## NEUROPHYSIOLOGY OF VISCERAL NOCICEPTORS BY ELECTROPHYSIOLOGY

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Gastrointestinal organs including esophagus are common sources of visceral pain that cause discomfort of many patients. Pathophysiologically this pain is attributed to hypersensitivity and hyperreactivity of nociceptive pathway. Our research group is specialized on molecular mechanisms of afferent nerve endings to detect painful stimuli. Except spinal afferent neurons projecting into esophagus, which are considered the major pain pathway from the esophagus, other groups of vagal nodose and jugular neurons (capsaicin-sensitive neurons) according to embryonic origin can contribute to visceral pain but the information on their sensory transduction is limited. We addressed the hypothesis that in the esophagus in contrast to nodose neurons the activation profile of spinal DRG neurons is similar to vagal jugular neurons with which they share the neural crest embryonic origin. Extracellular recordings of single unit activity were made in the isolated innervated guinea pig esophagus. The esophagus was dissected with preserved bilateral vagal innervation including jugular and nodose ganglia. The tissue was continuously perfused with Krebs solution. The recording aluminosilicate microelectrode was micromanipulated into the nodose or jugular ganglion and a distention-sensitive unit was identified when esophageal distention at 60 mm Hg evoked action potential discharge. The signal was amplified, filtered, digitally recorded (sampling rate 33 kHz) and analyzed (software TheNerveOfIt, PHOCIS). The single C-fiber is evaluated for response to mechanical stimuli (von Frey hair) and chemical stimuli. Extracellular recording is the most appropriate to address the hypotheses in this proposal as it fulfils complex mechanical, electrophysiological, and pharmacological characteristics of spinal and vagal afferent nerve endings in naïve animals and in animal model of inflammation. We conclude that the activation profile of the spinal DRG neurons innervating the guinea pig esophagus is consistent with the nociceptive function. Our data indicate that the activation profile of the nociceptive nerves in the esophagus is dictated by their embryonic origin. Thus the neural crest-derived spinal DRG and vagal jugular nociceptors share the activation profile distinct from the placodes-derived vagal nodose nociceptors by activation profile, neurotrophic regulation and expression of neurotransmitters.

*The study was supported by BioMed Martin (ITMS: 26220220187) and VEGA 1/0070/15.*

## METASTATIC DISEASE IN MICE IMMUNODEFICIENT MODELS

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The metastatic disease is a complex multistage process that is affected by local environment, hormonal and immune system. Because of complexity of the interactions between the tumor and its environment it is impossible to imitate reliably *in vitro*. *In vivo* models represent the relevant alternative. In our lab we conduct several studies dealing with metastasis. For induction of metastases we use established cell lines or primary tumor tissues from patients suffering from cancer. 1) In case of the colonization model tumor cells are systemically injected through the mouse tail vein. Cells subsequently home to lungs or lymph nodes. This model is characterized by rapid formation of metastases in the organs according to preferences of metastatic cells. 2) Spontaneous metastasis model imitates the natural course of the spread of metastatic disease, including whole metastatic cascade. Potential long latency of disseminated cells sometimes requires resection of the primary tumor to allow sufficient time for metastatic progression. Patient-derived xenografts (PDX) enable formation of spontaneous metastases from patient's tissue including inter-patient and intra-tumor heterogeneity. For PDX models, the fresh tissue or the tissue processed to single cell suspension, respectively, is used. In order to provide the growth of xenografts from different types of tumors, two lines of mice are used in our facility. Refinement tools are used to reduce the pain during projects. Inhalation anesthesia substitutes for the administration of anesthetic by injection, animals wake up immediately after surgery. Heating pad and ophthalmic ointment improve the convalescence after surgery. The per oral analgesia improves welfare during convalescence, and silver spray accelerates wound healing because of the oxygen access. The spray substitutes a plaster that limits mobility of animals. Refinement tools mentioned above help to perform our projects to comply with animal welfare requirements.

*The study was supported by VEGA grants: 2/0087/15, 1/0271/17, 2/0128/17, and 2/0124/17; APVV grants: APVV-0052-12, APVV-15-0697, APVV-16-0010 and APVV-16-0178.*

## DIFFERENCE IN SYSTOLIC PRESSURE VALUES AT INFLATION AND DEFLATION OF THE CUFF IN RATS WITH GOLDBLATT RENAL HYPERTENSION

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Clinically relevant animal models should mirror basic epidemiology data from real patients – age, sex, primary disease i.e. diabetes, hypertension... Hypertension is most frequent concomitant disease and is the cause of many cardiovascular diseases. For our needs (stroke study) we use Goldblatt renal hypertension model (GRHM). Briefly about GRHM: hypertension is induced by the stenosis (no total occlusion) of one (2K1S) or two renal arteries (2K2S) (Abbreviation: K=kidney, S=stenosis). Renal artery stenosis with renal hypoxia produce high level of angiotensin with all consequences - hypertension, initial hypertrophy of left ventricle, chronic remodelation of myocard and artery wall. Three-month-old, male, Wistar rats were divided in groups: 2K1S (n=9), 2K2S (n=5), KON (n=11). In the 2K1S left renal artery was stenotized; in the 2K2S both renal arteries and in KON group a sham surgery was performed. Four months after surgery the blood pressure was measured (tail artery, rats cuff, vascular doppler flow detection). The measurement was performed under isoflurane anesthesia. The measurement was repeated 3 times and systolic arterial pressure values in cuff inflation (SAPi) and cuffs deflation (SAPd) were recorded. SAPi values (mean ± SD) were in 2K1S group 140±29 mm Hg, in 2K2S 144±20 mm Hg and in KON group 113±13mm Hg. Differences in systolic pressure at inflation and deflation of the cuff (SAPi-SAPd) were 12±4 mm Hg in 2K1S group, 11±5 mm Hg in 2K2S group and 2±2 mm Hg in KON group. GRHM produce elevation of SAPi-SAPd compared to control group.

*The study was supported by the project no. LQ1605 (MEYS CR, NPU II).*

## TOXICITY OF WASTEWATER FROM HEALTH CARE FACILITIES ASSESSED BY ALTERNATIVE METHODS

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The aim of the water policy in the European Union is to promote sustainable water use, and a major ambition of the Water Framework Directive (2000/60/EC) is the long-term progressive reduction of contaminant discharges into the aquatic environment.The diferent chemicals and pharmaceuticals which are released into the environment mainly from health care facilities can have biological adverse effects on the environment and human health, if not treated in the wastewater

treatment plant (WWTP). Conventional WWTPs eliminate a great amount of compounds but often show a limited reduction of micropollutant concentrations. There is a great need for the development of suitable sensitive bioassays in order to characterize properly the possible residual toxicity of treated effluents. The purpose of this study was to determine toxicity of wastewater from hospitals in the Czech Republic using traditional and alternative toxicological methods. The pilot study comprised weekly dynamics of sewage ecotoxicity of treated wastewater from one large hospital in two different seasons (May vs. November). A detailed investigation of wastewater ecotoxicity, genotoxicity and reprotoxicity followed in five different hospitals. The toxicity classification system based on the calculation of TU (toxic unit) was applied to evaluate ecotoxicity. The seven following bioassays were used in this study: algal growth inhibition test with *Desmodesmus subspicatus* (EN ISO 8692), *Vibrio fischeri* luminescent test (EN ISO 11348-2), immobilization test with *Daphnia magna* (EN ISO 6341), *Allium cepa* assay, Bacterial Reverse Mutation Test – Ames Agar Plate Test (OECD TG 471), Comet assay (single-cell gel electrophoresis) and YES/YAS – Yeast Based Reporter Gene assay. In the pilot study, the wastewater ecotoxicity during one week showed no significant differences in separate working days, however, higher toxicity values were recorded in May compared to November. In the following study, samples from two of the five hospitals were classified as toxic (III. toxicity class), the others as non toxic (I. toxicity class). Genotoxicity has not been confirmed neither by Ames test, nor Comet assay in any sample. In several cases, wastewater samples exhibited agonist activity to the estrogen and androgen receptors.The study demonstrated different levels of toxicity of treated hospital wastewater. Variable sensitivity of individual bioassays for tested wastewater samples was recognized. It can be assumed that the results of Ames test, Comet and YES/YAS assays may be influenced by sample sterilization (by filtration) which might have caused a loss of genotoxic and reprotoxic activity as certain chemicals may be captured on the filters. The study will continue with optimization of sample preparation. A more extensive study including proposal for improvement of hospital wastewater treatment within the Czech Republic can be recommended with the aim to decrease the discharge of toxic chemicals into the local sewage system and environment.

*Supported by Ministry of Health, Czech Republic – conceptual development of research organization („National Institute of Public Health – NIPH, IN: 75010330“)*

## IN VITRO METHODS FOR IDENTIFICATION OF CHEMICALS WITH ENDOCRINE DISRUPTION POTENTIAL

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Global concerns have been raised in the last decades over the possible adverse effects resulting from exposure to chemicals with potential to interfere with the endocrine system in wildlife and humans. Interaction of exogenous substances with human receptors is a significant initiation event on molecular level that leads to various complex effects. The physiological receptor mechanism may be affected either by direct receptor binding of the exogenous ligand to the receptor, resulting in activation (agonistic activity) or inhibition (antagonistic activity), or by consequent modulation of associated signaling pathways regulation. Endocrine disruptors may act in low nanomolar concentrations similarly to endogenous hormones and have been associated with adverse effects, such as endocrine system disturbances and consequent developmental, reproductive and immune disorders. Sources of exposure to endocrine disruptors may be products coming from industry or agriculture and include consumer products, e.g. food packaging materials, household products, thermopaper, plastics, cosmetics or toys. Certain substances may be persistent, resulting in their bioaccumulation in the food chain as well as in the human organism and others, on the contrary, may be quickly metabolized and act for a limited time. These facts complicate the detection of negative effects of endocrine disruptors *in vivo*. Development and use of *in silico* screening tools and fast and cheap *in vitro* methods is therefore most effective for first-level screening of potential endocrine disruption, allowing for a rapid initial prioritization and sorting of chemicals for further evaluation in mechanistically based screens. In our recent studies of the interactions of chemicals with human estrogen and androgen receptors, the battery of available tests included the OECD QSAR Toolbox, Stably Transfected Transactivation In Vitro Assay to Detect Estrogen Receptor Agonists (OECD TG 455) and yeast-based microplate assay (in compliance with Draft ISO/DIS 19040). Selected bisphenols and phthalates were tested in a pilot study. *In vitro* results correlated well with *in silico* prediction for phthalates, while the predictions for bisphenols differed slightly. The results revealed that bisphenol A analogues should be further tested as they may show similar or higher activity *in vivo* comparing to bisphenol A, which has been recently legislatively regulated. Further studies involved testing of newly developed antimicrobials based on nanosilver and phtalocyanines or selected rare earth elements, which have not shown any endocrine activity. The *in vitro* methods demonstrated great advantage in hazard assessment of chemicals over *in vivo* studies as they enable to test separate substances without the background of complex hormonal system of a living organism subjected to a cocktail of potential endocrine disruptors from the environment.

*Supported by Ministry of Health, Czech Republic – conceptual development of research organization („National Institute of Public Health – NIPH, IN: 75010330“) and TE02000006 Centre for alternative environment friendly high effective polymer antimicrobial agents for industrial applications (ALTERBIO).*

## THE METHOD OF LONG-TERM CATHETERIZATION OF THE VENA JUGULARIS IN PIGS

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The pig is a valuable biomedical model, however, its great disadvantage is limited vascular access. The optimal solution is therefore to install a permanent intravenous catheter, which must be very resistant to damage and infection. The method has been verified in fourteen 3-month-old pigs of the Prestice breed. The 420 mm polyurethane catheter tube was surgically introduced using the Seldinger's technique. The part of the tube that was not inserted into the vein was threaded through a subcutaneously introduced trocar to the site behind the external ear, fitted with a simple plug made of a cut-off detrited 16G or 18G injection needle, which was clamped in the wing made of a folded piece of waterproof adhesive tape. This external part of catheter was then fixed by 3 sutures to the skin of the animal's nape, where it was well accessible and well protected from damage. To enhance the protection, the entire outer part of the catheter was overlapped with a tubular bandage and the outlet of the tube was continually treated with iodine ointment. The catheters were flushed daily with sterile 0.9 % saline and locked with 4 % citrate between frequent blood samplings, or with 30 % citrate between 1-day frequencies. Once a week, the catheter was locked with a 4 % citrate containing taurolidine in order to prevent infection. There was no occurence of infection or thrombus in any animal to disable the catheter. All the catheters remained fully functional in both directions and were removed at the experiment termination, except for the first three animals that damaged the catheter due to unsuitable placement of the catheter outlet. This catheter was well tolerated by the animals and allowed comfortable and stress-free blood sampling for up to 11 weeks.

*This study was supported by the National Sustainability Program I (NPU I) Nr. LO1503.*

## ANIMAL MODELS FOR COUGH RESEARCH

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Cough is the most important airway defensive reflex and it is also the most common symptom of many inflammatory diseases such as asthma and chronic obstructive pulmonary disease. So far, there is no effective treatment (excluding the primary disease) that would successfully suppress cough in subjects with significantly reduced quality of life due

to excessive coughing. The most important information about the neural pathways in cough reflex and its pharmacological regulation have been conducted in guinea-pigs, rats, rabbits, cats and dogs. From small laboratory animals, the best model seems to be awake guinea pig. Neurophysiology and neuropharmacology of the vagus nerve in guinea pigs is very similar to humans and opens possibilities for successful translational research. *In vitro* data suggest that the isolated guinea pig vagus nerve depolarises in response to tussive stimuli in a similar manner to the human isolated vagus. Although many studies have been performed in conscious rats, and cough sounds recorded, there is scepticism regarding the ability of these animals to cough that resembles the reflex seen in human. Murine model is also not suitable due to the lack of rapidly adapting receptors in the airways. Usually, conscious animals are placed into a transparent box or a plethysmograph where they are inhaling the aerosol of tussive agents and the count of coughs is recorded during a defined time period. Total number of coughs and cough latency are the usual outcomes. Inhalation of aerosols with increasing concentrations allows the detection of cough reflex threshold – a concentration that activates airway cough related afferents. Up-regulation or down-regulation of cough can be modelled by e.g. sensitization of animals by ovalbumin, leading to allergic airway inflammation, by exposure to the tobacco smoke, which provokes rather neutrophilic inflammation, or by ACE-i medication, which decreases the breakdown of neuropeptides in the airways. Nowadays, possibility of the use of SPF guinea pigs is being discussed, as it seems their cough response is significantly weaker that the cough response of animals from conventional environment adapted animals. Large laboratory animals such as cats or rabbits are effectively used as an anaesthetized tracheotomised model, even though the anaesthesia reduces reflex responses. In these models, cough is induced either by inhalation of the tussive substances or by mechanical stimulation of the airway mucosa. Cough is recognized based on a large burst of electromyogram activity in the diaphragm immediately followed by a burst of activity in the rectus abdominus muscle. Interpretation of the results must always consider the cough differences conserved across species and the results obtained in laboratory studies must be translated to the clinical conditions with understanding of all limitations of the used models.

*The study was supported by VEGA No: 1/0260/18.*

## PRETERM GNOTOBIOTIC PIGLET MODEL OF PRETERM INFANTS

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Preterm infants born with immature organ systems are very sensitive to different biological and environmental factors. Suitable animal models can be useful in investigating and understanding the effects of different conditions on the health of these vulnerable immunocompromised infants. In contrast to the human, the epitheliochorial placentation of the pig prevents the prenatal transfer of protective maternal immunoglobulins. Surgical-derived colostrum-deprived piglets are free of the maternal immunoglobulins, and the cells that are post-natal provided *via* colostrum. The preterm and term germ-free piglets were bred in sterile conditions and compared in an enterocyte development and intestinal morphology, tight junction proteins claudin-1 and occludin, pattern-recognizing receptors RAGE, TLR2, TLR4, and TLR9, coreceptors MD2 and CD14, adaptor molecules MyD88 and TRIF, and inflammasome NLRP3. Levels of inflammatory mediators IFN-α, IL-4, IL-6, IL-8, IL-10, IL-12/23 p40, TNF-α, IFN-γ, and HMGB1 in the intestine of the piglets were assessed. The ileum of the preterm germ-free piglets showed decreased lamina propria cellularity, reduced villous height, and thinner and less distinct stratification – especially muscle layer. Claudin-1 transcription was increased in the intestine of the preterm piglets, but transcriptions of pattern-recognizing receptors and adaptor molecules showed ambiguous trends between the groups. The levels of IL-6, IL-8, IL-10, and TNF-α were increased in the preterm ileum statistically nonsignificantly, but significantly in the colon. IL-12/23 p40 and IFN-γ were significantly higher in the preterm colon. Both blood plasma and intestinal HMGB1 levels were higher in the preterm group. The intestine of the preterm germ-free piglets showed "mild inflammation in sterile conditions". The preterm gnotobiotic piglet model of preterm infants can be used in studies of the impact of microbiota and nutrition on the development and health of the vulnerable immunocompromised preterm infants.

*This work was supported by grant 13-14736S of the Czech Science Foundation and the Institutional Research Concept RVO:61388971 of the Institute of Microbiology.*

## DETECTION OF ANTIBODIES AGAINST BORRELIA BURGDORFERI S.L. IN WILD-TYPE SMALL MAMMALS AND COMPARISON OF SUSCEPTIBILITY OF DIRECT METHODS (PCR AND CULTIVATION)

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The aim of this study was to determine the prevalence of antibodies against Borrelia burgdorferi s.l. in wild-type small mammals as well as to compare the sensitivity of direct detection methods (cultivation and PCR). Between 2010 and 2014, 691 wild small mammals were caught in three locations in the Czech Republic. Serum (n=351) or cardiac rinse (n=340) were examined with a modified indirect enzyme immunoassay (ELISA). Antibodies against B. burgdorferii s.l. were detected in 12 % (484/691) animals. The highest prevalence of 20 % was found in Clethrionomys glareolus (30/151), followed by 12 % (2/17) for Sorex araneus, 10 % (47/453) for Apodemus flavicollis, 6 % (2/31) for A. sylvaticus and 9 % (3/32) for A. agrarius, Microtus arvalis (n=6) and Talpa europea (n=1) were negative. A statistically significant difference ($p<0.05$) was found in the prevalence of C. glareolus and other animal species and prevalence by individual years varied (2-20 %). The seroprevalence did not differ statistically ($p\leq0.05$) according to families (Muridae 10 %, Microtidae 19 %), sex (males 10 %, females 14 %) and localities (Poodří 11 %, Moravský Kras 12 % and Mohelno 14 %). To compare the sensitivity of two direct methods, seventy random samples were used in 2012. B. burgdoferi s.l. was cultured in one sample (1.4 %), which was also negative in PCR and ELISA. Using PCR, B. burgdoferi s.l. detected in seven samples (10 %), two of them were dubious in ELISA. A total of 23 samples (11 positive and 12 dubious) had antibodies to B. burgdorferi s.l., but were also negative in PCR and cultivation. Based on the results, it is evident that B. burgdorferi s.l. circulating in the population of wild tiny in the given localities. The PCR method proved to be more sensitive compared to cultivation.