R, Liu B, Hu Y, Page A, Ramírez Á, Sharpe PT, Ohazama A. Excess NF-κB induces ectopic odontogenesis in embryonic incisor epithelium. J Dent Res. 2015 Jan;94(1):121-8.

Tooth anomalies in the mouse model of Ellis-van Creveld syndrome.

Clinical features of patients with Ellis-van Creveld syndrome include various dental anomalies. We showed that the absence of the cilial protein Evc in a mouse model causes hypo- and hyperplasia defects during first molar development resulting in microdontia, disruption of tooth segmentation and symmetry, as well as root fusions. Our data indicates that disrupted activities of the Shh pathway are the primary cause for the dental anomalies in patients with Ellis-van Creveld syndrome or Weyers acrodental dysostosis. Members of the Department have been invited as experts in the field of early tooth morphogenesis in normal and mutant mice. We processed embryological material for histology and made 3D reconstructions of developing teeth, analysed data and participated in manuscript writing and making figures.

Nakatomi M, Hovorakova M, Gritli-Linde A, Blair HJ, MacArthur K, Peterka M, Lesot H, Peterkova R, Ruiz-Perez VL, Goodship JA, Peters H. Evc regulates a symmetrical response to Shh signaling in molar development. J Dent Res. 2013 Mar;92(3):222-8.

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Transplantation Immunology, Tissue Engineering and Pharmacology

The team's research focused on three main areas: the study of immune reactions associated with cell-based therapy using stem cells in preclinical models (Department of Transplantation Immunology, head Prof. Holáň), preparation of novel drug delivery systems for tissue engineering and regenerative medicine for both in vitro and in vivo studies (Department of Tissue Engineering, head Prof. Amler) and screening of immunobiological activities of new compounds of various origin with the aim to identify prospective candidates for further preclinical studies (Department of Pharmacology, head Dr. Zídek)

Department of Transplantation Immunology

Limbal and mesenchymal stem cells were cultured in vitro on nanofiber scaffolds and transferred onto a damaged ocular surface in experimental animal models. The therapeutic effects of stem cells were evaluated by histology, immunohistochemistry and according to the ability to inhibit the expression of genes for proinflammatory cytokines IL-2, interferon-gama and IL-17, as well as inducible nitric oxide synthase. It was observed that stem cells significantly suppressed a harmful local inflammatory reaction and supported re-epithelialization and a healing process. The results thus demonstrate the ability of stem cells and nanofiber scaffolds to regenerate the ocular surface after injury. All the work and manuscript preparation was done by members of the Department of Transplantation Immunology. In one publication, other coworkers from the IEM assisted us with microscopy.

Holáň, V., Javorková, E.: (2013) Mesenchymal stem cells, nanofiber scaffolds and ocular surface reconstruction. Stem Cells Rev. Rep. 9(5), 609-619.

Čejková, J., Trošan, P., Čejka, Č., Lenčová, A., Zajícová, A., Javorková, E., Kubinová, Š., Syková, E., Holáň, V.: (2013) Suppression of alkali-induced oxidative injury to the cornea by mesenchymal stem cells growing on nanofiber scaffolds and transferred onto the damaged corneal surface. Exp. Eye Res. 116, 312-323.

Holáň, V., Javorková, E., Trošan, P.: The growth and delivery of mesenchymal and limbal stem cells using copolymer polyamide 6/12 nanofiber scaffolds. In: Wright, B. and Connon, C. J. (eds), Corneal Regenerative Medicine, Methods Mol. Biol., Humana Press – Springer, New York, London 187-199, 2013.

The role of cytokines in the development of regulatory T (Treg) and B (Breg) cells was described. TGF-beta was shown to be the main cytokine determining the development of Tregs, but this cytokine inhibited activation of IL-10-producing Breg. On the contrary, another two cytokines, IL-12 and IFN-gama, enhanced the development of Breg. Due to their experience with the study of cytokines, the study collaborated with a Polish partner (the Institute of Pharmacology) to study the role of cytokines in an experimental model of chronic depression. These studies showed distinct roles of cytokines in the development of Treg and Breg, and suggested new

approaches for targeted regulation of the immune system. All the work as well as the preparation of two manuscripts was done exclusively by members of our Department. In a cooperative publication with the Polish partner a significant part of the work (characterization of immunoregulatory molecules) was performed and evaluated in our Department.

Holáň, V., Zajícová, A., Javorková, E., Trošan, P., Chudičková, M., Pavlíková, M., Krulová, M.: (2013) Distinct cytokines balance the development of regulatory T cells and IL-10-producing regulatory B cells. Immunology 141, 577-586, 2014.

Kubera, M., Curzytek, K., Duda, W., Leskiewicz, M., Basta-Kaim, A., Budziszewska, B., Roman, A., Zajícová, A., Holáň, V., Szczesny, E., Lason, W., Maes, M.: (2013) A new animal model of (chronic) depression induced by repeated and intermittent lipopolysaccharide administration for 4 months. Brain Behav. Immun. 31, 96-104.

Holáň, V., Krulová, M.: (2013) Common and small molecules as the ultimate regulatory and effector mediators of antigen-specific transplantation reactions. World J. Transplant. 3(4), 54-61.

In collaboration with other colleagues from our Institute we participated in the characterization of mesenchymal stem cells (MSCs) in human models. In more detail, we characterized mouse bone marrow-derived MSCs and used them for ocular surface regeneration. Using a model of alkali-injured ocular surface we demonstrated that systemically administered MSCs selectively migrate to the site of injury and inhibit the inflitration of proinflammatory cells and attenuate the production of cytokines. The migratory and immunotherapy properties of MSCs can be potentiated by their preincubation with interferon-gama. The result shows the possibility to use systemically administered MSCs for the suppression of local inflammatory reaction. One publication was done exclusively by members of our Department, two other publications were prepared by other members of the IEM, with a significant methodological contribution made by members of our Department.

Koci Z., Turnovcova K., Dubsky M., Baranovicova L., Holan V., Chudickova M., Sykova E., Kubinova S.: Characterization of human adipose tissue-derived stromal cells isolated from diabetic patients´s distal limbs with critical limb ischemia. Cell. Biochem. Funct. 32, 597-604, 2014.

Zablotskii V., Lunov O., Novotná B., Churpita O., Trošan P., Holáň V., Syková E., Dejneka A., Kubinová S.: Down-regulation of adipogenesis of mesenchymal stem cells by oscillating high-gradient magnetic fields and mechanical vibration. Appl. Phys. Lett. 105, 103702, 2014.

Javorková E., Trošan P., Zajícová A., Krulová M., Hájková M., Holáň V.: Modulation of the early inflammatory microenvironment in alkali-burned eye by systemically administered interferon-γ treated mesenchymal stem cells. Stem Cells Dev. 23, 2490-2500, 2014.

Department of Tissue Engineering

Department has achieved significant progress concerning the preparation, recognition and functional modification of functionalized nanofibers. They have developed "smart functionalized nanofibers" for controlled and targeted drug delivery. The aim of the study was devoted to cell therapy and regenerative medicine, studying the effects of smart nanofibers both on mesenchymal stem cells and on differentiated cells (namely chondrocytes, osteoblasts, fibroblasts and keratinocytes). However, the main focus concerned the preparation of intelligent composite scaffolds for cell-free therapy. The latter approach seems to be very perspective not only from the point of view of basic research, but is also highly promising for clinical application. The cell-free approach is characterized by an ideal ratio between the therapeutic effect and price and, in addition, fewer obstacles prevent its application in humans. The authors concentrated on the development

of cell-free systems for controlled cell migration, followed by their proliferation and, subsequently, their differentiation. A high level of knowledge has been reached during the last five year period, making it possible to negotiate the practical application of the systems in clinical trials. Results connected with these topics have been published. The major part of the work and manuscript preparation were done by the members of Department of Tissue Engineering.

Buzgo M, Jakubova R, Mickova A, Rampichova M, Prosecka E, Kochova P, Lukas D, Amler E: Time-regulated drug delivery system based on coaxially incorporated platelet alpha granules for biomedical use. Nanomedicine-UK 2013 Jul; 8(7):1137-1154.

Mickova A, et al. Electrospun core/shell nanofibers: a promising system for cartilage and tissue engineering? Nanomedicine-UK (Lond). 2013 Apr;8(4):509-12.

The preparation of a biodegradable functionalized scaffold on a collagen foam basis with a microsystem prepared from functionalized PCL(poly-ε-caprolactone) nanofibers is undoubtedly among the most significant achievements of this team. The system has been proven to be suitable for mini-invasive application in the treatment of small and large osteochondral lesions. The team of the Department of Tissue Engineering played the major role in preparing and evaluating the data, and in writing the manuscript.

Amler, E., Filová, E., Buzgo, M., Prosecká, E., Rampichová, M., Nečas, A., Nooeaid, P., Boccaccini, A. R.: Functionalized nanofibers as drug-delivery systems for osteochondral regeneration. Nanomedicine-UK 2014 9(7): 1083-1094.

Functionalized nanofibers, can be successfully implemented into currently well-known systems to upgrade them onto a significantly higher level. A clear example is the application of functionalized PCL nanofibers upon the currently used polypropylene mesh. Synthetic chirurgical meshes have already become a current standard in the treatment of incisial hernia. However, application of such a net has not surprisingly decreased the risk of recurrence, but simply prolongs the time till re-operation. We have tested our newly developed systems and have proven that functionalized nanofibers served as an ideal drug delivery system as well as biomechanically active compound to facilitate fibroblast migration and proliferation. Synthetic mesh functionalized with PCL (Polycaprolactone) nanofibers and thrombocyte derivatives has been found to be a suitable means for treating and regenerating ventral hernias. The functionalized smart mesh has been found to be superior for cell adhesion (namely fibroblasts) and for triggering cell proliferation. Sustainable release of native growth factors has contributed significantly to the acceleration of tissue regeneration and to the appearance of a firm connection between the polypropylene mesh with tissue, namely at the critical points (edges) which seem to be the most common reasons for reoperation. Notably, polypropylene mesh has maintained all the advantages of the original polypropylene mesh. Additionally, the composite system has also kept all the positive properties of the functionalized nanofibers. In conclusion, application of this system has led to the facilitation and acceleration of hernia healing and to the development of a novel tissue with biomechanical parameters that is even fully comparable to healthy native fascia. This is a collaborative study in which the members of Department of Tissue Engineering contributed the major part of the work, evaluated the results and prepared the manuscript.

Plencner, M., East, B., Tonar, Z., Otáhal, M., Prosecká, E., Rampichová, M., Krejčí, T., Litvinec, A., Buzgo, M., Míčková, A., Nečas, A., Hoch, J., Amler, E.: (2014) Abdominal closure reinforcement by using polypropylene mesh functionalized with poly-ε-caprolactone nanofibers and growth factors for prevention of incisional hernia formation. *Int. J. Nanomed.* 9: 3263-3277

The preparation and testing of 3D scaffolds based on functionalized nanofibers is among one of the other important achievements of our group. We have identified an important problem that has hindered the broader application of nanofibers in tissue engineering – two dimensional system of nanofiber which could be optimal for *in vitro* tests, but which is far from optimal for *in vivo* application.. This three-dimensional system has been applied both *in vitro* to test adhesion and proliferation of mesenchymal stem cells and osteoblasts, but also *in vivo* for the regeneration of large bone defects. It is necessary to emphasize that force spinning as a newly emerging technology has been applied for the preparation of three dimensional nanofiber scaffolds. There are several advantages of this novel technique in comparison with classical electrospinning: there is no negative effect of a highly intensive electric field on the biological material and, moreover, polymers, that cannot produce nanofibers by classical electrospinning, can create the nanofiber structure. The majority of the work and manuscript preparation were done by the members of Department of Tissue Engineering.

Rampichová, M., Buzgo, M., Chvojka, J., Prosecká, E., Kofroňová, O., Amler, E.: (2014) Cell penetration to nanofibrous scaffolds: Forcespinning®, an alternative approach for fabricating 3D nanofibers. *Celll Adhes. Migr*. 8(1): 36-41.

Department of Pharmacology

As collaborators of the Centre for New Antivirals and Antineoplastics we have analysed the immunobiological properties of the widely recognised antivirals, acyclic nucleoside phosphonates, invented by Prof. A. Holý (Institute of Organic Chemistry and Biochemistry, Prague). The oral prodrugs of the prototype compounds, e.g., 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; *adefovir*), and 9-(*R*)-[2-(phosphonomethoxy)propyl]adenine [(*R*)-PMPA; *tenofovir*] have been approved by the FDA for the treatment of hepatitis B (Hepsera), and acquired immunodeficiency syndrome (AIDS) (Viread), respectively. Our results show that a number of acyclic nucleoside phosphonates possess immunostimulatory activity. The major finding is that tenofovir and some diaminopurines stimulate the production of chemokines RANTES and MIP-1a. These factors bind to specific receptors that are required for the penetration of HIV into cells. The enhanced production of chemokines can thus substantially suppress the infectivity of HIV. We have demonstrated that the activation of cytokine and chemokine secretion is mediated by the adenosine A_1 receptor. We have shown that acyclic nucleoside phosphonates represent a new generation of antivirals with combined anti-metabolic and immunomodulatory modes of action. The members of the Department of Pharmacology were responsibly for testing and evaluating the biological activity of new substances and contributed crucial aspects to the experiment and prepared the manuscripts.

Jansa, P., Baszczyňski, O., Dračínský, M., Votruba, I., Zídek, Z., Bahador, G., Stepan, G., Cihlar, T., Mackman, R., Holý, A., Janeba Z.: A novel and efficient one-pot synthesis of symmetrical diamide (bis-amidate) prodrugs of acyclic nucleoside phosphonates and evaluation of their biological activities. Eur. J. Med. Chemistry, 46: 3748-3754, 2011

Kostecká, P., Holý, A., Farghali, H., Zídek, Z., Kmoníčková, E.: Differential effects of acyclic nucleoside phosphonates on nitric oxide and cytokines in rat hepatocytes and macrophages. International Immunopharmacology, 12: 342-349, 2012.

Nekvindova, J., Contreras, J. A., Juvan, P., Fon, T. K., Anzenbacher, P., Zidek, Z., Kopecna Zapletalova, M., Rozman, D., Anzenbacherova, E.: Acyclic nucleoside phosphonates: a study on cytochrome P450 gene expression. Xenobiotica 44: 708-715, 2014.

We also participated in the grant project Interference of Probiotics with Factors Determining the Pharmacokinetics of Drugs. The results extended and brought about new data on the biological activities of probiotics from groups of both Gram-negative and Gram-positive bacteria. In addition to living and dead bacteria, corresponding lysates were also found to be strong activators of the production of cytokines, nitric oxide and prostaglandin PGE2. Lc lysate counteracts the LPS-triggered polarization of macrophages to the M1 (F4/80+CD206+IL-7R-) phenotype. The project launched the hitherto neglected question concerning the possible interactions of probiotics with enzymatic biotransformation and transmembrane drug transport. The in vitro experiments suggested that EcN has no significant impact on passive and active drug transport. The expression and activities of major enzymes of liver and gut metabolism of xenobiotics (cytochromes P450) were not substantially influenced in experimental animals treated orally with EcN or Lc. The presence of probiotics in the gut can however modulate drug pharmacokinetics. Enhanced gut biotransformation of 5-acetylsalicylic acid after EcN, and an approximate two-fold increase in bioavailability after Lc treatment of animals were observed. The results warrant further preclinical and clinical studies. We have analyzed and revealed novel molecular mechanisms of immunostimulatory properties of probiotic preparations. This part of the research is a collaborative effort in which members of the Department of Pharmacology performed a significant aspect of the research, contributed to the evaluation of the results and prepared the manuscripts.

Zídek, Z., Farghali, H., Kmoníčková, E.: Intrinsic nitric oxide-stimulatory activity of lipoteichoic acids from different Gram-positive bacteria. Nitric Oxide, 23: 300-310, 2010.

Zídek, Z., Kmoníčková, E., Kostecká, P., Tlaskalová-Hogenová, H.: Decisive role of lipopolysaccharide in activating the nitric oxide and cytokine production by the probiotic *Escherichia coli* strain Nissle 1917. Folia Microbiol. 55: 181-189, 2010.

Matušková, Z., Tunková, A., Anzenbacherová, E., Večeřa, R., Šiller, M., Tlaskalová-Hogenová, H., Zídek,Z., Anzenbacher, P.: Effects of probiotic *Escherichia coli* Nissle 1917 on expression of cytochromes P450 along the gastrointestinal tract of male rats. Neuroendocrinol. Lett. 31 (Suppl. 2): 46-50, 2010.

Other results from studies conducted within the lab have revealed new biological features of sarco-endoplasmic calcium ATP-ase (SERCA) inhibitors, *i.e*, their immunostimmulatory potential. Thapsigargin, a representative of a widely used experimental group of compounds, induced production of nitric oxide (NO), as well as the secretion of cytokines interferon-γ (IFN-γ) and interleukin-2 (IL-2). Similar activities were observed with other SERCA inhibitors, such as cyclopiazonic acid (CPA) and butyl-benzohydroquinone (BHQ). We have proved that the immunomodulatory effects of these SERCA inhibitors do not depend on the intracellular concentration of calcium. Non-specific inhibitors of calcium pumps (e.g. curcumin, artemisin, clotrimazole) did not show such an immunomodulatory profile. Chemical analysis of plant extracts revealed a new, hitherto unknown structural analogue of thapsigargin, trilobolide,that also possesses immunostimulatory activity. In order to study the structure-activity relationship, we designed and realized semi-synthetic modifications of trilobolide. The relatively low toxicity and remarkable immunostimulatory potential of these compounds warrant further preclinical studies. The members of Department of Pharmacology were responsible for the evaluation of the biological activity of the new substances and contributed crucial aspects to the experiment and prepared the manuscripts.

Kmoníčková, E., Harmatha, J., Vokáč, K., Kostecká, P. Farghali, H., Zídek, Z.: Sesquiterpene lactone trilobolide activates production of interferon-γ and nitric oxide. Fitoterapia, 81:1213-1219, 2010.
Černý, D., Lekić, N., Váňová, K., Muchová, L., Hořínek, A., Kmoníčková, E., Zídek, Z., Kameníková, L., Farghali, H.: Hepatoprotective effect of curcumin in lipopolysaccharide/D-galactosamine model of liver injury in rats: Relationship to HO-1/CO antioxidant system. Fitoterapia, 82: 786-791, 2011.
Harmatha, J., Budešínský, M., Vokáč, K., Kostecká, P., Kmoníčková E., Zídek Z.: Trilobolide and related sesquiterpene lactones from *Laser trilobum* possessing immunobiological properties. Fitoterapia, 89C: 157-166, 2013.

During the bilateral project between our Institute and the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, China, under the title Research and Development of New Immunomodulatory and Anti-Inflammation Agents from Traditional Chinese Medicine, we analyzed a number of extracts and identified compounds isolated from twelve herbs widely used in Traditional Chinese Medicine. They were investigated for their biologically and pharmacologically interesting properties. With only a few exceptions, the preparations proved to possess immunoinhibitory effects. The most interesting were the results obtained from compounds isolated from *Artemisia annua,* a herb used for the treatment of malaria. We found that sesquiviterpens artemisinin, dihydroartemisinin, artemisinic acid, arteannuin B, and flavonoids casticin and chrysosplenol D are potent inhibitors of angiogenesis. We suggested that this may be considered as one of mechanisms underlying the antitumour effects of *A. annua*. Phenolic compounds 4-hydroxybenzaldehyde and 3,4-hydroxybenzaldehyde isolated from the fern Osmunda japonica, used for the treatment of infections, proved to have immunostimulatory activity. All experiments were run in the IEM. We have used our original screening know-how which enables the assessment of both immunostimulatory and immunoinhibitory activity of any kind of compounds. The biological material from the Chinese partner was used in these studies, but all experimental work, evaluation of results and preparation of the manuscripts were carried out by the members of Department of Pharmacology.
Zhu, X., Yang, L., Li, Y., Zhang, D., Chen, Y., Kostecká, P., Kmoníčková, E., Zídek, Z.: Effects of sesquiterpene, flavonoid and coumarin types of compounds from *Artemisia annua* L. on production of mediators of angiogenesis. Pharmacol.l Rep., 65 (2): 410-420, 2013.
Zhu, X.-X., Li, Y.-J., Yang, L., Zhang, D., Chen, Y., Kmoníčková, E., Weng, X.-G., Yang, Q., Zídek, Z.: Divergent Immunomodulatory effects of extracts and phenolic compounds from the fern *Osmunda japonica* Thunb. Chin. J. Integr. Med. 19 (10): 761-770, 2013.
Zhang, D., Li, S.-B., Yang, L., Li, Y.-J., Zhu, X.-X., Kmoníčková, E., Zídek, Z., Fu, M.-H., Fang, J.: Two new C-methyl flavanones from the rhizomes and frond bases of *Matteuccia struthiopteris*. J. Asian Nat. Prod. Res. 15 (11):1163-1167, 2013.

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Auditory Neuroscience

The main research aims of the Department of Auditory Neuroscience AS CR are oriented towards investigations of the structure and function of the auditory system in animals and humans under normal and pathological conditions and during ontogeny and ageing.

Laboratory of Auditory Physiology and Pathology

Neuronal processing of simple tones and complex sounds

The functional organization of the individual fields of the auditory cortex (AC) in young rats on the basis of neuronal responses to acoustic stimuli was described. The AC is divided into the primary auditory cortex (AI) and three other core fields. The core fields are surrounded by a belt area. Neurons in the core fields have shown well defined characteristic frequencies (CF) in response to pure tone stimulation, neurons responding only to broad band noise (BBN) stimulation were found mostly in the belt area. Neurons with the shortest response latencies to BBN stimulation were found in layer 4 (L4) and layer 6 (L6) in the AI, while those with the longest latencies were found in the superficial layers (L1/2) of the belt area. The most dominant type of responses in all fields, as well as in all layers, was the pure onset response. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.

Profant O, Burianová J, Syka J (2013) The response properties of neurons in different fields of the auditory cortex in the rat. Hear Res, 296C: 51-59.

In cooperation with the Faculty of Mathematics and Physics, Charles University in Prague, a new statistical method was proposed for the estimation of neuronal response latency in the presence of spontaneous activity. A nonparametric estimator of the response latency based on repeated stimulations was developed by members of the Faculty of Mathematics and Physics, testing of the method using real data from inferior colliculus neurons and manuscript preparation were performed by J.Popelář from the Department of Auditory Neuroscience IEM AS CR.

Pawlas Z, Klebanov LB, Beneš V, Prokešová M, Popelář J, Lánský P (2010) First-spike latency in presence of spontaneous activity. Neural Comp. 22, 1675–1697.

The mechanisms of sound source localization based on interaural level differences in the lateral superior olive were modelled. Z.Bureš from the Department of Auditory Neuroscience IEM AS CR contributed by 50% to this work.

Bureš Z, Maršálek P (2013) On the precision of neural computation with interaural level differences in the lateral superior olive. Brain Res. 1536, 16-26.

We created a versatile, ready-to-use, and freely available software package in MATLAB to process data from in vivo two-photon calcium imaging. The software package is called a Two-Photon Processor. TPP includes routines to search for cell bodies in full-frame (Search for Neural Cells Accelerated; SeNeCA) and line-scan acquisition, routines for calcium signal calculations, filtering, spike-mining, and routines to construct parametric fields. Searching for somata in artificial in vivo data, our algorithm achieved better performance than human annotators. SeNeCA copes well with uneven background brightness and in-plane motion artifacts, the major problems that occur in simple segmentation methods. To the best of our knowledge, our segmentation algorithm is also the fastest one (e.g. artificial *in vivo* images with a resolution of 256 x 256 pixels containing ~100 neurons can be processed at a rate up to 175 frames per second). All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.

Tomek, J, Novák, O, Syka, J (2013) Two-Photon Processor and SeNeCA - A freely available software package to process data from two-photon calcium imaging at speeds down to several ms per frame. J Neurophysiol. 110(1): 243-256.

The representation of four typical guinea pig vocalizations in the auditory cortex (AI) of anesthetized guinea pigs was investigated with the aim to compare cortical data to the data already published for identical calls in subcortical structures – the inferior colliculus (IC) and medial geniculate body (MGB). Like the subcortical neurons, cortical neurons also typically responded to many calls with a time-locked response to one or more temporal elements of the calls. A comparison between the activity in the AI and those of subcortical structures showed a different transformation of the neuronal response patterns from the IC to the AI for individual calls: i) while the temporal representation of chirp remained unchanged, the representations of whistle and chutter were transformed at the thalamic level and the response to purr at the cortical level; ii) for the wideband calls (whistle, chirp) the rate representation of the call spectra was preserved in the AI and MGB at the level present in the IC, while in the case of low-frequency calls (chutter, purr), the representation was less precise in the AI and MGB than in the IC; iii) the difference in the response strength to natural and time-reversed whistle was found to be smaller in the AI than in the IC or MGB. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.

Šuta D, Popelář J, Burianová J, Syka J. (2013) Cortical representation of species-specific vocalizations in Guinea pig. PLoS One., 8(6):e65432. doi: 10.1371/journal.pone.0065432.

Lateralization of auditory functions in the rat auditory cortex

The left auditory cortex (AC) in humans is involved in the processing of temporal parameters of acoustical signals, specifically in speech perception, whereas the right AC plays the dominant role in pitch and melody perception. The lateralization of brain functions to individual hemispheres of the brain in experimental animals remains unresolved. In the previous study, using an avoidance conditioning procedure and lesioning of the AC, we demonstrated the dominance of the right AC in the discrimination of the direction of frequency modulation. In the present study the ability of rats to detect or discriminate a series of gaps in continuous noise under conditions of unilateral or bilateral reversible inactivation of the AC was examined (Rybalko et al., 2010). The results showed that muscimol-induced reversible inactivation of the left AC suppresses the ability of rats to discriminate between acoustical stimuli of different temporal parameters (duration or repetition rate), whereas inactivation of the right AC results in no change or only a mild decrease

in discrimination ability. The results demonstrated that the direction of frequency modulation discrimination (Spectral discrimination) was more deteriorated after right auditory cortex (AC) lesion, whereas discrimination of gap repetition rate in noise (Temporal discrimination) was significantly worsened following left AC inactivation. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.
Rybalko N, Šuta D, Popelář J, Syka J (2010) Inactivation of the left auditory cortex impairs temporal discrimination in the rat. Behav Brain Res., 209(1):123-30

Effects of early neonatal sound exposure

We demonstrated in a previous study that short noise exposure during the developmental period in rats (125 dB SPL BBN, 8 min, 14th postnatal day) produced significant changes in frequency selectivity of neurons recorded in the inferior colliculus (IC) in adult rats. In a later study, aside from the poorer frequency selectivity of IC neurons in the noise-exposed animals, the neuronal representation of sound intensity was significantly affected. Although the response thresholds were similar in the exposed and control rats, the IC neurons in the exposed animals had a longer first-spike latency, a narrower dynamic range, lower maximum response magnitudes and a steeper slope of the rate–intensity functions. The percentage of monotonic neurons was significantly lower in the exposed animals. The observed anomalies were confined to the mid- and high-frequency regions, whereas no significant changes were found in the low-frequency neurons. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.
Bureš Z, Grécová J, Popelář J, Syka J (2010) Noise exposure during early development impairs the processing of sound intensity in adult rats. Eur J Neurosci 32, 155–164,

The changes in the hearing function of noise-exposed rats were reflected also in morphological alterations in central auditory structures. Using the Golgi–Cox method we have found that the mean total length of the neuronal tree was larger in the IC and in the ventral division of the medial geniculate body (MGB) in 3-month-old rats exposed to noise as juveniles in comparison with non-exposed control rats. These findings demonstrate that early postnatal short noise exposure can induce permanent changes in the development of neurons in the central auditory system, which apparently represent morphological correlates of functional plasticity. The team of the Department of Auditory Neuroscience IEM AS CR designed the study and performed most of morphological analysis (60%). H.P.Lu performed part of the morphological analysis during her stay in the IEM AS CR (40%). Evaluation and interpretation of data as well as writing of the manuscript were done exclusively by the team from the Department of Auditory Neuroscience IEM AS CR.
Ouda L, Burianová J, Balogová Z, Lu HP, Syka J (2014) Structural changes in the adult rat auditory system induced by brief postnatal noise exposure. Brain Structure and Function, in press

Brief acoustic trauma during the critical period of development in rats modified the development of their auditory function. By monitoring the acoustic startle response (ASR), the transient hyper-reactivity to startle stimuli, manifested by a decrease of ASR thresholds and an increase of ASR amplitudes were observed. ASR enhancement was more pronounced at high frequencies and lasted for two weeks from the noise exposure. The histological control did not reveal loss of hair cells in adult exposed rats, however, the number of inner hair cell ribbon synapses was significantly decreased, especially in the high-frequency part of the cochlea. Brief

noise exposure during the critical period of postnatal development in rats resulted not only in altered development of ASR in young animals, but also in the anomalous processing of acoustic stimuli in the auditory system of adult rats. Although the hearing thresholds in control and noise-exposed rats were not different, a reduced strength of the ASR and different efficacy of prepulse inhibition (PPI) of ASR in exposed and control rats was observed. Our findings demonstrate that brief noise exposure in rat pups results in altered behavioral responses to sounds in adulthood, indicating anomalies in intensity coding and loudness perception. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.

Rybalko N, Bureš Z, Burianová J, Popelář J, Grécová J, Syka J (2011) Noise exposure during early development influences the acoustic startle reflex in adult rats. Physiol Behav. 102: 453-458.

In contrast to damaging loud noise exposure the structure and function of the auditory system may be positively influenced by using moderate acoustic stimulation, especially during the early postnatal developmental period. The effects of an acoustically enriched environment applied during the third and fourth week of life on the responsiveness of IC neurons in rats was demonstrated. Enrichment, comprising of a spectrally and temporally modulated complex sound reinforced with several target acoustic stimuli, one of which triggered a reward release, resulted in lower excitatory response thresholds, an increased frequency selectivity, larger response magnitudes, steeper rate–intensity functions and an increased spontaneous activity in adult rats in comparison with age-matched control rats. These findings indicate that a rich and stimulating acoustic environment during early development may permanently affect signal processing in the subcortical auditory nuclei, including the excitatory thresholds of neurons and their frequency and intensity resolution. All the work and manuscript preparation was exclusively done by members of the Department of Auditory Neuroscience IEM AS CR.

Bureš Z, Bartošová J, Lindovský J, Chumak T, Popelář J, Syka J (2014) Acoustical enrichment during early postnatal development changes response properties of inferior colliculus neurons in rats. Eur J Neurosci, Vol. 40, pp. 3674–3683.

Age-related changes of hearing

The most prominent area of our research is the study of changes in the auditory system during aging. We used an experimental approach that contrasts age-related changes of hearing function in a naturally aging strain of rats, Long Evans, and a strain with accelerated aging, Fischer 344. This approach revealed profound changes in the inner ear (collagen depletion, stria vascularis damage), specifically in Fischer 344 rats, while control Long Evans rats remained relatively intact. Importantly, both strains exhibited comparable age-related changes in the central part of auditory system in glutamate-decarboxylase, calretinin and SMI-32 in terms of protein levels and a number of immunohistochemically labeled neurons. This is an important finding since it speaks in favor of the hypothesis that the age-related neurochemical changes in the central auditory structures occur, at least partly, independent of the state of the periphery. All the work and manuscripts preparations were done exclusively by members of the Department of Auditory Neuroscience IEM AS CR.

Syka J (2010) The Fischer 344 rat as a model of presbycusis. Hear Res. 264(1-2):70-8. Epub 2009 Nov 10.

Ouda L, Burianova J, Syka J (2012). Age-related changes in calbindin and calretinin immunoreactivity in the central auditory system of the rat. Exp Gerontol. 47: 497-506.

On the behavioral level, in comparison with Long Evans rats, F344 rats showed larger age-related hearing threshold shifts, lower amplitudes of acoustical startle responses and less efficient prepulse inhibition. In a detailed analysis of the behavioral data we found no correlation between threshold shifts and prepulse inhibition efficacy that lent further some support to the idea that central age-related changes develop independently of the periphery. P.W.F.Poon contributed to the design of the experiment by suggesting some modifications of the behavioral method. Evaluation and interpretation of data as well as writing of the manuscript were done exclusively by the team from the Department of Auditory Neuroscience IEM AS CR.

Rybalko N, Bureš Z, Burianová J, Popelář J, Poon PWF, Syka J (2012) Age-related changes in the acoustic startle reflex in Fischer 344 and Long Evans rats. Exp Gerontol 47: 966-973.
Šuta D., Rybalko N., Pelánová J., Popelář J., Syka J. (2011) Age-related changes in auditory temporal processing in the rat. Exp. Gerontol. 46 739–746.

The use of nanoparticles for transporting active substances to the cochlea (neurotrophic factors, plasmids) for prevention and treatment of hearing disorders was tested in rats. The team of the Department of Auditory Neuroscience IEM AS CR designed the experiment and performed all physiological testing and morphological analysis. The team also played a major role in the evaluation and interpretation of data as well as in writing the manuscripts. Nanoparticles were prepared and supplied from Finland (S.Ranjan, R.Soon, P.K.J.Kinnunen) and Great Britain (T.A.Newman, A.H.Johnston) by our partners in the project NanoEar of the 6th FP.

Buckiová D, Ranjan S, Newman TA, Johnston AH, Sood R, Kinnunen PKJ, Popelář J, Chumak T, Syka J (2012) Minimally invasive drug delivery to the cochlea through application of nanoparticles to the round window membrane. Nanomedicine, 7 (9), 1339-1354.

In human studies, hearing thresholds over the extended frequency range 0.125-16 kHz were measured in a large sample of men and women aged 16-70 years using pure-tone audiometry to enable their normalization. All the work and manuscript preparation was done exclusively by members of the Department of Auditory Neuroscience IEM AS CR.

Jilek M, Suta D, Syka J (2014) Reference hearing thresholds in an extended frequency range as a function of age. J Acoust Soc Am. 136 (4),

Significant atrophy in the auditory cortex of elderly subjects with different degrees of presbycusis and metabolic changes in the auditory centers were revealed using magnetic resonance (MR) morphometry, MR spectroscopy and diffusion tensor imaging. MR spectroscopy showed a decrease of the main excitatory neurotransmitter glutamate, N-acetyl-aspartate and an increase of lactate in an elderly population without a significant effect on the degree of hearing loss. Morphometric measurements showed atrophy of gray matter in the auditory cortex of aged population expressed by changes in the thickness, surface and overall volume of the involved gyri. Atrophy was present to a lesser extent also in the white matter of the auditory pathway. Stimulation with pink noise showed changes in the age related lateralization recorded by fMRI. Young adults dominantly activated left AC, whereas in the elderly activation by sound shifted towards the right AC. Most results from human studies revealed only low effects of the hypofunctional inner ear stimulation on the auditory cortex. MRI measurement and data analysis were performed in the MRI clinic in the IKEM, audiometric measurements and manuscripts preparation were performed in the IEM AS CR

Profant O, Balogová Z, Dezortová M, Wagnerová D, Hájek M, Syka J. (2013) Metabolic changes in the auditory cortex in presbycusis demonstrated by MR spectroscopy. Exp Gerontol. 2013 Aug; 48(8):795-800.
Profant O, Škoch A, Balogová Z, Tintěra J, Hlinka J, Syka J (2014) Diffusion tensor imaging and MR morphometry of the central auditory pathway and auditory cortex in aging. Neuroscience. Feb 28; 260:87-97.

Laboratory of Synaptic Physiology

Two lines of research were carried in the lab during the period 2010 - 2014. The first one was aimed at molecular mechanisms of modulation of inhibitory G-protein coupled receptors (GPCRs) by specifically interacting proteins. We studied GABAB and melatonin receptors. GABAB receptors are activated by GABA, the main inhibitory neurotransmitter in the brain. We have shown that native GABAB receptors are high-molecular-mass complexes consisting of a GABAB1 subunit, GABAB2 subunit and members of a subfamily of the KCTD proteins (named after their K+ channel tetramerization-domain). KCTD proteins associate tightly with the carboxy terminus of GABAB2 as auxiliary subunits of GABAB receptors and determine the number of the receptors at the cell surface as well as the pharmacology and kinetics of the receptor response. We studied molecular mechanisms of KCTD12-induced desensitization of GABAB receptor activated K+ currents. We have found that the desensitization results from a dual interaction of KCTD12 with the G protein: constitutive binding stabilizes the heterotrimeric G protein at the receptor, whereas dynamic binding to the receptor-activated Gβγ subunits induces desensitization by uncoupling Gβγ from the effector K+ channel. We have further demonstrated that the KCTD12-induced desensitization is under physiological conditions regulated by a constitutive phosphorylation of serine-892 in GABAB2 subunit. Serine-892 phosphorylation by protein kinase-A (PKA) rearranges KCTD12 at the receptor and slows the KCTD12-induced desensitization. As GABAB receptor inhibits PKA activity, this cross regulation of serine-892 phosphorylation and KCTD12 activity sharpens the response during repeated receptor activation. In addition, GABAB receptor activity was shown to be regulated by ionotropic glutamate receptors (iGluRs). Activation of NMDA subtype of iGluRs promotes dynamin-dependent endocytosis of GABAB receptors by Ca2+/Calmodulin-dependent protein kinase II (CaMKII) mechanism. CaMKII associates with GABAB receptors in vivo and phosphorylates serine 867 (S867) in the intracellular carboxy terminus of the GABAB1 subunit. Overall, these results indicated that GABAB receptors are endowed with both fast and slow mechanisms of homologous and heterologous desensitization which prevents excessive receptor influences on neuronal activity. Finally, we characterized the molecular complex of the melatonin MT(1) receptor, which couples with the regulator of G-protein signaling 20 (RGS20). We proposed a model wherein one G-protein and one RGS20 bind to separate protomers of MT(1) dimers in a pre-associated complex that rearranges upon agonist activation. Our data extend the concept of asymmetry within GPCR dimers, reinforce the notion of receptor specificity for RGS proteins and highlight the advantage of GPCRs organized as dimers in which each protomer fulfils its specific task by binding to different GPCR-interacting proteins. These experiments were done in tight collaboration with the laboratories of Prof. Bettler (Department of Biomedicine, University of Basel, Switzerland), Prof. Fakler (Institute of Physiology, University of Freiburg, Germany) and Prof. Jockers (Institut Cochin, Universite Paris Descartes, France). We were responsible for the electrophysiology and immunohistochemistry parts of these projects.

*Schwenk J, *Metz M, *Zolles G, *Turecek R, Fritzius T, Bildl W, Tarusawa E, Kulik A, Unger A, Ivankova K, Seddik R, Tiao JY, Rajalu M, Trojanova J, Rohde V, Gassmann M, Schulte U, Fakler B and Bettler B (2010) Native GABAB receptors are heteromultimers with a family of auxiliary subunits. Nature 465, 231-235. *Contributed equally to this work.
Guetg N, Abdel Aziz S, Holbro N, Turecek R, Rose T, Seddik R, Gassmann M, Moes S, Jenoe P, Oertner TG, Casanova E and Bettler B (2010) NMDA Receptor-Dependent GABAB Receptor Internalization via CaMKII Phosphorylation of Serine 867 in GABAB1. Proc. Natl. Acad. Sci. USA 107, 13924-13929.
Maurice P, Daulat AM, Turecek R, Ivankova-Susankova K, Zamponi F, Kamal M, Guillaume J-L, Bettler B, Galès C, Delagrange P and Jockers R (2010) Molecular organization and dynamics of the melatonin MT1 receptor/RGS20/Gi protein complex reveal asymetry of receptor dimers for RGS and Gi coupling. EMBO J, 29, 3646-3659.
Aburi M, Rives ML, Han Y, Kralikova M, Urizar E, Yano H, Javitch JA (2011) Crosstalk between receptors: challenges of distinguishing upstream from downstream mechanisms. In: RSC Drug Discovery Series No. 8, G Protein-Coupled Receptors: From Structure to Function. Edited by Jesus Giraldo and Jean-Philippe Pin. Published by the Royal Society of Chemistry. Chapter 12, 255 - 268.
Ivankova K, Turecek R, Fritzius T, Seddik R, Prezeau L, Comps-Agrar L, Pin J-P, Fakler B, Besseyrias V, Gassmann M and Bettler B (2013) Up-regulation of GABAB Receptor Signaling by Constitutive Assembly with the K+ Channel Tetramerization Domain-containing Protein 12 (KCTD12). J. Biol. Chem., 288, 24848-24856.
Turecek R, Schwenk J, Fritzius T, Ivankova K, Zolles G, Adelfinger L, Jacquier V, Besseyrias V, Gassmann M, Schulte U, Fakler B and Bettler B (2014) Auxiliary GABAB receptor-subunits uncouple G-protein βγ-subunits from effector channels to induce desensitization. Neuron, 82, 1032-1044.
*Adelfinger L, *Turecek R, Ivankova K, Jensen AA, Moss SJ, Gassmann M and Bettler B (2014) GABAB receptor phosphorylation regulates KCTD12-induced K+ current desensitization. Biochemical Pharmacology 91, 369-379. *Contributed equally to this work.

The second line of experimental work conducted in the lab has been focused on the role of GABAergic and glycinergic inhibition in the mammalian auditory system. Specifically, we studied properties of presynaptic and postsynaptic glycine and GABAB receptors in the medial nucleus of trapezoid body (MNTB), one of the brainstem auditory nuclei. We demonstrated two functionally different glycine receptor populations in the rat MNTB. Postsynaptic receptors formed α1/β-containing clusters on somatodendritic domains of MNTB principal neurons, colocalizing with glycinergic nerve endings to mediate fast, phasic postsynaptic inhibition. In contrast, presynaptic receptors that trigger a slow facilitation of glutamate release in the calyx of Held nerve terminals were composed of dispersed, homomeric α1 receptors. The results suggested that specific targeting of glycine receptor β subunit produces segregation of glycine receptor subtypes involved in two different mechanisms that modulate synaptic strength. Next, we examined the precise distribution of glycine receptors in the rat glutamatergic calyx of Held nerve terminal using high-resolution pre-embedding immunoelectron microscopy. We found that presynaptic glycine receptors co-localized with potential sources of their endogenous agonists. Interestingly, one of the sources appeared to reside in postsynaptic principal neurons (PNs). We further investigated this possibility using electrophysiology techniques and found that glycine could actually be released from somatodendritic parts of PNs by a reverse action of glycine transporter 2 (GlyT2), triggered by

bursts of postsynaptic action potentials. This indicated a novel mechanism of retrograde modulation of excitatory synaptic transmission at central synapses. Overall, the results supported the activation of presynaptic glycine receptors by glycine spillover and also the existence of an activity-dependent mechanism regulating the surface distribution of α homomeric GlyRs in axonal terminals of central neurons. Finally, we investigated the role of presynaptic GABAB receptors in the MNTB in vivo. Our data showed that pharmacological activation of the receptors can reduce sound-evoked synaptic transmission in the MNTB, although the ambient GABA concentration in the auditory brainstem is too low to activate the receptors significantly. These experiments were done in collaboration with the laboratories of Prof. Borst (Department of Neuroscience, University Medical Center Rotterdam, the Netherlands) and Prof. Kulik (Institute of Physiology, University of Freiburg, Germany). We were responsible for the electrophysiology and immunohistochemistry parts of these projects.

Hruskova B, Trojanova J, Kulik A, Kralikova M, Pysanenko K, Bures Z, Syka J, Trussell LO and Turecek R (2012) Differential distribution of glycine receptor subtypes at the rat calyx of Held synapse. J. Neurosci., 32, 17012-17024.

Wang T, Rusu SI, Hruskova B, Turecek R and Borst JGG (2013) Modulation of synaptic depression of the calyx of Held synapse by GABAB receptors and spontaneous activity. J. Physiol., 591, 4877-4894.

Kralikova, M.; Turecek, R. (2013) Modulation of excitatory synaptic transmission by the somatodendritic release of glycine in the rat medial nucleus of the trapezoid body. Program No. 230.14. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013.

Trojanova J, Kulik A, Janacek J, Kralikova M, Syka J and Turecek R (2014) Distribution of glycine receptors on the surface of the mature calyx of Held nerve terminal. Front. Neural Circuits 8:120. doi: 10.3389/fncir.2014.00120.

Research Report of the team in the period 2010–2014

Institute	Institute of Experimental Medicine of the CAS, v. v. i.

Scientific team	Genetic Ecotoxicology

The research of The Department of Genetic Ecotoxicology is related to the study of the impact of air pollution on human health using new methods of molecular epidemiology. Model regions for this tasks were selected in Prague, Moravian Silesian Region and Ceske Budejovice (Southern Bohemia, control region). Other research activities focus on studying the effects of air pollutants on model cell lines. Polluted air was classified by the International Agency for Research on Cancer as carcinogenic to humans. Thus, the investigation of the organism's protective mechanisms against the negative effects of air pollutants is of high importance as it may deliver useful information that may potentially improve human health.

We analyzed the frequency of stable and unstable chromosomal aberrations in Prague mothers and their newborns during the winter season, 2007-2008, when the levels of carcinogenic polycyclic hydrocarbons in the air were elevated. Cytogenetic analysis by whole chromosome painting #1 and #4 was for the first time applied in paired mother-newborn samples. We used a completely new method for analysis based on fully automated searching and scanning of metaphases. The obtained results showed the significant differences between groups in frequencies of stable aberrations, while the levels of unstable aberrations reflected the same exposure to pollutants during pregnancy. We consider the frequency of stable chromosomal aberrations in newborns of 31-40 year old mothers which were five times higher in comparison to newborns of 20-30 year old mothers to be an important finding, because the higher frequency of chromosomal aberrations is generally associated with a higher risk of cancer in the future. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.

Rossnerova A, Balascak I, Rossner P Jr,Sram RJ: Frequency of chromosomal aberrations in Prague mothers and their newborns. Mutat. Res. 699 (2010):29-34. IF 4.440

We studied the association between oxidative damage to placental DNA (levels of 8-oxo-7,8-dihydro-2´-deoxyguanosine, 8-oxodG), exposure to air pollution during pregnancy, genetic polymorphisms in 94 selected genes and adverse pregnancy outcomes (intrauterine growth retardation-IUGR, low birth weight-LBW). Adverse pregnancy outcomes were associated with increased levels of oxidative damage. Multivariate regression analyses confirmed that elevated 8-oxodG levels are the only factor associated with the risk of IUGR. LBW was associated with oxidative damage to DNA, the child´s gender, the mother´s smoking habits, and haplotypes in the promoter of the *MBL2* gene (mannose-binding lectine 2). The role of air pollution in IUGR and LBW seems to be minor. The association between the *MBL2* gene and the risk of LBW was not previously known. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.

Rossner P Jr, Tabashidze N, Dostal M, Novakova Z, Chvatalova I, Spatova M, Sram RJ: Genetic, biochemical and environmental factors associated with pregnancy outcome in newborns from the Czech Republic. Environ. Health Perspect., 119(2011):265-271. IF 6.123

Air pollution causes oxidative damage to macromolecules, chromosomal aberrations and changes in gene expression. The levels of oxidative stress markers [8-oxo-7,8-dihydro-2´deoxyguanosine (8-oxodG), 15-F_{2t}-isoprostane(15-F_{2t}-IsoP), protein carbonyls] and cytogenetic parameters [genomic frequency of translocations (F_G/100) and percentage of aberrant cells (%AB.C.)] were studied in subjects living in Prague and in the heavily polluted Ostrava regions. We also compared the expression of genes participating in base excision repair (BER) and non-homologous end-joining (NHEJ) in 64 subjects from Prague and 75 subjects from Ostrava. The levels of air pollutants (benzo[a]pyrene (B[a]P); carcinogenic polycyclic aromatic hydrocarbons (c-PAHs); benzene) measured by personal monitors were significantly elevated in Ostrava compared to Prague ($p<0.001$). However, no differences in biomarkers of oxidative stress between the two locations were observed. Multivariate analyses revealed that subjects living in Ostrava had increased *XRCC5* expression. 8-oxodG levels were associated with levels of vitamins C and E. Our results suggests that air pollution by c-PAHs affects *XRCC* gene expression, which probably protects subjects from Ostrava against the induction of a higher frequency of translocations; elevated vitamin C and E levels in the Ostrava subjects decrease the levels of 8-oxodG. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
Rossner PJr, Uhlirova K, Beskid O, Rossnerova A, Svecova V, Sram RJ: Expression of XRCCS in peripheral blood lymphocytes in upregulated in subjects from a heavily polluted region in the Czech Republic. Mutation Research 713(2011):76-82. IF 4.440

We performed a comprehensive analysis of transcriptome alterations induced by smoking in maternal and fetal cells (from peripheral blood PL, placenta PL, cord blood UCB, N smokers=20, N nonsmokers=52). Gene expression profiles were assayed by Illumina Expression Beadchip v3 for analysis of 24,526 transcripts. A comparative analyses defined a significant deregulation in 193 genes in PB, 329 genes in PL, and 49 genes in UCB smokers. The deregulated genes were mainly related to xenobiotic metabolism, oxidative stress, inflammation, immunity, hematopoiesis, and vascularization. The study demonstrated that maternal smoking causes significant changes in transcriptome of placental and fetal cells that deregulate numerous biological processes important for growth and development of the fetus. Department of Genetic Ecotoxicology prepared the project proposal, organized the study in hospitals, collection of material, processing of biological samples, evaluation of results, preparation of manuscript (60%).
Votavova H, Merkerova-Dostalova M, Fejglova K. Vasikova A, Krejcik Z, Pastorkova A, Tabashidze N, Topinka J, Veleminsky M Jr, Sram RJ, Brdicka R: Transcriptome alteration in maternal and fetal cells induced by tobacco smoke. Placenta. 32(2011):763-770. IF 2.985

We studied genetic damage in human lymphocytes measured using automated image analysis of micronuclei (MN) in a group of 178 mothers and their newborns from two locations in the Czech Republic. The three-month mean concentration of B[a]P before delivery was lower in Prague in comparison with Ceske Budejovice: 1.9±0.5 ng/m^3 vs. 3.2±0.2 ($p<0.001$). The opposite trend was found for PM2.5 and benzene: 27.0±2.5 μg/m^3 and 2.5±0.5 μg/m^3 vs. 24.5±0.7 μg/m^3 and 2.1±0.8 μg/m^3 ($p<0.001$) for Prague vs. Ceske Budejovice, respectively. The frequencies of

MN per 1000 binucleated cells were 8.35±3.06 vs. 6.47±2.35 ($p<0.001$) for mothers from Prague and Ceske Budejovice, respectively, and 2.17±1.32 vs. 3.82±2.43 ($p<0.001$) for newborns from Prague and Ceske Budejovice, respectively. The results suggest that the different sensitivity of the study groups to various mixtures of carcinogenic pollutants could be affected by significant differences in lifestyle factors. Possible higher genetic damage was analyzed in newborns of smoking mothers, and the birth weight of this group was 7.4% lower ($p<0.05$) in comparison with the newborns of nonsmoking mothers. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
Rossnerova A, Spatova M, Pastorkova A, Tabashidze N, Veleminsky M Jr, Balaščak I, Solansky I, Sram RJ: Micronuclei levels in mothers and their newborns from regions with different types of air pollution. Mutation Research. 715(2011):72-78. IF 4.440

Personal exposures to volatile organic compounds (VOCs) were measured in three industrial cities in the Czech Republic, Ostrava, Karvina and Havirov, while the city of Prague served as a control. Office workers from Ostrava and city policemen from Karvina, Havirov and Prague were monitored in the winter and summer of 2009. Only adult non-smokers participated in the study (N=160). Radiello® diffusive passive samplers were used to measure the exposure to benzene, toluene, ethylbenzene, meta- plus para-xylene and ortho-xylene (BTEX). The average personal BTEX exposure levels in both seasons were 7.2/34.3/4.4/16.1 $\mu g/m^3$, respectively. The benzene levels were highest in winter in Karvina, Ostrava and Prague: 8.5, 7.2 and 5.3 $\mu g/m^3$, respectively. The indoor environment, ETS (environmental tobacco smoke), cooking, a home heating fireplace or gas stove, automobile use and being in a restaurant were important predictors for benzene personal exposure. Ostrava`s outdoor benzene pollution was found to be a significant factor, increasing the exposure of the Ostrava study participants to VOCs in winter ($p<0.05$). All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
Svecova,V., Topinka, J., Solansky, I., Sram, R.J.: Personal exposure to volatile organic compounds in the Czech Republic. J.Expo.Sci.Environ.Epidemiol. 22(2012):455-460. IF 3.050

To extend our knowledge on the molecular effects of tobacco smoke exposure in pregnancy, we analyzed transcriptome alterations in passive smokers (PS) and compared them with nonsmokers (NS). Using Illumina Expression Beadchip with 24,526 transcript probes, gene expression patterns were assayed in placentas from PS (N=25) exposed to ETS in the course of pregnancy and non-exposed counterparts (N=34) and in cord blood cells from their newborns. A total of 158 genes were significantly deregulated in placentas of PS compared to NS, whilst cord blood of the newborns of PS displayed differential expression of 114 genes encoding mainly adhesion molecules and regulators of immunologic response. This study demonstrates that even low dose exposure to ETS during pregnancy leads to significant deregulation of transcription in placental and fetal cells. This study was part of our project, we organized the collection of biological material and questionnaires in hospital, detection of cotinine, isolation of RNA, validation of microarray data by qRT-PCR, evaluation of results, as well as the preparation of the manuscript (60%).
Votavova HG, Dostalova,-Merkerova M, Krejcik Z, Fejglova K, Vasikova A, Pastorkova A, Tabashidze N, Topinka J, Balascak I, Sram RJ: Deregulation of gene expression induced by environmental tobacco smoke exposure in pregnancy. Nicotine Tob. Res. 14 (2012):1073-1082. IF 2.805

Acute respiratory infections are common in children below 5 years old and recent studies suggest a possible link with air pollution. This study was conducted in Teplice and Prachatice districts, Czech Republic. Children were followed from birth to 4.5 years of age. The results demonstrate an association between NO(x) and respiratory infections that are sufficiently severe. The evidence, if causal, is of concern to public health because acute respiratory illnesses are common in preschool children. The continuous follow up of child health from birth to 10 years old was performed by the Department of Genetic Ecotoxicogy. This study was designed and conducted by our research team, as was the preparation of the manuscript. R. Ghosh and J. Joad did the statistical evaluation of the effect of genetic polymorphisms, I. Herz-Picciotto participated in the manuscript (80%).

Ghosh R, Joad J, Benes I, Dostal M, Sram RJ, Hertz-Picciotto I: Ambient nitrogen oxides exposure and early childhood respiratory illnesses. Environ Int. 39 (2012):96-102. IF5.664

The Northern Moravia Region (Silesia) is characterized by high concentrations of c-PAHs due to industrial air pollution. Exposure to B[a]P (benzo[a]pyrene) in Ostrava-Radvanice is the highest in the EU. Children from this part of the city of Ostrava suffered higher incidence of acute respiratory diseases in the first year of life.

Gene expression profiles in leukocytes of asthmatic children compared to children without asthma were evaluated in groups from Ostrava-Radvanice and Prachatice. The results suggest the distinct molecular phenotype of asthma bronchiale in children living in polluted Ostrava region compared to children living in Prachatice.

The effect of exposure to air pollution on biomarkers in newborns was analyzed in Prague vs. Ceske Budejovice, two locations with different levels of pollution during the winter season. B[a]P concentrations were higher in Ceske Budejovice. DNA adducts and micronuclei were also elevated in cord blood in Ceske Budejovice in comparison to Prague. The study of gene expression profiles in cord blood showed differential expression of 104 genes. Specifically, biological processes related to immune and defense response were down-regulated in Ceske Budejovice.

Our studies demonstrate that air pollution significantly affects child health. Especially noticeable is the increase of respiratory morbidity. With the development of molecular epidemiology, we can further evaluate the health risk of air pollution using biomarkers. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.

Sram RJ, Binkova B, Dostal M, Merkerova-Dostalova M, Libalova H, Milcova A, Rossner P Jr, Rossnerova A, Schmuczerova J, Svecova V, Topinka J: Health impact of air pollution to children. Int J Hyg Environ Health 216(2013):533-540. IF3.276

We investigated the impact of air pollution on a cohort of subjects living in Ostrava with a cohort of subjects from Prague in three sampling periods (winter 2009, summer 2009, winter 2010). To evaluate DNA damage in subjects from both locations we determined the level of DNA adducts in peripheral blood lymphocytes using the 32P-postlabeling method. Multivariate analyses conducted among subjects from Ostrava and Prague revealed that exposure to B[a]P and PM2.5 significantly increased levels of B[a]P-like DNA adducts in the Ostrava subjects, but not in subjects from Prague.

Further we studied the effect of exposure to PM2.5, benzene and B[a]P on oxidative stress markers (8-oxo-7,8-dihydro-2‘-deoxyguanosine 8-oxodG, 15-Ft-isoprostane 15-2Ft-IsoP and

protein carbonyls) and cytogenetic parameters. Multivariate analyses conducted separately in subjects from Prague and Ostrava showed a negative association between the frequency of micronuclei and concentration of B[a]P and PM2.5 in both regions. A positive relationship was observed between lipid peroxidation and air pollution; protein oxidation seems to be positively affected by PM2.5 in both regions. All the work and manuscript preparation was done exclusively by members of the Department of Genetic Ecotoxicology.

Rossner P Jr., Svecova V, Schmuczerova J, Milcova A, Tabashidze N, Topinka J, Pastorkova A, Sram RJ: Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part I. Bulky DNA adducts. Mutagenesis 28 (2013):89-95. IF 3.497

Rossner PJr, Rossnerova A, Spatova M, Beskid O, Uhlirova K, Libalova H, Solansky I, Topinka J, Sram RJ: Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part II. Chromosomal aberrations and oxidative stress. Mutagenesis 28 (2013):97-106. IF 3.497

We studied the differences in methylation pattern (27,578 CpG sites in 14,495 genes were analyzed per subject) of 200 children from two regions in the Czech Republic with different levels of air pollution (Ostrava vs. Prachatice). Other factors like length of gestation, birth weight, breastfeeding or smoking are also related to a final methylation pattern which has an impact on the function of genes. It was the first paper on DNA methylation in asthmatic children. Results demonstrate a significant impact of different environmental conditions on the DNA methylation patterns of children from the two regions. Further, we observed differences in DNA methylation pattern depending on gender and urinary cotinine levels. This study is in majority an original work of the Department of Genetic Toxicology. Our team covered the following activities: collection of samples and health records, DNA isolation, bisulfite treatment, methylation analysis by 27K BeadChips and manuscript preparation. Statistical analysis were done by Genedata Company (90%).

Rossnerova A., Tulupova E, Tabashidze N, Schmuczerova J, Dostal M, Rossner P Jr, Gmuender H, Sram RJ: Factors affecting the 27K DNA methylation pattern in asthmatic and healthy children from locations with various environments. Mutation Research 741-742 (2013):18-26. IF 4.440

Personal exposure to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) was described in detail for the first time in Moravian Silesian Region Czech Republic (Ostrava, Karvina and Havirov, Prague served as control). The levels by far exceed the EU (WHO standard) limit of 1 ng/m3 of B[a]P and pose a significant risk for human health. In this study, we examined personal exposure to c-PAHs and tested it for associations with potential predictor variables collected from questionnaires, addressing life style factors and day-to-day activities. We found outdoor concentration, ETS exposure, home heating fuel of coal, wood or gas, frequency of exhaust fan use, cooking, and commuting by car to be the main determinants of personal exposure. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.

Svecova V, Topinka J, Solansky I, Rossner P Jr, Sram RJ. Personal exposure to carcinogenic polycyclic aromatic hydrocarbons in the Czech Republic. J.Expo.Sci.Environ. 23(2013): 350-355. IF 3.050

To confirm or refute the hypothesis that the morbidity of children (from birth to age 5 years old) born and living in the heavily polluted (PM2.5, B[a]P) eastern part of Ostrava (Radvanice and Bartovice) was higher than the morbidity of children living in other parts of the city, we studied

children from 10 pediatricians in 5 districts of Ostrava. We report on acute illnesses in 1535 children of Czech ethnicity in the first 5 years of life. Over 45% were upper respiratory infections. In the first year of life, the incidence of URI in 183 children in the eastern area - 372 illnesses/100 children/year - was more than twice as high as in the other 3 areas of Ostrava. From birth to 5 years old, pneumonia, tonsillitis, viral and intestinal infections were also several times higher in children living in the eastern part of Ostrava. The children born and living in the eastern part of the city of Ostrava had, from birth to 5 years old, significantly higher incidence rates of acute illnesses compared to children in other parts of Ostrava. They also had a higher prevalence of wheezing, atopic dermatitis and allergic rhinitis. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
Dostal M, Pastorkova A, Rychlik S, Rychlikova E, Svecova V, Schallerova E, Sram RJ: Comparison of child morbidity in regions of Ostrava, Czech Republic, with different degrees of pollution: a retrospective cohort study. Environ Health. 2013,12:74. doi: 10.1186/1476-069X-12-74. IF 2.710

A random sample of 1133 children born between 1994 and 1998, in two districts of the Czech Republic was followed-up from birth, of which 793 were genotyped. Pediatric records were abstracted for respiratory illnesses. We examined six single nucleotide polymorphisms (GSTM1, GSTP1, GSTT1, CYP1A1 Mspl, EPHX1 exon 3 and 4) and one (EPHX1) diplotype. The EpHX1 low activity diptotype consistently imparted greater susceptibility to bronchitis from second-hand tobacco smoke (SHS), polycyclic aromatic hydrocarbons (PAHs) and PM2.5. Each of these three classes of exposure also showed an elevated risk for bronchitis in the presence of either one or two histidines at exon 3 and exon 4 EPHX1. Additional effect modifiers include CYP1A1 and GSTT1. Several xenobiotic metabolizing genes may modify the impact of SHS, PAHs and PM2.5, on acute bronchitis in preschool children. This study was designed and conducted by our research team, as was the preparation of the manuscript. R. Ghosh and J. Joad did the statistical evaluation of the effect of genetic polymorphisms, I. Herz-Picciotto participated in the manuscript (80%).
Ghosh R, Topinka J, Joed JP, Dostal M, Sram RJ, Hertz-Picciotto I: Air pollutants, genes and early childhood acute bronchitis. Mutation Res 749(2013):80-86. IF 4.440

We investigated the effect of complex mixtures containing genotoxic as well as non-genotoxic carcinogens on model human cell lines (HEL12469, A549). We used toxicogenomics and analyses of a panel of conventional markers of genotoxic and non-genotoxic effects to analyze mechanisms of action of the individual model PAHs, their binary and complex artificial mixtures and EOMs collected in localities differing in their extent of environmental pollution. The most important findings of the project can be summarized in two points: 1. Transcriptional changes induced by organic compounds bound to respirable fraction of ambient air aerosols suggest a crucial role of the AhR-receptor. 2. Genotoxicity of B[a]P in an artificial mixture of PAHs is several-folds lower than that of B[a]P alone. This finding is important for the evaluation of toxic effects of PAH mixtures and their risks for human health. It means that the calculations of total risk based on the toxic equivalents for individual PAHs, which are founded on the assumption of the additivity of the effects, are not correct. The team of the Department of Genetic Ecotoxicology performed all the analysis of global and specific gene expression changes and multiple genotoxicity markers (DNA adducts, Oxidative damage to DNA). The team also played a major role in the evaluation and interpretation of data as well as in the writing of the manuscripts (90%).

H. Líbalová, K. Uhlířová, J. Kléma, M. Machala, R.J. Šrám, J. Topinka, Global gene expression changes in human embryonic lung fibroblasts induced by organic extracts from respirable air particles, Particle and Fibre Toxicology, 2012, 9:1-16. IF 6.990
H. Líbalová, S. Krčková, K. Uhlířová, A. Milcová, J. Schmuczerová, M. Ciganek, J. Kléma, M. Machala, R.J. Šrám, J. Topinka Genotoxicity but not the AhR-mediated activity of PAHs is inhibited by other components of complex mixtures of ambient air pollutants, Toxicology Letters 2014, 225, 350-357. IF
H. Líbalová, S. Krčková, K. Uhlířová, J.Kléma, M. Cigánek, P.Rossner, Jr., R.J. Šrám, J. Vondráček, M. Machala, J.Topinka, Analysis of gene expression changes in A549 cells induced by organic compounds from respirable air particles, Mutation Research /Fundamental and Molecular Mechanisms of Mutagenesis 2014, 770, 94-105. IF 4.440

We studied the effects of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and extractable organic matter (EOM) from particulate matter collected from polluted air on DNA damage and induction of relevant DNA repair pathways (nucleotide excision repair, NER; non-homologous end-joining, NHEJ) in human embryonic lung fibroblasts (HEL12469 cells). We observed no effect on DNA repair activity measured 24h after application of the tested compounds. We concluded that EOM components other than PAHs play a likely role in the DNA damage response in HEL12469 cells. Due to non-consistent results and the generally weak NER response of the cells we assume that HEL12469 cells are less suitable for genotoxicity testing of the compounds requiring metabolic activation for their mechanism of action. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
P. Rossner, Jr., A. Mrhalkova, K. Uhlirova, M. Spatova, A. Rossnerova, H. Libalova, J. Schmuczerova, A. Milcova, J. Topinka, R.J. Sram, Nucleotide Excision Repair Is Not Induced in Human Embryonic Lung Fibroblasts Treated with Environmental Pollutants, Plos One, 8 (2013). IF 3.534

We further developed methods to study DNA damage and repair. Although methods of measurement of activity of NER and NHEJ have already been published, they have never been used in our department. We have successfully modified them and set them up for specific samples and conditions in our lab. The cytogenetic methods applied in the project are unique. Automated image analysis of micronuclei has never been used in binucleated embryonic lung fibroblasts. Also, FISH analysis of stable chromosomal aberrations based on the painting of six chromosomes that cover about 36% of the genome was reported for the first time in this model cell line. All these methods are important tools of DNA damage and repair detection and will be applied in our future projects. All the work and manuscript preparation was exclusively done by members of the Department of Genetic Ecotoxicology.
P. Rossner, Jr., A. Rossnerova, O. Beskid, N. Tabashidze, H. Libalova, K. Uhlirova, J. Topinka, R.J. Sram, Nonhomologous DNA end joining and chromosome aberrations in human embryonic lung fibroblasts treated with environmental pollutants, Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis, 763-764 (2014) 28-38. IF 3.497