4. znanstveno-stručni simpozij Hrvatskog društva za znanost o laboratorijskim životinjama (CroLASA) i
3. zajednički skup CroLASA-e i Slovenskog društva za laboratorijske živali (SLAS) s međunarodnim sudjelovanjem

POKUSNE ŽIVOTINJE U ZNANSTVENIM ISTRAŽIVANJIMA

2025

LABORATORY ANIMALS IN SCIENTIFIC RESEARCH

4th Congress of Croatian Laboratory Animal Science Association (CroLASA) and
3rd joint CroLASA and Society for Laboratory Animals of Slovenia (SLAS) Congress with international participation

Zagreb, 05. i 06. veljače 2025. / Zagreb, February 5th and 6th, 2025

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4. znanstveno-stručni simpozij Hrvatskog društva za znanost o laboratorijskim životinjama i 3. zajednički skup CroLASA-e i SLAS-a s međunarodnim sudjelovanjem

“Pokusne životinje u znanstvenim istraživanjima”

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“Laboratory Animals in Scientific Research”

Zagreb, 05. – 06. 02. 2025.

RADIONICE / WORKSHOPS

05. veljače 2025. / February 5th, 2025

KRATKA RADIONICA/MINI-WORKSHOP CARLOS OSCAR SORZANO:

Optimizacija dizajna pokusa / Optimization of experimental design

06. veljače 2025. / February 6th, 2025

MatTek 3R RADIONICA / MatTek 3R WORKSHOP

Silvia Letasiova: Test iritacije kože primjenom 3D rekonstruiranog tkivnog modela epidermisa čovjeka / Skin Irritation Test using 3D reconstructed tissue model of human epidermis

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CONTENT

Programme

SRIJEDA 5. VELJAČE 2025 / WEDNESDAY 5TH FEBRUARY 2025

VRIJEME / TIME	TEMA / TOPIC
8:00 – 9:00 Predvorje / Entrance hall	REGISTRACIJA / REGISTRATION
9:00 – 9:10 Velika dvorana / Large hall	OTVARANJE SIMPOZIJA / OPENING CEREMONY
9:15 – 9:45 Velika dvorana / Large hall	PLENARNO PREDAVANJE / PLENARY LECTURE: Bojan Polić: Upotreba laboratorijskih životinja u imuno-metaboličkim istraživanjima / The use of laboratory animals in immunometabolic research
9:50 – 10:20 Velika dvorana / Large hall	PLENARNO PREDAVANJE / PLENARY LECTURE: Diana Cash: Pretkliničko oslikavanje mozga u neuroznanosti i translacijsko otkrivanje lijekova / Preclinical brain imaging in neuroscience and translational drug discovery
10:20 – 10:40 Predvorje / Entrance hall	PAUZA ZA KAVU / COFFEE BREAK
10:45 – 12:20 Velika dvorana / Large hall	SIMPOZIJ / SYMPOSIUM: Animalni modeli, metode istraživanja i primjena 3R načela na laboratorijskim životinjama / Animal models, research methods and application of 3R principles on laboratory animals

- ILIJA BRIZIĆ

Trajno pripremljena mikroglija ograničava reaktivaciju latentnog citomegalovirusa na račun neuronske sinaptičke povezanosti / Persistently primed microglia restrict the reactivation of latent cytomegalovirus at the expense of neuronal synaptic connectivity

- DAIVA BALTRIUKIENĖ

Pristupi sljedeće generacije: Uloga NAM-a u smanjenju broja laboratorijskih životinja / Next-Generation Approaches: The Role of NAMs in Reducing Laboratory Animal Use

- KRATKA RADIONICA/MINI-WORKSHOP CARLOS OSCAR SORZANO:

Optimizacija dizajna pokusa / Optimization of experimental design

12:25 – 13:25
Predvorje / Entrance hall

RUČAK / LUNCH

13:30 – 14:55
Velika dvorana / Large hall

SIMPOZIJ / SYMPOSIUM:

Zakonodavstvo, obrazovanje i unaprijeđenje standarda vezanih uz rad s laboratorijskim životinjama / Legislation, education and standard improvement related to the work with laboratory animals

- MARTINA PERŠE

Osnovno i kontinuirano obrazovanje kroz prizmu Direktive 2010/63/EU i smjernica FELASA-e / Basic and Continuing Education through the Lens of Directive 2010/63/EU and FELASA guidelines

- SREĆKO GAJOVIĆ

Pretvorba suvremenih standarda provođenja pokusa na laboratorijskim miševima i štakorima u nova prostorna rješenja na primjeru BIMIS-a - Biomedicinskog istraživačkog središta Šalata / Conversion of modern standards of conducting experiments on laboratory mice and rats into new spatial solutions on the example of BIMIS - Biomedical Research Center Šalata

- DAMIR KOVAČIĆ

Rušenje prepreka u prevladavanju gluhoće: od životinjskih modela do neuroelektroničkih sučelja usred izazova istraživanja na životinjama u Hrvatskoj / Breaking barriers in overcoming deafness: from animal models to neuroelectronic interfaces amid challenges in animal research in Croatia

- TATJANA PIRMAN

Utjecaj genetske pozadine, prehrane i spola na pH crijeva i profil hlapljivih masnih kiselina u debelih i mršavih miševa / Influence of genetic background, diet and sex on gut pH and volatile fatty acid profile in fat and lean mice

15:00 – 15:30
Velika dvorana / Large hall

PLENARNO PREDAVANJE / PLENARY LECTURE:

Kirk Leech: Istraživanje na životinjama: Vrijeme je za razgovor! / Animal Reseach: Time to Talk!

15:35 – 16:35
Mala dvorana / Small hall
Predvorje / Entrance hall

POSTER SEKCIJA / POSTER SECTION

PAUZA ZA KAVU / COFFEE BREAK

16:40 – 18:40
Velika dvorana / Large hall

SIMPOZIJ / SYMPOSIUM:

Dobrobit laboratorijskih životinja / Welfare of laboratory animals

- URTE JAEH

Razmišljanje unutar okvira: Razvojni pristup metodama otkrivanja patogena glodavaca / Thinking Inside the Box: An Evolving Approach to Rodent Pathogen Detection Methods

- NGAIRE DENNISON

Praćenje zdravlja životinja bez upotrebe sentinelnih životinja/ Non-animal health monitoring

- MARIJA HEFER

Nadzor kaveza na daljinu: prednosti, nedostaci i mogućnosti /Home Cage monitoring: advantages, disadvantages and prospects

- DAVOR VIRAG

TheBehaviourForum.org: Prvi online forum za diskusiju o bihevioralnim pokusima i dobrobiti laboratorijskih životinja / TheBehaviourForum.org: The first online forum for discussing behavioural experiments and laboratory animal welfare

- VLADIANA CRLJEN

Etički prihvatljivi načini obuzdavanja pokusnih životinja / Ethically acceptable ways of restraining experimental animals

ČETVRTAK 6. VELJAČE 2025 / THURSDAY 6TH FEBRUARY 2025

VRIJEME / TIME	TEMA / TOPIC
8:00 – 9:00 Predvorje / Entrance hall	**REGISTRACIJA / REGISTRATION**
9:00 – 10:35 Velika dvorana / Large hall	**SIMPOZIJ / SYMPOSIUM:** In vitro alternative kao zamjena za pokuse na životinjama / In vitro models as alternatives to animal testing • MAJA SABOL Zašto su 3D in vitro modeli budućnost u istraživanja raka? / Why are 3D in vitro models the future of cancer research? • IVAN ALIĆ Napredni in vitro modeli: snažan alat za primjenu 3Rs načela / Advanced in vitro models: a powerful tool for 3Rs principle • MARKO PENDE Pročišćavanje tkiva i svjetlosno snimanje. Dva snažna alata za cjelovitu 3D analizu / Tissue-clearing and light-sheet imaging. Two powerful tools for whole system 3D analysis • SILVIA LETASIOVA Mogu li 3D rekonstruirani modeli tkiva ljudskog epidermisa zamijeniti testiranje na životinjama? / Can 3D reconstructed human epidermis tissue models replace animal testing?

10:40 – 11:40 Mala dvorana / Small hall Predvorje / Entrance hall	POSTER SEKCIJA / POSTER SECTION PAUZA ZA KAVU / COFFEE BREAK
11:45 – 12:45 Mala dvorana / Small hall	KVIZ / QUIZ
12:45 – 13:35 Velika dvorana / Large hall	DODJELA NAGRADE ZA NAJBOLJU POSTERSKU PREZENTACIJU / THE BEST POSTER PRESENTATION AWARD ZATVARANJE SIMPOZIJA / CLOSING CEREMONY GODIŠNJA SKUPŠTINA CROLASA-E / ANNUAL MEETING OF CROLASA
13:05 – 14:05 Predvorje / Entrance hall	RUČAK / LUNCH
14:10 – 16:10 Mala dvorana / Small hall	MATTEK 3R RADIONICA / MATTEK 3R WORKSHOP Silvia Letasiova: Test iritacije kože primjenom 3D rekonstruiranog tkivnog modela epidermisa čovjeka / Skin Irritation Test using 3D reconstructed tissue model of human epidermis

Keynote lectures

THE USE OF LABORATORY ANIMALS IN IMMUNOMETABOLIC RESEARCH

Bojan Polić[1]

[1]*Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia*

The development of chronic metabolic diseases, such as diabetes or metabolic dysfunction-associated steatotic liver disease (MASLD), is most often a complex disorder involving the interaction of multiple systems, leading to damage to individual organs and impairment of their function. To determine the mechanisms of interaction between the systems involved in the pathophysiological spiral of these diseases, it is necessary to use experimental disease models. For this purpose, we use laboratory animals, particularly genetically modified mice, which allow us to identify these mechanisms and later extrapolate them to clinical medicine. For the past ten years, my group has been intensively studying the interactions between the immune and endocrine (metabolic) systems in the development of type II diabetes and MASLD under conditions of obesity and/or viral infections. The basis of these conditions is the activation of the immune system due to metabolic disorder and/or viral infections, the secretion of certain cytokines that deregulate the endocrine system, and lead to an inflammatory process that subsequently damages organs and tissues. In this lecture, I will present the methods of modeling the mentioned metabolic diseases and the use of various genetically modified mice to identify the complex mechanisms of disease initiation and development.

Keywords: *type II diabetes, MASLD, NK cells, T cells, viral infections, obesity*
Founds: *Grants of the Croatian Scientific Foundation to BP (IPCH-2010-10-8440 & IP 2024-05-9583)*

BOJAN POLIĆ

Professor Bojan Polić earned his medical degree from the University of Rijeka, Croatia, where he also pursued his PhD in the Department of Physiology and Immunology. He began his academic career at the Department of Physiology, Immunology, and Pathophysiology as a PhD candidate, working in the research group of Professor Stipan Jonjić (1991–1994), before continuing as an assistant in the same department. In 1996, he transitioned to the Department of Histology and Embryology (together with Professor Jonjić), where he worked as an assistant, and after completing his doctorate in 1996, as a senior assistant.

At the end of 1997, he undertook a postdoctoral position with Professor Klaus Rajewsky at the Institute for Genetics at the University of Cologne (1997–2000, plus an additional six months in 2001), where he worked on the role of the αβ T Cell Receptor in maintaining T cells. Upon returning to Croatia in 2000, he became an assistant professor at the Department of Histology and Embryology, where he established his own research group. At the same department, he was promoted to full professor in 2008 and full professor with tenure in 2013. Since 2021, he has been the head of the department.

In 2003, he introduced targeted gene mutation and the production of genetically modified mice at the Faculty of Medicine. He also played a key role in establishing the Laboratory Mice Breeding and Engineering Center (LAMRI) under the leadership of Professor Jonjić. He has been the director of the center since its inauguration in 2004. His major scientific interests include immune responses involved in the development of insulin resistance and Type 2 diabetes mellitus in obesity / viral infection, and the biological roles of the NKG2D receptor.

Currently, he serves as the elected president of EFIS (European Federation of Immunological Societies) and has been a member of its Executive Board since 2022. Starting next year, he will assume the role of Executive President.

PRECLINICAL BRAIN IMAGING IN NEUROSCIENCE AND TRANSLATIONAL DRUG DISCOVERY

Diana Cash[1]

[1] *The BRAIN (Biomarkers Research and Imaging for Neuroscience) Centre, Neuroimaging Department, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, e-mail: diana.cash@kcl.ac.uk*

The BRAIN Centre at King's College London was established in 2017 as a preclinical neuroimaging facility specialized in collaborative neuroimaging research with academia and industry. Researchers at the BRAIN Centre are committed to exploring forward and back-translational research by employing brain imaging techniques in order to elucidate the mechanisms and treatments for a range of disorders and pathologies, such as dementia, schizophrenia, unhealthy ageing, autism, Parkinson's, stroke, and more. This lecture aims to introduce the range of activities conducted at the Brain Centre and showcase examples of their work in experimental neuroscience, as well as contribution of non-invasive imaging toward the principles of 3R. Using model of inflammatory demyelination in mice it will be demonstrated how multimodal in vivo MR imaging and spectroscopy can provide an intricate characterization of pathology, complementing neurochemistry and histology findings. It will be also shown how this approach can confirm treatment efficacy and address the mechanisms of action, crucial for clinical relevance. Another example will include monitoring the efficacy of anti-Alzheimer's medication in the rodent models using comparative analysis of MR, histology, and behaviour measures. Using a transgenic rat model, we have developed a sensitive method to detect amyloid plaques that enables us to measure changes over time and thus quantify the drug's efficacy in the living animals. Finally, a pipeline for characterizing functional effects of CNS active compounds on the brain will be presented. This methodology involves quantifying brain blood flow and resting-state functional connectivity to extract unique fingerprints of compounds, such as ketamine, clozapine and psilocybin, with

which novel drugs can then be compared. The plans to combine these techniques with EEG recordings will additionally be discussed.

Acknowledgments and/or funding: *Medical Research Council, The Wellcome Trust, Jazz Pharmaceuticals, Sir Jules Thorn Trust*
Keywords: *preclinical neuroimaging, drug discovery, cerebral blood flow, brain activity, translational research*

DIANA CASH

Diana Cash (BSc Mol Biology - Birkbeck, MSc Neuroscience - KCL, PhD Neuroimaging – KCL) is a Senior Lecturer (Associate Professor), and Director of the preclinical neuroimaging facility The Brain Centre (https://brain-imaging.org) at the Institute of Psychiatry, Psychology and Neuroscience (IOPPN), King's College London (KCL).

Diana is an expert researcher with over two decades of career in neuroimaging. Throughout, she has spearheaded a variety of brain imaging projects that leverage state-of-the-art MR imaging and spectroscopy, PET, as well as other in vivo and in vitro methods (including behaviour, EEG, histology, and autoradiography) in experimental models of neuro-psychiatric disorders. Diana championed the establishment of The BRAIN Centre in 2017, which serves as a world-class facility specialising in collaborative neuroimaging research with academia and industry. Diana and colleagues from the Neuroimaging department are committed to exploring forward and back-translational research aimed at elucidating the mechanisms and treatments for a range of disorders and pathologies, such as dementia, schizophrenia, unhealthy ageing, autism, Parkinson's, stroke, and more.

ANIMAL RESEACH: TIME TO TALK!

Kirk Leech[1]

[1]*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 550 institutions where institutions have collectively agreed to commitments on pursuing greater openness with the public. 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 engage actively with these pressures and needs to make a stronger and clearer public case for the use of animals in research where applicable. 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.

EARA is a communications and advocacy organisation with offices in London, Brussels and Lisbon. It was established in 2014 through the collaboration of seven bio-medical

research organisations, it now has a global membership of 200 institutions on five continents.

Keywords: *transparency, welfare, public information*

KIRK LEECH

Kirk has been the Executive Director of the European Animal Research Association since its creation in 2014. EARA has grown quickly to be the largest advocacy organisation in Europe. Previously Kirk worked in government affairs for the Association of the British Pharmaceutical Industry. Prior to that he acted as a consultant for the White House Writers Group a strategic communications consultancy based in Washington, DC, founded by a group of former US Presidential Speechwriters. Kirk was engaged to advise on improving public opinion on the environmental, economic and cultural impact of a new goldmine in Transylvania, Romania. Earlier to this Kirk worked for a tribal rights organisation working in rural Gujarat India on influencing public opinion on the economic benefits of the Narmada dam and in opposing the imposition of wildlife sanctuaries on tribal land. Kirk is a regular writer and presenter in the European media with over 500 articles and appearances.

Invited lectures

PERSISTENTLY PRIMED MICROGLIA RESTRICT THE REACTIVATION OF LATENT CYTOMEGALOVIRUS AT THE EXPENSE OF NEURONAL SYNAPTIC CONNECTIVITY

Andrea Mihalić[1], Daria Kveštak[1], Berislav Lisnić[1], Fran Krstanović[1], Shirin Hosseini[2,3], Katarzyna M. Sitnik[4,5], Mijo Golemac[1], Vanda Juranić Lisnić[1], Ahmad S. Rashidi[6], Isabell Bochow[2], Alessia Arossa[7], Milena Furione[8], William J. Britt[9], Georges M.G.M. Verjans[6,] Martin Korte[2,3], Luka Čičin-Šain[4,10], Stipan Jonjić[1,11] and Ilija Brizić[1]

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[3]*Helmholtz Centre for Infection Research, Research Group Neuroinflammation and Neurodegeneration, Braunschweig, Germany*

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[5]*Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria*

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[9]*Department of Pediatrics, University of Alabama at Birmingham, Birmingham, USA*

[10]*Centre for individualized Infection Medicine, a joint venture of the Helmholtz Centre for Infection Medicine and Hannover Medical School, Hannover*

[11]*Department of Biomedical Sciences, Croatian Academy of Sciences and Arts, Rijeka, Croatia*

Microglia are myeloid cells that reside within the central nervous system (CNS), where they maintain homeostasis under normal, non-pathological conditions. In addition, microglia also perform numerous immune functions upon different pathogenic stimuli, including CNS infections with various neurotropic viruses. Herpesviruses establish a lifelong latent infection from which they reactivate intermittently upon waning of immune control. The role of microglia in preventing reactivation of latent herpesviruses remains unclear. In this work, we used congenital cytomegalovirus (CMV) infection as a model to investigate the impact of a persistent virus infection of the brain on microglia. We show that mouse CMV (MCMV) latency in the CNS is associated with permanent microglial priming. The changes induced by persistent infection include continuous, interferon-gamma-dependent microglia activation and extensive transcriptional reprogramming at the single-cell level, leading to the expansion of a microglia subset associated with latent infection. Notably, the maintenance of microglia in a primed state provides enhanced control of latent infection and superior recall response but is associated with excessive loss of synaptic dendritic spines mediated by primed microglia. Altogether, our results indicate that latent CMV infection in the brain causes perturbation of microglial homeostasis, which leads to chronic neuroinflammation that successfully restricts virus reactivation but simultaneously compromises neuronal synaptic connectivity in the brain.

Acknowledgments and/or foundation: *This work has been fully supported the Croatian Science Foundation under the project numbers HRZZ-PZS-2019-02-7879 and HRZZ-IP-2022-10-3371.*

Keywords: *cytomegalovirus, microglia, synapses*

NEXT-GENERATION APPROACHES: THE ROLE OF NAMS IN REDUCING LABORATORY ANIMAL USE

Daiva Baltriukienė[1]

[1]*Department of Biological Models, Institute of Biochemistry, Life Sciences Center, Vilnius University, daiva.baltriukiene@bchi.vu.lt*

The development and adoption of new approach methodologies (NAMs) are transforming the landscape of biomedical research and regulatory testing, offering innovative alternatives to traditional animal-based studies. NAMs encompass advanced in vitro models, computational simulations, organ-on-a-chip technologies and other cutting-edge tools that aim to provide reliable, human-relevant data while minimising the use of laboratory animals. This presentation will review the latest advances in NAMs, highlighting their potential to enhance scientific accuracy, reduce ethical concerns and adhere to the 3Rs principle: Replacement, Reduction and Refinement. Key case studies will be highlighted to illustrate successful integration of NAMs in toxicology, pharmacology, and disease modelling. In addition, the presentation will address the challenges and opportunities in terms of regulatory acceptance, funding and interdisciplinary collaboration required to accelerate the transition to animal-free research paradigms.

Keywords: *New Approach Methodologies (NAMs), in vitro models, animal-free research*

BASIC AND CONTINUING EDUCATION THROUGH THE LENS OF DIRECTIVE 2010/63/EU AND FELASA GUIDELINES

Martina Perše[1]

[1]*Faculty of Medicine University of Ljubljana, martina.perse@mf.uni-lj.si*

The welfare of animals used in scientific research and the quality of animal studies are fundamentally influenced by the expertise and competence of the personnel directly involved in animal experimentation. Directive 2010/63/EU identifies specific functions in animal experimentation (i.e., (a) carrying out procedures, (b) designing procedures and projects, (c) taking care of animals, and (d) killing animals) and requires that individuals performing these functions are adequately educated, trained, and competent. In addition to these functions, the Directive sets out other tasks and roles (such as a person responsible for the welfare and care of animals, a person responsible for information, training, and competence, a designated veterinarian, a member of an animal welfare body, an inspector, and a project evaluator). However, all of these tasks and roles are involved in animal research at various levels and indirectly impact animal welfare and the quality of animal studies; therefore, appropriate training and competence apply to these tasks and roles as well. FELASA was the first to recognize the need for appropriate training and competence for individuals working with laboratory animals. In the 1990s, FELASA published a series of recommendations for basic education and training (E&T) of personnel involved in animal experimentation and established an accreditation system to harmonize E&T courses across EU countries. In the 2010s, FELASA revised the established E&T recommendations and accreditation system to meet the requirements of the Directive (courses became modular in design, species-specific, and focused on learning outcomes) and prepared Guidelines for Continuing Education for Persons Involved in Animal Experiments, also known as Continuing Professional Development (CPD). Currently, there is no consistency regarding CPD across EU countries. To

harmonize CPD for professionals involved in the Laboratory Animal Science (LAS) field (functions, tasks, and roles), the Commission established a Working Group to prepare guidelines on the CPD framework. Recently, FELASA released new recommendations to help implement a harmonized CPD strategy. The talk aims to present the recently prepared documents.

Keywords: *legislation, education and training, competence, continuous professional development*

CHALENGES IN DESIGNING A FACILITY FOR THE LABORATORY ANIMALS – AN EXAMPLE OF FUTURE BIMIS – BIOMEDICAL RESEARCH CENTER ŠALATA

Srećko Gajović[1], Siniša Škokić[1], Jadranka Bubić Špoljar[1], Carlo Demaldè[2]

[1]*University of Zagreb School of Medicine, BIMIS – Biomedical Research Center Šalata, Zagreb, Croatia*

[2]*Tecniplast S.p.A., Buguggiate (VA), Italy*

The animal research is in the constant need for improvement and adaptation. There is a strong pressure to improve the experiments on the animals applying the most recent approaches of 4R (i.e. Replace, Reduce, Refine and Responsibility). This is accompanied by ethical and legal regulations, and as the most important aspect, with increased requirements of the scientific rigor, being codified by the internationally recognized guidelines. The Croatian animal research is not an exemption to the widespread difficulties of the laboratories to reach the necessary standards. When designing the animal facility of the new research infrastructure BIMIS – Biomedical Research Center Šalata, being an integral part of the University of Zagreb School of Medicine, we attempted to identify the challenges and provide solutions when creating the facility layouts. The methodology involved the internal discussions of the dedicated team, literature analysis and external discussions with the equipment producers and colleagues involved in running other animal facilities or performing animal research (e.g COST Action CA20135 TEATIME, animal facilities in Genoa, Milan, Prague, and Ljubljana). The research on laboratory animals represents the core activity of the BIMIS. The researchers need to access to animals to perform the experiments and collect the animal samples (to be analyzed in other BIMIS laboratories). To achieve the best standards only mice and rats are considered to be housed on site. The facility is divided in 4 zones, the

zone dedicated to the SOPF breeding and with the most restricted access, the zone dedicated to holding the animals during the experiment, the zone for the researchers to perform the experiments, and the technical zone separated from the animals, to be accessed by the corresponding technical personnel. The dedicated routes for the necessary procedures and appropriate barriers for entry and exit in/out of the clean environments were organized. In accordance with the anti-allergic principles, the repetitive procedures are to be performed using the automation and robots. Moreover, the animals will be home-cage monitored in the digital individually ventilated cages. The design to be realized will rely on everyday routines, education and motivation of the users. Another future challenge would be the cost to maintain the functionality of the facility. We argue in favor of centralized high-quality facilities rather than distributed individual approaches.

Acknowledgements: *We acknowledge COST Action CA20135 Improving biomedical research by automated behavior monitoring in the animal home-cage (TEATIME).*
Funding sources: *European Union through the European Regional Development Fund, under Grant Agreement KK.01.1.1.09.0033 project Biomedical Research Center Šalata – BIMIS, and University of Zagreb Grant AneMišAkcija.*
Keywords: *animal housing, scientific rigor, home cage monitoring.*

THINKING INSIDE THE BOX: AN EVOLVING APPROACH TO RODENT PATHOGEN DETECTION METHODS

Urte Jaeh[1], Ken Henderson, PhD, MS[2], Cheryl Perkins[3],

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Soiled bedding sentinels (SBS) have been traditionally used for health monitoring in laboratory rodent facilities, but their effectiveness has been challenged by modern housing systems and increased biosecurity measures. This this presentation describes a study aimed to evaluate and optimize alternative sentinel-free methods for detecting rodent pathogens in individually ventilated cages (IVC). The investigation compared six different contact filter media types under four treatment schedules for detecting pathogens in soiled bedding from pet shop mice. The experimental design involved mixing pet shop mouse bedding with specific pathogen-free CD-1 mouse bedding at a 1:5 ratio to simulate low prevalence conditions. Contact media were exposed to bedding through manual agitation following different schedules: monthly collection with separate or combined testing, weekly collection with monthly combined testing, or continuous use throughout the three-month study period. Results demonstrated that contact media detected significantly more pathogens (28-29 different agents) compared to traditional soiled bedding sentinels (10 agents). The optimal protocol involved weekly agitation of contact media maintained throughout the study period, with two specific media types showing superior performance. The study identified 42 different agents among pet shop mice and 31 among contact sentinel mice using various diagnostic methods. The contact media approach eliminated the need for live sentinel animals, supported the 3Rs principles (Replacement, Reduction, Refinement), and provided improved pathogen detection compared to traditional methods. This sentinel-free methodology offers a standardized, efficient, and more humane approach to health monitoring in laboratory

rodent facilities while addressing the limitations of traditional sentinel programs in modern housing systems.

Acknowledgments: *Not provided in the source material.*
Keywords: *laboratory animals, health monitoring, soiled bedding sentinels, contact media, pathogen detection, rodent housing*

REPLACING SENTINEL ANIMALS

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Research facilities that house rodents have traditionally used soiled bedding sentinel health-monitoring programs to detect rodent pathogens that could affect research results. Whilst the use of such sentinels has been instrumental in the past, there are limitations for the method in terms of accuracy and the types of organisms that can be detected. In addition, the approach requires the use of live animals, which can add to the risk of compassion fatigue for staff, and can be costly and time consuming. Environmental health monitoring (EHM) is an alternative that has many advantages and overcomes some of these limitations. EHM allows the surveillance of rodent colonies for pathogenic organisms, without the use of animals, by replacing live animal sentinels with methods of sampling from dust, debris, and/ or pooled soiled bedding. These samples are then analysed by PCR, giving high sensitivity and specificity. EHM is now recognized for its efficacy in detecting multiple rodent pathogens when used as an adjunct or complete replacement to soiled bedding sentinel health monitoring programs. This presentation will discuss the types of EHM available for different caging systems and the benefits of changing to EHM.

Keywords: *Sentinels, Environmental Health Monitoring, Replacement,*

HOME CAGE MONITORING: ADVANTAGES, DISADVANTAGES AND PROSPECTS

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The Home Cage (HC) is a recently developed technology that enables continuous 24/7 monitoring of experimental animals, primarily mice. Its design was motivated by the 3Rs principles (Reduction, Refinement, and Replacement), aiming to improve animal welfare, enhance experimental reproducibility, generate large datasets, minimize data gaps, and maximize the use of experimental time. Technically, HC systems consist of cages equipped with cameras and sensors that monitor light, sound, temperature, movement, food consumption, and excretory outputs. These sensors can be paired with implanted devices in animals to allow real-time tracking of metabolic parameters. Data collected from the cage and animals are analysed using advanced statistical methods, machine learning, and artificial intelligence, and the system's adaptability enables diverse experimental setups. HC systems are currently employed in behavioural phenotyping, circadian rhythm studies, drug efficacy and safety testing, metabolic and toxicological research, chronic disease monitoring, and investigations of social behaviors. Despite these benefits, HC systems face significant challenges, the primary one being their high cost, which has led to the increasing use of DIY solutions, raising concerns about standardization. Additional issues include the diversity of available equipment, sensor sensitivity, calibration difficulties, and synchronization challenges. Software limitations, such as licensing and accessibility, further hinder broader use, while the vast amounts of dana generated require bioinformatics expertise that smaller research teams may lack. To address these barriers, the development of affordable and standardized sensors, user-friendly modular systems, and more open-source software and hardware solutions is essential. Collaborative equipment sharing, cost-sharing strategies, and online support communities could further enhance the accessibility and utility of HC technology for

smaller research groups. Although HC systems resolve many experimental issues, the translational value of their findings to human research remains a key question.

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Keywords: *Home Cage, animal welfare, 3Rs principles, behavioural phenotyping, big data, open-source solutions*

THEBEHAVIOURFORUM.ORG: THE FIRST ONLINE FORUM FOR DISCUSSING BEHAVIOURAL EXPERIMENTS AND LABORATORY ANIMAL WELFARE

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The Behaviour Forum is, to our knowledge, the first online Forum for discussion of laboratory animal behaviour. It was developed with the aim of improving conditions, methodologies, and study designs by facilitating networking and collaboration with other

experts and commercial representatives in the field. It is an outcome of the TEATIME EU COST Action project focusing on improving biomedical research through automated monitoring of animal behavior in home cages where laboratory animals spend most of their lives (www.cost-teatime.org). However, the Forum's scope is broader, and content related to laboratory animal behavior in general is appropriate. The Forum's vivid activity is due to the active participation of over a thousand users. The main category fostering the exchange of experiences is the Q&A category, where users discuss many aspects of animal studies, such as animal welfare, experiment planning, establishing new methods, and analysis of complex data. Other categories include Guides and Tutorials, Community News, Events, Meetings and Training, and Job Opportunities. Since its launch in July 2023, the Forum has seen continuous activity, especially in the form of detailed descriptions of experiences from day-to-day practice, and thoughtful, well-argued positions in discussions. As such, the Forum is becoming an indispensable tool for all researchers working with laboratory animals.

Note: *Co-authors are listed alphabetically.*

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Keywords: *networking, laboratory animal welfare, COST, home cage monitoring, experimental design, data analysis*

ETHICALLY ACCEPTABLE WAYS OF RESTRAINING EXPERIMENTAL ANIMALS

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Using animals in research requires restraint for blood sampling or injection. It is important to choose the method that will least influence homeostasis. Restraining methods can affect results. Mouse and rat are the most used animals in basic research. Each one requires specific way of restraining. Animals can be awake or anesthetized. Here are presented methods to restrain awaken mice or rats in ethically acceptable way. The usual method to restrain a mouse is making a skin fold from the back of the neck and expose the abdomen while grasping the tail with a little finger. The cup or cylinder method can be used to move the mouse from the cage to a work surface or another cage without adverse effect. To avoid gavage, non-injectable substance should be dissolved in milk or other drink solution that is tasteful to mice or rats. Restraining the rat with one hand is done by placing each finger of the hand to surround the head and upper limbs holding the animal still. By turning the rat, the abdominal surface is exposed to give the injection. Other method to restrain the rat is using the Camille position where upper part of the body is wrapped in cloth. The rat will stand still and the tail is exposed for blood sampling or injection. I.P. injection can be given while holding the rat with both hands close to the body of a person. In this position the abdominal wall of animal is relaxed and injection is less painful. The animal does not resist such a procedure. Novel method is restraining device with two cylinders of different diameters for blood sampling from the rat tail or injection. Hiding in litter or in a cylinder is a common behaviour of rats. Using restraint device with two cylinders is in accordance with rat's natural behaviour. Cylinders can be used for enrichment in the cage making them familiar to rats. Additionally, placing a rat in a restraining device does not affect glucose levels, which can increase during stress. The use of a standard Ketamine and Xylazine

combination for anaesthesia has a stress-inducing effect raising the animal's glucose level. Laboratory animals can be restrained in different ways depending on the planned procedure. Choosing a restraining method that will be least stressful or aversive for animal is highly important.

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Keywords: *laboratory animals, restraining by hands, restraining device*

WHY ARE 3D IN VITRO MODELS THE FUTURE OF CANCER RESEARCH?

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Cancer research is a complex and exhaustive process which includes many steps, from the research of basic mechanisms, through investigation of signalling pathways, identification of actionable protein targets, design and synthesis of potential therapeutics, and finally multiple steps of testing in vitro and in vivo. Cell culture is used as a first step in preclinical testing, as it is fast, cost-effective, and easy to use for fast screening. However, many compounds fail upon introduction to animal models, as cell culture cannot reliably represent the complex environment found in vivo, such as cell morphology, cell-cell contacts, molecular gradients, microenvironment and immune response, or predict potential side-effects on different organ systems. Therefore, 3D cell models with increasing complexity, from the most basic spheroid cultures to various models of in vitro organs, are the next step in efficient preclinical setting. This talk will introduce various types of 3D in vitro models being used or tested today, including models developed from immortalized cell lines and patient material/primary cultures. Even the simplest 3D in vitro models demonstrate differences in behaviour and response to treatment, and this increases with rising complexity of the model. The application of 3D models contributes to the 3R principle in using animal models, as research done on such 3D systems more closely resembles in vivo conditions. Even though animal models still cannot be completely excluded from the research process, introduction of 3D in vitro systems can provide an additional step of preclinical validation that can reduce animal suffering and increase the success rate of novel therapeutics.

Acknowledgments and/or foundation: *HrZZ project HHGrow3D IP-2022-10-6672*
Keywords: *3D in vitro models, cancer, preclinical testing, spheroids*

ADVANCED IN VITRO MODELS: A POWERFUL TOOL FOR 3RS PRINCIPLE

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In modern biomedical studies, particularly in neuroscience, numerous advanced in vitro models have been developed over the past twenty years, significantly reducing the use of animals in research. While a living organism remains irreplaceable, as no in vitro model can completely replicate the events occurring in an animal model, these models are continuously improving and becoming increasingly accurate. One major advantage of human in vitro models is their human origin and euploid number of genes. Notably, the genes located on human chromosome 21 are distributed across three chromosomes in mouse model of Down Syndrome. In our laboratory, we utilize various cell lines derived from both human and animal sources, with isogenic iPSCs being the most fundamental. From these iPSCs, we generate neural stem cells, neurons, astrocytes, cerebral organoids, spheroids and finally assembloids. Our models effectively replicate the processes in the human brain of individuals with Down syndrome as well as euploid controls, encompassing aspects such as development, differentiation, migration, and neurodegeneration. In our research group, we focus on analysing human cell morphology, branching, neurite diameter, synaptic activity, and cortico-striatal connections in vitro. In conclusion, while animal models cannot be entirely replaced, in vitro models, particularly 3D structures like organoids, serve as invaluable tools for many studies. Based on our findings, there is no perfect model for research; a comprehensive understanding requires both in vitro and in vivo data generated from gyri-cephalic and

lissencephalic brains. However, we have developed a robust platform for in vitro analysis and reduced animals completely.

Keywords: *iPSCs, organoids, assembloids, Down Syndrome*

TISSUE-CLEARING AND LIGHT-SHEET IMAGING. TWO POWERFUL TOOLS FOR WHOLE SYSTEM 3D ANALYSIS

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Abstract: Tissue-clearing techniques, combined with light-sheet microscopy, have emerged as powerful tools for biological imaging. However, the wide variety of tissue-clearing methods raises the question of which protocol is most suitable for specific applications. Additionally, the substantial volume of data generated by light-sheet imaging presents significant challenges in data processing, analysis, and storage. Dehydration-based tissue-clearing methods offer notable advantages, including ease of sample handling, effective clearing of both soft and hard tissues, and reduced imaging data due to smaller sample sizes. In contrast, water-based approaches provide better preservation of tissue morphology and transgenic fluorescent protein (XFP) signals, while facilitating subcellular imaging due to the ability to work with larger sample sizes. Here, we propose a tissue-clearing method that combines the benefits of both approaches

within the same sample, allowing modulation of tissue size as needed. This dual approach enables whole-mount visualization and high-resolution imaging of specific regions in the mouse brain. Furthermore, we introduce an updated version of the mesoSPIM (mesoscale selective plane illumination microscope) light-sheet system. This updated version incorporates a set of modified, high-numerical-aperture, long-working-distance, large-field-of-view objectives. Lastly, we present a data analysis pipeline that integrates virtual reality in the final step for neuronal tracing. Together, our tissue-clearing approach and mesoSPIM imaging system enable interrogation of the brain connectome with scalable sample size and resolution.

Acknowledgements: *MDI Biological Laboratory Light Microscopy Facility, which is supported by the Maine INBRE grant (GM103423) from the NIGMS at the NIH.*
Keywords: *Tissue-clearing, Light-sheet microscopy, virtual reality connectome analysis*

CAN 3D RECONSTRUCTED HUMAN EPIDERMIS TISSUE MODELS REPLACE ANIMAL TESTING?

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The potential of substances (chemicals, cosmetics, ingredients and formulations) to cause effects such as corrosion or irritation to skin 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. 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 regulatory toxicology. RhT-based assays for skin irritation/corrosion and phototoxicity testing are validated and regulatory accepted at the OECD TG 431, 439, 498 and ISO 10993-23. 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.

Keywords: *3D tissue epidermis models, alternatives, replacement of animals, skin corrosion, skin irritation*

Oral presentation

INFLUENCE OF GENETIC BACKGROUND, DIET, SEX ON GUT PH AND VOLATILE FATTY ACID PROFILE IN FAT LEAN MICE

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In laboratory mice, the analysis of volatile fatty acids (VFA) in the intestinal content reveals the effects of diet and genetic background on digestibility and microbiota. A high-fat diet typically reduces total VFA production due to decreased carbohydrate fermentation and may alter the VFA ratio, often increasing propionic acid and decreasing acetic acid. Obese mouse lines generally exhibit higher VFA levels due to enhanced fermentation efficiency, linked to a microbiota that more effectively converts nutrients into energy, contributing to obesity. In contrast, lean mouse lines tend to have lower VFA levels or a different ratio (e.g., higher acetic acid), reflecting reduced microbial energy efficiency. VFAs thus serve as indicators of dietary and genetic influences on metabolic processes and the propensity for obesity or leanness. Our study investigated the effects of genetics, diet and sex on VFA content and pH of caecum content in two polygenic mouse models: Fat (FLI) and Lean (FHI) lines. Mice were fed a high-fat diet (HFD) or a low-fat diet (LFD). The results revealed statistically significant differences in caecal pH between Fat and Lean male mice on an HFD, with diet showing a significant effect in Lean males. In females, neither diet nor genotype significantly affected caecal pH levels. There were also greater differences in VFA content in male mice than in female mice. A statistically significant difference was in genotype, higher propionic and butyric acid content in Fat mice on HFD, but not on LFD. In Lean mice, the effect of diet was evident, where propionic and butyric acid content was significantly higher in LFD compared to HFD, The data highlight the complex interplay between diet, genotype and

sex in shaping gut pH and VFA profiles, with implications for understanding metabolic adaptations and energy utilization.

Keywords: *Lean mice, Fat mice, LFD, HFD, pH, VFA, Caecum*

BREAKING BARRIERS IN OVERCOMING DEAFNESS: FROM ANIMAL MODELS TO NEUROELECTRONIC INTERFACES AMID CHALLENGES IN ANIMAL RESEARCH IN CROATIA

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The success of cochlear implants (CIs) owes much to decades of research using animal models such as guinea pigs, mice, and rat pups, which have been instrumental in understanding the electrode–neural interface and optimizing CI designs. This study investigates the potential of micro- and nano-engineered platforms to enhance the integration of spiral ganglion neurons (SGNs) with CI electrodes. SGNs were extracted from P5–P7 rat pups and adult guinea pigs using conventional and otosurgical approaches. They were cultured on silicon micro-pillar surfaces (MPS) with pillar widths of 1–5.6 µm and spacings of 0.6–15 µm, micro-patterned complementary metal-oxide-semiconductor (CMOS) electrode arrays with 0.8–1.6 µm pillar spacings, and two-dimensional (2D) nanomaterials, including graphene variants and hexagonal boron nitride. SGNs were analyzed morphologically and functionally using fluorescence imaging, machine-learning-assisted image processing, and Fluo-4 calcium imaging. Results showed that MPS and CMOS arrays promoted SGN survival, alignment, and neurite growth, with CMOS arrays enabling direct SGN–electrode contact and functional stimulation. 2D nanomaterials significantly altered SGN morphology, enhancing neurite growth, branching, and alignment. Notably, rat pups and guinea pigs demonstrated robust neuronal responses across all substrates, highlighting their complementary roles in auditory neuroelectronic research. These findings emphasize the

importance of animal models in advancing CI technology and underscore their role in bridging the gap between preclinical and clinical applications. However, the regulatory framework for animal research in Croatia often limits innovative and multidisciplinary approaches, requiring reform to better support advanced auditory neuroscience.

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Keywords: *Spiral ganglion neurons, cochlear implants, neuroelectronic interfaces, animal models, silicon micro-pillar surfaces, CMOS arrays*

Poster presentations

MODEL FOR MORBID OBESITZ – LONG-TERM KOTOGENIC DIET IN C57BL/6 MICE

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The ketogenic diet (KD), characterized by high-fat, low-carbohydrate intake, induces ketosis – a metabolic state where the body utilizes fat as the primary energy source. KD can lead to significant weight loss, reduced appetite, and improved metabolic health. It has been extensively studied for its therapeutic potential in various metabolic and neurological disorders such as type 2 diabetes, obesity, epilepsy, and neurodegenerative diseases. Recent studies have highlighted the neuroprotective effects of KD, demonstrating its ability to reduce pathology and improve outcomes in animal models of diseases like Alzheimer's and Parkinson's. Additionally, KD has shown promise in modulating behavior and cognitive functions in rodent models. Meanwhile, its long-term effects are still not fully researched. Given the diverse effects of KD, the specific composition of the diet is crucial for its efficacy. Particular attention should be paid to the sources and composition of fat. Certain fatty acids are known to promote inflammation, while others have anti-inflammatory effects. Additionally, there are fatty acids that do not play a role in the inflammatory process at all. Therefore, understanding the precise constitution of KD is essential for optimizing its benefits and minimizing potential adverse effects. The aim of this study was to establish our own mouse model prone to obesity and metabolic syndrome on the KD to determine the effect of long-

term KD (in particular, on body weight, spleen, and tissues with high energy needs – brain, muscle, liver). The study included 39 three month old C57BL/6 mice of both sexes (19 males (M) and 20 females (F)). Mice were randomly assigned to the control (K) (10 males and 10 females) or experimental group (D) (9 males and 10 females). While K was fed with standard chow (Altromin, C1324), D was fed with KD for 12 weeks (Altromin, C1084). KD had 84 % fat, 11 % protein and 5 % carbohydrates. The animals were weighted weekly till the end of the experiment and percentage change of body weight was calculated. Suprisingly, D group significantly gained weight. DM showed a significant increase in body weight after the 4th week of KD, while DF showed the same only after the 7th week. Also, DF had significanly increased spleen (Mann-Whitney U test, $p<0.001$), indicating an inflammatory process. In conclusion, KD affected males faster than females but it exerted stronger immune reaction in females. The underlying cause of these changes remains unknown and warrants further investigation.

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Keywords: *ketogenic diet, obesity, inflammation, animal model*

PRENATAL EXPOSURE TO A-CYPERMETHRIN DOES NOT AFFECT SEX HORMONE STATUS OF PUBERTAL FEMALE WISTAR RAT OFFSPRING

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During the past decades, along with an increased evidence of female reproductive disorders concern about the possible reproductive effects of environmental chemicals with endocrine- disruptive properties, including pesticides, has risen. Many studies reported that sex steroid hormones present an important target for the action of endocrine disruptive chemicals. As one of the most commonly used pyrethroid insecticide today, α-cypermethrin (α-cyp) is widely used in agricultural and veterinary as well as in different domestic applications. The endocrine disruptive effects of pyrethroid insecticide are proven, but there are no evidences about α-cyp effects on female sex hormones. Thus, the aim of this study was to investigate the effects of prenatal exposure to α-cyp on sex hormone levels of pubertal female rat offspring. Pregnant Wistar rats were exposed from 6th to 21st day of gestation (DG) to α-cyp per os in three doses (1, 10 and 19 mg/kg bw/day, respectively), diethylstilbestrol as positive control, corn oil as solvent control and water as negative control. After confirmation of the puberty onset, the blood for hormone analyses in serum of female offspring was sampled. The estradiol and testosterone levels were measured in serum by the enzyme-linked immunosorbent assay (ELISA) using commercial kits and according to the standard protocol supplied by

the kit manufacturer. Our results showed no changes in estradiol and testosterone serum levels of pubertal female offspring prenatally exposed to α-cyp. However, further research is needed to elucidate endocrine disruptive effects of this pyrethroid insecticide on female reproduction in different exposure scenarios.

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Keywords: *pyrethroid insecticide, gestational exposure, female rat offspring, estradiol, testosterone, ELISA*

TF50 EXTRACT AMELIORATES METABOLIC SYNDROME AND IMPROVING GLUCOSE TOLERANCE IN WESTERN DIET-FED MICE

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Metabolic syndrome, a major driver of obesity, raises the risk of cardiovascular disease and type 2 diabetes mellitus. Studies link high fat and carbohydrates consumption with the development of obesity. Rodents fed similar diets exhibit metabolic changes resembling those seen in humans, making them valuable models for studying metabolic syndrome. We aimed to examine the impact of FT50, a plant extract, on the progression of metabolic syndrome and type 2 diabetes, focusing on its effects on obesity, glucose tolerance, and insulin resistance. It is believed that FT50 impede the breakdown and absorption of carbohydrates in the gastrointestinal tract. By lowering blood sugar, these properties may provide therapeutic benefits. To evaluate this, we subjected C57BL/6J male mice to a long-term (12-week) Western diet enriched with simple carbohydrates. This dietary regimen induced a full-blown metabolic syndrome in mice, characterized by significant weight gain, partially compensated type 2 diabetes mellitus with glucose intolerance and insulin resistance during intraperitoneal glucose and insulin tolerance tests. Incorporating FT50 in the Western diet prevented the weight gain observed in the group fed solely the Western diet. Furthermore, FT50 not only prevented the onset of glucose intolerance and insulin resistance but also improved glucose tolerance and insulin sensitivity. These results suggest that FT50 may mitigate metabolic dysregulation by interfering with glucose absorption in the gastrointestinal tract and modulating insulin

signaling in target tissues. Additionally, FT50 supplementation exhibited lower fecal carbohydrate concentrations and pH, suggesting that the supplement may have altered the gut microbiota to utilize carbohydrates more efficiently. The findings demonstrate significant potential in advancing the development of plant extracts as viable antidiabetic agents for human therapeutic applications.

Keywords: *Metabolic syndrome, Western diet, type 2 diabetes*

BEYOND LABORATORY MAMMALS: WILD LIZARDS AS MODEL ORGANISMS IN BEHAVIORAL AND (NEURO)PHYSIOLOGICAL RESEARCH

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Standardized rearing environment, the norm in contemporary neuroscientific research, fails to provide a true representation of behaviors present in wild populations, thus motivating the exploration of unconventional vertebrate models. Reptiles, birds, and mammals have a common amniotic ancestor, making them interesting in comparative, evolutionary, and neurophysiological studies. Lizards have a simpler brain, well-suited for investigating the fundamental mechanisms of primal behavior. The insights gained from studying lizards could potentially be applied to other amniotic vertebrates. In our research, we use lizards from the family Lacertidae, the most diverse lizard family currently in expansion in Europe, therefore interesting from the evolutionary perspective; their abundance also makes them suitable for field sampling. We do research in the field of neuroethology and are currently investigating the monoamine regulation of competitive behavior in coexisting populations of lizards Podarcis siculus and Podarcis melisellensis. Lizards pose special challenges in capturing them from the wild, transportation to housing facilities and ensuring their proper care. Factors such as cage

size, substrate, temperature, and humidity are not standardized, and a specialized illumination-temperature system is necessary. Diet in captivity consists of live food, which needs to be obtained from a certified supplier and maintained alive, along with calcium and vitamin supplementation. Additionally, there are no standardized methods in behavioral research or specific reagents for molecular studies. Despite these problems, the advantages of investigating an animal from the wild outweigh the difficulties. We were able to standardize the caring and welfare of the animals, while, as for any model, respecting the 3Rs principle as well as the PREPARE, ARRIVE and STRANGE guidelines. We want to share with the wider scientific community the experience of introducing an unconventional animal model into laboratory research.

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Keywords: *reptiles, laboratory animals, behavior, neuroscience*

DECODING THE HEDGEHOG: UNRAVELING TUMOR-STROMA INTERACTIONS IN HEAD AND NECK CANCER IN 3D MODELS

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Head and neck cancers rank as the seventh most commonly diagnosed cancers worldwide, with rising incidence rates posing a significant global health challenge. This heterogeneous group of malignancies primarily arises in the upper respiratory and digestive tracts and is frequently diagnosed at advanced stages. Among these, head and neck squamous cell carcinomas (HNSCCs) are notable for their intricate interaction with the tumor microenvironment, where lymphocyte infiltration serves as a critical biomarker, while tumor-stroma ratio is being investigated as a potential prognostic biomarker. The Hedgehog-GLI signaling pathway, often upregulated in head and neck cancers, plays a pivotal role in regulating processes such as cell proliferation, survival, differentiation, and cancer-stroma communication, with elevated pathway activity correlating with poorer patient prognosis. To address the need for models that accurately reflect microenvironmental factors, we developed 3D spheroid cultures of oral fibroblast cell line HorF and oral cancer cell line Detroit 562. Using protein arrays for 108 secreted cytokines and chemokines in the supernatant of spheroid cultures, we analyzed aberrant protein expression following Hedgehog pathway hyperactivation via GLI2 transfection in fibroblasts and cancer cells. Comparative analysis between fibroblasts and cancer cells revealed 10 commonly expressed proteins, nine uniquely expressed in fibroblasts, and one exclusive to cancer cells. In fibroblasts, GLI2 transfection did not induce novel protein expression but led to the upregulation of 6 and downregulation of 12 proteins. In cancer cells, transfection resulted in the induction of one protein, with 6 proteins showing upregulation and 3 proteins showing downregulation of expression. These

findings highlight the differential impact of Hedgehog pathway hyperactivation on fibroblasts and cancer cells, emphasizing the importance of microenvironmental context in HNSCC progression. Our 3D model provides a promising platform to further dissect these interactions and identify potential therapeutic targets for this challenging malignancy.

Acknowledgments and/or foundation: *HrZZ project HHGrow3D IP-2022-10-6672*
Keywords: *head and neck cancer, 3D in vitro models, spheroids, protein array*

UNCOVERING OVARIAN CANCER SECRETOME USING 3D SPHEROID CELL MODELS

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Ovarian cancer is one of the most common malignant tumors of the reproductive organs and has the highest mortality rate among all gynecological malignancies. Treatment of ovarian cancer consists of chemotherapy and surgical debulking. Despite the availability of many therapies, acquired resistance remains a significant challenge. The limited number of established disease markers, along with the high variability and diversity of ovarian cancers, complicates the development of new effective treatment options. Since 2D cell models cannot mimic the tumor microenvironment and lack cell-cell and cell-matrix interactions, new models for research are necessary. Three-dimensional (3D) cell cultures provide an improved model of biological mechanisms that could aid the development of more effective treatments, ultimately improving patient outcomes. 3D models offer several advantages, including cost-effectiveness, simple establishment, exclusion of animal use, gradients of oxygen, pH, and nutrients. Evaluation of drug efficacy using spheroids often involves monitoring changes in spheroid shape and volume as primary indicators of therapeutic effects. Cytotoxicity during treatment disrupts cell-to-cell and cell-to-matrix interactions, leading to cell aggregation disruption. In this study, we used the ovarian carcinoma cell lines OVCAR-8, OVCAR-3, OVSAHO, IGROV-1, and SKOV-3 to establish 3D spheroid cell cultures. Spheroid formation was monitored microscopically over five days to determine the optimal cell number for further experiments based on observed morphology. Images of the spheroids were captured at 24, 48, 72, and 96 hours, and subsequently processed using the FIJI program to analyze size. Additionally, spheroids were analyzed with a three-color system for detection of live and dead cells, to determine the viability of the spheres during culturing. Based on morphology and measurements, the OVCAR-8 cell line was selected as the

most appropriate for further research. The OVCAR-8 cell line represents high-grade serous adenocarcinoma. One of the aims of this study is also to investigate which growth factors are under the control of the Hedgehog signaling pathway. To achieve this, the adherent OVCAR-8 cell line was transfected with GLI2 gene and subsequently turned into spheres. Using the formed spheres, a protein array for a panel of 108 secreted cytokines and chemokines was performed, and proteins of interest were detected. Results indicated that the expression of four secreted proteins is altered after GLI2 transfection, indicating them as potential novel transcriptional targets of the GLI2 protein.

Acknowledgments and/or foundation: *HrZZ project HHGrow3D IP-2022-10-6672*
Keywords: *ovarian cancer, secretome, 3D spheroid models, Hedgehog signaling pathway*

ALTERNATIONS OF PERINEURONAL NETS (PNNS) IN THE HIPPOCAMPUS OF ADULT RATS AFTER MODERATE, NON-INVASIVE, SHORT-TERM PERINATAL HYPOXIC INJURY

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We investigate the impact of moderate non-invasive short-term perinatal hypoxic injury in rats on morphology, distribution, number, and molecular components of perineuronal nets (PNNs) and GFAP-positive astrocytes in the hippocampus in adult rats. In total, 30 Wistar Han (RccHan: WIST) rats were separated from dams on the first postnatal day (P1) by gender and randomly classified into the control or hypoxic group. The hypoxic and control groups comprised an equal number of females and males (3 females and 3

males per experiment). They were placed in a heated hypoxic chamber together with nest and dams' cage bedding, a thermometer, and a hygrometer and subjected to hypoxic conditions: partial pressure of oxygen (pO2) 9.7325 kPa (73 mm Hg); atmospheric pressure (pATM) 46.6628 kPa (350 mm Hg) or control conditions: pO2 21.1983 kPa (159 mm Hg); pATM 101.3250 kPa (760 mm Hg) for 2 hours. Immediately after hypoxia, all rat pups were marked by a permanent toe tattoo, returned to the cage with the dams, and used later in the study. Animals were sacrificed at the age of 3.5 months for histological and immunohistochemical analysis of molecular components of PNNs and their colocalization around parvalbumin-positive neurons (PV+), as well as for GFAP-positive astrocyte expression (GFAP+). In the post-perinatal hypoxia animal group, there was a significant increase in PNNs (WFA-positive) expression in the hippocampus compared to control animals, as well as a significant increase in the number of PV+ neurons and parvalbumin, aggrecan, neurocan, and versican proteins expression. The expression of the GFAP+ astrocytes in the hippocampus was also increased in the post-hypoxia animals. The 3R principles (Replacement, Reduction, and Refinement) were applied in the research design and while experimenting, as well as the 4A's of responsible experiments on laboratory animals – 1) Awareness of the suffering experienced by the experimental animal during the experiment, 2) to Assess all procedures on animals to 3) Avoid or 4) Alleviate the suffering of the animals in the experiment.

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Keywords: *GFAP, PV, aggrecan, neurocan, versican*

CENTRAL CONTROL OF SYMPATHETIC NERVE ACTIVITY IN A RAT: THE ROLE OF SPINAL CORD AND FOREBRAIN CENTERS

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Central control of sympathetic nerve activity (SNA) has been studied extensively in the past. There is an abundance of information available about the central control of regional SNA by the brainstem and lower brain centers, however, the influence of forebrain or spinal centers, on sympathetic drive is less well understood. Some higher brain structures influence the activity of sympathetic preganglionic neurons directly while others modulate it via medullary relay centers. Additionally, spinal cord is an important site for control of SNA but sympathetic recordings obtained in spinal animals have not been frequently studied. The purpose of this study was to test the effect of forebrain and spinal centers on splanchnic sympathetic nerve activity (sSNA) in male Spraque-Dawley rats. All the experiments presented in this study were performed at Wayne State University School of Medicine. Accordingly, all protocols and surgical procedures were reviewed and approved by the Wayne State University Institutional Animal Care and Use Committee and were performed in accordance with the Guide for the Care and Use of Laboratory Animals endorsed by the American Physiological Society and published by the National Institutes of Health. SNA was recorded in urethane anesthetized, pancuronium paralyzed, and ventilated rats before and after performing precollicular decerebration. Time and frequency domain of SNA was analyzed before and after decerebration in (i) intact, (ii) baroreceptor denervated, and (iii) chronically spinalized rats. For time domain parameters: overall integrative SNA, burst amplitude, and burst frequency were quantified before and after decerebration while for frequency domain parameters: power spectral density and coherence analyses were used to quantify frequency and correlation as a function of frequency, in sympathetic and hemodynamic recordings. In both intact

and baroreceptor denervated animals, precollicular decerebration resulted in an immediate and dramatic increase in SNA of 126% ± 34% and 93% ± 38%, respectively. Spinal cord injury markedly altered the phenotype of SNA and decerebration induced increases in SNA were not observed in chronically spinalized rats. The effect of precollicular decerebration on SNA firing frequency and coherence between SNA and hemodynamic parameters is presented. These data suggest that forebrain structures provide tonic descending inhibition of regional SNA and that this inhibition exists independently of central baroreflex processing. Spinal centers are capable of maintaining sympathetic tone but clustering of action potentials is markedly affected by the spinal cord injury.

Funding source: *Startup funds.*
Keywords: *sympathetic nerve activity, forebrain centers, spinal cord*

ULTRASONIC VOCALIZATIONS AS A MEASURE OF ANHEDONIA IN A RAT MODEL OF ENDOGENOUS DEPRESSION

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The sucrose preference test (SPT) is the single most used measure for anhedonia in rodents. Still, it is criticized as imprecise and limited. 50-kHz ultrasonic vocalizations (USVs) showcase positive hedonic response to various stimuli. Rodent depression models, including the Wistar Kyoto rat line, exhibit reduced basal 50-kHz vocalizations. How such animals vocalize in response to rewards remains unknown. To test if 50-kHz USVs can be a marker for anhedonia, we compared control Wistar rats (24 males, 6 weeks old) with the Wistar Kyoto rats (24 males, 6 weeks old). Animals underwent two behavioral tests: the sucrose preference test (SPT) and the conditioned place preference test (CPP), during which USVs were recorded. SPT was performed twice, before and after CPP. In SPT, each rat resided in a separate cage for 12 hours, equipped with two 250 mL bottles, one filled with water and other with 2 % w/v sucrose solution. For the CPP, each animal was treated (s.c.) with either amphetamine (1 mg/kg, 1 mL/kg), morphine (1 mg/kg 1 mL/kg), or saline (1 mL/kg) for eight days with one practice per day. In each practice (20 minutes), the drug was paired with a randomly assigned chamber or saline with the other. USVs were recorded after the first and fourth reward administration. On test day, the animal was put in the middle chamber, and time spent in the drug-paired chamber was evaluated. One-way and repeated measures two-way ANOVAs showed significant differences for the number of vocalizations in all amphetamine treated animals. Vocalizations in Wistar rats significantly increased from basal measurements to the fourth administration, showcasing steady growth. On the

other hand, vocalizations in Wistar Kyoto rats decreased after first administration (not significantly), and significantly increased following the fourth administration. During the CPP test, no significant differences for time spent in the drug-paired chamber vs. the saline-paired chamber were observed. In SPT tests, a significant difference between the lines was observed after the first test. Wistar rats drank significantly more sucrose solution compared to Wistar Kyoto rats. No effect of drugs was observed after the second SPT test. These results indicate that measures of 50-kHz USVs provide more specific information about reward response in the depression model than traditional behavioral tests. Still, caution should be applied when inferring further conclusions due to the limited power of this study.

Keywords: *ultrasonic vocalizations, depression, anhedonia, Wistar Kyoto*

INTEGRATING LIVE CELL CALCIUM IMAGING AND TISSUE DAMAGE ASSESSMENT IN A NOVEL MODEL OF ACUTE PANCREATITIS

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Acute pancreatitis (AP) is a condition characterized by acute inflammation of the exocrine pancreas, resulting in autodigestion and destruction of exocrine tissue. The dynamic interplay between exocrine and endocrine functions is critical, as damage to acinar cells can influence endocrine function and vice versa. Despite its importance, our understanding of these interactions remains limited. Traditional histological methods are not compatible with live-cell imaging, highlighting the need for alternative techniques to simultaneously assess tissue damage and live cell activity. In this study, we introduce a novel approach that combines the commercial fluorescence LiveDead assay for tissue damage evaluation with live cell calcium imaging to assess endocrine function. To investigate this, we induced AP in adult male NMRI mice using repeated cerulein injections. Pancreatic tissue slices were prepared and stained with a calcium indicator dye to monitor calcium dynamics in beta cells via confocal microscopy. Concurrently, the

same slices were stained using the LiveDead assay to evaluate tissue damage. To validate this method, traditional histological analysis using hematoxylin and eosin staining was performed on tissue samples from a separate cohort of animals with AP. AP was found to increase the active time of beta cell oscillations in response to glucose stimulation during the stable plateau phase of their response. Histological analysis revealed classic signs of AP, including pancreatic edema, necrosis, vacuolization, and inflammatory infiltration. Notably, no significant regional differences in tissue damage were observed, and the islets of Langerhans remained histologically intact. The LiveDead assay showed strong correlation with traditional histological methods in assessing pancreatic damage. This study demonstrates that AP increases beta cell activity in response to glucose and highlights the utility of the LiveDead assay for evaluating AP in the same tissue slices used for calcium imaging. This dual approach provides valuable insights into the spatial relationship between exocrine damage and islet function in AP.

Acknowledgments: *The work presented in this study was financially supported by the Slovenian Research Agency Programs I0-0029 and P3-0396, and projects J3-9289, N3-0133, N3-0170 and J3-3077, as well as by National Research, Development and Innovation Office (SNN134497 to VV).*
Keywords: *acute pancreatitis, calcium imaging, LiveDead assay, pancreatic tissue slices, histological analysis*

BRAIN-REGION DEPENDENT STRESS INDUCED INSULIN CONCENTRATION INCREMENT AFTER INTRANASAL ADMINISTRATION

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Intranasal (IN) administration is a non-invasive method for delivering drugs to the central nervous system (CNS). Nasal cavity's large absorption surface and rich vascularization, as well as the nasal mucosa's close proximity to the brain, ensure rapid delivery of drugs to the CNS and allow drug concentrations in cerebrospinal fluid (CSF) to exceed those in plasma. This way, the blood-brain barrier and hepatic first-pass metabolism are bypassed, systemic uptake and related peripheral side effects are minimazied and bioavailability of the drug is higher. The exact mechanism by which drugs reach the brain remains unclear, but it is hypothesized that they travel along the olfactory and trigeminal nerves. IN use of regular insulin has shown efficacy in clinical trails for Alzheimer's disease (AD) and mild cognitive impairment. One of the main goals of this research is to clarify and explore the time-dependent insulin concentration in the rat brain following intranasal administration of insulin or saline. Male Wistar rats were given intranasally regular insulin (2 IU) or saline and were sacrificed 3, 7.5 and 15 minutes after administration (6 animals per group). Control animals were sacrificed without intranasal administration. Insulin concentration were measured in plasma, cerebrospinal fluid (CSF), respiratory and olfactory epithelia (RE and OE), hypothalamus (HPT), parietal cortex (PC), temporal cortex (TC) and hippocampus

(HPC). Insulin immediately distributes to all observed brain regions after intranasal insulin administration and is instantly utilized and/or metabolized. The most pronounced insulin concentration increment was found immediately (3 min) after saline administration in PC (6.3 fold higher). In addition, more than 2 fold increment was found in: OE (2.5) and HPT (2.6) 7.5 min after administration; HPC (2.8) and TC (3.0) 15 min after saline administration. Insulin concentration was found unaltered in plasma, CSF and RE after saline administration. Handling and intranasal administration induces stress in rats, seen as an acute insulin concentration increment suggesting increased entrance or production of insulin in the brain. Insulin increment after saline administration was inconsequential in comparison to insulin administration (up to 12 fold higher). In addition, insulin dose was likely too high and caused its transport back to epithelia through unknown mechanisms, which could be of relevant significance for human dose reduction.

Acknowledgments: *This work was supported by the Croatian Science Foundation under the project number HRZZ-2022-10-1895 and DOK-NPOO-2023-10-3354, University of Zagreb project (10106-23-2516) and co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).*
Keywords: *intranasal administration, insulin, rat*

REVERSAL OF DIET-INDUCTED BETA CELL DISFUNCTION IN TYPE 2 DIABETES WITH CALORIC RESTRICTION

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Diet-induced obesity (DIO) mouse models are a pivotal research model for studying pathophysiology of type 2 diabetes mellitus (T2D). Currently used models have some inherent methodological drawbacks such as beta cell plasticity when used at an early age, and the composition of the diet used to induce T2D. Clinical studies by Taylor et al. hint at effective T2D remission with caloric restriction in humans, however limited mechanistical explanation is available at the level of beta cell function. To address that void, we therefore constructed a novel mouse model of DIO that more closely reflects T2D in humans. Male and female C57BL/6J mice were fed a western diet for 12 weeks starting from the age of 12 weeks, after which they exhibited a T2D phenotype in the form of increased body weight, fasting hyperglycemia, impaired glucose clearance during intraperitoneal glucose tolerance test ipGTT and increased insulin resistance during intraperitoneal insulin tolerance test (ipITT). Implementing 7 days of caloric restriction (35 % of the caloric intake of the control group) completely reversed the diabetic phenotype, with normalization of body mass, normalization of glucose handling and insulin sensitivity. To provide a mechanistical explanation for both the DIO and remission following caloric restriction at the level of beta cell function and glucose sensitivity, we performed functional multicellular confocal calcium imaging on acute

pancreatic tissue slices. A left shift in the glucose dependence was detected in the DIO group, which together with hyperglycemia could account for hyperinsulinemia observed in vivo. Short term caloric restriction completely reversed the compensatory left shift in beta cells and decreased their oscillatory activity. Our findings further elucidate the impact of caloric restriction on T2D and our model provides a novel platform for studying T2D.

Keywords: *Type 2 diabetes, beta cells, caloric restriction*

FELASA WORKING GROUP: PAIN ASSESMENT IN MICE

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The species most commonly used in research is the mouse, and since many experiments involve procedures that can cause pain, standardized pain treatment protocols for mice are highly valued. Nevertheless, there is a constant need for refinement and adaptation. The diversity of mouse strains makes the detection of pain conditions more complex, as pain-related behaviors and clinical signs can vary by strain, experimental model, and genetic variation. There is a need for defined guidelines for the management of pain in mice undergoing potentially painful procedures. FELASA has recognized this and formed a group of experts to review current pain management strategies and produce a review article with guidelines and recommendations for the proper recognition, assessment and relief of pain in experimental mice. As part of the working group, a questionnaire was created to assess the attitudes of personnel involved in mouse models in which analgesia or other analgesic methods are used to relieve pain in mice. This research on analgesic treatments in mice provided valuable data on current strategies. Based on these data and new insights into pharmacologic options, this group will propose methods for administering analgesics, techniques for evaluating analgesic efficacy, and options for pain-management refinement in mice.

Keywords: *mice, pain, treatment, standardisation*

NINE ATEMPTS OF ARBOVIRUS ISOLATION USING SUCKLING MICE

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Arboviruses are an ecological group of viruses characterized by the specific biological transmission from a competent hematophagous arthropod to a warm-blooded vertebrate including humans. Isolation of infectious viruses is not always successful using cell lines most often due to very low viral load or inhibitory effect of the samples used for inoculation. In such cases suckling mice can be used, as they have an immature immune system and are very susceptible to pathogens. From 2017 to 2024 9 attempts of arbovirus isolation were performed in the animal facilities, following all appropriate biosafety level measures. Altogether, isolation attempts were performed, of Zika virus (1X), Tick-borne encephalitis virus (TBEV) (1X), Trojica virus (2X), West Nile virus (WNV) (3X) and Sindbis virus (2X). A total of fifty-one 5/6-day old BALB/c suckling mice (provided by Medical Experimental Centre, Ljubljana, Slovenia) were included. In each experiment the litter was housed with a female mouse in a cage in controlled environmental conditions. Suckling mice were inoculated intracerebrally with 20 µL of inoculum (samples from 3 patients, 3 virus isolates, 2 brain homogenate passages and 3 mosquito pools, respectively), except a control group of 1 suckling mouse that was not inoculated. Mice were monitored daily according to the clinical scoring system used to determine humane endpoints. On average 8±3 days (maximum 14 days) post-inoculation, mice were euthanized with CO2. Brains from suckling mice and blood from female mice were collected for analysis. Total nucleic acids were isolated for detection of individual arbovirus by real-time RT-PCR (rt RT-PCR). Out of nine attempts, there were two successful virus isolations. First the historical TBEV isolate was successfully re-isolated in all 3 inoculated suckling mice and also detected by rt RT-PCR in the blood of the female mouse after 4 days post inoculation. Second, WNV was successfully isolated 7 days post inoculation from a pool of mosquitoes in one suckling mouse (4 were inoculated) and

was also detected in the blood of the female mouse, by rt RT-PCR. Zika virus, Trojica virus and Sindbis virus isolation were unsuccessful. Virus isolates are very important for human virology diagnostics and research; for virus characterization, pathogenesis studies and development of diagnostic tests and protocols.

Foundation: *Slovenian Research and Innovation Agency Programme; Host-Parasite Relationship (P3-0083). All procedures involving animals were approved by the National Ethical Committee and the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection. Animal care and treatment were conducted in accordance with the institutional guidelines and international laws and policies (Directive 2010/63/EU on the protection of animals used for scientific purposes).*
Acknowledgments: *We would like to acknowledge and thank Tea Knapič and Jana Šušnjara for helping us with field work and mosquito identification, Klara Bohinc and Alenka Pintar for their help with laboratory work, Jana Turk and Katja Potočnik for mosquito identification.*
Keywords: *virus isolation, arboviruses, suckling mice, virology*

Workshop

CARLOS OSCAR SORZANO[1]: THE NEED FOR STATISTICAL EXPERIMENTAL DESIGN IN EXPERIMENTS WITH ANIMALS

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Proper experimental design is essential for ensuring reliable, reproducible, and ethically sound research when working with animals. Despite best intentions, suboptimal study planning—such as inadequate statistical power, insufficient sample size calculations, and poorly chosen experimental endpoints—can compromise both the validity of findings and the welfare of the animals involved. This talk will highlight common pitfalls in experimental design for animal studies and demonstrate how robust statistical methods and thoughtful research planning can significantly enhance outcome validity. By addressing challenges such as randomization, blinding, and appropriate control selection, researchers can reduce bias and uncertainty in their work. Moreover, adopting strong statistical frameworks helps to refine experiments, thus minimizing the number of animals required while improving the overall quality of the data collected. Attendees will leave with practical guidelines on implementing statistical best practices that can lead to more trustworthy results and more responsible use of laboratory animals.

Key words: *statistical experiment design; sample size calculation; factorial design*

MATTEK 3R WORKSHOP

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SILVIA LETASIOVA: Skin Irritation Test using 3D reconstructed tissue model of human epidermis

MatTek has been a global leader in innovative in vitro human tissue models for over 25 years. Their advanced skin, ocular, oral, respiratory, and intestinal models are widely used in the cosmetics, chemical, pharmaceutical, and household product industries to ensure safety and efficacy without animal testing.

The workshop is led by Silvia Letasiova. She is the managing director and senior scientist at MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia. She has background in biochemistry and microbiology and holds a doctoral degree in biochemistry. Her main field of interest is the development and production of highly reproducible and predictive 3D reconstructed tissue models for in vitro topical toxicity testing (i.e. skin/eye irritation, corrosion, phototoxicity and sensitization) thus contributing to reduction & replacement of in vivo testing. Silvia is a member of ESTIV (EU Society of Toxicology in vitro), SETOX (Slovak Toxicology Society), EUSAAT (EU Society for Alternatives to Animal Testing) and a member of US Society of Toxicology.

Within the workshop she will cover Skin Irritation Test using 3D reconstructed tissue model of human epidermis. Skin irritation refers to the production of reversible damage to the skin following the application of a test substance. Understanding the potential of topically-applied chemicals or formulations to induce skin irritation (hazard) is an important consideration in their safety evaluation. The EpiDerm Skin Irritation Test was developed and designed to predict skin irritation potential of neat test substances in the context of identification and classification of skin irritation hazard according to the EU classification system (R 38 or no label) and the UN GHS system. The EpiDerm Skin

Irritation Test allows for the discrimination between irritants of category 2 and non-irritants. MatTek offers the EpiDerm Skin Irritation Test as a GLP or non-GLP service.

Key words: *skin irritation; 3D reconstructed tissue model; in vitro topical toxicity testing*

Author Index

A

B

C

Č

D

E

F

G

H

I

J

K

L

M

N

O

P

R

S

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T

V

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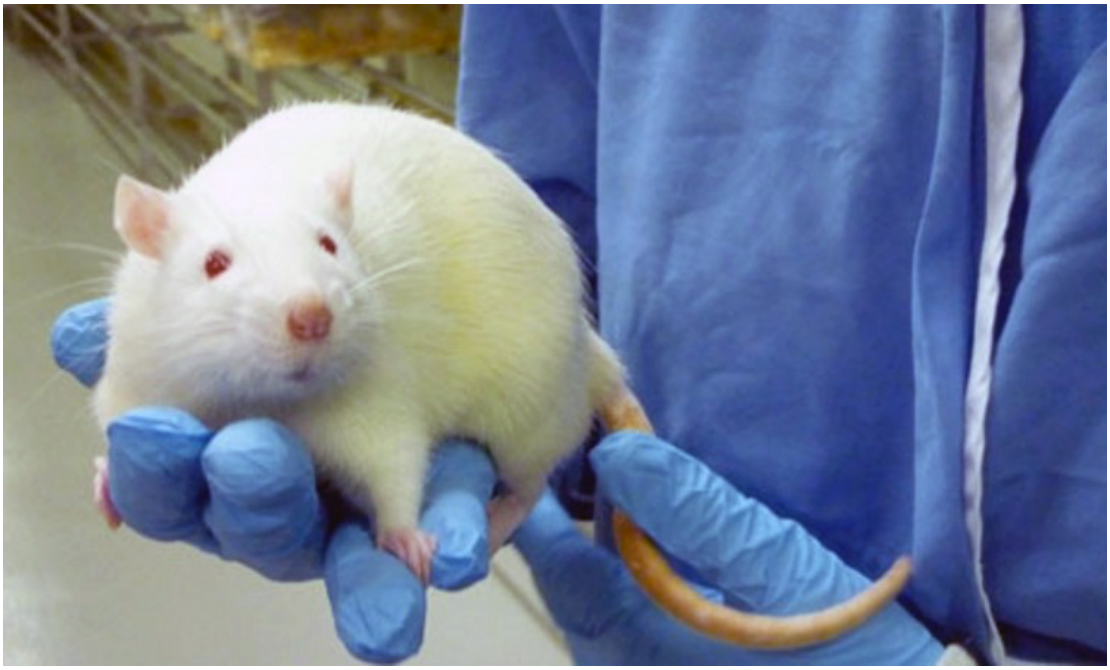
Uspjeli smo opremiti gotovo sve znanstvene i istraživačke ustanove na području Hrvatske, koje su na bilo koji način povezane sa laboratorijskim životinjama.



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