SABV SYMPOSIUM 2024

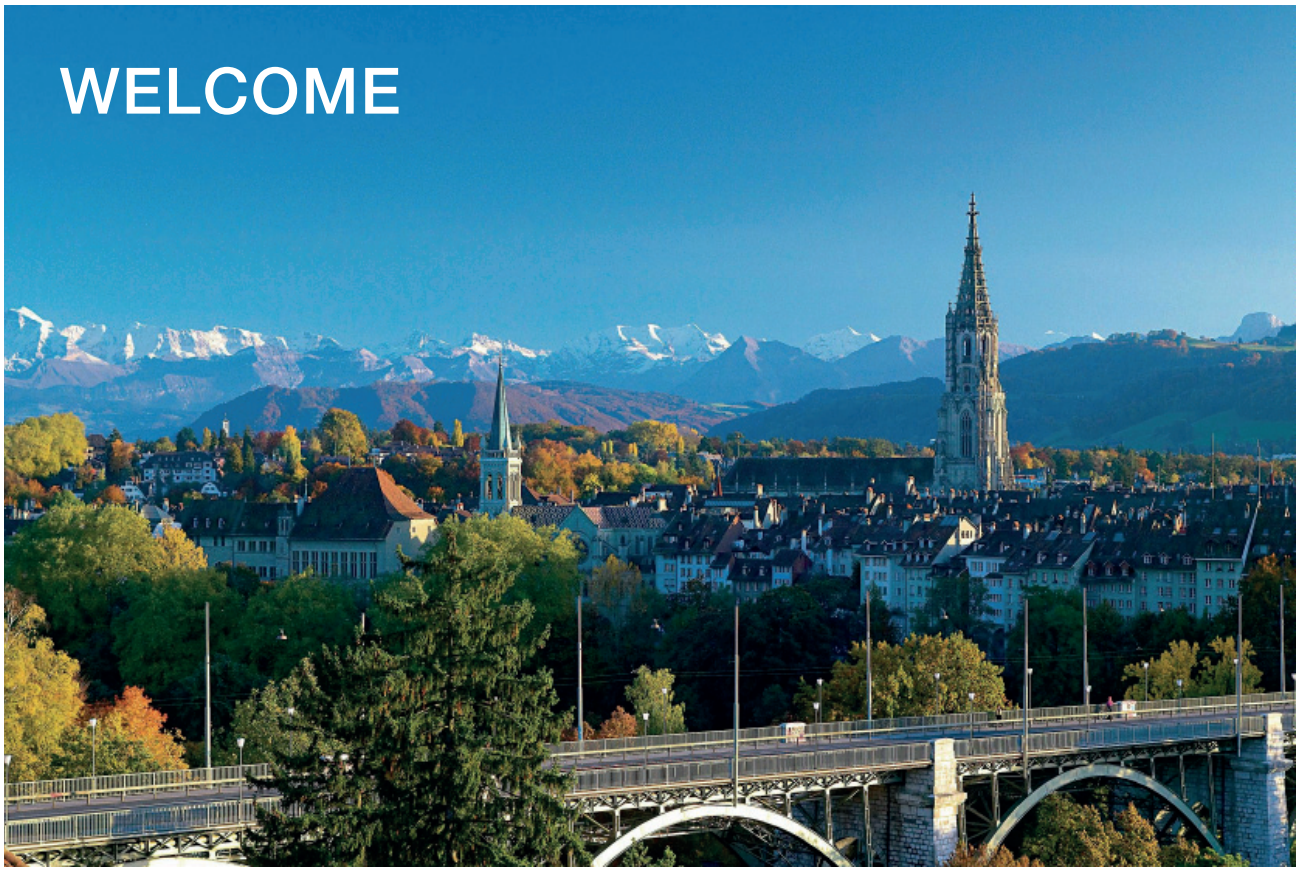
Sex as a Biological Variable (SABV)

Addressing Challenges and
Paving Future Steps in
Basic and Preclinical Research

12 - 14 June 2024
Bern, Switzerland

UNIVERSITÄT BERN

WELCOME



Dear Colleague,

It is a great pleasure to welcome you to the **SABV Symposium 2024** in Bern, following the success of our inaugural SABV Symposium in 2022. This international symposium is dedicated to raising awareness of the importance of the SABV initiative and providing evidence-based guidance for integrating SABV into research practices effectively. The symposium features lectures by internationally renowned experts from various disciplines, who are presenting current evidence on how sex influences biological processes from the molecular to the organismal level. The program also includes presentations from selected abstracts, poster sessions, and practical workshops on non-invasive estrous cycle evaluation and statistical analysis for experimental designs incorporating both sexes.

I hope you'll enjoy the symposium and find it inspiring for your research, broadening your perspective on sex as a biological variable. Wishing you a great and productive time in Bern, and I warmly thank you for participating.

Ivana Jaric

We would appreciate it if you could help us improve our SABV symposium by taking a short survey to give us feedback. The survey will also help authorities consider if symposium on this topic and workshops like those presented here should be organized in the future as continuing education courses.

Scan to open SABV Symposium EVALUATION FORM

FUNDED BY:

Swiss National Science Foundation

SNSF Scientific Exchanges grant (Grant number 224763) to Ivana Jaric

3R Swiss 3R Competence Centre

3RCC grant
Best practice guidance for including sex as a biological variable in animal research (grant number OC-2020-004) to Ivana Jaric and Hanno Würbel

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SGV swiss laboratory animal science association

Swiss Laboratory Animal Science Association (SGV)

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IMPORTANT INFO

Wi-Fi

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Instructions for guests:

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Symposium venues

Date	Venue
12. June (Wednesday)	Cupola room Hochschulstrasse 4
13. June (Thursday)	
14. June (Friday)	Main lecture hall Länggassstrasse 124c

Continuing Education Certificate

The SABV Symposium 2024 has been accredited as **2 days of continuing education** for study directors and experimenters by the Federation of Swiss Cantonal Veterinary Officers (VSKT).
Study directors and experimenters working in Switzerland can obtain the certificate **ONLY if they have signed the list of attendance at the registration desk** at the beginning and at the end of each symposium day.

The workshops will be recognized for half a day of continuing accreditation.

Wednesday 12. June 2024

8.30-9.00	Registration	
9.00-9.15	**Dr. Ivana Jaric** ***University of Bern*** ***Switzerland***	**Welcome address and symposium intro**
9.15-9.30	**Prof. Dr. Hanno Würbel** ***University of Bern*** ***Switzerland***	**Embracing diversity in animal research – SABV is a matter of inclusion and good research practice**
9.30-10.15	**Prof. Dr. Paola Palanza** ***University of Parma*** ***Italy***	**How many letters in animal research? The role of the 4th S for Sex**
10.15-10.45	Coffee break and posters viewing	
10.45-11.30	**Prof. em. Dr. Günter Vollmer** ***Technische Universität Dresden*** ***Germany***	**Role of life stage and metabolism on sex specific outcomes in experimental animal studies**
11.30-12.15	**Prof. Dr. Cheryl McCormick** ***Brock University*** ***Canada***	**Challenges investigating sex-specificity and sex differences in behaviour in developmental studies**
12.15-12.50	**Mikail Nidaa, MD** ***University of Zurich*** ***Switzerland***	**Sex Differences in the Brain-Heart Axis in the Setting of Acute Mental Stress**
12.50-13.05	**Anja Helmer** ***Inselspital, University of Bern*** ***Switzerland***	**Sexual dimorphism in cardiac graft recovery in a rat model of donation after circulatory death** (short talk selected from abstracts)
13.05-14.05	Lunch (Restaurant Grosse Schanze, Parkterrasse 10)	
14.05-14.40	**Dr. Marija Glisic** ***University of Bern*** ***Switzerland***	**Conceptual Framework for Sex and Gender Disaggregated Research in the Field of Predominantly Male Conditions: Spinal Cord Injury as a Case in Point**
14.40-15.25	**Adj. Prof. Dr. Leena Strauss** ***University of Turku*** ***Finland***	**Sex Steroid Biosynthesis – Deciphering Mouse and Human Pathways**
15.25-16.10	**Prof. Dr. Ivan Nalvarte** ***Karolinska Institutet*** ***Sweden***	**Sex hormones and sex differences in Alzheimer's disease**
16.10-16.40	Coffee break and posters viewing	
16.40-17.25	**Prof. Dr. Natalie Rasgon** ***Stanford University*** ***USA***	**Neuronal insulin signaling associated with sex-specific phenotype in mood disorders**
17.25-17.40	**Dr. Elena Kutsarova** ***Max Planck Institute for Brain Research*** ***Germany***	**The proteomic profile of the midbrain periaqueductal gray: impact of sex and social environment** (short talk selected from abstracts)

Thursday 13. June 2024

SABV SYMPOSIUM 2024

9.00-9.45	**Prof. Dr. Thorsten Buch** ***University of Zurich*** ***Switzerland***	**Inclusion of Both Sexes in a Single Animal Experiment: Best Practice Guidance for Statistical Design and Analysis**
9.45-10.25	**Dr. Antonella Santuccione Chadha** ***Women's Brain Project*** ***Switzerland***	**Integrating Sex as a Biological Variable in Neuroscience: *In vitro* aspects**
10.25-10.55	Coffee break and posters viewing	
10.55-11.40	**Prof. Dr. Charlotte Cornil** ***University of Liège*** ***Belgium***	**Role of neuroestrogens on social behavior**
11.40-12.25	**Prof. Dr. Christina Dalla** ***National and Kapodistrian University of Athens*** ***Greece***	**Sex differences in models of depression and anxiety: GPER1 as a new psychopharmacological target**
12.25-12.40	**Prof. Dr. Nicole Gervais** ***University of Groningen*** ***Netherlands***	**The role of sex and ovarian hormones in sleep loss-induced hippocampal deficits** (short talk selected from abstracts)
12.40-12.55	**Dr. Stefan Rudloff** ***Inselspital, University of Bern*** ***Switzerland***	**Exploring sex-related disparities in different segments of the renal tubule** (short talk selected from abstracts)
12.55-14.00	Lunch (Restaurant Grosse Schanze, Parkterrasse 10)	
14.00-14.45	**Dr. Jodi Pawluski, PhD, HDR** ***Université de Rennes*** ***France***	**Beyond SABV: The importance of reproductive experience on the adult brain**
14.45-15.30	**MSc Zoé Bürger** ***University of Tübingen*** ***Germany***	**Feeling stressed? Stress response differs in women using intra-uterine or oral hormonal contraception and naturally cycling women**
15.30-16.15	**Prof. Dr. Natalie Tronson** ***University of Michigan*** ***USA***	**Beyond sex differences: Hormone contraceptive modulation of stress**
16.15-16.45	Coffee break and posters viewing	
16.45-17.25	**Dr. Ivana Jaric** ***University of Bern*** ***Switzerland***	**The importance of environmental and hormonal factors in implementing the SABV in rodent research**
17.25-17.35	**Dr. Paolo Cinelli** ***Swiss Laboratory Animal Science Association (SGV)***	Presentation of Swiss Laboratory Animal Science Association (SGV)

Friday 14. June 2024

SABV SYMPOSIUM 2024

9.00-9.40	**Dr. Ivana Jaric** ***University of Bern*** ***Switzerland***	**Estrous cycle as a crucial indicator of female health outcomes and tips on how to access it**
9.40-10.10	**Adj. Prof. Dr. Leena Strauss** ***University of Turku*** ***Finland***	**Estrous cycle determination by deep learning-based method on Aiforia® platform**
10.10-10.40	Coffee break	
10.40-11.40	**Strauss, McCormick, Dalla, Pawluski, Tronson, Rasgon, Jaric**	**Round table discusion**
11.40-12.00	**Dr. Bernhard Voelkl** ***University of Bern*** ***Switzerland***	**Statistical accommodations for the inclusion of sex**
12.00-12.10	**Dr. Ivana Jaric**	**Closing remarks**
12.10-13.30	Lunch - only for the speakers and participants of the workshops (Mensa vonRoll, Fabrikstrasse 8)	

13.30-17.30

Workshop 1: Estrous cycle

Dr. Ivana Jaric

13.30-17.00

Workshop 2: Statistical analysis

Dr. Bernhard Voelkl

IMPORTANT: Please bring your own laptop with R installed.

SPEAKERS

Prof. Dr. Hanno Würbel, University of Bern, Switzerland

Embracing diversity in animal research – SABV is a matter of inclusion and good research practice

Male bias in animal research is partly rooted in male chauvinism, partly in a misguided methodological strive for precision and reproducibility. While the chauvinist motivation is easy to dismiss, the methodological fallacy inherent to focussing on one sex only proves much harder to overcome.

Prof. Dr. Paola Palanza, University of Parma, Italy

How many letters in animal research? The role of the 4th S for Sex

We all appraise the importance of the 3Rs (3R = reduction, refinement, replacement) in animal research. In the perspective of robust face, predictive and construct validity of animal models, we also need to carefully consider the 3Ss (3S = species, strain, setting). However, to disentangle the hidden variability in experimental data we must take into account the fourth S of Sex as a fundamental factor to unravel differential responses to experimental variables and vulnerability to disease.

Prof. em. Dr. Günter Vollmer, Technische Universität Dresden, Germany

Role of life stage and metabolism on sex specific outcomes in experimental animal studies

This presentation will summarize key insights gained from animal studies investigating the impact of natural compounds (botanicals) on hormone dependent target tissues. Special emphasis will be given on the role of life stages using the female rat as an experimental model and on metabolism test material in pharmacological studies using complex botanical mixtures. Overall evidence is provided that these physiological processes contribute to sex specific variations in experimental outcomes.

Prof. Dr. Cheryl McCormick, Brock University, Canada

Challenges investigating sex-specificity and sex differences in behaviour in developmental studies

In this talk, I will highlight some of the milestones in the understanding of sexual differentiation of the brain; given such knowledge, the question is why have so many researchers avoided investigating females. I will use old and new data from my lab to illustrate the challenges in, and value of, incorporating both sexes into one's research program.

Mikail Nidaa, MD, University of Zurich, Switzerland

Sex Differences in the Brain-Heart Axis in the Setting of Acute Mental Stress

Investigating the gender gap in cardiovascular disease management, our prospective study delves into the amygdala and myocardial responses to acute mental stress, alongside the hypothalamus-pituitary-adrenal axis and sympathetic nervous system responses. In our clinical study, we explore the connections between stress-related neural activity and mental stress, shedding light on potential gender-based differentials in cardiovascular diseases.

Dr. Marija Glisic, University of Bern, Switzerland

Conceptual Framework for Sex and Gender Disaggregated Research in the Field of Predominantly Male Conditions: Spinal Cord Injury as a Case in Point

Sex and gender are critical factors in shaping the landscape of disease, disability, and death. Inclusive research that considers both sex and gender is vital to improving health outcomes for both women and men. This is particularly problematic for diseases and health conditions that primarily affect men. Spinal cord injury (SCI) serves as a good example, with approximately 80% of affected individuals being male. Therefore, addressing the sex and gender gap in SCI research poses many challenges. In a collaborative effort involving Swiss Paraplegic Research, University of Bern, Charité – Universitätsmedizin Berlin, Harvard University, and University of Lucerne, we developed a framework for prioritizing sex/gender-disaggregated research in the SCI field. We hope that this framework can serve as a model to guide future research directions for health conditions where women are in the minority, ensuring that everyone benefits from scientific advancements, regardless of sex and gender.

Adj. Prof. Dr. Leena Strauss, University of Turku, Finland

Sex Steroid Biosynthesis – Deciphering Mouse and Human Pathways

Mice are widely used as models to study defects in reproduction and in many sex steroid-dependent diseases, such as breast and prostate cancer. Although the production of sex steroids and their regulation is mostly similar in mice and humans, there are also differences that have to be considered while conducting translational research. In this lecture, I will present the most significant steroidogenic pathways in mice and humans, and discuss their similarities and differences.

Adj. Prof. Dr. Leena Strauss, University of Turku, Finland

Estrous cycle determination by deep learning-based method on Aiforia® platform

The stage of the estrous cycle can be determined by cytology through the microscopic examination of vaginal smears from mice. This is a time-consuming process since studies often involve high numbers of mice and many samples over many weeks. I will present a deep learning AI-based method for the automated classification of estrous cycle stages.

Prof. Dr. Ivan Nalvarte, Karolinska Institutet, Sweden

Sex hormones and sex differences in Alzheimer's disease

Two thirds of all patients suffering from Alzheimer's disease are women. A combination of factors may attribute to this sex difference, including environment, sex chromosomes, and sex hormones. My laboratory studies the effect of the female sex hormone estrogen on healthy brain aging, with focus on the neuroprotective effects of estrogen receptor beta. Understanding the sex differences in Alzheimer's disease can bring new knowledge to its etiology and can suggest new sex-specific preventive recommendations to combat this disease.

Prof. Dr. Natalie Rasgon, Stanford University, USA

Neuronal insulin signaling associated with sex-specific phenotype in mood disorders

While sex differences in presentation, clinical course, and treatment outcomes in mood disorders are well described, specific biomarker signatures remain to be elucidated. Here, I will present the data from my group showing that 'in vivo' neuronal insulin signaling can serve as a predictor of sex-specific phenotypes in mood disorders.

Prof. Dr. Thorsten Buch, University of Zurich, Switzerland

Inclusion of Both Sexes in a Single Animal Experiment: Best Practice Guidance for Statistical Design and Analysis

Differences between the sexes have long been neglected in biomedical research. This was often based on the assumption that results from one sex can simply be applied to the other sex. One prominent reason for the use of only one sex in basic and translational animal research is the pressure to reduce the number of animals according to the "3R" principle (3R = reduction, refinement, replacement). Thus, scientists currently find themselves in the dilemma of having to minimize animal consumption while still detecting a treatment effect and having to maximize insight by including both sexes. Here, I will present a statistical approach to effectively design studies that incorporate both sexes, while conforming to the requirements of ethical principles and financial limitations.

Dr. Antonella Santuccione Chadha, Women's Brain Project, Switzerland

Integrating Sex as a Biological Variable in Neuroscience: *In vitro* aspects

In order to achieve and maintain brain health, it is crucial to recognize and comprehend the role of sex and gender in the field of neuroscience. Men and women often exhibit differences in psychiatric and neurological disorders, which must be taken into account for effective treatments. However, historical research has frequently neglected these differences, leading to an incomplete understanding of these disorders. Fortunately, recent guidelines from organizations such as the National Institutes of Health and the European Commission emphasize the importance of considering sex as a biological variable (SABV). These guidelines have significantly impacted experimental design, analyses, and reporting across both preclinical and clinical studies, with researchers now recognizing sex as one of the instrumental factors to consider. In this context, I will discuss the most common neurodegenerative diseases with a sex and gender lens, and discuss different aspects related to integrating SABV into in vitro experiments.

Prof. Dr. Charlotte Cornil, University of Liège, Belgium

Role of neuroestrogens on social behavior

Different sex hormones are required for the activation of sexual behavior in males and females (testosterone in males vs estradiol and progesterone in females). The action of testosterone on male sexual behavior is mediated in part by its conversion into an estrogen within the brain, a reaction catalyzed by the enzyme aromatase. This talk will focus on how neuroestrogens control male sexual behavior but will also discuss what neuroestrogens might do in females.

Prof. Dr. Christina Dalla, National and Kapodistrian University of Athens, Greece

Sex differences in models of depression and anxiety: GPER1 as a new psychopharmacological target

Affective and anxiety disorders significantly burden individuals and society, with a higher prevalence in women. Recently, G protein-coupled estrogen receptor 1 (GPER1) has been implicated in depression/anxiety, with a suspected overlap with the rapid antidepressant effects of glutamatergic drugs such as esketamine. I will present recent data from my group contributing to the identification and characterization of a potential new target for developing novel treatments for anxiety and affective disorders. Our studies underscore the importance of considering sex in preclinical research to improve the efficacy of antidepressant and anxiolytic treatments.

Dr. Jodi Pawluski, PhD, HDR, University of Rennes, France

Beyond SABV: The importance of reproductive experience on the adult brain

In the study of SABV we are often focused on similarities and differences between males and females, however it is important to take into account how life experiences, such as parenting, interact with sex/gender to impact neurobehavioral outcomes in adulthood. This talk will highlight how reproductive state and parenting impact the adult brain and point to the value of incorporating these experiences in SABV research.

MSc Zoé Bürger, University of Tübingen, Germany

Feeling stressed? Stress response differs in women using intra-uterine or oral hormonal contraception and naturally cycling women

Intra-uterine devices (IUDs) are worldwide the third most used method for birth control. Although data shows that exogenous hormones impact women's mental well-being, behaviour and brain functions, research on the impact of hormonal IUDs (LNG-IUDs) are still scarce. In this presentation, I will focus on longitudinal data in women using LNG-IUDs. Specifically, I report results on stress response in women with different hormonal status, namely women using the LNG-IUD (containing the progestin levonorgestrel, but no synthetic oestrogen), women using combined hormonal contraceptives orally (OCs; containing both a synthetic oestrogen and a progestin) and women with a natural menstrual cycle (NC). Using the Maastricht Acute Stress Test we induced stress in a female human sample and assessed exogenous and endogenous sex hormones as well as multi-dimensional stress indicators such as salivary cortisol, subjective stress, heart rate and skin conductance. Our results show group differences in stress response depending on the stress indicator, which are further associated with endogenous sex hormone concentrations. Research on hormonal contraception and the separation by hormonal contraception type is urgently needed to understand the complex relationship with mental well-being in detail, as distinct administration routes and hormonal composition of the method seem to impact women's stress responses in different ways.

Prof. Dr. Natalie Tronson, University of Michigan, USA

Beyond sex differences: Hormone contraceptive modulation of stress

With rising attention to including females in basic research and animal models of disease, and a growing attention to data on women's health, several things are becoming clear. First - sex matters, and underlying mechanisms of pathological processes can differ even when symptoms appear the same. Second, "females" are not a single category, and sex/gender-specific experiences and exposures also influence disease outcomes. In this talk, I will focus on hormone contraceptives, their role in modulating stress, and the importance of incorporating hormone contraceptive exposure in animal models of disease.

Dr. Bernhard Voelkl, University of Bern, Switzerland

Statistical accommodations for the inclusion of sex

Including both sexes in an experimental study requires adjustments of the statistical analysis. At the planning stage the question arises, how the inclusion of both sexes affects the study design and the power calculation for determining the required sample size. For data analysis several options exist –from separate analyses of the sexes to different ways of joint analyses. The pros and cons of these different options will be outlined.

Dr. Ivana Jaric, University of Bern, Switzerland

The importance of environmental and hormonal factors in implementing the SABV in rodent research

The underrepresentation of female mice has led to the establishment of the SABV policy, mandating equal representation of both sexes in basic and preclinical biomedical research. However, its practical implementation in animal facilities has raised questions about whether to house male and female mice together or in separate rooms. Although rarely reported, this crucial aspect of the animals' environment can significantly influence physiology and behavior, consequently impacting experimental outcomes. I will discuss the effects of housing male and female mice in setups with or without odor cues from conspecifics on neurobehavioral and reproductive outcomes, offering insights into best practices for mouse housing and experimental design when implementing SABV.

Dr. Ivana Jaric, University of Bern, Switzerland

Estrous cycle as a crucial indicator of female health outcomes and tips on how to access it

Recognizing the rodent estrous cycle as a crucial welfare indicator faces challenges due to a historical bias toward male animals in the scientific community. However, while the SABV initiative is well-intentioned, it has overlooked the importance of the estrous cycle as a variable, despite it being well-known that ovarian hormone status can profoundly impact brain function, cardiovascular health, and metabolic responses. In this talk, I will discuss why the estrous cycle is an important variable to consider, when it is useful to track it, and present different methods of assessing it.

SHORT TALKS SELECTED FROM ABSTRACTS

SABV SYMPOSIUM 2024

Sexual dimorphism in cardiac graft recovery in a rat model of donation after circulatory death

Anja Helmer 1,2,3, Alexia Clavier 1,2,3, Selianne Graf 1,2,3, Maria Arnold 1, Joyce Wüthrich 1, Adrian Segiser 1,2, Matthias Siepe 1, Sarah Longnus 1,2

1 Department of Cardiac Surgery, Inselspital Bern University Hospital, Bern, Switzerland
2 Department for BioMedical Research, University of Bern, Bern, Switzerland
3 Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

Transplantation of hearts with donation after circulatory death (DCD) is one possibility to increase cardiac graft availability. However, DCD hearts are subjected to potentially harmful conditions during, and even before the onset of warm ischemia, in the donor. In response to ischemic injury and the adaptation to altered substrate availability in the heart, sex differences have been identified, but little is known in the context of DCD heart transplantation.

Hearts from sham-operated males and females, as well as ovariectomized (OVX) Wistar rats underwent simulated DCD followed by heart explantation and ex-vivo perfusion. Cardiac recovery was evaluated with measurements of ventricular and endothelial/vascular function, metabolism, and cell death.

Preliminary results suggest that female hearts show increased tolerance to ischemiareperfusion injury (IRI) and overall graft recovery compared to male and OVX hearts. Notably, recovery of left ventricular function and the efficiency of oxygen consumed per work performed were greater in female hearts compared to males and OVX. This sexual dimorphism may result from differences in the heart's ability to adapt to metabolic stress and altered energy-substrate availability caused by exposure to conditions of simulated DCD and IRI.

These findings advance our understanding of sexual dimorphism of cardiac grafts in response to ischemia in the setting of DCD and should enable the improvement of the DCD transplantation protocol. Importantly, the identification of sex-specific alterations in cardiac energy metabolism in response to DCD-induced IRI will help in the identification of novel sex-specific targets for the tailoring of cardioprotective strategies.

The proteomic profile of the midbrain periaqueductal gray: impact of sex and social environment

Elena Kutsarova 1, Kristina Desch 1,2, Petros Chalas 1, Imke Wüllenweber 2, Genesis Rosiles 1, Julian D. Langer 1,2, A.Vanessa Stempel 1

1 Max Planck Institute for Brain Research, Frankfurt am Main, Germany
2 Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Instinctive behaviours such as defence, feeding and reproduction have evolved across animal phyla and ensure survival of both the individual and its kin. A brainstem region central to the initiation of virtually all instinctive behaviours is the periaqueductal gray (PAG). Although stereotyped to some degree, the expression of instinctive behaviours exhibits significant variability within and across individuals. Previous work has mostly focussed on behavioural variability arising from plasticity mechanisms occurring in forebrain regions projecting to the PAG, including the hypothalamus, amygdala and cortex. Here, we asked whether the PAG itself possesses the molecular machinery to support synaptic plasticity and neuromodulation.

We used a label-free LCMS-based proteomics approach with data-independent acquisition to compare tissue from the PAG and two other highly plastic brain regions: neocortex and hippocampus. We analysed differences in the PAG proteome of male and female mice in two housing conditions (single- versus group-housed) as a means to induce plasticity.

This comprehensive proteomic dataset, containing ~10,000 proteins per sample, allows us to posit that 1) the PAG expresses all proteins critical for postsynaptic plasticity and 2) changes in the social environment lead to significant differential expression of the majority of the PAG proteome. Third, we identified specific protease inhibitors, linked to the extracellular matrix, that are strongly upregulated in males throughout the brain.

These proteins have been associated with Alzheimer's disease (AD) and may point to a molecular mechanism of sex-specific AD vulnerability. In summary, we reveal how sex and social environment influence the proteome of the PAG.

The Role of Sex and Ovarian Hormones in Sleep Loss-Induced Hippocampal Deficits

Susanne Hazenberg, Luisa Epifani, Robbert Havekes, Peter Meerlo, **Nicole J. Gervais**

Groningen Institute for Evolutionary Life Sciences, University of Groningen, The Netherlands

Sex differences are observed across species in both sleep and memory. While sleep loss adversely affects hippocampal memory and supporting processes, few studies have explored whether these effects are modulated by sex and age. Importantly, young females might be protected by the neuroprotective effects of elevated oestradiol. Whether higher oestradiol levels shield females from the memory and synaptic plasticity deficits induced by sleep loss remains unknown. The goal of the present study was to test whether females with elevated estradiol show reduced consequences of sleep loss.

Young adult male and female C57B16/J mice were tested on working (spontaneous alternation task) and long-term memory (object location memory) following either six hours of sleep deprivation (SD), or spontaneous sleep (non-sleep deprived controls). Female experience SD either during the early morning of proestrus, or estrus.

Preliminary results from a subset of the sample revealed a non-significant reduction in alternating behaviour in males after SD. Females in proestrus performed significantly worse than males under control conditions ($p = .029$) and exhibited a non-significant increase in alternating behaviour compared to SD proestrus females. A one-sample t-test showed a significant decrease in object location memory in SD males compared to male controls. Data collection is ongoing, and includes dendritic spines analysis, as well as a second cohort of 36 mice. Findings in relation to planned projects will be discussed.

Exploring sex-related disparities in different segments of the renal tubule

Stefan Rudloff*, Murielle Spahr, Uyen Huynh-Do

Clinic for Nephrology and Hypertension, University of Bern and University Hospital Bern, Bern, Switzerland
*corresponding author: stefan.rudloff@unibe.ch

The kidney is a complex organ that consists of thousands of miniscule filter-tubule-units (nephrons). Along its course, a nephron can be divided into multiple segments, each with its own morphology and physiological function, which together perform a variety of vital functions. Loss of nephrons (e.g., age or drug toxicity) can lead to chronic kidney disease (CKD), the distribution of which is far from equal between the sexes: the overall prevalence is higher in women, while more men require renal replacement therapy. The aim of this study is to explore the molecular patterns underlying this difference using specific nephron segments.

Kidneys of 10-12 week old male or female mice were subjected to a novel tissue sorting protocol developed in the lab, yielding large amounts of highly pure nephron segments. These segments were then analyzed by bottom-up proteomics.

Segments of the proximal tubule (gross reabsorption of renal filtrate) or the distal convoluted tubule (finetuning of many electrolytes) showed sex-related differences in energy and drug metabolism. Conversely, no sex difference was found in segments of the thick ascending limb of the loop of Henle, which is involved in urine concentration.

These findings suggest that females might better cope with the exposure to certain drugs, especially those that are metabolized in part by enzymes involved in lipid metabolism. In males, these enzyme might be preoccupied by breaking down fatty acids, thus leading to higher drug concentrations in the kidney for a longer period, which could propel the progression of CKD.

POSTER 1

Role of CBS/H2S pathway in the pathogenesis of neurobehavioral dysfunction in a mouse model of Down syndrome

Lucia Janickova, Olivier Bremer, Theodora Panagaki, Csaba Szabo

Pharmacology, Faculty of Science and Medicine, University of Fribourg, Switzerland

Down syndrome (DS) is a genetic disorder caused by trisomy of all or part of human chromosome 21. The extra copy of chromosome 21 leads to an upregulation of more than 600 genes, including gene coding the cystathionine-β-synthase (CBS), responsible for the biological production of hydrogen sulfide (H2S).

In order to investigate the functional role of the CBS/H2S pathway in the pathogenesis of DS, we used the transgenic DS mouse model - Dp(17)3Yey/+. Localization of CBS was detected in the whole brain, predominantly in astrocytes. Anatomical and morphological alterations of astrocytes were determined by immunohistochemistry, followed by 3D reconstruction and quantitative image analyses. Morphological analysis of astrocytes showed reactive astrogliosis, evidenced by increased number, size, and branching of astrocytes. Increased expression of CBS in DS mice was also detected by Western blot, and it was more pronounced in the brains of female DS mice, than in male DS mice. DS mice exhibited normal locomotion and exploratory behavior but exhibited impaired spatial learning in a T-maze test and impaired recognition memory in the novel object recognition test, with a more severe defect in females. Treatment of mice with a prototypical CBS inhibitor - aminooxyacetate - AOAA, improved neurobehavioral function and astrocyte morphology in females as well as males.

We demonstrated that increased expression of CBS and the consequent overproduction of H2S contributes to the pathophysiological neurological events and that pharmacological inhibition of CBS may be of potential future therapeutic utility in DS condition.

The research was supported by the Lejeune Foundation-20070750.

POSTER 2

Characterization of a rat model of DCD to investigate sex differences in cardiac ischemic tolerance

Alexia Clavier 1,2,3, Selianne Graf 1,2, Anja Helmer 1,2,3, Maria Arnold 1,2 Deborah Lagger 1, Manuel Egle 1,2,3, Matthias Siepe 1, Sarah Longnus 1,2

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2 Department for BioMedical Research, University of Bern, Bern, Switzerland
3 Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

DCD (Donation after Circulatory Death) hearts are subjected to possible harmful conditions during and before the onset of warm ischemia in the donor. In response to cardiac ischemic injury, sex differences have been identified but these differences in DCD have received little research interest. We aimed to establish and characterize a rat model of DCD to evaluate sex differences in cardiac ischemic tolerance.

Male, female, and ovariectomized (OVX) rats Wistar rats were anesthetized, and DCD was simulated followed by 22 minutes of functional warm ischemia (FWIT). To characterize both the model and cardiac responses to in situ warm ischemia, blood samples were obtained at baseline (BL), FWIT onset, and at the end of ischemia (EI).

Body and heart weight of male rats were significantly higher compared to female and ovariectomized animals ($p < 0.05$ for all). Circulating levels of glucose, free fatty acids, calcium, and potassium were significantly higher at baseline and at FWIT in males vs. females and OVX.Intervals between WLST-FWIT were similar between sexes.

We have successfully established a rat model of DCD to investigate sex differences in cardiac ischemic tolerance. Furthermore, our model of rat DCD is suitable for detecting differences in circulating factors during DCD protocol. These findings may contribute to better knowledge about sexual dimorphism in response to ischemia in a DCD setting and should enable the improvement of the DCD protocol in a sex-specific manner.

POSTER 3

Sex matters in Systemic Sclerosis: males and females are equal but not the same!

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Systemic sclerosis (SSc) is an autoimmune disease marked by connective tissue abnormalities, fibrosis, vascular remodelling, and immune responses affecting various organs. Despite its higher prevalence in females (female-to-male ratio exceeding 5:1), males with SSc often experience a more severe form of the disease. The gender-specific differences in disease manifestation suggest a potential impact of sex-related factors in SSc development. Our objective is to explore this gender disparity in disease manifestation and uncover the role of sex hormones in SSc.

Using Fra-2 Tg mouse model; an established model for SSc, we investigated the influence of sex on the disease phenotype. First, we looked for sexual dimorphism in male and female Fra-2 mice, and we observed a more severe phenotype in females. To assess the role of sex hormones, we performed castration experiments. Surprisingly, ovariectomy did not altered disease course in females, whereas orchiectomy exacerbated the phenotype in males. Subsequent experiments involved testosterone replacement in castrated mice, resulting in a significant improvement in disease phenotype among orchiectomized Fra-2 Tg mice. This improvement was observed in enhanced lung function, improved hemodynamic, reduced fibrosis, reduced inflammation, and alleviated vascular remodelling in the lungs.

These findings suggest a potential link between testosterone levels and disease severity in male Fra-2 mice, underscoring the significance of sex hormones in SSc pathology, and our next avenue is to investigate this in patients.

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POSTER 4

The implementation of a Rodent Model of Gender-Affirming Hormone Therapies to Identify Susceptibility and Vulnerability of Transgender People in the Chemical Risk Assessment

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Gender-affirming hormone therapy (GAHT) for transgender (TG) men involves testosterone treatment and for TG women estrogen plus antiandrogens. Due—but not limited—to the lifelong lasting of GAHT, usually TGs show different and specific susceptibility and vulnerability compared to general population, including the response to chemical contaminants present in daily life; in particular, the endocrine disrupters (EDs) - affecting hormonal and metabolic processes - can share targets and mechanisms with GAHT. Since the endocrine system of TGs is overstimulated by the GAHT and potentially by EDs, it's reasonable to hypothesize that TG health deserves special attention in the frame of toxicological risk assessment.

In this context, the development of innovative animal models to mimic GAHT have a high priority. Aims of project are to provide robust data for hazard identification of TGs, leading to a more reliable risk assessment and to study potential long-term consequences of GAHT.

The first results identified: i) suitable dose of testosterone to be used in long-term study of (de) feminizing-masculinizing model; ii) suitable dose of estradiol plus cyproterone acetate to be used in long-term study of (de)masculinizing-feminizing model; iii) high-quality biomarkers to support the success of (de)masculinizing or (de)feminizing HT to obtain a reliable animal model for TGs; iv) "new" (and neglected) targets affected by GAHT.

Further experiments are planned to better characterize both models. The implementation of models mimicking GAHT for risk assessment is critical to support clinical studies and to filling data gap in order to ensure accurate and personalized care for TG people.

Reference

[1] Tassinari R, Maranghi F. Int J Environ Res Public Health. 2021 Nov 30;18(23):12640. doi: 10.3390/ijerph182312640. Rodent Model of Gender-Affirming Hormone Therapies as Specific Tool for Identifying Susceptibility and Vulnerability of Transgender People and Future Applications for Risk Assessment.
[2] Tassinari R et al. Cells. 2023 Feb 1;12(3):474. doi: 10.3390/cells12030474. Risk Assessment of Transgender People: Development of Rodent Models Mimicking Gender-Affirming Hormone Therapies and Identification of Sex-Dimorphic Liver Genes as Novel Biomarkers of Sex Transition.
[3] Tassinari R et al. 2023 Risk assessment of transgender people: implementation of demasculinizing-feminizing rodent model including the evaluation of thyroid homeostasis. (in preparation)

POSTER 5

The rat juvenile model to study sex-related metabolic alterations induced by pesticides: the example of Acetamiprid

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The juvenile rat model is a reliable tool to evaluate hazards specifically targeted to children health. Rodents are immature and treated during the peri-pubertal period, resembling the childhood phase of life; particularly vulnerable/susceptible to contaminant effects. Besides the usual parameters of toxicological studies, potentially relevant endpoints specific for peri-pubertal period are included. Acetamiprid (ACE) is a widely used insecticide with potential risks for humans. Limited information are available on the potential metabolic effects and the sex-based susceptibility in children.

ACE hazard and mode of action is evaluated in 8 sex/group rats administered by gavage (28 days) during peri-pubertal period (post-natal day (23-60). Doses of 0, 0.07, 0.7 and 7 mg/kg body weight (bw)/day are derived from the NOAEL (7.1 mg/kg in 2-year dietary study), based on reduced bw (females) and liver lesions (male).

Preliminary data showed increased feed consumption at the higher dose levels (0.7 and 7 mg/kg) without changes of bw gain in female rats, identifying a potential sex-specific metabolic effects to be confirmed. Increased AW (0.07 and 0.7 mg/kg) and RW (0.07 mg/kg) of adrenals and kidney RW (0.07 and 7 mg/kg) were also observed. Male rats showed reduction of adrenals AW and RW (7 mg/kg) and pancreas AW (0.7 mg/kg).

Preliminary results in juvenile rats confirmed kidneys as sex-specific target of ACE – as in adults -and identified adrenals as new target in both sexes. The ongoing analysis will clarify the metabolic effects and potentially highlight the different susceptibility to ACE toxicity linked to sex and age.

POSTER 6

Evaluation of reported claims of sex-based differences across meta-analyses: A meta-research study

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Background: The consideration of treatment effect differences between men and women are an important topic across very different interventions, diseases, and medical specialties. However, evidence highlights the poor quality of conducting and reporting of sub-group analyses. **Aim 1:** Determine how often claims of sex-based differences in treatment effects are reported in the abstracts of meta-analyses of randomized controlled trials. **Aim 2:** Determine which sex-based differences in treatment effects have formal statistical support and which reflect different types of fallacious claims. **Aim 3:** Evaluate how often sex-treatment interaction claims that have statistical support in meta-analyses are maintained in updated meta-analyses on the same topic. **Aim 4:** Evaluate how often sex-treatment interactions that have statistical support are discussed and lead to differential treatment recommendations in men and women in UpToDate.

Methods: We assembled published meta-analyses of randomized trials that had any mention of sex or gender (male and/or female, men and/or women) subgroup or related analysis in their abstract. Among them, we determined how many had prominently made claims of sex-based differences in treatment effects in their abstracts. These meta-analyses were examined in depth to determine whether these claims reflect genuine sex-treatment interactions or fallacious claims and categorized the frequency of different fallacies. For those with genuine statistical support, we will search to identify if the most recent updated meta-analysis on the same topic corroborates the sex-treatment interaction findings, and we examine whether they are taken into account and discussed in the UpToDate.

Results: In total 221 manuscripts fulfilled the eligibility criteria and made some sort of claims for any sex-based interaction in the abstract. Of them, 99 manuscripts made negative claims, and 23 mentioned a sex/gender subgroup analysis, but did not report results in the abstract. 99 meta-analyses made 113 positive sex-treatment interaction claims. Of them 26 claims across 20 articles had genuine statistical support with a p-value <0.05. 1/20 meta-analyses had an update, with results being corroborated and 4/26 claims were mentioned in UpToDate. 87 fallacious claims were made across 82 articles. 42/87 claims were based on meta-regression, 26/87 had significant effects in one sex but without significant differences between groups and 9/87 claims had larger effects in one sex, without significant difference between groups and 10/87 had other types of fallacies. Only 8/82 studies provided a p-value for interaction and only 5/82 provided the necessary data to re-run the analysis.

Conclusions: Most of the positive sex-difference claims of meta-analyses of randomized controlled trials lack formal statistical support and do not provide the necessary data for replication.

POSTER 7

Sex-specific neurometabolism in young rats with hepatic encephalopathy: a 1H magnetic resonance spectroscopy study

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Type C hepatic encephalopathy (HE) is a decompensating event of chronic liver disease (CLD) characterized by motor and cognitive impairment that can evolve into coma and death[1]. Despite growing evidence of men and women being deferentially affected by the disease[2,3], the lack of preclinical studies on potential sex differences prevents a comprehensive understanding of HE pathophysiology. The aim of this work was to study the elects of sex on the neurometabolic profiles of young rats with HE. To do so, we used in vivo magnetic resonance (MR) spectroscopy, a powerful MR modality that allows one to access brain metabolic content non-invasively.

The bile duct ligated (BDL) rat model of type C HE was used. Liver enzymes and systemic blood biomarkers were measured longitudinally to follow disease progression. MRS acquisitions were performed at week 6 post BDL surgery to compare metabolite concentrations in the hippocampus and striatum of young male versus female rats with HE (female SHAM/BDL: N=9/8, male SHAM/BDL: N=9/11, 2-way ANOVA).Similar neurometabolic profiles in male and female rats with HE were observed (increase in glutamine, decrease in glutamate and osmolytes), with additional sex-specific effects of HE: with disease, ascorbate decrease was stronger in male BDL (vs female BDL),
GABA decrease was stronger in female BDL (vs male BDL) and creatine decrease was stronger in male BDL (vs female BDL).

Impact: the study of sex differences at the preclinical level could open a new window of investigation for patients with HE.

References

1 Vilstrup et al., J. Hepatol., 2014
2 Caudle et al., J Pediatr Gastroenterol Nutr, 2012
3 Pemmasani et al., Eur. J. Gastroenterol. Hepatol., 2023

POSTER 8

Magnetic resonance spectroscopy: a non-invasive imaging modality to investigate the impact of sex on metabolism

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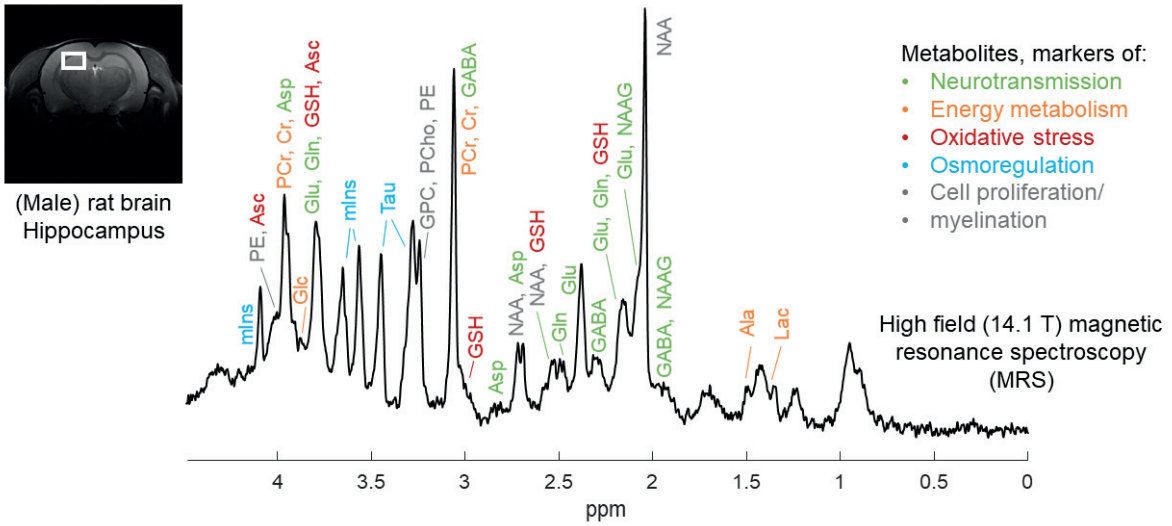
In vivo magnetic resonance spectroscopy (MRS) is a non-invasive imaging modality, performed with traditional magnetic resonance imaging (MRI) scanners, that allows one to measure the local content of metabolites from a tissue. In the brain for example, MRS provides valuable information on a variety of cerebral functions, probing metabolites involved in neurotransmission (glutamate, glutamine, GABA), osmoregulation (taurine, myo-inositol), energy metabolism (lactate, glucose), oxidative stress (ascorbate, glutathione), or myelination/cell proliferation (N-acetylaspartate, choline).

Whereas there is culminating evidence that metabolism differs between men and women, both in healthy and pathological conditions, MRS has hardly been applied so far in the context of SABV questions. With this poster, we aim at presenting this imaging methodology and will highlight some preclinical and clinical applications where MRS has provided fundamental insights. We hope to further discuss the potential of extending the use of MRS to study the impact of sex on metabolic alterations in disease.



Ala: alanine, Asc: ascorbate, Asp: aspartate, Cr: creatine, GABA: γ-aminobutyric acid, Glc: glucose, Gln: glutamine, Glu: glutamate, GPC: glycerophosphocholine, GSH: glutathione, Lac: lactate, mIns: myo-inositol, NAA: N-acetylaspartate, NAAG: N-acetylaspartylglutamate, PCho: phosphocholine, PCr: phosphocreatine, PE: phosphorylethanolamine, Tau: taurine

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