FELASA'S MISSION AND ROLE AT THE EUROPEAN AND GLOBAL LEVEL

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FELASA

The Federation of European Laboratory Animal Science Associations (FELASA) was founded in 1978. Since then, the federation has had the mission to further all aspects of laboratory animal science (LAS), by advocating responsible scientific research with emphasis on animal welfare with the 3Rs principle as the guiding star. This mission is accomplished by collaboration and networking with bodies at both the European and global level, by establishing working groups for the publication of guidelines and recommendations, by accrediting LAS courses, and by organizing scientific conferences. This presentation will discuss the mission and the accomplishments in detail, as well as discuss the role of FELASA for its members and for LAS in the future.

FELASA-EFAT WORKING GROUP: HARMONISATION OF EDUCATION, TRAINING AND CPD FOR LABORATORY ANIMAL CARETAKERS AND TECHNICIANS

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Competent, confident and caring laboratory animal caretakers, technicians and technologists (LAS staff) are vital for good animal welfare, high quality science and a secure Culture of Care. This requires high quality education, training, supervision and Continuing Professional Development (CPD) of LAS staff. However, there is a lack of harmonization regarding how this education and training is conducted among European countries, nor are there recommendations adapted to Directive 2010/63/EU. Therefore, FELASA and EFAT established a working group with the task of establishing recommendations for education, training and CPD for LAS staff. The working group established five different levels (LAS staff levels 0-4), defining the required level of competence and attitude, as well as suggesting educational requirements for reaching each level. Defining these levels should help to ensure that appropriate educational and CPD activities are in place, and enable employers and LAS staff to determine the level and career stage attained. Furthermore, proper assessment of competencies and effective CPD schemes for all relevant staff should be established. Regulators should support this by setting standards for competence assessment and ensuring that they are consistently applied. In addition, establishments should involve the LAS staff in defining and developing the Culture of Care. The Animal Welfare Body should be involved and have oversight of education, training and CPD. These recommendations will contribute to harmonization and increased quality of education, training and CPD, as well as provide clearer career pathways for LAS staff, helping to ensure high standards of animal welfare and science.

ANIMALS FOR BETTER HUMAN HEALTH

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Modern medicine treats according to the principles of evidence-based medicine, i.e. according to the valid results of properly conducted research. In the research chain of new pharmacotherapy possibilities, research without a testing phase in animals, in the vast majority of laboratory mice or rats, is unthinkable. The basic reaction to administration to a living organism, the determination of a lethal dose, and the like is determined. The reaction of a living organism, due to the number of biological variables, cannot be reliably determined by estimation even with the use of modern IT methods and programs. Without the animal testing phase, testing on volunteers (humans) cannot be started for ethical reasons. In the case of direct testing on humans (inexcusable from an ethical point of view), there could be significant, even fatal damage to the health of these probands, if this phase is „skipped" there could be, and with a high probability would be, significant damage to the health of the recipients of these preparations.

BEHAVIORAL PHENOTYPING OF GPR 75 KO MICE

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G protein receptor 75 (GPR 75) plays an important role in brain inflammation, CNS homeostasis, and psychiatric disorders like schizophrenia (SCZ). GPR75 belonging to the superfamily of GPCRs, with unknown biological functions (oGPCRs), significantly downregulated in patients suffering from SCZ. For this reason, the aim of the study was to develop a line of GPR 75 knockout mice to be used in behavioral tests related to SCZ. Behavioral phenotyping involves a wide variety of standardized test batteries and approaches, including general health, body weight, body temperature, coat and whisker appearance, and assessment of neurological reflexes. GPR75 KO mice were generated by homologous recombination. All studies compared female and male GPR75 KO mice with wild-type (WT) mice. The animals were kept under SPF conditions. For behavioral phenotyping, groups of 24 mice, male and female KO and WT were created, in which body weight was monitored starting at 3 weeks of age, monthly, until 34 weeks of life, feed consumption, as well as hematological and biochemical profiles. Statistical evaluation was performed using GraphPad 9.4.1, and comparisons between groups were analyzed by ANOVA with the Bonferroni multiple comparison test. Differences were considered statistically significant when $P<0.05$. Regarding the evolution of body weight depending on the age of the animals, a statistically significant difference ($P<0.0001$) was observed in the case of all GPR 75 vs. WT groups, in the sense that both males and WT females showed greater increases in body weight. WT mice registered higher food consumption, with statistically significant differences ($P<0.0001$) to GPR 75 KO. The feed consumption of WT mice has a corresponding with WT body weight. The hematological parameters showed a statistically significant difference ($P<0.01$) of hemoglobin level and the number of red cells The biochemical profile, in the case of both categories of analyzed animals, did not show any differences. In conclusion, GPR 75 KO mice, compared to WT, are hypophagic, with a healthy hematological and biochemical profile. These results suggest that GPR75 inhibition in mice can safely decrease energy intake and body fat without affecting the animal's overall condition. Next, we aim to study the effect of the identified ligands and modulatory compounds in wild-type and deficient mice, by using complex mouse SCZ behavior models.

The research leading to these results has received funding from the EEA Grants 2014-2021, under Project contract no. 34 / 2021 named "Next generation of drugs targets for schizophrenia".

DEVELOPMENT OF A MOUSE LESION MODEL FOR TESTING INNOVATIVE NANOCELLULOSIC TREATMENTS

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The study of the healing of various skin lesions requires the choice of an appropriate animal model, and in translational research, no animal fully

predicts all clinical outcomes. However, the mouse is the most commonly used, although there are differences between murine and human skin repair. The aim of the study was to evaluate the skin regeneration process in a mouse model, by excision of a skin flap, followed by the application of an innovative nano cellulosic treatment. 20 BALB/c males, aged 10 weeks, were included in the study, divided into a control group and a treatment group. Under general deep anesthesia (a cocktail of Ketamine and Acepromazine), the animals were subjected to a surgical intervention that involved the excision of a skin flap, with a diameter of 10 mm from the dorsal scapular region. In the control group, the wound was covered with Tegaderm dressing, and the treatment group was tested with patches covered with nano cellulosic particles. In both groups, the patches were changed once every two days, an interval that coincided with the measurement of the diameter of the wounds. After 14 days of clinical monitoring, the animals were euthanized by an overdose of anesthetic, and samples of scar tissue were taken for histopathological examination. The results showed that mice provide a satisfactory animal model for healing skin wounds, and from the analysis of clinical data we noticed that the rate of skin regeneration, after the first 7 days after excision, was 51.05 % in the case of the treatment group compared to 39.87 % in the control group. At the end of the study, the initial diameter of the wounds was significantly reduced, in the case of both groups, reaching from 200 mm^2 to 17±3 mm^2 in diameter. These results will be complemented by the histopathological examination of the scar tissue samples collected to confirm the effectiveness of the applied nano cellulosic treatment. In conclusion, BALB/c mice met the needs of the study, the wound healing process being close to the human one. The innovative nano cellulosic treatments have proven their effectiveness and thereby contribute to the optimization of current therapeutic protocols.

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2021-2559, "Biopolymeric structures obtained by plasma treatment for wound healing".

IMPLICATIONS OF ADVANCED GENOME ENGINEERING TECHNIQUES ON REFINEMENT IN ANIMAL RESEARCH

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CRISPR/Cas9 methodology has revolutionized generation of genetically modified organisms. From an engineering point of view, creating knock-out animals is no longer a challenge. However, constitutive gene knock-outs often result in lethality or development of harmful phenotypes. To overcome this issue, we have implemented a refined engineering system, which allows for the depletion of proteins in a time-controlled manner. In this system, a small degrader tag (FKBP) is added to the endogenous protein but remains inactive until the addition of the ligand (dTAG). Once the ligand is added, the fusion protein is ubiquitinated and degraded. The aim of the project was to assess efficiency of knock-in FKBP mouse line generation, influence of the tag on mouse phenotype and the kinetics of protein depletion after addition of the ligand. 8 mouse lines were generated using CRISPR/Cas9 methodology, FKBP tag was added N- or C-terminally. All targeted genes were embryonic lethal in as homozygotic KOs or displayed severe harmful phenotype. Zygotes were microinjected with gRNA, Cas9 mRNA and dsDNA repair template and transferred to surrogate mothers. F0 mice were genotyped, sequenced, and founders were once backcrossed. Primary cell lines were generated from F1 generation mice to assess the function of FKBP/dTag system. Finally, dTag was added *in vivo* to FKBP/FKBP mice to study the effect of the depletion of studied proteins. Efficiency of insertion of FKBP tag was very high, around 13 % of pups born after microinjections had a correct mutation. In most cases the addition of the tag had no effect on embryo survival and mice well-being. After addition of dTAG depletion of the tagged protein was fast and efficient both *in vitro* and *in vivo*. In conclusion, we show a very efficient method for the generation of knock-in animals with a degrader tag. Such models can open new opportunities to study essential genes with knockout are embryolethal or display harmful phenotype. Implementing such models could open opportunities for novel research and improve laboratory animals' welfare**.**

MONITORING HEALTH PARAMETERS IN ANIMAL MODELS TO EVALUATE 3D BIOPRINTED PERSONAL ARTIFICIAL TISSUES FOR HYPOSPADIAS AND URETHRAL STRICTURES

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Complex hypospadias and urethral strictures are difficult conditions that lead to severe dysfunction, impaired urinary flow, and other complications. Autologous skin or buccal mucosa grafts are commonly used for reconstruction, but these tissues are limited, and surgery may result in prolonged morbidity. Bioengineered artificial tissue would provide additional options for the treatment of extensive recurrent urethral lesions. In this study, multilayer artificial urethral tissue was fabricated using 3D bioprinting technology. The bioprints were tested *in vivo* in an experimental rabbit model. During the 3-month postoperative follow-up, weight, temperature, secretion, behavior, wound healing, laboratory samples, urethrograms, and final histology were evaluated. Scaffold composition was gradually improved to optimize tensile strength and sutureability. Urethrograms showed the advantage of the cell-loaded scaffold. Weight loss and leukocyturia were found to be the most important factors in predicting scar formation.

This project has received funding from European Regional Development Fund No. 01.2.2-LMT-K-718-03-0087 under a grant agreement with the Research Council of Lithuania (LMTLT).

COST ACTION – IMPROVING BIOMEDICAL RESEARCH BY AUTOMATED BEHAVIOUR MONITORING IN THE ANIMAL HOME-CAGE (TEATIME). JOINT ACTIVITIES IN SEARCH OF SOLUTIONS TO ADVANCE ANIMAL WELFARE

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Aside the ethical imperative of protecting the welfare of animals used for scientific purposes, reducing stress and distress can improve the reliability and reproducibility of animal studies. An important factor to consider is providing animals with the best husbandry conditions, catering to species-specific needs, allowing them to express natural behaviours as much as possible, and minimizing environmental stress. There has been an increase in interest on laboratory animal welfare in recent decades, as shown by the growing number of studies on this topic, including studies focused on the behavior of laboratory animals. However, direct observation of animals raises many technical limitations, one of which being the impact on natural behaviour by the presence of humans. Typical behavioural tests only allow a glimpse to a specific moment in the animal's life, under stressful conditions, arising from removal from the home cage, separation from cage mates, introduction to a new environment, and interaction with the researcher. Home-cage monitoring (HCM) systems can avert these caveats, by allowing 24/7 observation of the animals activity and social behavior in a familiar environment, as well as retrieval of physiological data, including body weight, food and water consumption, and body temperature variations. Such systems, however, also have limitations that need addressing. Firstly, long-term observation provides massive amounts of data, the analysis of which can be very time-consuming. Moreover, HCM users require specific skills and knowledge of each HCM system, in order to correctly obtain the behavioural information, adequately analyze the data, and draw well-informed conclusions, based on a deep understanding of laboratory animal behavior, as well as the capabilities and limitations of each system. In order to make the most of the possibilities offered by this technology, a consortium of laboratory animal scientists, ethologists, veterinarians, animal welfare officers, and researchers working with laboratory animals gathered under the auspices of the COST Action TEATIME: "Improving biomedical research by automated behavior monitoring in the animal home-cage".

TEATIME aims to widen and improve the use of home-cage monitoring systems, through dedicated training schools, expert guidelines, peer-reviewed papers, free online webinars (https://www.youtube.com/@cost_teatime6948), and by supporting young researchers with grants to visit laboratories using HCM systems throughout Europe. The ultimate goal is to improve both the reproducibility of animal research and the 3Rs – particularly Reduction and Refinement – through use of HCM, through sharing of knowledge and experiences between scientists, and building meaningful scientific cooperation. The COST TEATIME program is open to all those interested in animal behaviour and welfare, and everyone is welcome to join at any stage of the project. Visit us at https://www.cost-teatime.org/.

CIRS-LAS – AN EASY WAY TO INCREASE TRANSPARENCY AND IMPROVE ANIMAL WELFARE

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Numerous approaches are realized in the last years to steadily increase the animal welfare in laboratory animal science. While the 3R principle can be seen as a standard practice in animal experiments, husbandry and breeding, the slow development of transparency initiatives shows that there is still some way to go. Transparency includes both general information about animal experiments and their necessity as well as a critical examination of animal experiments and possible incidents and errors. While the former represent a transparent approach to the public, incidents mainly concern those who work with experimental animals. However, as in all fields, dealing with mistakes in laboratory animal science is difficult and associated with fears of blame and possible punishment. For this reason, CIRS-LAS (Critical Incident Reporting System in Laboratory Animal Science) was created as a way to report incidents and errors anonymously if desired. These reports are collected in a database and are available to registered persons to inform themselves about incidents, for example in the run-up to an experiment. The report requests information about the involved animal, the incident and the background, possible reasons for the incident and gives the possibility to provide improvements. CIRS-LAS.org is web-based and open to all individuals working with laboratory animals worldwide. The entry of a case report is associated with the reflection of an error, whether self-caused or observed. In both cases, possible refinement strategies can be actively considered and lead to improved animal welfare (Refinement) and more transparency in laboratory animal based research. The search in the database can prevent the repetition of a failed trail and thus reduce the number of laboratory animals (Reduction). The collection and statistical evaluation of case reporting data can provide indications of possible increased risks with regard to a research area or an animal species. At the same time, it can be shown where sources of error can be found in order to eliminate them if possible. CIRS-LAS was developed to provide an easy way to the transparent handling of errors and incidents. Worldwide access is intended to enable networking of all those working in laboratory animal science in order to exchange information on incidents, errors and measures to avoid them. Talking about mistakes should no longer be associated with blame and punishment, but rather with the desire to improve animal welfare through open communication and constructive discussions.

GNOTOBIOLOGY: CURRENT TECHNOLOGIES AND WELFARE CONSIDERATIONS

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The gnotobiology research has exponentially grown in the last few years due to the important role of host–microbiota interactions in the study of several metabolic and autoimmune diseases. The use of axenic mice, which are rendered free of microorganisms, is a straightforward approach to investigate the overall contribution of the microbiota in such interactions. A Historical Overview of Gnotobiology, with some of the most important events that lead to this day, will be presented, followed by the current technologies in use (from equipment to shipment of axenic rodents). As an example of the technologies in use, the Axenic Facility of Instituto Gulbenkian de Ciência (IGC) will be shown. A dedicated platform for axenization of different mouse strains reared, maintained, and manipulated in multi-cage isolators, highly well-established since 2005, with an annual capacity of 400 axenic mice being produced for internal requests and for the international community, in the frame of the INFRAFRONTIER-EMMA EU consortium. In 2013, the increased demand of gnotobiology experiments by the researchers at IGC led to the development of a Gnotobiology Facility equipped with racks of Individually Ventilated Cages (IVC) under positive pressure, namely the ISOcage P system. This system is designed to achieve the technical features of an isolator, allying the ergonomic, flexibility and density advantages of an IVC. Currently, the Gnotobiology Facility hosts all experiments performed on axenic mice, while the Axenic Facility is exclusively dedicated to the production of axenic mice and axenization of new strains. Since the implementation of the Gnotobiology/Axenic Facility, more than 560 different gnotobiology experiments were performed, with animals being submitted to various procedures, from gavage and regular feces collection to sophisticated surgeries. To finalize, considerations and improvements regarding gnotobiotic mice welfare will be demonstrated. No specific or detailed recommendations for gnotobiotic mice welfare are available, apart from the general ones for rodent facilities, which lead us at IGC to develop our own response for situations inherent to gnotobiotic housing or husbandry conditions. We have changed to colored bottoms and to use cage lids, changed the bedding, have been using different nesting and environmental enrichment materials according to cage type (breeding, stock, experimental), diminished the exposure to light and noise inside isolators, and refined our protocol of fostering fetus inside an isolator for germ-free derivation, avoiding foster mother stress and nest disturbance. An acclimatization period is also in place when transferring mice from our production isolators to the ISOcages. By changing simple elements, we have achieved better breeding performances, higher adoption rates on germ-free rederivations, and have greatly decreased the stress of experimental animals. Better welfare for sure means high gnotobiotic standards and a general good research outcome.

INHALED VOLTAGE-GATED SODIUM CHANNEL NAV1.7 AND NAV1.8 INHIBITORS SUPPRESS COUGH IN A GUINEA PIG MODEL

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Cough is one of the most important airway defensive mechanisms for lung health. However, in the case of cough dysregulation, this reflex can become excessive, irritable or troublesome as seen in respiratory diseases, and this is considered as pathological cough. Respiratory nodose Aδ- and jugular C-fibres are involved in the regulation of cough. The available data suggest that changes in primary sensory neuron excitability may contribute to the development of cough hypersensitivity. Unfortunately, available antitussive therapy still has a limited efficacy. Since voltage-gated sodium channels (Na_Vs) are required for action potentials initiation and conduction irrespective of the stimulus, Na_Vs became a promising neural target. We recently showed that inhalation of $Na_V1.8$ blocker A-803467 (3 mM) and $Na_V1.7$ blocker *PF-05089771* (100 μM) administered alone caused about 50 to 60 % inhibition of cough. In this study, we focused on the evaluation of inhaled mixture of $Na_V1.8$ blocker A-803467 (1 mM) and $Na_V1.7$ blocker *PF-05089771* (10 μM) in lower concentrations on cough in guinea pigs (Dunkin Hartley, male, 250-350 g, obtained from Innovo, Iszaszeg, Hungary) tussive challenge model. We used a standard method for cough provocation using an aerosol of capsaicin (50 μM) or citric acid (0.4 M) according to the ERS guidelines on the assessment of cough (Eur Respir J 2007;29:1256-1276). Prior to the beginning of the experiment, guinea pigs were adapted to laboratory conditions (they were placed in the whole-body plethysmograph) by inhalation of PBS aerosol for 5 min at least twice on different days of the week. An experimental group was pretreated with the aerosol (created by nebulizer connected to the plethysmograph) of $Na_V1.7$ and $Na_V1.8$ inhibitor mixture for 10 min followed by inhalation of capsaicin or citric acid aerosol together with inhibitors for 5 min. The interval between

cough challenge was 10 days and the duration of sub-experiment was approximately 5 weeks. No analgesic treatment was required and no respiratory distress was observed. Inhaled solution of A-803467 (1mM) and PF-05089771 (10 μM) mixture inhibited the capsaicin-induced cough by ≈60 % (7.9±0.54 vs. 2.9±0.5, n=13) compared to vehicle and without changes in respiratory rate. Similarly, inhaled A-803467 and PF-05089771 aerosol significantly suppressed the citric acid-induced cough bursts by ≈ 65 % (6.8±0.54 vs. 2.4±0.23, n=15) and no changes in respiratory rate were observed. These results are consistent with electrophysiological studies where action potential discharge in jugular C-fibres was inhibited by selective blockers of Na_Vs channels. Our previous and present studies indicate that $Na_V1.7$ and $Na_V1.8$ may present promising therapeutic targets for antitussive therapy.

Supported by VEGA 1/0041/23.

HUMANELY ENDING LIFE OF LABORATORY RATS WITH CO_2

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In 2019, around 5.4 million mice and 900,000 rats have been euthanized for research purposes in the European Union and 400,000 mice and 64,000 rats in Switzerland. Carbon dioxide (CO_2) is the most commonly used gas agent for killing laboratory rodents. Rodents are aversive to CO_2 and it is assumed that they feel stress and discomfort when exposed to it. A recent systematic review of available literature on this subject showed highly variable impacts of CO_2 on rodent welfare. Thus, additional studies are needed to accurately evaluate the impact of CO_2 euthanasia on the welfare of laboratory animals. This study aimed to compare the physiological impacts of two different CO_2 concentrations during euthanasia in rats. 2 months-old females and males Sprague Dawley rats were used for this study. To provide a robust multifaceted approach, several state-of-the-art techniques were combined to assess different physiological parameters at the same time, in the same animal. All animals were implanted with DSI (Data Science International) radiotelemetric transmitters to acquire EEG and EMG and cardiovascular parameters (ECG, blood pressure), body temperature and activity. Thereafter, they were placed one by one in whole body plethysmography transparent chambers gradually filled with either 30 % or 70 % CO_2 to evaluate the respiratory function during euthanasia. Video recordings were acquired in parallel to investigate facial expressions and behavior. After death, blood was collected to analyze stress parameters (ACTH, corticosterone, adrenaline, noradrenaline), as well as lungs and upper airways for future examinations. We determined that rats exposed to 70 % CO_2 lost righting reflex and became motionless sooner than rats exposed to 30 % CO_2 (56 vs. 91 s). Only 8 % of the animals euthanized with 70 % CO_2 became motionless before manifesting gasping compared to 45 % of counterpart rats in the 30 % CO_2 group. The cessation of neocortical activity occurred faster with 70 % than 30 % CO_2 (86 vs. 162 s). The mean blood pressure increased in both groups immediately after CO_2 exposure but decreased faster with continued exposure in the 70 % CO_2 group. The heart rate decreased rapidly after CO_2 influx in both groups, but the decrease was significantly faster in the 70 % CO_2 group. Respiratory rate and tidal volume significantly increased after CO_2 influx in both groups with the increase of respiratory rate being significantly higher for the 30 % CO_2 group. No significant differences were observed between groups for adrenaline and noradrenaline plasma levels. Corticosterone levels were higher in the 70 % CO_2 group compared to 30 % CO_2 group. Our results suggest that 70 % CO_2 euthanasia is faster than 30 % CO_2 but animals might encounter more discomfort when gasping before the loss of righting reflex.

THE ALTERNATIVE METHODS IN ANIMAL USE WITHIN RESEARCH AND EDUCATIONAL INSTITUTIONS IN GREECE: THE FIRST DATA

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The most valuable of the three terms commonly known as the Three Rs (3Rs), the internationally established principles formulated by W.M.S. Russel and R.L. Burch in 1959, is Replacement. It is defined as the alternative methods that avoid or substitute the use of animals for research and training purposes. This includes absolute replacement, for example by *in vitro* and *in silico* models, as well as relative replacement by invertebrates, due to their lower potential for perception of pain. Despite the undeniable scientific benefits of animal model use, the raise of profound ethical concerns among the members of the scientific and public community demands the development of a transition strategy in order to move from existing established methods to alternative ones. Nevertheless, accessing up-to-date information on replacement tools can be a challenge. With regards to Greece, there is no official statistical database for the alternative methods used in the country's various Institutions. In this study we demonstrate the first initiative to gather data regarding the current status of animals' alternatives used in Research and Education in Greece. A questionnaire survey was designed and will be distributed to the correspondent personnel in research and educational institutes as well as Greek hospitals, with user authorization. The collection and the analysis of the surveys results will present an overview of the percentage and the types of non-animal methods used among research and training facilities in the country before the construction of a relevant, official database.

EXPERIMENTAL MURINE MENINGITIS MODEL WITH NEISSERIA MENINGITIDIS, SEROGROUP B

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Experimental animal models for meningitis have been proven useful to study both the pathogenetic mechanisms and to test innovative therapies of the disease. The aim of the study is the development of a murine model of meningitis induced by *Neisseria meningitidis*, serogroup B, isolated from human patients, which will later be used for testing new drugs. The experiment consisted of two stages: the standardization of the bacterial inoculum and the induction of meningitis with an inoculum obtained by nephelometry. *Neisseria meningitidis* was grown on chocolate agar medium. The inoculations were made using brain-heart infusion broth (BHI) supplemented with Iron Dextran, establishing the concentrations for the experiment of 10^7, 10^6, 10^5 CFU/ml. The animals who were included in the experiments come from the SPF Animal Facility of the Cantacuzino Institute, Bucharest. 20 BALB/cN, mice, female, aged 10 weeks, maintained in SPF conditions, were used and were divided into 3 groups, depending on the bacterial concentration received plus the control group. 10 µl of the inoculum was injected into cisterna magna by stereotaxic frame (Harvard Apparatus), in previously anesthetized mice with Ketamine and Xylazine. The animals were monitored for 7 days, and in the first 2 days after inoculation, infected animals health deteriorated accompanied by a decrease in body weight, diarrhoea, photophobia and lethargy. Towards the end of the study, the health of the animals improved, the hematological examinations indicating only the presence of a moderate level of inflammation. The animals were euthanized by cervical dislocation and 4 types of samples were taken: 10 µl of cerebrospinal fluid inoculated on a chocolate agar, pieces of brain tissue that were spread on a chocolate agar plate, the rest

of the brain that was placed in 12 ml of BHI and some brain samples for histological analysis. After incubation of the samples accordingly, *Neisseria meningitidis* could not be isolated from any of the animals. In conclusion, higher concentrations of the bacterial inoculum are needed, capable of reproducing meningitis, without endangering the animal's life, thereby allowing the testing of new treatments.

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number ERANET- EURONANOMED – 3, ANTINEUROPATHO within PNCDI III.

THE WELFARE OF ZEBRAFISH

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The growing use of the zebrafish as a vertebrate model for addressing a diverse range of scientific questions is paralleled by continuous efforts to elucidate the welfare needs of the species. Stressed fish or fish in pain in an experiment will have negative effects on the result, or drastically reduce the robustness of the experimental data. The welfare of zebrafish can be impacted by daily husbandry routines and scientific procedures. Priority and constant attention should be attributed to health monitoring and biosecurity measures as well as refinements targeting improvements in anaesthetic procedures and the development of least-invasive analgesic regimes. But how to measure fish welfare? Survivorship, growth, health, reproductive performance, levels of blood/body cortisol, behaviour and affective states are common indicators of fish welfare mainly during husbandry practice. The steady rise in the plethora of experimental procedures carried out on zebrafish should be also utilized to improve the understanding of fish welfare, i.e. by adapting welfare scoring systems for different experimental procedures; and developing efficacious analgesic regimes and protocols to alleviate their welfare impact. The scientific challenge is today represented by the standardization of health status and welfare provisions, which stem from a comprehensive understanding of the animal's physiology and adequate application of experimental techniques. Scientists, animal welfare officers and veterinarians specializing in this area are the principal actors in the implementation of the 3Rs and in setting the welfare of zebrafish as integral component of the research needs.

STATISTICAL DATA AS A TOOL OF 3RS

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The tasks of Animal Welfare Bodies (AWB) are defined by the European Union (Directive 2010/63/EU of the European Parliament and of the Council; Articles 26 and 27) and national legislations (in Hungary the Decree 40/2013 of the Hungarian Government; Article 39). Furthermore, defined duties, the University of Debrecen Committee of Animal Welfare, is participating actively in collecting and reporting statistical data to the official authority, the National Food Chain Safety Office, Hungary. We would like to present the last eight years period data (2015-2022) at the University of Debrecen on the alternation in the number of projects (authorized vs. authorized active projects), the changes in the total number of experimental animals (conventional and genetically altered animals) by species, used in research and for education and training, the actual severity (mild, moderate, severe and non-recovery). We also demonstrate the changes in the estimation of the severity of the experimental procedures (given by the researchers during the project application procedures vs. the severity given by the authority) and the retrospective severity assessment evaluation at the end of the project. The aim of our presentation is to show how the summarization and analysis of statistical data can help the work of animal welfare bodies and, through this, the activities of principal investigators and research participants, the planning of experiments, their retrospective evaluation, and their correct severity classification.

PERCEPTION OF THE IMPORTANCE OF TEACHING ABOUT LABORATORY ANIMAL SCIENCE TO PROFESSIONALS OF HEALTH GRADUATION COURSES IN SÃO LUÍS – MA, BRAZIL

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In vivo experimental research has existed for centuries and has brought many benefits to human and non-human species. Among those benefits, we can mention the discovery of vaccines, treatments, and knowledge about anatomical features of the various species. However, for many years, experiments were performed without due regulation. In Brazil, with the approval of the Arouca Law (11794/2008), the breeding and use of animals in research, teaching and testing throughout the national territory are now submitted to this Law. Consequently, there was a need to improve aspects and techniques of teaching, breeding and research. Currently, the harmonization of teaching practices and experimental protocols are prioritized. Laboratory Animal Science (LAS) is a multidisciplinary subject and like other areas is in the search of improvement in skills and competencies. The aim of this research was to evaluate the degree of knowledge on Laboratory Animal Science of future teachers, researchers, and health professionals in the state of Maranhão, Brazil, since this discipline is not offered in normal curricular courses. Questionnaires were applied to 249 students from three Universities in Maranhão: students of biomedicine, biological sciences, pharmacy, medicine, and veterinary. The results demonstrate that the careers which are the focus of this research show fragile training in the area of Laboratory Animal Science (LAS) with little knowledge about technical responsibilities concerning breeding facilities and animal research. It was concluded that LAS needs to be a mandatory curriculum discipline for the courses of Biological Sciences and Veterinary and an elective course for Biomedicine, Pharmacy, and Medicine in order to give these professionals enough knowledge to better work in research, teaching, and testing when using experimental animals.

LABORATORY ANIMALS AS SUBJECTS OF THE ADMINISTRATIVE PROCEDURE

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The legislation dealing with animal protection is a very big issue included many sources of law. This problematic is constantly evolving. We have to know a lot of legal norm, when we want to use the animal for the scientific or education purposes. Many rules and obligations we have to follow if we want to expand knowledge which can make better of the natural environment and make better condition for live to humans and animals. We have to demonstrate our understanding to take care about animal and do not cause the animal a level of pain, suffering and distress more than it is necessary. But we must know more. The project and its authorization are subject to the procedures of administrative authorities. This administrative procedure has its own rules and principles, which we should know. Which form of the project proposal is actual? Who is the competent authority? What can I do, when the project proposal is not perfect? When I can start the project? The answers of some of these questions are said in Act No. 246/1992, but in Act No. 500/2004 too and the explanation of main and basic principles of the administrative procedures is the aim of this presentation.

NUTRITION OF LABORATORY ANIMALS: BALANCING WELFARE CONSIDERATIONS WITH RESEARCH STUDY REQUIREMENTS

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A nutritionally balanced diet is important both for the welfare of laboratory animals and to ensure appropriate experimental design and

results. Feed nutrition and intake, nutrient absorption and utilization can be affected by many factors. Environmental factors (for example, bedding, disturbing stimuli, temperature, light cycles, or other environmental conditions), physical form of diets (pelleted, extruded diets), stage of life (for example, growth, pregnancy, or lactation), genetic background (strain differences in nutritional requirements), and specific research conditions are all key factors that can change the requirements for ensuring the proper nutrition of laboratory animals. Experimental procedures may cause stress or otherwise alter food and water intake. The aim of our study was to describe approaches to the nutrition of laboratory animals during specific experimental procedures in order to prevent the development of poor nutritional status. We focused on nutrition of irradiated mice where the gastrointestinal syndrome and sialadenitis are two major problems that can cause bad nutritional condition of mice. Also paralysis of limbs and inability to move after application of various compounds or viral agents may lead to inability to ingest food. A common problem during research projects is that administration of therapeutics in water or test substances in diet can lead to dehydration and anorexia. We also were concerned with the nutrition of transgenic mice with weak teeth, we were comparing different types of pelleted diets. Animal welfare and appropriate nutrition are important priorities for biomedical research study design and its quality and reproducibility. This study provides some key concepts in nutrition to prevent the occurrence of pathological and behavioural disorders in animals during the specific experimental study design in the biomedical research. Better animal welfare can be achieved when nutrition of laboratory animals is better adapted to the specific research study design.

KOREA NONHUMAN PRIMATES RESOURCES BANK (KNHPRB) IN SEOUL NATIONAL UNIVERSITY HOSPITAL MARMOSET MODEL NETWORK CENTER (SNUH-MMNC)

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Recently, while the demand for non-human primates as laboratory animals has increased, difficulties in animal supply, cost burden, and lack of research facilities have become obstacles to research activation. In order to overcome these limitations and promote collaboration among domestic researchers, Korean Ministry of Food and Drug Safety established Laboratory Animal Resources Bank, and designated the Seoul National University Hospital Marmoset Model Network Center (MMNC) as a nonhuman primates resources bank which nationally manages and shares nonhuman primate-derived resources such as fixed tissues, paraffin blocks, sectioned slides, serum samples, primary cells, freezing sperms, etc. SNUH MMNC fulfils securing marmoset resources, standardizing marmoset breeding and quality management, and development of marmoset disease models to support researchers who need primate research. Korea NonHuman Primates Resources Bank (KNHPRB) stored 432 resources derived from cynomolgus monkey, rhesus monkey, and marmoset in 2022. These resources includes samples of normal animals, Parkinson's disease model, osteoporosis model, marmoset wasting syndrome samples, etc. These activities are expected to provide academic support and high-quality laboratory animal (primate)-derived resources to researchers who wish to conduct primate research in the field of biomedical research. Nonhuman primate resource bank provides more easily access to valuable primate research and more effective way for expanding ethical experiments that may potentially reduce the numbers of required laboratory animals.

LAS INTERACTIVE – AN ONLINE INFORMATION AND TRAINING PLATFORM FOR LABORATORY ANIMAL SCIENCE

Exner, C., Linklater, N.
LAS interactive GmbH

Every person who works with laboratory animals must have the appropriate expertise, be knowledgeable about the 3Rs (reduction, replacement, refinement) and undergo regular continuing professional development (CPD). Among other things, the harmonization of Europe-wide training is one aspect of education and training in laboratory animal science (LAS). Modular course structures and species-specific focal points are intended to help ensure that training is not only tailored to requirements, but also to animal welfare. To advance this, web and digital training resources enable the LAS community to share their collective knowledge and expertise and they offer the opportunity to address this in shared, open platforms or by interlinking different efforts by e.g., common login-portals or forums where people share training experiences and didactics. LAS interactive is such a collaborative approach. Contributors of LAS interactive are experts from different areas of animal research from universities, research facilities and industry partners. LAS interactive has been available for more than 15 years. It was originally developed at the University of Marburg with funding from the German Federal Ministry of Education and Research and the German Research foundation. To ensure the continuance of the platform, a company (LAS interactive GmbH) was established in 2019, with the Philipps-University of Marburg as a shareholder. Presently, LAS interactive consists of the freely available information platform vtk online and fee based online courses (las campus). The platform includes information on 16 species, such as mice and rats but also other species such as bats, dogs, spiny mice, songbirds and others. LAS interactive is structured according to the modular system suggested by the EU Expert Working Group on Education and Training in LAS and is further being built up to include topics that have not yet been addressed in the modular scheme, such as working with hibernators or to include specific techniques such as telemetry. Techniques are illustrated by pictures and videos of live animals or by introducing teaching alternatives and interactive elements. The content of the platform is currently available in three languages (German, English, French) but can easily be adapted to further translations. The future aim of LAS interactive is (in collaboration with experts from other institutions) to expand the content to more species such as wildlife and farm animals, outlining the different challenges when working with these animals in the laboratory or field. By providing information that links species specific needs with research methods, legislation and the 3Rs, LAS interactive promotes the best practice for working with animals in research. In collaboration with 3R-SMART (https://3r-smart.de) we are developing a common training portal for both the LAS community and efforts to replace animal experiments. In line with the idea to disseminate and advance knowledge about the 3Rs we are working on developing a 3R curriculum that can be integrated in laboratory animal science courses to support the development of 3R-competencies.

DESIGNING A ZEBRAFISH HEALTH MONITORING PROGRAM

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This presentation is about providing insights in developing a health monitoring program for zebrafish. The program must aim to detect both infectious and non-infectious diseases, including pathogenic and non-pathogenic conditions and quarantine procedures to prevent the spread of disease within the colony and to new colonies. We will examine how different sample types are impacting diagnosis and how prevalence and institutional prevalence are important in determining the sample size. The goal of this presentation is to contribute to the development of effective measures for the prevention and control of diseases in zebrafish.

INNOVATIONS IN THE ANIMAL FACILITY; HOW LABORATORY ANIMAL TECHNICIANS INFLUENCE LABORATORY ANIMAL SCIENCE

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The profession of animal care has a long history and as changes have occurred throughout laboratory animal science, the profession has increasingly changed. Hardly any animal house can be run without laboratory animal technicians. In addition to the basic care of the animals, they are increasingly responsible for the performance, control and documentation of animal experiments. Hardly any other professional group is so well trained in dealing with animals that innovations in the sense of the 3Rs are often inspired by this professional group. I would like to give a brief overview of the measures that have resulted from good animal care. The presentation will provide an overview of the introduction of tunnel handling, a presentation of the adaptation of cage changing (less is more) and an overview of the tools that are significantly influenced by animal care. Changes in routine are sometimes difficult to implement but worthwhile. I would like to encourage laboratory animal technicians and the management to share their ideas with the community and not to stop thinking about the 3Rs. I would like to thank every laboratory animal technician who inspired me to do just that.

PHENOTYPING SPONTANEOUS IN CAGE ACTIVITY AND PLACE PREFERENCE IN INBRED AND OUTBRED MICE USING DVC®

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In cases where research outcomes are impacted by animal activity, a good baseline is needed against which to compare any changes following an experimental invention or a genetic modification, to understand, for example, the efficacy of a drug or the reliability of the animal model, including mutants (1). This presentation provides an overview of some of the most widely used wild-type mouse strains peculiarities that may impact experimental results and data interpretation. The presentation focuses on the longitudinal characterization of circadian spontaneous in cage activity, from post weaning stage to early adulthood in C57BL/6NCrl (inbred), BALB/cAnNCrl (inbred) and CRL:CD1(ICR) (outbred) mice. Assessment was conducted on both males and females, group-housed in Digital Ventilated Cages (Tecniplast), over a 24-h period for 2 months, in two different periods of the year to reduce the seasonality effect. Recorded locomotor activity was analysed based on different and commonly used circadian metrics (1). Results demonstrate differences in the circadian activity of the three analysed strains, when comparing inbred versus outbred as well as inbred strains (C57BL/6NCrl versus BALB/cAnNCrl). Further analysis obtained at a later stage on the same data set allowed us to appreciate differences under further, different behavioural perspectives, when assessing cage change impact, space preference in the cage and toilet position, as well as hygienic needs. In conclusion, we were able to clearly describe and confirm differences between strains in spontaneous activity, with CRL:CD1(ICR) being the most active and dynamic strain, yet with the narrowest spatial activity, C57BL/6NCrl being the most susceptible to environmental stimuli and BALB/c being the least active strain, showing a spatial activity which is more dispersed in the cage. Furthermore, DVC® technology is a powerful and promising tool not only when considering its potentials for experimental design, but also when considering data analysis over time.

The authors are grateful to CNR-IBBC/EMMA/Infrafrontier/IMPC for technical assistance with mouse colonies. Further acknowledgements go to Charles River Laboratories Italy for providing the mice and Tecniplast SpA for providing DVCs.

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TEAM MANAGEMENT – THE KEY TO PROPER ANIMAL WELFARE

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To improve and maintain a good level of animal welfare, a key role is played by the staff involved in daily activities. The team needs people who have knowledge, can take decisions regarding animals and procedures, are empowered to do so, but at the same time understand the responsibility to act according to the rules. According to the country, the environment (e.g. industry vs. academia vs. public institutes), and the settings (large company vs. small start-up) there are differences regarding i) the culture on how to manage personnel (e.g. how to reimburse extra time, promotions, but also performance reviews); ii) which are the stakeholders involved (HR, unions, other group categories); iii) and opportunities for further educational development. During this presentation, I will introduce practical strategies on how to improve animal welfare by acting on the team of technicians, animal care-takers and veterinarians, i.e. the staff belonging to the Animal Facility. Despite the abovementioned differences, basic actions can be implemented, at different levels, even in less organized structures.

1. In the case of multicultural teams, be aware that cultural differences can be a barrier in establishing some rules, that might seem too strict or at least look strange in another culture. Clarifying what's 'your norm' will allow the other party to at least be aware of (if not totally accepting) the situation and related differences.
2. Clarify which are the expectations and goals of the department/service provider, and how performances are monitored and reviewed. Even if the HR department doesn't have a system in place, the animal facility management should be able to discuss regularly how each person is performing. One-to-one meetings will give the possibility to both the person involved and the manager to think about his performance, which are the strongest and weakest skills, if there are behaviors that are not accepted and what is encouraged. This will also be a good time to provide feedback to the manager. Of course, it would be important that both parties are trained on how to have this kind of conversations. Moreover, when setting up a new team, or hiring new members, it's easier to clarify from the very beginning how the process will work. The real challenge is when the new manager enters a team with already set-up dynamics.
3. Whenever possible, share reasoning for decisions. There will always be somebody who doesn't agree with management decisions, but providing the logic behind that will give context. For some practical aspects, the team should be allowed to take the decision and understand the consequences of that.
4. Push for training and continuous professional development. Exposing the team to different point of views would trigger internal discussions and help to further improve animal welfare in the facility. Nowadays, there are several opportunities that don't require investments (e.g. free webinars).

All these activities require time to spend on personnel management, but on the long run these strategies will have a positive effect on animal welfare and facility management.

BENEFITS AND SAVINGS OF A MICROBIOLOGICAL MONITORING PROGRAM BASED ON EAD TESTING

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The introduction of Exhaust Air Dust (EAD) testing by PCR in a health monitoring (HM) program of an animal facility equipped with

individually ventilated cages can bring several benefits. These include the improved detection of rodent infectious agents, the reduction/replacement of the animals used as sentinels, labor, time, and cost savings. The authors manage a 600 m^2 mouse facility housing around 6,000 ventilated cages, used both for breeding and experimental activities. The facility was opened in 2007 and its HM program was based on traditional soiled bedding sentinels (SBS). In 2016, the efficacy of PCR testing of EAD samples was evaluated in comparison to serology, bacteriology and parasitology carried out on SBS. The results of this test, together with the reports published by different groups, encouraged the authors to include EAD testing in the HM program of the facility. A hybrid program was developed and run for a few years, allowing comparative evaluations between EAD testing Vs SBS. The analysis of the results obtained showed that PCR testing of EAD samples confirmed or outperformed the results obtained by traditional methods on SBS, thus, supporting the authors in the decision to develop a new program, completely SBS-free. This new approach allowed the replacement of a significant number of animals used as sentinels (more than 700 every year) resulting both in ethical and economic benefits. Moreover, the new sentinel-free HM program allowed time and labor savings associated with the simplified samples preparation. Finally, this new solution contributed also to the reduction of the emotional fatigue of the staff of the facility.

IMPROVING ANIMAL MODEL TRANSLATION, WELFARE AND OPERATIONAL EFFICIENCY WITH APPROPRIATE HOUSING AND INFRASTRUCTURE DESIGN

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This session will highlight the latest, best-in-class solutions and trends for rodent housing and infrastructural designs that will allow you new tools and strategies for next-level operational and research outcome success. Current and future rodent housing units limitations and benefits, as well as, new automation strategies that improve animal model translation by elevating animal welfare are emphasized and discussed. Critical accompanying infrastructure systems and designs that provide for safe, secure, cost-effective, and high-quality operations are also presented. The main areas of automation review include: research, holding room, housing units, cage wash and linkage logistics. Each area will be discussed in detail with the key emphases on animal welfare, staff safety, staff efficiencies, cost reductions and quality outcomes and improvements for animal model translations. The target audience includes all individuals engaged in research involving animal models, including animal facility managers and directors, lab animal and research technicians, staff, veterinarians, IACUC members, and scientists.

INTRODUCING INTERNATIONAL COUNCIL FOR LABORATORY ANIMAL SCIENCE (ICLAS)

Helppi, J.
International Council for Laboratory Animal Science (ICLAS), Belgium

The International Council for Laboratory Animal Science (ICLAS) is a scientific organization dedicated to advancing human and animal health and well-being by promoting the ethical care and use of animals in research worldwide. ICLAS consists of National Members, Scientific/Union Members, Institutional Members, Associate Members, and Affiliate Members. National Members are represented by a government agency or ministry concerned with research at a national level. Scientific Members are most often national or regional associations for laboratory animal science. ICLAS members are part of a network of national and international organizations that promotes basic harmonization in standards for Laboratory Animal Science, with a priority of encouraging quality animal-based science in developing nations. Through ICLAS, members have a voice in respected international science unions and agencies such as the World Organization for Animal Health (OIE, WOAH), the International Science Council (ISC), the Council of International Organizations in Medical Science (CIOMS) and the United Nations Educational, Scientific, and Cultural Organization (UNESCO). A successful outcome of these collaborations is the publication of the CIOMS-ICLAS International Guiding Principles for Biomedical Research Involving Animals. On a practical level, ICLAS can assist its members financially, for example ICLAS can support member associations with costs of a training session or congress. The ICLAS Europe Regional Committee offers travel grants for individuals to learn new skills, and train-the-trainer grants to assist with development of training courses in laboratory animal science. Harmonization and promotion of standards of laboratory animal quality, care, and ethical consideration benefit the ability to collaborate internationally, and improve confidence in accepting science protocols conducted across nations. Research facilities and laboratories participating in the ICLAS Laboratory Animal Quality Network (LAQN) and Performance Evaluation Program (PEP) are directly assisted in validating the microbiological and genetic quality of their research animals.

SERVICE-ORIENTED MANAGEMENT OF AN ANIMAL FACILITY BENEFITS EVERYONE (SCIENTISTS, ANIMAL CARETAKERS AND ADMINISTRATION)

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The management of animal facilities is becoming increasingly challenging, mainly due to the increased demands of scientists, and many animal facilities are under pressure to provide more than just standard animal housing. Appropriate management techniques applied in the day-to-day operation of well-designed and constructed animal facilities can lead to better motivation of staff and more effective operation of the facility. This would result in more work being done with fewer staff who are better trained and motivated, leading to fewer mistakes and ultimately better animal welfare. The Biomedical Services of the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, uses a full-service ideology in its animal facility that goes beyond standard housing and husbandry of experimental animals to include comprehensive technical services including techniques such as tissue biopsies, blood and organ collection, perfusions, colony management and database management, to name a few. All services are provided on a fee-for-service basis, with a comprehensive costing matrix providing a powerful management tool for animal facility management. Animal care staff are thoroughly trained from day one to provide the best possible service, and much emphasis is placed on creating a good team atmosphere, flat hierarchies and motivated staff. No employee works only in animal care, even trainees are involved in the technical service on a daily basis. Our facility also goes to great lengths to build inspiring and fruitful relationships with the researchers who use the animal facility. This presentation will summarize the above principles and explain how our animal facility is managed and organized. It will also include some solid data on costs, the number of animal caretakers needed for different tasks and much more. In short, this presentation will provide various solutions to some of today's management challenges, but also take a look at the possible challenges of the near future.

CUSTOMIZED EDUCATION AND TRAINING PROGRAM FOR LABORATORY ANIMAL MANAGER IN SEOUL NATIONAL UNIVERSITY HOSPITAL

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Department of Experimental Animal Research in Seoul National University Hospital (SNUH-DEAR) was certified as GLP facility by KFDA (2003), fully accredited by AAALAC international (2007), and designated as an Excellent Animal Testing Facility by KFDA (2012). As the demand for animal testing increases in the biomedical research field, the importance of quality control, housing system, and animal welfare of experimental animals has also increased. And, the quality of research and the welfare of experimental animals greatly depend on the competence of laboratory animal managers as well as researchers

involved in the care and use of the animals. In accordance with the implementation of the Animal Protection Act (2008) and the Act on Experimental Animals (2009) made to strengthen the ethics and reliability of animal testing by reinforcement of 3Rs (Replacement, Reduction, Refinancing), strict operation of Animal Ethics Committee, and systematic training of laboratory animal managers. In order to secure the ethicality of animal experiments and to strengthen the capabilities of laboratory animal managers, SNUH-DEAR has performed the customized education and training programs for 20 years. Education and training are conducted through On-the-Job-Training (OJT) according to the proficiency and job duties of laboratory animal managers.

Level 1 (Education of new employee): Legal and guidance education are provided as essential education training for new users.

Level 2 (Training for new assignee): Training on SOP and guidelines for the relevant assignment when a new position is assigned.

Level 3 (Occupational Training for experienced Worker): All employees of each job are regularly provided training to improve their ability to perform their duties.

Common education: All employees of each job are provided once a week according to the education and training plan.

OJT program includes entire subjects such as genetics and breeding, housing and care, macro and micro environment, facilities and equipment management, drug management, safety management, veterinary care, etc. And the effects of education and training were monitored by QA/PAM. This customized education of experimental animal managers is improving 3Rs compliance, animal welfare, and research reliability.

STRAIN DIFFERENCES IN RESPONSE TO ANALGESIA IN MICE

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The laboratory mouse is the most common animal studied in the field of pain and analgesia. In this study we compared the effects of opioid and non-opioid analgesia in five strains of mice using hot plate and tail-flick methods. Our study aimed to find some correlations to guide the choice of the most effective analgesic in an experimental animal model. The results of this study show relevance in experiments that assume the partial abolition of pain sensitivity. The study consisted in detecting the thermal-pain sensitivity threshold pre- and post-analgesia by direct exposure of the animals to the thermal stimulus. The obtained values allowed the assessment of the effectiveness of the installed analgesia. Five mouse strains, B6129PF2/J, B6.Cg-Kit$^{W\text{-}sh}$/HNihrJaeBsmJ, WB/ReJKit^{W}/J, BKS.Cg-m+/+Lepr^{db}/J, B6129S2-Thbs1^{tm1Hyn}/J, purchased from Jackson Laboratory, were used for the experiments. Each experimental group consisted of ten adult animals, males and females. Both non-opioid analgesics were administered per os, 100 mg/kg Paracetamol and 500 mg/kg Metamizole sodium. The synthetic opioid Tramadol was administered intraperitoneally at 40 mg/kg, while Fentanyl was injected subcutaneously at a concentration of 0.25 mg/kg. All drugs were administered once for each test, and two days off between tests. All five strains of mice treated with Fentanyl showed significant differences in the reaction time in both tests, tail-flick and hot plate, in comparison with the baseline values. Tramadol elicited significant analgesic activity depending on the strain and the test. The significant differences was observed for B6.Cg-*KitW-sh*/HnihrJaeBsmJ mice treated with Tramadol when evaluated by tail-flick. B6129PF2/J mice presented significant differences in the reaction time in both tests when received Tramadol. WB/ReJ KitW/J and BKS.Cg-m+/+Leprdb/J mice presented significant differences in the reaction time in hot plate test when received either Tramadol or Metamizole sodium. Observation in all strains treated with Metamizole sodium did not give any significant analgesic effect in comparison with baseline when tested by tail-flick. Paracetamol induced significant analgesic effect just for B6129S2-*Thbs1tm1Hyn*/J mice evaluated by tail-flick test. In conclusion, Fentanyl and, in a lower degree, Tramadol produced profound analgesia in all strains of mice, while Paracetamol had the lowest analgesic effects. The different response to analgesics of different strains, as well as the differential effect of distinct classes of analgesics, are parameters that must be taken into account in experimental animal models.

This work was partially supported by Core Program, implemented with the support NASR, projects nos. 23.16.02.02 and 31PFE/2021.

SEOUL NATIONAL UNIVERSITY HOSPITAL MARMOSET MODEL NETWORK CENTER (SNUH MMNC) IN KOREA

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Recently, the demand of non-human primates for biomedical research has been increased for several decades. Although macaque monkeys such as Cynomolgus and Rhesus have been widely used in NHP research, they have disadvantages such as securing husbandry space, low reproductive efficiency, difficulties in handling and risk of zoonotic infections. In comparison, common marmoset (*Callithrix jacchus*) has emerged as an attractive NHP to overcome the limits of macaque monkeys. Marmoset has several advantages in breeding, handling, diseases and small body size. For these reasons, US, China, Japan, and Europe countries regard marmoset as a crucial strategic bio-resource and are striving to secure marmoset and establish infrastructure. However, in Korea, domestic colony and breeding system for marmoset and related infrastructure were not well established. In 2021, Korean National Bio-Resource Project was launched and SNUH MMNC project was selected to strategize the marmoset as a national bio-resource. SNUH MMNC project has carried out for three years, and the ultimate goals are: 1. Securing marmoset resources, 2. Standardizing marmoset breeding and quality management systems. 3. Development of marmoset disease model. For these goals, SNUH MMNC team is composed of laboratory animal medicine, microbiology, clinical disease, obstetric and genetically engineering experts. Each expert communicates regularly and hold SNUH MMNC symposium twice a year. Also SNUH MMNC organized a public relations team to promote the role of MMNC to the veterinary and biology major students, and researchers through on-offline. The marmoset resource bank will be operated to encourage researchers to utilize the marmoset for research. We expect that these efforts will develop the quality of biomedical science and contribute to the improvement of public health of Korea.

CLINICAL SCORING OF SYMPTOMS OF CHEMICALLY INDUCED COLITIS IN EXPERIMENTAL RATS DURING COLONIZATION OF THE GUT PROTIST BLASTOCYSTIS

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Blastocystis appears to be a very common intestinal protist found in humans and animals, but its role in health and effect on the microbiome remain poorly understood. While Blastocystis is sometimes associated with inflammation and gastrointestinal symptoms, increasing number of studies show that this protist is often present in asymptomatic people and is more common in health than in disease. The inconsistent view of Blastocystis in health and diseases reflects persistent gaps in our knowledge about factors that influence host colonization and its interaction with the gut microbiome. In our study, we directly tested the impact of Blastocystis ST3 colonization on the immune system and the gut bacterial microbiota alone, and in combination with colitis induced by a single per-rectal application of DNBS (58 g/l in 50 % EtOH). Here, we focus on the clinical scoring of the health status of the experimental rats prior and after colitis induction during Blastocystis colonization. We experimentally inoculated outbred female Wistar rats (SPF, 13 weeks old, from Envigo RMS B.V.) with Blastocystis ST3, and then induced colitis after three weeks (short-term exposure experiment) and after three months (long-term exposure experiment) of colonization. We

monitored the intensity of inflammation in colonized rats compared to the control group based on the cytokine's gene expression, macroscopic and microscopic observation, clinical data, and the bacterial microbiome. We monitored weight loss, stool consistency, hematochezia, and after dissection macroscopically evaluated the length and inflammation of the large intestine. We also qualitatively observed other clinical signs of colitis, including apathy and dull coat. Our results show no effect of the short-term colonization on gut inflammation, but the long-term exposure to *Blastocystis* ST3 appears to promote a faster recovery of rats from colitis. We detected a significant reduction in inflammatory markers (TNFα, IL-1β) and in pathology two days after colitis induction in the colonized group, as well as improvement of clinical scores in this group. In case of the short-term colonization, we observed no difference in clinical measures and macroscopical evaluation of the intestine between colonized and non-colonized group. In contrast, in terms of long-term colonization, we found worse health indicators in *Blastocystis*-colonized rats compared to control groups after colitis induction, but these trends were reversed immediately the following day. One day after colitis induction, colonized rats showed significantly greater weight loss and shortening of intestine, more pronounced apathy and dullness of coat as well as significantly more pronounced gut inflammation, observed macroscopically. Surprisingly, the next day the health status of colonized rats improved considerably in contrast to control group. They manifested a significant weight gain, hematochezia appeared less intense, and a reduction of intestinal inflammation and significant extension of the large intestine were observed. For more detailed results on gut bacterial microbiome, see our publication Billy *et al.* (2021). Overall, our results suggest that long-term colonization of *Blastocystis* ST3 may be protective against intestinal inflammation by promoting faster recovery. These results may translate to humans, who are also colonized by *Blastocystis* for long time periods.

3RS CENTRE CZECH REPUBLIC AT NATIONAL INSTITUTE OF PUBLIC HEALTH IN PRAGUE

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The Directive 2010/63/EU is implemented in the Czech Republic in the Act No. 359/2012, amending Act No. 246/1992, on the protection of animals against cruelty. The harmonized legislation covers the protection of animals used for scientific purposes and for toxicological testing within the principles of 3Rs, i.e. reduction of animals in tests, limitation of their suffering by refinement of procedures and replacement of animal testing by alternative toxicological methods. The 3Rs Centre Czech Republic is a public establishment seated at a governmental institution, i.e. the National Institute of Public Health in Prague (NIPH). The 3Rs Centre Czech Republic is actively promoting the 3Rs principles and contributing to the fulfillment of 3Rs strategy. Currently, it functions as a multidisciplinary network comprising National Reference Centres (NRC), National Reference Laboratories (NRL) and the Unit for Alternative Toxicological Methods at the Centre of Toxicology and Health Safety, at NIPH. Thanks to the active multidisciplinary approach, advanced technologies and equipment, the 3Rs Centre Czech Republic covers all three principles of the 3Rs strategy. To cover the Replacement principle, the NRL for Experimental Immunotoxicology focuses on scientific research, development, validation and standardization of New Approach Methodologies (NAMs), such as *in vitro* methods based on 3D tissues and cell lines. The Unit for Alternative Toxicological Methods implements validated NAMs and provides professional testing of chemicals, cosmetics, consumer products and medical devices in compliance with ISO standards, accreditation and GLP quality systems. The dissemination of the 3Rs concept is realized by active participation at national and international events, organized by e.g. EUSAAT, ESTIV, SETOX (Slovak Toxicology Society), PROKOS (Association of producers, importers and distributors of cosmetics and their ingredients), CLASA (Czech Laboratory Animal Science Association), etc. Members of the 3Rs Centre Czech Republic are involved in international societies such as EUSAAT, ESTIV, serve as experts for OECD, ECHA and EURL-ECVAM. Members of the 3Rs Centre Czech Republic participate in authorization of experimental projects in cooperation with the animal welfare committee at the Ministry of Health, ensuring that the approved projects fulfill the 3R principles required by the Directive 2010/63/EU. With regard to scientific quality/translatability, the Ministry of Agriculture of the Czech Republic has nominated NIPH as the contact point to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation (PARERE) and the National Reference Laboratory for Experimental Immunotoxicology as a specialized laboratory to the network of qualified laboratories participating in validation of NAMs (EU-NETVAL). The presented poster summarizes the range of activities of the 3Rs Centre Czech Republic.

Supported from ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860) and by Ministry of Health, Czech Republic - conceptual development of research organisation („National Institute of Public Health - NIPH, IN: 75010330").

THE HUNGARIAN PUBLIC'S OPINION ON LABORATORY DOGS

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In Hungary, people have very strong negative feelings about animal testing. Animal rights activists regularly try to raise public awareness by showing experiments on dogs, but they usually do so by distorting the number, severity and purpose of these experiments. The aim of this study was to assess the general awareness and opinion of people about experiments and examinations on dogs in the laboratory in the form of an online questionnaire. The respondents were given 26 questions to examine their opinions and emotional involvement. The response was voluntary and anonymous. Out of the 510 responses received, 509-507 responses per question could be evaluated. Our hypothesis that humans see the world of animal experimentation darker than reality seems to be confirmed. Our respondents found the protection of the rights of experimental animals important (98.4 %), while 21.8 % thought that there was no veterinary supervision in an experiment using dogs, and 23.6 % thought that their caregivers could abuse laboratory dogs. Nearly three-quarters of respondents (64.5 %) did not know what kind of person could perform an experiment on dogs, and 18.9 % thought it was irrelevant that the animals in the experiment were healthy. Our hypothesis was that respondents show a more negative attitude if the subject of the experiment is not a mouse but a dog. Drug testing in mice was considered unethical by 53.8 % and testing in dogs was considered unethical by 72.2 %. In general, people are not fully aware of the legal framework for an animal experiment, not everyone knows what is mandatory for the dogs in an experiment, and that most experiments on dogs are designed to protect human health.

THE IMPORTANCE OF OPENNESS IN VETERINARY EDUCATION

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It is a serious problem worldwide that the general public is not informed about animal experiments in professional forums, but instead form their opinions from social media or from emotional articles of animal/environmental protection organisations. As a result, there are many misconceptions circulating among the public, which greatly increases the rejection of animal experimentation. According to our research, people don't know what is considered animal experimentation, which species are used, and they also greatly overestimate the amount of suffering and pain involved. They don't know that it is governed by rules, let alone how strict these rules are. Unfortunately, the same phenomenon is also typical for veterinary students, which we experience whilst teaching Laboratory Animal Science in Budapest. When students arrive, they don't understand why a veterinarian needs to learn about laboratory animals, and the attitude "I would never do such

a job" is common. Animal experiments are often identified with animal cruelty, therefore attitudes are generally negative. We are continously analysing the knowledge and attitudes of Hungarian and international students through questionnaires, and we are also investigating the knowledge of the Hungarian population and where they get their information. In the current study we asked first, second and third year veterinary students about different topics from the field of animal experimentation. We received 97 answers. There is a considerable difference between the opinions of first-year students and second- and third-year students who have already received laboratory animal science education. For example while 60.8 % of first year students think that it is unacceptable to cause pain to laboratory animals, only 5.6 % of third year students think the same. And while first years students significantly overestimate the number of severe experiments. in later years most students have a more realistic view of the subject. The fact that a 15-hour course can make such a difference is certainly a promising sign that, with the right openness and information, it could be possible to improve the attitude of the public towards laboratory experiments relatively quickly.

OVERVIEW OF THE RECENT ADVANCES IN ENVIRONMENTAL ENRICHMENT FOR FISH IN THE SCIENCES

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Environmental enrichment is an important aspect of caring for fish used in scientific research. Fish, like any other animal, have complex behavioural, social, and cognitive needs that must be met in order to ensure their welfare. Environmental enrichment refers to the provision of stimuli that encourage natural behaviours, such as foraging, swimming, and social interaction. Enrichment can take many forms, including the provision of hiding places, plants, and other structures that mimic a fish's natural environment. Research has shown that environmental enrichment can have a positive impact on the health and behaviour of laboratory fish, leading to more accurate and reliable results in scientific studies. For example, studies have shown that providing fish with more space, hiding places, and social interaction can lead to reduced stress levels and improved immune function.
In practice the following enrichment was tested for use in fish welfare support:
1. Providing hiding places: Hiding places such as rocks, plants, and other structures can provide fish with a sense of security and help to reduce stress levels.
2. Adding plants and natural materials: Live or artificial plants and other natural materials such as wood or rocks can provide fish with a more natural environment and opportunities for foraging and exploring.
3. Varying water flow and current: Mimicking natural water flow and current patterns can create a more stimulating and natural environment for fish.
4. Offering toys and puzzles: Providing toys and puzzles such as floating objects, mirrors, and food puzzles can provide mental stimulation and prevent boredom.
5. Providing appropriate tank size and group composition: Ensuring that fish have enough space and social interaction is important for their physical and mental health. Proper tank size and group composition can help to reduce aggression and stress in fish.
6. Providing a varied diet: Offering a varied and species-appropriate diet can help to keep fish healthy and provide mental stimulation through foraging and hunting behaviors.
7. The use of light with different wavelengths, for instance, the use of red light is proven to be beneficial for tilapia in terms of growth rate and survival.
8. Playing music underwater – it has been proven that music can also improve the welfare of fish.
Overall, our understanding of fish welfare and how it can be maintained using environmental enrichment is still growing. However, it is important to acknowledge that fish are the most diverse group of vertebrates and as a result, not all types of enrichment may be suitable for all species. A species-specific approach is necessary when considering the enrichment of the fish environment for animals used for scientific research.

RESEARCH IN VETERINARY CLINICAL PRACTICE. ON THE BOUNDARY BETWEEN EXPERIMENTS AND TREATMENT

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Research in veterinary clinical practice differs fundamentally from translational research, since the animal represents a model for itself and the research species is identical to the target species. This facilitates ethical consideration, since the problem of transferability does not exist. In most cases, the expected benefit is also foreseeable in time and can be transferred to humans, for example, in questions about OneHealth. Attention should be drawn to a topic that many practicing veterinarians may not be fully aware of. Veterinary practitioners are allowed to perform any intervention, if there is a curative necessity. The same procedure performed by the same person but exclusively for scientific interest is legally classified as an animal experiment, which has to be applied for at the respective competent authorities. Criteria for classifying an animal experiment include interventions that serve directly or indirectly a scientific purpose (i.e. the production, extraction, storage and reproduction of substances, products and organisms or the removal of organs and tissues) or interventions on animals for educational and training purposes in combination with the possibility of this causing pain, suffering, distress or harm to the animals. With the help of some practical examples, it will be demonstrated how fine the line between an animal experiment and a clinical trial which is not subject to authorization is, and how decisions can be found. In the early planning stage of a study, it should be discussed, if an approval has to be applied for. Even if the procedure performed is not classified as an animal experiment in the legal sense, but as a clinical treatment, which may lead to interesting findings that could be published as a case report or similar, many journals request an ethical statement for all studies involving animals. If available, the file number of the approving authorities can be added. Animal welfare officers are often confronted with such situations and are asked for advice. Therefore this issue should be brought into the focus and discussed.

USE OF LABORATORY MICE IN DIABETOLOGY

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The intensity of IBMIR (Instant Blood-Mediated Inflammatory Reaction) depends on the amount of tissue factor (TF) molecules on the islet cells. At the same time, TF is an important growth factor stimulating islet graft revascularization. To reduce the intensity of IBMIR and to improve the early function of islet graft, we tested the possibility of the short-term inhibition of TF synthesis in islet cells using RNA interference. Male Brown Norway rats (250-270 g) served both as pancreatic islet (PI) donors and recipients. PI were isolated using collagenase digestion according to standard protocol. After overnight cultivation PIs were transfected with anti-TF siRNA (s130189, ThermoFisher Scientific, USA) using lipofection (Lipofectamine RNAiMAX, 50 nM; n=6) or microporation (Neon, 2 pulses, 950 V, 20 ms, 200 nM; n=6). After 24 h, treated PIs were transplanted to portal vein of streptozotocin diabetic animals in marginal dose (2 PI/g). Both methods led to a comparable decrease in TF mRNA expression – microporation of siRNA reduced the amount of TF mRNA by 76/55 %, lipofection by 75/70 % after 24/48 h, respectively. Both methods led to a significant decrease in TF protein expression as proven by western blot and a significant reduction of liver ischemia after PI transplantation as proven by MRI. PIs normalized glycemia of all recipients transplanted with lipofected PIs but none with microporated PIs. In conclusion, antiTF-siRNA transfected by microporation efficiently reduced the amount of TF for 24 and 48 h but did not improve the function of transplanted PIs. AntiTF-siRNA transfected by lipofection efficiently reduced amount of TF for 24 h and significantly improved the function of transplanted PIs. Microporation likely caused the Off-target effect in PI graft.

SUPPORTING THE 3RS BY INCREASING CATHETER PATENCY DURATION

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Access to the vascular system is a critical aspect of many research studies. Whether for the infusion of compounds or blood withdrawal, an ample period of catheter patency is necessary for medium to long-term studies. Multiple factors affect catheter patency. The most important being catheter tip placement. The catheter tip must be positioned in the ideal location for optimal patency. The second factor is the use of a closed system. Studies have demonstrated that a closed system using the Vascular Access Button™ (VAB™) has a major impact in extending the patency duration. Catheter material and tip profile also affect patency. Polyurethane (PU) is the material of choice and a round catheter tip minimizes damage to the lining of blood vessels. Sterility of components is another factor. Non-sterile components such as catheters and solutions may lead to the formation of biofilms at the catheter tip. The last factor is the positive pressure technique when locking the catheter. Utilizing positive pressure reduces the probability of clot formation within the catheter. Increasing the duration of catheter patency supports the 3Rs by Reduction of the number of animals enrolled in medium- to long-term studies. The VAB™ also endorses the 3Rs by Refinement as it improves animal welfare. With the addition of the magnetic metal cap on its port, group housing is possible allowing animals to engage in social behavior. Furthermore, the VAB™ reduces animal handling and associated stress resulting in better research results.

ANIMAL RESEARCH: TIME TO TALK! HOW OPENNESS AND TRANSPARENCY ABOUT THE WAYS IN WHICH ANIMALS ARE USED IN SCIENTIFIC AND MEDICAL RESEARCH CAN IMPROVE PUBLIC ACCEPTANCE AND UNDERSTANDING

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European Animal Research Association (EARA)

In a growing number of countries, public and private research institutions have made the bold decision to adopt new persuasive practices and policies to engage with the public on the benefits and achievements of using animals in scientific and biomedical research. In Europe there are now eight National Transparency Agreements on animal research in Spain, Portugal, Belgium, France, Germany, Netherlands, Switzerland and the UK involving close to 500 institutions where research institutions have collectively agreed to commitments on pursuing greater openness with the public. A similar agreement exists in New Zealand with work progressing in Australia and the United States. These commitments are that institutions: will be proactive in seeking opportunities to explain when, how and why they use animals in research; will provide information to the media and the general public about the conditions under which research using animals is carried out and will explain the benefits obtained from using them compared to other methods of research; will develop initiatives that generate greater public knowledge and understanding about the use of animals in scientific research; will place an animal welfare statement on their institution's website. The belief is that being more open and transparent about their use of animals in research will help improve public understanding and acceptance of the use of animals for scientific purposes. The need for a collective commitment is also important. There is simultaneously growing political pressure in the USA and Europe to transition towards 'animal free science'. The research community needs to make a stronger and clearer public case for the use of animals in research. This presentation will evaluate the experience in these countries of greater openness on the use of animals in research, and explain why we need to talk more openly about animal research.

MATTEK WORKSHOP – SKIN IRRITATION TEST (SIT) ACCORDING TO OECD TG 439 USING 3D RECONSTRUCTED TISSUE MODEL OF HUMAN EPIDERMIS

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EpiDerm 3D human tissue model is used across a diverse range of applications including safety and risk assessment, and biological efficacy. Simple protocols and the evaluation of early cellular endpoints allow researchers to acquire data in few days. EpiDerm, a Reconstructed Human Epidermis (RHE), is ready-to-use, highly differentiated 3D tissue model consisting of normal, human-derived epidermal keratinocytes (NHEK) cultured on specially prepared tissue culture inserts cultured at the air-liquid interface (ALI) and allows for the evaluation of topically applied compounds, chemicals, cosmetic/personal care product ingredients and final formulations. With multiple ECVAM validations and OECD accepted test guidelines, EpiDerm is a proven *in vitro* model system for chemical, pharmaceutical and skin care product testing. EpiDerm SIT is a validated and accepted method under OECD TG 439, EpiDerm Skin Corrosion test as OECD TG 431 and EpiDerm phototoxicity test as OECD TG 498. The workshop will provide a brief overview of *in vitro* 3D reconstructed human tissue models and their use in toxicology and pharmacology as well as the practical demonstration of EpiDerm Skin Irritation Test (SIT) according to OECD test guideline 439 (TG 439). Participants will have a hands-on opportunity to practice the EpiDerm SIT with actual tissue models. The workshop is suitable for everybody who would like to practice the method, consult a specific problem or get information, as well as those who are just considering the use of *in vitro* models in their research. Number of participants for practical work is limited to 40.

RECONSTRUCTED HUMAN EPIDERMIS AS AN ETHICAL ALTERNATIVE FOR TOPICAL TOXICITY TESTING

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The potential of substances (chemicals, cosmetics, ingredients and formulations) to cause effects such as corrosion or irritation to skin and eye is a concern of toxicologists in their assessments of possible worker and consumer safety issues. Moreover, national and international regulatory agencies require that substances are labelled as to the toxicity potential to skin or eye. To prevent the unnecessary use of animals for the above-mentioned purposes, EU as well as US regulations recommend the use of 'alternative' *in vitro* tests methods "whenever appropriate and feasible". Since reconstructed human tissue (RhT) models closely mimic native tissues, they can be used for reliable estimation of hazard and risk related to human health. Tests with RhT models for topical toxicity testing are cost-effective, deliver faster and more reproducible results than many of the traditional *in vivo* assays. Their characteristics can be precisely controlled by established Quality Assurance procedures to assure long-term reproducibility, which is important in the regulatory toxicology. RhT-based assays for skin and eye irritation/corrosion and phototoxicity testing are validated and regulatory accepted at the OECD TG 431, 439, 492, and 498. A number of *in vitro* RhT-based methods have completed pre-validation testing (genotoxicity) or are ready to enter the pre-validation process in the near future. They enable testing without excessive need for laboratory animals, which is of great importance for REACH as well as for EU cosmetic legislation. This presentation will describe currently available RhT-based assays for topical toxicity testing. Approaches to the development, validation and implementation of these assays into regulatory systems and testing strategies will be discussed.

DIVERSITY IN PRECLINICAL STUDIES

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Diversity is defined as any dimension that can be used to differentiate groups and people from one another. In clinical trials, diversity is characterized by age, disability/comorbidity, weight, sex/gender,

ethnicity, religion, sexual orientation, education, and national origin. While the approach in clinical trials is to increase diversity, the focus in preclinical trials is the exact opposite, by attempting to reduce diversity as much as possible. This approach is so strongly applied in animal research and is being investigated. Just two years ago, Nature published a paper indicating that the field of preclinical pain research is male biased (Mogil, Nature Rev 2020;21:353-365). Most neurology research is performed in young-adult animals in one species (normally in rodents), one strain and one sex. This presentation will stress the translatability of this approach to the clinic. Using specific case studies in neurology, this presentation will review the contribution of several diversity parameters in understanding disease mechanism and drug development.

USING ANIMAL MODELS TO STUDY AND DEVELOP THERAPIES FOR PAIN

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Rodents are commonly used to study the pathophysiological mechanisms of pain. However, pain is a unique disease since it encapsulates the emotional experience of unpleasant events. This important aspect of the pain definition is difficult to quantify in humans and certainly cannot be evaluated accurately in animals. In animals, especially in rodents, many behavioral methods were developed to detect pain-related responses or nociception. These behavioral methods can be divided into stimulus-evoked or non-stimulus evoked (spontaneous) nociception based on whether the application of an external stimulus is used to elicit a withdrawal response. Stimulus-evoked responses, which include manual and electronic von Frey, Randall-Selitto and the Hargreaves test, were the first to be developed and continue to be prevalent. However, in recent years concerns over the clinical translatability and subjectivity of stimulus-evoked nociception have led to the development and increasing implementation of non-stimulus evoked methods, such as grimace scales, burrowing, weight bearing and gait analysis. Lately, *in vivo* electrophysiology was also added as a monitoring method of pain, which is particularly important due to the objectivity of this method. This presentation will provide an overview and discussion on the advantages and disadvantages of the most common methods of pain assessment in rodents.

MONITORING GROUP ACTIVITY OF HAMSTERS AND MICE AS A NOVEL TOOL TO EVALUATE COVID-19 PROGRESSION, CONVALESCENCE, AND RVSV-ΔG-SPIKE VACCINATION EFFICACY

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The COVID-19 pandemic initiated a worldwide race toward the development of treatments and vaccines. Small animal models included the Syrian golden hamster and the K18-hACE2 mice infected with SARS-CoV-2 to display a disease state with some aspects of human COVID-19. A group activity of animals in their home cage continuously monitored by the HCMS100 (Home cage Monitoring System 100) was used as a sensitive marker of disease, successfully detecting morbidity symptoms of SARS-CoV-2 infection in hamsters and in K18-hACE2 mice. COVID-19 convalescent hamsters rechallenged with SARS-CoV-2 exhibited minor reduction in group activity compared to naive hamsters. To evaluate the rVSV-ΔG-spike vaccination efficacy against SARS-CoV-2, we used the HCMS100 to monitor the group activity of hamsters in their home cage. A single-dose rVSV-ΔG-spike vaccination of the immunized group showed a faster recovery than the nonimmunized infected hamsters, substantiating the efficacy of rVSV-ΔG-spike vaccine. HCMS100 offers nonintrusive, hands-free monitoring of a number of home cages of hamsters or mice modeling COVID-19.

EFFECTS OF BEDDING VOLUME ON TEMPERATURE AND HUMIDITY REGULATION INSIDE CAGE MICROENVIRONMENT OF LABORATORY MICE

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Deep bedding in cages not only has been preferred by mice but also has been shown to have positive effects on animal's thermoregulation and has been characterized as an efficient method for reduction of thermal stress in laboratory mice. The aim of the study was the creation of a cage microenvironment that includes thermal insulation and humidity reduction, using larger bedding volumes along with an effective enrichment method for mice. During the 4-week experimental trials, 16 female wt (wild type, 129SV) and 16 female des-/- (desmin null) mice along with 16 male wt and 16 male des-/- mice (64 mice in total) were allocated into groups of low bedding volume (0.4 l) and high-volume bedding conditions (1.6 l) respectively. Two filter top cages, each one containing four mice, were assigned to every group. Corn cob bedding was used. Temperature and humidity were monitored daily in both room and cages for the whole duration of the experimentation. Every two weeks, consumption of food and water and as well as animals were weighed; bedding soiling condition was photographed and the percentage of soiled surface was calculated using *ImageJ*, along with the behavioural observation that took place before the biweekly cage change using Freymann's (2015) ethogram of home cage behaviors. The results showed that cage temperature was not significantly different between the two housing conditions for all groups. High correlation was detected between room and cage temperature in all housing conditions. Cage humidity was significantly lower in all groups with high volume bedding. Bedding soiling in cages surface with high bedding volume was statistically lower ($P<0.05$) and increased digging activity was observed in the same cages. Nevertheless, mice body weight and food consumption showed no variation between high and low volume bedding conditions. In conclusion, animals body weights and food consumption remained unaffected by varied bedding volumes. No difference was detected between the intra-cage temperatures in two different volume bedding conditions compared with the room temperature. High correlation of intra-cage with the room temperature was also determined. No significant difference of intra-cage temperatures was detected between low and high bedding volume groups. Intra-cage humidity levels were less correlated with humidity levels of the room. A statistical decrease in intra-cage humidity was detected in high bedding volume groups in comparison to low bedding volume groups. Finally, as was to be expected, the additional benefit of using higher bedding volume was that it provides for more hygienic environment.

ENDOCRINE RESPONSES TO MODERATE ALTITUDE HYPOXIA IN PREGNANT ILE DE FRANCE SHEEP WITH LOW OR HIGH BASAL HEMATOCRIT LEVELS

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The objective of the presented study was to investigate hormonal responses to mild hypoxia. Thirty Ile De France ewes were selected according to their hematocrit levels and were allocated into 3 groups: low hematocrit (LHct) (hematocrit range – 19.7-27.9 %), high hematocrit (HHct) (hematocrit range – 32.0-36.9 %) and mean hematocrit (MHct) (hematocrit range – 28.3-29.8 %). Immediately after shearing, ewes were transported from the Institute farm (altitude 500 m) to a mountain pasture (altitude 1440 m). Blood samples were taken by jugular venepuncture at the following time points: before transportation (baseline level), on day 7, 20 and 42 after the transport. The traits investigated were blood cortisol, thyroid hormones (T3 and T4), growth hormone, reticulocyte count and lactate. Moderate altitude exposure resulted in significant increase on day 7 in plasma cortisol levels in LHct ewes ($P<0.05$). Cortisol levels increased significantly on d 20 in HHct and MHct ewes ($P<0.05$) compared to baseline levels. Thyroxine

levels in LHct and MHct ewes were significantly higher on day 7 as compared to baseline levels (P<0.05). Triiodothyronine in LHct and HHct ewes declined significantly at d 20 compared to baseline level (P<0.05). Growth hormone levels declined significantly in HHct ewes on d 42 as compared to baseline levels (P<0.05), while in LHct and MHct ewes remained unchanged. Corrected reticulocyte count was significantly higher in HHct ewes compared to LHct ewes at d 7 (P<0.05). There was a general trend of a slight decrease in T3 and T4 levels in all ewes at d 20 and d 42. Blood lactate levels increased significantly in LHct (P<0.001) and HHct ewes (P<0.05) at d 7 compared to baseline levels. Lactate levels on d 20 and d 42 in LHct ewes and HHcrt ewes declined but remained significantly higher compared to baseline levels (P<0.05). There were significant correlations on day 7 between: GH and T3 (r=0.503; P<0.05); GH and lactate (r=0.574; P<0.01); T3 and lactate (r=0.517; P<0.05). In conclusion, adaptation of shorn pregnant ewes to moderate altitude hypoxia was associated with a slight decrease in basal metabolism accompanied by a slight increase in lactate level.

This work was financially supported by Bulgarian National Science Fund (grant KP-06-H26/2, 04.12.2018).

ANIMAL RESEARCH: FROM EMOTION TO COMMUNICATION

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Animal research has contributed to many of the medical advances we now take for granted. We have probably all benefited from vaccines and antibiotics to prevent and treat infections, and anesthetics used in all forms of surgery. Medicines can now overcome serious conditions such as diabetes, asthma, and high blood pressure. Medicines and vaccines for pets and livestock rely just as much on animal research as human medicines. In addition, the majority of the medicines that vets prescribe are actually derived from those used in humans. The general public's opinion is divided on the use of animals in research. In part, this could be due to a lack of openness and transparency about why and how animals are used in research, and a lack of awareness of the stringent rules and regulations under which such experiments are performed. Universities (as well as other research players) could be more vocal about the benefits which animal use brings to both basic and applied research. The scientists have historically been reticent to speak openly about their animal research or to open their animal facilities to the public in any way. Consequently, most of the available information has come from those opposed to animal research. This imbalance has led to suspicion and lagging public support for this work. Science is, potentially, the best source for the evidence needed to answer to the benefits of human and veterinary biomedical research. Public outreach has become an issue of growing importance for science. The scientists are often very willing to communicate their research, but can lack confidence and a nuanced approach in communicating this sensitive topic. Active communication between researchers and society is necessary for the scientific community's involvement in developing science- based policies. However, the trust of the public in scientific processes needs to be earned and kept, which will require inclusive, self-reflecting, honest and inspiring science communication. The development of a strategy for communication concerning the use of animals in basic and applied research, especially biomedical research is essential.

IMPLEMENTING THE DIRECTIVE 2010/63/EU IN THE ABSENCE OF NATIONAL LEGISLATION

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The protection and welfare of animals involved in research is an area covered by a wide range of EU legislation. Animal studies, whether for the purposes of basic research, for drug development, for higher education and training, for studying environmental/ecological factors or for the testing of chemicals or new food additives, should be carried out in compliance with EU legislation, in particular, Directive 2010/63. On the other side, animal research is undertaken in Georgia as in non-EU member states, that yet have no laws or regulations controlling such practice. These countries lack systematic norms for ethical assessment of scientific proposals, monitoring and reporting of research involving laboratory animals. As well, husbandry and housing standards, personnel training, animal health monitoring are imperfect or absent. All of the above negatively affects the quality, reliability and credibility of the research. As a result, the data obtained are often called into question, which makes it difficult, on the one hand, both to publish them and to involve scientists in international projects that are guided by strict international regulations. One possible solution to the present challenges might be the voluntary agreement of scientists to follow international regulations. This, in turn, necessitates collaboration in the selection of the most appropriate transitional standards, as well as their availability in local languages. To overcome the existing problems, one of the possible solutions may be the voluntary agreement of scientists to follow international rules. However, this in turn implies the need for coordination in the selection of the most appropriate transitional standards, as well as their availability in local languages. The implementation of such coordination can be carried out by a non-governmental organization that brings together specialists in the country who are familiar with international practice. Georgian Association for Laboratory Animal Science (GALAS), the only LAS association in South Caucasus, currently helps local scientists in solving arising challenges *via* workshops and training and provides state-of-the-art information on the Directive 2010/63 standards regarding animal research focused on the compliance of research plan and methodology with internationally recognized ethical norms (justification of the number of animals, minimization of pain and distress, adequate methods of euthanasia). GALAS, in particular, aided in the establishment of Ethical Committees on Animal Research at leading biomedical institutions, including one Interinstitutional Animal Care and Use Committee, which now controls all ABSL-2 and ABSL-3 research in the country. Several seminars and training sessions were offered for researchers and members of the Committees. All interested parties may now access a set of obligatory regulations available in local language on the GALAS website. As well, a draft change to the existing legislation is also being developed for submission to the government.

COMMUNICATION AND HEALTH CONSIDERATIONS IN MOUSE PHENOTYPING FACILITIES

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During the last years, the rapidly evolving technologies have become valuable tools in research. Mouse phenotyping is usually performed in core facilities, which provide centralized access, sophisticated equipment and expertise, offering the opportunity of longitudinal, quantitative, and sometimes even non-invasive monitoring of disease progression in different animal models, leading to reduction of variability and significant animal reduction, by replacing phenotyping methods and assays that require euthanasia. The most commonly used facilities provide services like *in vivo* imaging (endoscopy, x-ray imaging, optical imaging, ultrasound imaging, computed tomography, etc.), metabolic phenotyping, behavioral phenotyping, hematology and clinical chemistry, as well as histopathology. For mouse phenotyping facilities, effective communication is an essential tool that ensures that the management team (scientists-in-charge, operational managers, technicians), the animal welfare team and the researchers (internal, external, students) are on the same page at all times. Successful communication is necessary at different stages, such as the experimental design, the animal transfer (and the health considerations in different facility set-ups), the services delivery and the reporting and may be achieved *via* standardized procedures, which provide specifications and step-by-step instructions for all operations and activities. Good communication plays one of the most crucial roles in operating mouse phenotyping facilities and achieving high-quality research.

We acknowledge support of this work by the project "InfrafrontierGR/Phenotypos" (MIS 5002135) which is implemented under the Action "Reinforcement of the Research and Innovation

Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

THE PIG AS AN ANIMAL MODEL IN CARDIAC ELECTROPHYSIOLOGY

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Cardiac arrhythmias are the most common heart disorders in the human population. Catheter ablation is currently the main method of their treatment. It is possible to prevent the occurrence or persistence of arrhythmia by creating a set of ablation lesions in the heart. Currently, radiofrequency energy (RFA) is commonly used for ablation. Since RFA has some risks, other methods of cardiac ablation are being developed. Most recently, the use of so-called irreversible electroporation (IRE) is being tested, which should have less complications compared to RFA. This testing is realized in cooperation between the International Clinical Research Center in Brno (ICRC Animal Center) and the Veterinary Research Institute in Brno, where pigs are used as animal models. The purpose of the article is to present anesthetic and monitoring procedures during cardiac electrophysiological intervention. Crossbred pigs were used for testing (white farm pig x landrace, females, 50 kg). An intramuscular administration of the mixture ketamine + xylazine + tiletamine + zolazepam (TKX mixture) was used as premedication. Pigs were intubated and maintained on inhalant isoflurane anaesthesia. Before IRE energy applications, butorphanol was administered intravenously. Once the animals were under general anaesthesia, electrodes for a 12-lead ECG were placed on the animal's chests to monitor the cardiac rhythm. A sensor for measuring oxygen saturation was placed on the animal's tongue. A thermometer was inserted into the oesophagus in order to measure body temperature and potential thermal damage to the oesophagus during ablation. An accelerometer was placed above the sternum to monitor potential muscle contractions. Catheters were inserted through the left and right femoral arteries and veins as follows: catheters designed for intracardiac ECG sensing, voltage mapping, and endocardial ablation were inserted into the right atrium. A catheter designed for invasive blood pressure measurement was inserted into the femoral artery. Catheters were introduced into the left atrium *via* a transseptal puncture, which was performed with the guidance of an intracardiac echocardiographic probe or *via* the femoral artery. The introduction of catheters and the verification of their correct position were controlled using X-rays and 3D mapping system. After the end of the experiment, animals were thoroughly monitored till complete recovery. Based on the results, anaesthetic and analgesic protocols and intraoperative monitoring procedures used by the ICRC Animal Center Brno and the Veterinary Research Institute in Brno proved to be safe for experimental pigs used for cardiac electrophysiological interventions. Out of 40 animals in several studies there were no anaesthetic complications. Complications related to cathetrisations were rare with frequency below 5 %.

The research of IRE was supported by the European Regional Development Fund – Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and by the Ministry of Agriculture (RO0523).

EDUCATING FOR THE 3RS - GOING BEYOND MANDATORY FUNCTIONS A-D TRAINING

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Aiming to stimulate discussion, in this talk I will highlight two challenges that I argue merit more attention as we move from teaching minimum requirements for procedures with animals towards an integrated approach to educate researchers in the 3Rs of Replacement, Reduction and Refinement into their research. In the context of researcher training in biology and biomedicine, work with laboratory animal science stands out as distinct from other laboratory methods that a fledgling scientist needs to learn. For people who will do experiments on animals, training is a requirement. In particular where this requirement is laid down in law, such as in the European Union (Directive 2010/63/EU, Article 23), there is great investment in developing training resources and standardizing assessment methods. But a too narrow focus on this as training to i) fulfill a legal requirement ii) for researchers working with animals is problematic. Let us look at each of the two aspects: Training as a legal requirement: While there is great merit in efforts to standardize legally required training as an effort towards harmonizing implementation of Directive 2020/63/EU, thinking about this training as primarily a way to fulfill legislation may be counterproductive. The question of how to best use animals in research is not comparable to the question of how to avoid occupational hazards or environmental contamination when working with radiation or with hazardous substances, other contexts for which training is a legal requirement for research professionals. Such training is important to prevent harmful side effects of research, but it does not address central aspects of research quality in the way training in how to use animals in research does. It is crucial that training in laboratory animal science is planned and organized in a way that appeals to researchers as professional development and not as an obligation to fulfill and get out of the way. Training for researchers working with animals: Appropriate training is not limited to technical understanding and skills in setting up models and running them in the laboratory, but also includes conceptual and scientific understanding of what a good model is and which research questions can be answered and what mechanistic understanding can be gained from using it. This knowledge is crucial for appropriate implementation of the 3Rs (Replacement, Reduction, Refinement) of animal experiments. If the 3Rs are only part of training in Laboratory Animal Science, for researchers who are already planning to use animal models in their research, there is a risk that the Replacement potential is not fully realized.

ADVANCED REPORTING MODULE FOR AQUATIC ANIMALS IN ZEBRABASE

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Thanks to the numerous experimental advantages and rapidly evolving new techniques, small fish species are constantly gaining popularity in many research areas. With more than 1000 laboratories using zebrafish as a model organism and many more using other small fish species, the question of proper tracking and reporting of the animals used in research is becoming more critical than before. There are certain aspects that make fish tracking more challenging and complex compared to rodents. The major difference is that groups of animals must be tracked as opposed to the individuals, which are typically the basic trackable unit in rodents. The aquatic facility manager may face the challenge of tracking tens of thousands of animals from the moment of fertilization until their death, which can be a very laborious task. Moreover, from the onset of protected developmental stage, all experimental procedures have to be recorded in detail and summarized in periodical reports. Zebrabase (zebrabase.org) is a non-profit project aiming at small fish species and other aquatic animals housed in tanks. It is a dedicated and scalable database solution that allows to track animal groups and their rearrangements, and is capable of storing the breeding history as well as creating animal usage reports. Recently, a brand new Animal reporting module has been implemented in Zebrabase that allows users to track and report all the experimental procedures, including severity assessment. System for tracking and evaluating lines with a genetic burden and reporting of the genotyping events is also implemented. The Animal reporting module is tailored specifically to animals tracked in groups and provides an easy-to-use tool for every researcher or aquatic facility manager.

INCREASING INSTITUTIONAL ENGAGEMENT FOR THE REDUCTION OF THE NUMBER OF DISCARDED ANIMALS USING THE LEAN SIX SIGMA METHODOLOGY IN AN ANIMAL BREEDING FACILITY

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Most research institutions in Brazil are governmental and have their own laboratory animal breeding and experimental facilities. Animal vendors are rare and Brazilian researchers in general do not have to pay for the laboratory animals they use. Thus, most of them do not know the cost of each mouse they receive. Working in breeding facilities, veterinarians, managers, and technicians must deal with the rodent surplus every month. To reduce rodent discard we initiated a project using the Lean Six Sigma Methodology, which mapped the mouse production process, established a baseline measurement of the performance of the process, analyzed it to identify potential causes of waste, and implemented appropriate countermeasures to improve the process. Aside from the root causes for the overproduction linked to colony management, we demonstrated that the clients' orders by weight were also an important cause of the surplus. We presented the results to the director of the facility, to the IACUC, and to the Laboratory Animal Users Committee. Recognizing that people in research areas do not understand that breeding facilities are guided by processes, we clarified for them how the animal production system is designed, with a flow of people, materials, and animals. Then, we demonstrated the impact of the animal orders by weight, showing the frequency distribution of weights for every age and sex of the most used outbred stock in our institution, the Swiss Webster, and how many animals are discarded to fill only one regular order. We showed examples, such as that of ordering a cohort of mice of just one gender with a weight range of only 2 g, which requires the production of six times as many mice as an order only by age. We showed the cost, space, person-hours of technician time required, and the ethical issues involved. Aside from the resistance of some investigators in changing their orders, all of them and the IACUC members were quite surprised about the impact of the orders on the surplus and became committed to reviewing their protocols and real needs of animals' characteristics to reduce the impact on animal welfare and meet the 3Rs. Two years after the beginning of this project we can keep five times fewer females in the production colony to attend the research and could reduce the surplus of this stock. We find that transparency, communication, and the continuous use of this methodology can boost efficiency, lower operational costs, improve the sustainability of the facility, and most of all, help breeding facilities to attend the 3Rs.

NOT ALL INDIVIDUALLY VENTILATED CAGING SYSTEMS ARE CREATED EQUAL

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The scientific community utilizes the IVCs or Individually Ventilated Caging systems in a general manner. However, the use of these general terms does not adequately describe the caging systems. The type of IVC utilized may impact facility design, operational planning, and, most importantly, study reproducibility. The presentation will review the history of rodent caging systems including IVCs and a formal classification system for IVCs that encompasses an array of different approaches and technologies, each with different risk/benefit tradeoffs. There are six parameters in the classification system including airflow mechanics, rack ventilation, air change rates, cage design, intra-cage air flow dynamics, and other parameters when assessing microenvironmental conditions. These six parameters should be considered when evaluating IVC systems and the methods employed to meet performance specifications and comparing different system types between studies.

REFINEMENT OF STREPTOZOTOCIN-INDUCED DIABETIC MODEL IN RATS

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Streptozotocin (STZ) is an antibiotic which is widely used in inducing experimental diabetes mellitus in rodents. By destroying pancreatic beta cells STZ induces type I diabetes mellitus. According to current protocol the most frequently used procedure is to administer one dose of STZ (40 to 70 mg/kg) to rats aged 8 to 10 weeks. Many investigators use a single dose of approximately 65 mg/kg to establish diabetes using the procedure described in this protocol. The aim of the study was to reduce the lethality in rodents during induction type I diabetes mellitus in rats with relatively low dose of streptozotocin. 8-12-week albino rats (b.w. 250-300 g) were used for the experiment. The rats were housed in groups in plastic cages with stainless-steel grid tops and kept under standard conditions (t 22±2 °C, humidity 50 %, 12/12 hr. light/dark cycle), with free access to food and water. Rats were randomly divided in three groups, 5 animals (1 female and 4 male) in each. Females were nulliparous and non-pregnant. Experimental animals were fasted 6-8 h prior to STZ treatment, water was provided normally. Single dose of STZ (65 mg/kg; 35 mg/kg, 30 mg/kg) was used in rodents to generate type I diabetes mellitus. STZ was injected once intraperitoneally using 1-ml syringes and 25-G needles. Diabetes was defined when fasted blood glucose level was equal or greater than 300 mg/dl. Blood glucose was measured by standard glucometer kit (One Touch Glucometer, Life Scan Inc.) daily for the first three consecutive days and then every other day. Rats with blood glucose levels of at least 300 mg/dl were retested the following day. Diabetes onset was defined when fasted blood glucose level reached 300 mg/dl for 2 consecutive days. The experimental protocol was approved by the Tbilisi State University, A. Natishvili Institute of Morphology Ethical Committee. According to the results it appeared that a twofold reduction in the dose of STZ decreased the total mortality of animals during the experiment by 60 %. At the same time, the period of time required to reach the required blood glucose level did not change. In conclusion, our modifications to STZ-induced diabetic model in rodents allow to significantly reduce mortality rates in routine experiments.

SOURCES OF VARIABILITY IN ANIMAL MODELS AND THEIR INFLUENCE ON TRANSLATIONAL POTENTIAL

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Biomedical research has an ultimate role to improve prevention, diagnostics and treatment of human diseases, which are decreasing the quality of life *via* deterioration of the health status. Therefore, animal models should replicate real human diseases or pathological conditions and the data obtained in animal studies should have comprehensive translational potential or straightforward clinical application. Biomedical research outputs conducted on animals are, despite rigorous standardization and validation, influenced by known/controlled and unknown/uncontrolled variables all of which are decreasing statistical significance of the research outcomes and quite common strategy to avoid statistical insignificance is exclusion of outlier results from the research process (there is some confusion about the uncontrolled variables and outlier results, these two are not synonymous). Although this exclusion makes the samples more homogenous and data less variable, this process considerably reduces translational potential of animal studies denying their significance. One of the most obvious and often ignored biological variable is the sex bias in biomedical research. Exclusion of female mammals from research, which compromised health of women, by excluding Also the previously accepted strategy of using SPF or germ-free animals is questioned in the light of the information about the microbiome importance. Considering the animal welfare principles, scientists should apply 4Rs rather than 3Rs into the practice, where the last R stands for "rational" approach. This work discusses variables such is interspecies difference, sex, season, diurnal variation and microbiome status which should be rather considered than

ignored, because human diseases are affecting both sexes (there are more important aspects of sex bias that deserve attention and they should be reflected in the presentation).
ARRIVE guidelines https://arriveguidelines.org/arrive-guidelines
PREPARE guidelines https://norecopa.no/media/7893/prepare_checklist_english.pdf

Supported by VEGA 1/0041/23.

ZEBRAFISH EMBRYOS AND LARVAE IN BEHAVIORAL STUDIES

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Before conducting any biological laboratory test that involves living animals, it is crucial to consider whether the experiment is necessary, ethical, feasible, and whether it may cause harm to the animals. To promote responsible animal experimentation, the 3R-principle has become a vital scientific standard worldwide. In recent decades, the increased use of living animals in laboratory investigations, for both basic science and regulatory purposes, has highlighted the need to develop alternative methods in accordance with the 3R-principle to reduce the number of experiments on mammals. As a result, scientists have investigated other vertebrate species with favorable biological properties. One of the most well-established and notable species is the zebrafish (Danio rerio), which is a small laboratory ray-finned fish with outstanding biological characteristics. Zebrafish embryos and early larvae have shown potential as a replacement for adult, higher vertebrate model species due to their low cost, high throughput, and complex behavioral repertoire only a few days after fertilization. Zebrafish embryos have been proposed as an *in vitro* animal model to bridge the gap between simple assays based on cell or tissue culture and biological validation in whole animals such as rodents. Drug screenings using zebrafish embryo-larval stages align with the 3Rs principle and can replace other vertebrates in studies involving all organic systems. Another advantage of using zebrafish embryos and early larvae as a model is that they exhibit a complex behavioral repertoire only a few days after fertilization. This includes various forms of locomotion, feeding, and responses to sensory stimuli. These behaviors can be studied in a high-throughput manner using automated tracking systems, providing a powerful tool for drug discovery and toxicology screening. In our laboratory, we use zebrafish embryos and larvae in a number of behavioral tests in the study of anxiety, epilepsy, and regeneration. Overall, zebrafish embryos and larvae offer a promising model organism for behavioral studies. Their rapid development and cost-effectiveness make them an attractive option for researchers. With the development of automated systems for data analysis, high-throughput screening of drugs and chemicals is now possible, which could lead to the discovery of new therapeutic drugs. But what is also very important, it allows to limit research using adult, higher vertebrates.

THE STEREOTACTIC APPROACH IN BRAIN DISEASE SCIENCE

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The animal facility team of the Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry of Palacky University wants to share its experience using the stereotactic apparatus in two types of research studies: brain tumors and brain infections. In the beginning, it is essential to define stereotactic surgery and the main applications of this approach. Stereotactic surgery is a minimally invasive form of surgery in which three-dimensional systems of coordinates are used to chart the locations of precise spots in the body so surgeons can perform actions such as injections, stimulations, biopsies, or radiosurgery. Stereotactic surgery pinpoints affected body areas for treatment so that the surrounding tissue is not damaged (Ruth 2023). Stereotactic surgery is based on a Cartesian coordinate system, which implies that any point in space may be determined by three right-angled planes defined as the x, y, and z axes (Jankovic *et al.*, 2021). The standard stereotaxic instruments include 3-axes, a left-hand or dual manipulator arm, mouse or rat snout clamp and tooth bar, dual-sided ear bars, and a corner clamp probe holder. The main applications of stereotactic surgery in rodents are introducing fluids directly to the brain or implanting cannulae and microdialysis probes. Stereotactic surgery is used in animal research studies to target specific brain sites and directly introduce pharmacological agents to the brain, which otherwise may not be able to cross the blood-brain barrier (Geiger *et al.*, 2008). We use stereotactic surgery to create rodent brain tumors and brain infection models. Both procedures are relatively the same in the techniques and methods of anesthesia, surgery, injection, fixation, and manipulation with animals. They differ in the composition of suspension you inject into the brain in a volume of up to 5 µl in mice and up to 10 µl in rats. In the first case, it is a suspension of tumor cells. In the second case, it is inoculum. The reason for creating these models is the research of anticancer therapies or brain infection therapies focused on developing and progressing related pathophysiological changes, including inflammation and loss of adequate response or function to stimulus. Meningitis is often caused by a bacterial or viral infection that moves into the cerebral spinal fluid. All aspects of the animal studies met the acceptance criteria for laboratory animals' care and experimental use. The Animal Research Committee of the Palacky University in Olomouc approved protocols. We discovered stereotactic inoculation and injection of tumor cells into the brain is an effective way to prepare accurate and reliable laboratory animal models for studying brain diseases.

The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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THE 3RS IN TRANSITION

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Since the first publication of the 3Rs by Russell and Burch in 1959, Rs have been added or definitions modified. The addition of the 'R' of responsibility and the contemporary definitions of the 3Rs presented by the National Centre for the 3Rs in the UK are most notable examples. The contemporary definition of Replacement is 'accelerating the development and use of predictive and robust models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals'. Indeed, the art of science is to pose the right research question to the right model or combination of models, to know the strengths and weaknesses of each of the models used, and to strive for continuous model improvement. However, a recent survey among scientists by the Biotechnology and Biological Sciences Research Council in the UK (BBSRC) found that there is generally a tendency to use the model that is available rather than the best model for the question, and that researchers tend to stick to what they know (1). Similar results were reported by Del Pace *et al.* (2). Does this mean that there is a research practice that is at odds with the 3Rs and public demand to end animal experimentation in Europe? I don't think it is that black and white, but a repositioning is needed both for the sake of science and the animals.

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THE ETHICAL CHALLENGES OF HUMAN ORGANOIDS CREATION AND USE IN BIOMEDICINE

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The creation and use of human organoids implies the existence of biobanks storing human biological materials (e.g. organs and a wide range of tissues and cell types, collected within framework of healthcare) used to produce the induced pluripotent, embryonic or adult stem cells, required for creation of organoids. Organoids are *in vitro* generated complex 3D constructs of human or animal origin, incorporating advances in stem cell biology and genome editing, designed to physiologically mimic tissues/organ functions. The development of Organ-on-a-Chip (OoC), single or multiorgan, brings technology and biology together. OoC devices are part of the family of Microphysiological Systems (MPS) containing living engineered organ structures in a controlled dynamic microenvironment of a fit-for-purpose microfluidic device, capable of capturing the function or dysfunction of organs they mimic. Historically, MPS lack formal validation and wider regulatory acceptance and are typically used as exploratory tools to guide internal decision-making across many different applications, for fundamental research, precision medicine and regenerative medicine. They are expected to lead to the more human-relevant approaches in biomedical research, more effective pre-clinical evaluation of new drugs efficacy and safety and to refine, reduce and replace the animals in pre-clinical testing and biomedical research, supporting cross species translational research. The initial focus predominantly favored human based complex *in vitro* models (CIVM). Currently, the creation of animal based CIVMs are driven by the need to reproduce species-specific effects and to determine species concordance using CIVM counterparts, i.e combining data from human and animal research studies. The side effect of the progress in biomedical research is the emergence of innovative technologies and the technology-specific ethical and governance issues. The ethics in biotechnology and organoid biobanking should cover the topics and potential consequences of genome editing and cloning and should focus on the adequate consent procedures in clinical and research applications of organoids, guaranteed donor privacy, legal ownership of health data and organoids and any patent resulting from it, including commercialisation. In the biobanking context, the tissue type and its subsequent transformation (e.g. into intellectual property) highly influence the value the donor may put on their bodily material. The “people with data” are recruited into research projects through tissues and information donations (e.g. access to health records of patients with rare diseases) where their contributions are subsequently repurposed for research use, encouraged by appeals to the public good. The public thus contributes to the large scale data and, information about patients’ characteristics, diseases, epidemiological, environmental, lifestyle and societal data, all require new approach to biobanks samples manipulation and their processing as they represent genetic and functional links to the donors. Donor has a substantial and active role in the development of governing structures and is active research participant and as such enables researchers to focus on the issues that matter to people (cloning, use of animals in research), to reduce research waste and to build science the public can trust.

BUSINESS CONTINUITY PLAN FOR ANIMAL EXPERIMENT FACILITIES IN SEOUL NATIONAL UNIVERSITY HOSPITAL DURING COVID-19

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Due to the corona pandemic, many people infected around the world, and the policies such as social distancing have been implemented in South Korea. Policies are available for acute disasters such as earthquakes, fire and floods, but little is available in case of laboratory animal facilities under the COVID-19 pandemic that imposed major restrictions on the free movement of people. Laboratory animal facilities had to find a good way to safeguard the welfare of animals in their care, to prevent animal suffering, to manage, to control the infected animal takers. Department of Experimental Animal Research in Seoul National University Hospital (SNUH-DEAR) prepared the Business continuity planning (DEAR-BCP), which is a work response plan for each level of manpower reduction. SNUH-DEAR is an animal experiment facility with a variety of animals including rodents, rabbits, large animals and nonhuman primates, and its core tasks are divided into animal management, non-clinical research, and research administration support team. “DEAR-BCP” has prepared countermeasures according to individual, animal species, and area based on response plans for each level of manpower reduction according to each core task. The level of manpower reduction was classified into 1~5 %, 5~20 %, and 20 % or higher. In the event of vacancies in each range, measures were prepared for vacation, working hours, and adjusting the scope of work processing. In preparation for individual vacancies, the first and second job agents were designated, and in the event of multiple vacancies, the contents of the readjustment work were adjusted for each animal species and area. Separately, all employees of animal experiment facilities also prepared personal responses such as separation of movement paths, minimization of personal contact, and separation and use of public spaces during work. SNUH-DEAR maintained from 1 to 5 % reduction in manpower, but if these measures are considered when installing and designing animal experiment facilities, animal experiment management will be efficiently maintained in the new pandemic era, and these measures can be adopted in future pandemics that lead to restricted movement of staffs.

ADVANCED RESEARCH AND DEVELOPMENT CENTER FOR EXPERIMENTAL MEDICINE: “PROF. OSTIN C. MUNGIU” - INFINITE SOLUTIONS

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Advanced Research and Development Center for Experimental Medicine: “Prof. Ostin C. Mungiu” – CEMEX is built upon over 40 years of expertise and experience in fundamental and applied biomedical sciences, pharmacology and pain research. Completely renovated and modernized in 2016, under a $10 mil project, co-funded by the 2007-2013 European Regional Development Fund, Priority Axis 2, CEMEX has become an interdisciplinary research facility for laboratory animals with the state-of-the-art technologies, a regional and national reference unit, comparable to similar structures in European and international countries. CEMEX is now an integrated research and development department of the Grigore T. Popa University of Medicine and Pharmacy, providing a variety of services for many sectors, including human or animal healthcare, pharmaceutics or biotechnology. The state-of-the-art infrastructure and highly qualified and passionate team allow various *in vitro*, *in vivo* and *ex vivo* studies, in accordance with the requirements imposed by the international regulations, for investigation of new diagnostic/therapeutic methods/new drugs in all phases of the preclinical development.

This work was supported by a grant of the Ministry of Research, Innovation and Digitalization, CNCS-UEFISCDI, project number PN-III-P1-1.1-PD-2021-0466, within PNCDI III.

SELECTIVE CANNABINOID RECEPTOR TYPE 2 AND DONEPEZIL COMBINED THERAPY IN ALZHEIMER’S DISEASE – PRELIMINARY DATA

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As one of the major healthcare challenges worldwide, Alzheimer’s disease (AD) is a detrimental brain disorder of a multifactorial nature, with an etiopathogenesis still not completely clear, influenced by epigenetic and genetic variants combined with environmental and lifestyle factors. With over 50 million people afflicted by AD globally, and total worldwide payments of caring for these patients estimated at

$1 trillion in 2020, acetylcholinesterase (AChE) inhibitors remain the mainstay treatment option, providing only symptomatic relief without slowing the disease progression. The cholinesterase inhibitors that interact simultaneously with AChE (catalytic and peripheral sites) and amyloid-beta (Aβ) plaque deposition coupled with added properties such as antioxidant action, neuroprotective activity or endocannabinoidergic system display the potential of ameliorating the cognitive deficit in AD by restoring cholinergic activities. The endocannabinoid system is composed of at least two well-described cannabinoid receptor 1 (CB1) and receptor 2 (CB2). CB1 receptors are widely expressed in the central nervous system, where they regulate the main functions of the brain. Recent studies have suggested that CB1 receptor-specific agonists have potential therapeutic properties in AD at low non-psychotropic doses. However, major attention in AD has been paid to specific CB2 receptor agonists due to their lack of psychoactive properties, even though CB2 receptors are mainly expressed in the immune system, with relatively low expression in neurons. Interestingly, it has been found that CB2 receptors are selectively overexpressed in cells associated with Aβ enriched neuritic plaques in AD samples from postmortem human brains. In light of all these data, exogenous and endogenous cannabinoids represent an attractive and promising target for the treatment of AD. So far, no results have been published to determine the effects of donepezil and CB2 agonists' co-administration on AD-relevant behaviors and brain pathology. The aim of this study was to evaluate the effects of long-term donepezil-cannabinoids with CB2 selectivity co-treatment in AβPP/PS1 mice, a transgenic model of AD that mimics the progressive cognitive deficiency and neurodegenerative process, in pre-symptomatic and early stage of the symptomatic phase of the disease. *In vivo* studies supported by cognitive performance evaluation, multimodal imaging, histology and immunohistochemistry analysis were used to meet the objectives of this study, investigate the preclinical efficacy of long-term co-treatment associations, evaluate the impact of therapy on cerebral metabolism, identify the acetylcholine-related changes and amyloid-β quantification in an animal model. Translating the selective CB2 agonists into the clinic is not an easy challenge, but identifying innovative approaches for treating millions of AD people holds the promise of improving their daily life and CB2 agents might just prove to be effective, due to their modulation of the endocannabinoid system, as well as their effect on neuroinflammation, Aβ clearance, cell viability in the presence of Aβ, and glucose uptake in brain.

This work was supported by a grant of the Ministry of Research, Innovation and Digitalization, CNCS-UEFISCDI, project number PN-III-P1-1.1-PD-2021-0466, within PNCDI III; and "Grigore T. Popa" University of Medicine and Pharmacy, grant number 4715/25.02.2021.

ESTABLISHING PATIENT DERIVED XENOGRAFT MODELS USING IMMUNODEFICIENT MICE IN THE STUDY OF VARIOUS PANCREATIC CANCERS

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Pancreatic cancer (PC) is a dismal oncological disease with an ever-increasing incidence and high morbidity and mortality rates. Therapeutic options are limited and the five-year survival rate is 7-20 %. In comparison with other cancers, there was no distinct progress in therapy over the last few decades. Most patients with PC die as a result of tumor cells and their microenvironment developing resistance to treatment. The goal of our research is to contribute to the personalization of PC therapy using *in vivo* models. We have introduced patient derived xenografts (PDX) into immunodeficient NOD/SCID and NU/NU mice of both genders. The PDX model was used as it encourages the study of the entire PC tissue (tumor and its microenvironment) of an operated patient with PC removed. All experimental work with animals was carried out, implemented in compliance with and governed by the existing regulations and guidelines for the breeding and experimental use of animals in accordance with Act No. 246/1992 Coll. and performed by persons with authorization to work with experimental animals in a specific pathogen-free environment of Experimental Animal Welfare Department, National Institute of Public Health. Measurements and therapy application were done under general isoflurane inhalation anesthesia. Procedures of approved experimental projects "Experimental model of an immunodeficient mouse with human pancreatic cancer" (MZDR 37099/2021-5/OVZ) were followed. Animals were housed in a specific pathogen free environment and provided with immunodeficient mouse feeding and fluids ad libitum. The study was approved by the Ethics Committee of the UHKV. Different types of conventional and experimental cytostatics were applied among each group of mice intraperitoneally. We have successfully established three PDX models with different PC subtypes (acinar, adenosquamous, ductal adenocarcinoma) applied subcutaneously into the dorsal part of mice. Success rates were in adenosquamous carcinoma 88.75 % (71/80), acinar carcinoma 97.56 % (40/41) and ductal adenocarcinoma 100 % (2/2). These PDX models are subsequently being used to study optimal therapeutic regimes in PC.

USING APPETITIVE INSTEAD OF AVERSIVE MOTIVATION TO TRAIN MICE FOR SPATIAL LEARNING IN THE BARNES MAZE

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The Barnes-maze is a spatial learning paradigm which is based on the innate fear of rodents from large open spaces and their drive to hide. However, the apparatus itself is often not aversive enough to provoke the hiding response, so additional factors (strong light, threatening sounds or odours) are often used to motivate the animals. However, this aversive motivation also causes stress to the animals. With appetitive motivation, e.g. using food as reward, these stressful circumstances can be avoided but in this case fasting may be necessary, which itself is also a stress factor. The objective of this study was to establish a Barnes-maze learning paradigm with appetitive motivation but without fasting by applying palatable food as a reward. We used 12 C57BL/6J and 12 NMRI male mice in two experiments. NMRI mice were own breeding, C57BL/6J obtained from Janvier Labs, Le Genest-Saint-Isle, France. The Barnes-maze was a circular metal table (1 m diameter) with twenty holes (5 cm) evenly spaced along the perimeter. Under one of them we placed the escape box where the mice could hide (target hole). Extra maze cues were placed in the room. Chocolate cereal was used as a reward. We habituated the mice to the chocolate cereal in their home-cage for 2 nights. Then the animals were put in the escape box with a piece of reward and placed in the middle of the maze for 20 and 10 min on two consecutive days. After the habituation period the maze-learning started. At the beginning of a trial the mouse was placed in the middle of the maze and was allowed to move around and find the target hole for 5 minutes. There were 2 trials a day. The experiment took 22 days with NMRI mice and 20 days with C57BL/6J mice. The learning criterium was finding the target hole with less than 1 hole error. When the animal reached this criterium we changed the target hole location. Mice needed to re-learn the new position with the same criterium. We measured the latency to find the target hole (LT), latency to reach the first visited hole (LF), initial error (distance of the first visited hole from the target hole, IE). NMRI mice reached the criterium in trial 31, C57BL/6J mice in trial 21. LT showed a steep decrease until trial 5 (C57BL/6J) or trial 4 (NMRI) and a slow gradual decrease afterwards. During the trials LF remained less than 6 s. IE decreased proportionally with time. After changing the box location LT significantly increased while LF remained the same. IE pointed to the original target hole. Mice learned the new target location within 12 trials (NMRI) or 18 trials (C57BL/6J). In conclusion, palatable food effectively motivated the mice to acquire spatial navigation in the Barnes-maze. The contextual part of the task

was mastered quickly, while the exact localization of the target hole required more time.

Funding sources 2017-1.2.1-NKP-2017-00002; FIKP 2020; TKP2021-EGA-25.

3R AND WELFARE PERSPECTIVE OF A RAT NON-INVASIVE HYPOXIA MODEL FOR NEUROSCIENCE AND OTHER RESEARCH APPLICATIONS

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Perinatal hypoxia and premature birth are common causes of neurological disorders and other impairments of physiological function in newborns and children, often with consequences present later in the life of adults. Regarding this, we wanted to create a non-invasive model of moderate perinatal hypoxia in rats and then use different research methods to research specifically the disturbances in brain development and its consequences. We designed a multimodal research approach with 3R principles applied that allowed us to gain insight into mild and moderate brain deficits using a minimal number of laboratory animals and the same animals in multiple experimental procedures. Since an alternative model is not available, reducing the number of animals, reusing animals, and refining the procedure in conducting the experiments were imperative. The same animals were used for behavior and then in a non-return experiment for MRI and histological investigations. The refinement of this research was the least invasive methods of hypoxia and animal marking, the usage of the nest and mothers' cage bedding in all experiments, and the shortest possible separation of pups from dams. The application of modern knowledge about the welfare of laboratory animals – health supervision, better housing conditions, education of the personnel and the experimenter, and application of modern methods aimed at reducing pain, suffering, and/or anxiety of laboratory animals are our standard practices when animals are used in scientific research. In study 48 of *RccHan:WIST* rats at postnatal day 1 (P1) were sex-determined, weighed, and randomly divided into hypoxic or control groups, keeping both sexes represented equally per experimental group (4 females and 4 males). Rat pups were placed in a hypoxic chamber and subjected to hypoxic (8 % O_2 in 92 % N_2) or normal conditions (21 % O_2 and 78 % N_2) for two hours. Subsequently, all rat pups were marked by a permanent toe tattoo. Twelve pups per group were decapitated immediately, and blood was collected to analyze the acid-base status using an i-STAT Alinity gas analyzer. Twelve pups per group were used for behavioral studies from P3 to P15 to investigate possible deficits in various reflexes, motor, sensory, memory, and learning disorders. These animals afterward were used for brain MRI at P15. Eight animals per experimental group were scanned using 7TMRI with several imaging modalities. All MRI-scanned animals were then sacrificed, and the brains were isolated for immunohistochemical labeling. Examination and analysis of brain sections were performed in search of histological indicators of brain injury at the molecular, cellular, and tissue level. The acid-base status measurements have confirmed a moderate shift from aerobic to anaerobic metabolic state in animals after hypoxia. The early postnatal behavior shows a significant increase in body weight and mild motor and sensory deficits in animals subjected perinatally to hypoxia. Histological and immunobiological labeling show no significant changes in cortical cyto- or histo- architectonics. The MRI-volumetric measurements indicate some increase in the volume of neocortical regions in hypoxic animals. The results obtained with this optimized research design were sufficient for statistical analysis and firm scientific conclusions.

Funding by project CSF-IP-2019-04-3182 and project GA KK01.1.1.01.0007.

ANIMAL MODELS IN NEUROSCIENCE WITH FOCUS ON BEHAVIORAL ASSESSMENT

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Neurological disorders are numerous and can be divided into different categories, mainly the ones in the realm of neuropsychiatric disorders and the rest with no psychiatric profile. The presentation will display the existing animal models (from roundworms and sea hares, to zebrafish, finches, and other vertebrate mammals) used for research of neuropsychiatric maladies. Representative diseases of this category, such as schizophrenia and depression, will be discussed. The difficulty of these ailments lies not only in the search of the most suitable experimental model but in the evaluation of their outcome. Although new imaging technologies have made the pathogenesis and/or the diagnosis of the disorders easier to find, the behavioral tests and their set up are still very controversial. The existing tests, such as the forced swimming and the tail suspension tests will be discussed from the point of view of efficacy and animal welfare. Other methods to evaluate stress in animals, such as the cortisol levels in saliva, will also be discussed. The talk will try to give newer perspectives in the use of animals for neuropsychiatric disorders along with their behavioral assessment and elucidate the details for the researchers focusing on this subject.

COPING BETTER WITH THE EMOTIONAL COST OF WORKING WITH LABORATORY ANIMALS

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Research on relevant, carefully designed and controlled animal models is an indispensable part of biomedical science. But working with laboratory animals may have a negative impact on the researchers, technicians, veterinarians and support staff, who may experience a significant emotional cost of compassion fatigue and moral stress when performing their different roles in research. This "cost of caring" is mainly caused by the connection we form with the laboratory animals and the empathy we feel for them. The effects of this condition can manifest as mental, behavioral, and even physical changes, such as apathy, irritability, depression and isolation, that in extreme cases may lead to health deterioration and addictive behaviors. These effects may in turn negatively impact work morale, staff turnover and even animal welfare. The aim of this talk is to improve our understanding of the causes and symptoms of these conditions, and to provide suggestions and resources for addressing these issues, highlighting better ways to cope and mitigate them.

IN VIVO EFFICACY OF NEW MDR-REVERSAL TAXANE ANALOGS PRESELECTED BY IN VITRO METHODS

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Taxanes are widely used anticancer drugs. However, resistance of cancer cells to conventional taxanes (paclitaxel, docetaxel) is a serious problem in the successful treatment of solid tumors. New experimental taxanes (Stony Brook taxanes; SB-Ts) seem to be potent agents against solid tumors with drug-resistant phenotype. The aim of this study was to

use *in vitro* methods for evaluation of the activity of SB-T taxanes in cancer cell lines and select and analyze just the most effective agents in subsequent *in vivo* preclinical models. At first, *in vitro* efficacy, proliferation, apoptosis of tested cell lines and uptake of SB-Ts in sensitive and resistant models of ovarian cancer cells were investigated. Simultaneously, changes in gene expression profiles connected with the treatment of SB-Ts in cancer cell lines were analysed. SB-T taxanes demonstrated 50 to 1000-times higher cytotoxicity than paclitaxel in resistant tumor cells. Resistant tumor cells accumulated significantly higher amount of SB-T taxanes than paclitaxel. The observed difference was, to a certain extent, due to the ABC transporter-dependent taxane transport. Furthermore, SB-T taxanes were effective at 20-times lower concentrations compared to paclitaxel in blocking of cell cycle at G_2/M. Accordingly, SB-T-taxanes seem to be potential candidates for the treatment of classical taxane-resistant tumors. For the most efficient taxane analogues SB-T-121605 and SB-T-121606, their effect and toxicity *in vivo* was also analysed using mouse cell derived xenograft (CDX) models. The incorporation of SB-T-121605 and SB-T-121606 into the regimens containing paclitaxel was effective in suppressing tumor growth in ovarian carcinoma resistant CDX mouse models at small doses (≤3 mg/kg), where their adverse effects were decreased. In addition, mRNA profile changes measured using Affymetrix arrays were evaluated in resistant ovarian carcinoma cells and tumors after the treatment with paclitaxel and SB-Ts. We have found significant differences in mRNA profiles linked with the effect of SB-Ts and identified key pathways and genes associated with the molecular mechanism of action of these taxanes (e.g. SLC transporters, NOTCH and AhR signaling pathways, CPS1 and TRIP6 genes). On the basis of *in vitro* technologies, the most effective SB-T analogs SB-T-121605 and SB-T-121606 were selected for evaluation of their effectivity in mouse *in vivo* model of highly resistant ovarian carcinoma. Both SB-Ts were effective in supression of tumor growth. In addition, deregulation of gene expression profile linked to taxane treatment revealed candidate pathways and genes, which are under active investigation, at present, as potential therapeutic targets in human cancers.

This study was supported by projects of the Czech Science Foundation no. 21-14082S, the Czech Ministry of Education, Youth and Sports: INTER-ACTION, project no. LTAUSA19032, by the Grant Agency of Charles University in Prague, program Cooperatio "Surgical Disciplines" no. 207043 and by the National Institutes of Health (NIH), U.S.A. grant R01 CA103314.

3R-SMART - AN ONLINE PLATFORM TO PROMOTE EDUCATION AND TRAINING IN THE 3RS

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It is a common understanding that animal experiments may only be carried out if no alternatives are available. One of the EU's declared aims is to replace procedures involving live animals in the long term (Recital 10 of Directive 2010/63/EU). Furthermore, the "principle of 3Rs" (Reduction, Replacement, Refinement) should be systematically considered in the implementation of the Directive (Recital 11 Directive 2010/63/EU) and the use of animals for scientific or educational purposes should only be considered if there is no non-animal alternative (Recital 12 Directive 2010/63/EU). In order to achieve these goals in the long term, it is not only necessary to develop appropriate alternative test procedures, but also to anchor training on the 3Rs or alternative methods in animal experimentation training. Furthermore, transparent information must be provided on the use of alternative and supplementary methods to animal experimentation. Against this background, 3R-SMART (https://www.3r-smart.de) was designed as an information and training platform on replacement and supplementary methods to animal experiments. The platform is funded by the German Federal Ministry of Education and Research as part of it funding scheme for alternatives to animal experiments. The platform has been realized in the Content Management System typo3. The platform addresses scientists and technical staff working with laboratory animals as well as interested third parties. The open access platform highlights specific efforts to replace, reduce, or refine animal experiments by providing instructive texts, explanatory videos, or recordings of lectures, 3R news and upcoming events as well as a 3R forum (registered users). Interactive maps introduce 3R centers in Germany and Europe. As part of the project, the video-based guide for preparing non-technical project summaries was developed by the German Center for Risk Assessemnt. The web presence is "collaborative": contributions from third parties can be easily integrated. In the future, the website will be expanded to include online training (including exams) and a forum on 3R topics. In collaboration with LAS interactive (https://las-interactive.de) a common training portal about LAS and alternatives to animal use will be developed. In line with the idea to disseminate and advance knowledge about the 3Rs we are working on developing a 3R curriculum that can be integrated in laboratory animal science courses to support the development of 3R-competencies. This way 3R-SMART forms an interface between animal research and alternative methods and can provide significant and sustainable support for a wider implementation of the 3Rs.

ASSESSMENT AND REFINEMENT OF THE WELLBEING OF MICE DURING METABOLIC CAGE HOUSING

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Animal experiments are necessary in research – especially for drug development – but experimental procedures involving laboratory rodents, in particular mice, must constantly be refined to improve animal welfare. Refinement of housing conditions in the home cage (e.g. providing nesting material) is regulated by law, but for metabolic cages only few regulations (e.g. floor area, cage height and recovery period) apply. Metabolic cages are frequently used to collect feces and urine samples, up to a few days continuously, in laboratory rodents for biological or biochemical analyses. These housing conditions are challenging for mice and are known to stress the animals: single housing, grid floor instead of wooden bedding, absence of enrichment and shelter materials, smaller cages, and powdered food. In the metabolic cages laboratory mice are exposed to relatively harsh environmental conditions, which affect the mice' homeostasis and behavior, so that further improvements are needed for laboratory mice housed under these conditions. So far, the few studies investigating the stress levels of laboratory mice in metabolic cages showed an increase in blood pressure, heart rate and corticosterone levels. Additionally, it can be assumed that particularly mice kept in metabolic cages are constantly exposed to a cold stress. Stress and metabolism are closely related, and we hypothesize that the current conditions of metabolic cage housing have a negative impact on the homeostasis of the physiological metabolism of mice. Our project aims to compare the stress levels of mice during standard metabolic cage housing with the following conditions a) hourly adaptation, b) thermoneutral environment temperature c) providing a shelter/nesting material and d) providing a visual barrier. To objectively assess stress levels, male and female C57BL/6J mice were implanted with HD-X11 transmitters (Datasci. Int, Saint Paul, USA), acquiring heart and respiratory rate, blood pressure and body core temperature measurements. Behavior and activity were continuously tracked with video cameras. Additionally, different biochemical stress parameters (e.g. corticosterone) will be analysed in urine and feces samples. Our preliminary results indicate differences between the different metabolic cage conditions. Under all conditions mice lost around 5 % of their body weight and never returned to baseline levels. Furthermore, mice maintained their day-night cycle, in terms of food/water intake and feces/urine excretion – which was comparable in all the conditions during the experimental day. Heart rate, respiratory rate and systolic blood pressure were reduced in the thermoneutral group. Whereby the body core temperature was reduced to 33 °C for individual mice in the control and adaption group. Further work will involve activity/behavioural analysis, additional housing conditions and quantification of stress parameters. Overall, our study confirms that simple changes in the setup of a metabolic cage experiments might help to improve the wellbeing of mice.

CHANGES AND TASKS FOR LABORATORY ANIMAL RESEARCHERS EDUCATION SYSTEM DURING COVID-19 PANDEMIC: THE IMPLICATIONS FOR POST-COVID ERA

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The value of preclinical research with laboratory animals is becoming more prominent in these days, and training and education system for researchers is also important for ethical and scientific research. Department of Experimental Animal Research in Seoul National University Hospital (SNUH-DEAR) was established in 1998, and certified as GLP facility by KFDA in 2003, fully accredited by AAALAC international in 2007, and designated as an Excellent Animal Testing Facility by KFDA in 2012. For the maintenance of a world-class animal testing system and environment based on ethics and reliability, SNUH-DEAR has conducted compulsory training and education system for animal experiments researchers since 2009. However, COVID-19 pandemic disrupted essential research, and researchers faced unexpected changes such as social distancing, and group education became impossible. SNUH-DEAR provided non-face-to-face or hybrid education on-line under the COVID-19 era.
1) “Animal Experimental Techniques Hand-on Workshop” partially changed to on-line workshop and practice was conducted with a limited number of people in a large space in order to minimize contact between people.
2) “Ethical Animal Experiment Education Seminar” was held as a face-to-face video seminar *via* Zoom system.
3) “New User Guidance Training” was conducted with a limit on the number of researchers who installed partitions in the seminar room and wore masks.
4) The 'LMO research activity worker education' has been converted to full online education.
SNUH-DEAR continued to deliver valuable education during the COVID-19, which can help researchers to understand the concepts of animal protection, animal welfare, basic skills for the ethical handling, scientific use, and ways to relieve animal stress during animal testing. SNUH-DEAR will continue to strive to provide educational programs for researchers to consider the ethics of laboratory animals and to cultivate animal experiments.

EUROPEAN COLLEGE OF LABORATORY ANIMAL MEDICINE – A CAPSTONE OF A LABORATORY ANIMAL VETERINARIAN CAREER

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A career in laboratory animal medicine entails many particularities and opportunities. The subject is still not well promoted or understood by veterinary faculties and society, however, the demand for specialized laboratory animal veterinarians is rising. The educational pathway, after graduating the veterinary school, usually includes a seminar for Module 24 of the common European education and training framework which explains the basics of the designated veterinarian duties, further seminars on laboratory animal science and medicine, possibly a larger course on Module 24 or a masters’ degree or national specialization in laboratory animal science and/or medicine, and/or a relevant research PhD. This tiered pathway is proposed in the ESLAV/ECLAM/LAVA/EVERI recommendations (Poirier *et al.,* 2014), and it also emerges naturally once a veterinarian begins working in this field and seeks continuing professional development. The capstone of a laboratory animal veterinarian career is a Diploma of the European College of Laboratory Animal Medicine, which constitutes the highest European qualification, certified by the European Board of Veterinary Specialization (EBVS), which additionally awards the title EBVS (TM) European Veterinary Specialist in Laboratory Animal Medicine to all active Diplomates. The incentives to pursue this are multiple: personal satisfaction, completion of a final certification to validate work and study efforts, goal to reach the highest veterinary specialization, ability to opt for job positions which require expertise, entering a collegial network of experts. ECLAM residents and diplomates are also actively involved in networking, council and committee work for ECLAM college to further promote the specialty and continuously improve training and opportunities. Reaching the Diplomate status signifies the peak of studies and opens the doors to another stage of this career, as a certified specialist in laboratory animal medicine.

OPPORTUNITIES AND CHALLENGES OF A COMPACT AND BUSY PRECLINICAL LABORATORY

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The operation of a compact and busy preclinical laboratory comes with many challenges but also presents some surprising opportunities. Running a mouse and rat animal facility and full laboratory with a procedures room, multiple imaging modalities, radiochemical and cells lab, in a 100 m^2 space has proven very functional from a managerial, organizational and multitasking aspect, as everything and everyone is close and easy to coordinate. Space limitations mainly affect washing, waste and supplies storage, adding heavy big equipment such as a biosafety cabinet, and hosting staff and occasional visitors. Operating the animal facility necessitates thorough sterilizations of cages and equipment in order to avoid cross-contamination due to receipt of animals from different breeders and health status. Procedure rooms are strategically organized to fit many needs, with the help of modern compact experimental equipment. The all in - all out operation, ability of close supervision, proximity of all laboratories, and good collaboration of everyone involved overcomes the confined space and allows for a rather convenient workflow. Radioprotection is addressed by using appropriate equipment and organizing the work accordingly, and exposure limits can be kept to the minimum for the public (1 mSv/year). Our experience proves that with good organization, resourcefulness and teamwork, less is more!