TEATIME

COST Action TEATIME

Meeting Tartu 23rd & 24th September 2024

Young Researchers Workshop

V Spa Conference Centre
V SpaHotell OÜ, Riia 2, Tartu, 51004, Estonia

DAY-1 – 23.9.2024

AGING

Chair – Vootele Voikar

09:45 - 10:30 Plenary lecture: Aging should not be rushed - Eero Vasar (University of Tartu, Estonia)

10:30 - 10:50 Assessment of Age-Related and Cognitive Activity in Two Inbred Mouse Strains Using Mouse Matrix and IntelliCage - Mahvish Faisal (University of Tartu, Estonia)

Eero Vasar, (Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia):
Ageing Should Not Be Rushed: Exploring Schizophrenia as a Model of Accelerated Aging

Recent scientific advancements have identified key hallmarks of aging, categorized into three groups. Primary hallmarks, such as genomic instability and telomere attrition, gradually accumulate over time and contribute directly to the aging process. Antagonistic hallmarks, including oxidative stress and cellular senescence, represent the body's response to damage and play a more complex role in the progression of aging. Integrative hallmarks, such as stem cell exhaustion and altered intercellular communication, become dominant when the damage from primary and antagonistic hallmarks can no longer be effectively managed, ultimately determining the rate at which aging accelerates.
This presentation explores whether schizophrenia can be conceptualized as a model of accelerated aging, contributing not only to a decline in quality of life but also to premature mortality. Evidence shows that despite targeted treatments, patients experiencing first-episode psychosis (FEP) often develop metabolic syndrome, leading to severe somatic complications. Chronic schizophrenia is further characterized by heightened oxidative stress and chronic low-grade inflammation, both of which are known to promote accelerated aging.
Emerging research highlights the potential of interventions like taurine and metformin in mitigating the hallmarks of biological aging. Taurine has been identified as a significant marker in FEP patients, while metformin, widely used in metabolic disorders, shows promise in reducing aging-related damage. The role of taurine and metformin in improving long-term outcomes for individuals with chronic schizophrenia warrants further investigation, offering potential avenues to slow down or reverse the accelerated aging observed in this population.
This study aims to shed light on the intersection between schizophrenia, biological aging, and therapeutic strategies that could improve patient prognosis by addressing the underlying mechanisms of accelerated aging.

Mahvish Faisal (University of Tartu):
Assessment of Age-Related and Cognitive Activity in Two Inbred Mouse Strains Using Mouse Matrix and IntelliCage

Aging is associated with progressive cognitive decline and alterations in behavior, often linked to underlying neurobiological changes. Rodent models have been instrumental in advancing our understanding of the neural mechanisms driving these age-related changes, particularly in the domains of cognitive flexibility, anxiety, and circadian rhythms. Investigating age-related phenotypic differences in two inbred mouse strains, C57BL/6J, and 129S2/SvPasCrl, utilizing novel RFID-based systems—Mouse Matrix and IntelliCage—to assess locomotor and cognitive behaviors under minimally intrusive conditions allow for precise analysis of activity patterns and cognition without disrupting natural behaviors. The IntelliCage system facilitates cognitive and motivational assessments in a naturalistic, group-housed environment, providing insights into behavior under realistic conditions. Key findings reveal significant strain- and age-specific variations in locomotor activity, anxiety-like behaviors, circadian rhythms, and adaptability to environmental changes. Aged mice exhibited reduced activity and impaired anticipatory behaviors, underscoring the impact of aging on circadian regulation. The 129SV strain showed higher anxiety and slower adaptation to environmental modifications, indicating reduced cognitive flexibility compared to the C57BL/6J strain, which demonstrated more rapid adaptation. These results emphasize the importance of strain-specific behavioral and cognitive phenotyping in translational research on aging and neuropsychiatric disorders. The advanced home-cage monitoring systems used in this study provide robust platforms for high-throughput, unbiased assessment of complex behavioral phenotypes in rodents, enhancing the reproducibility and welfare of animal research.

BEHAVIOUR

Chair – Anna Kiryk

11:30 - 11:55 Animal behavior as biomarkers in disease research - Anna Kiryk (Nencki Institute of Experimental Biology, Poland)

11:55 - 12:20 Influence of sex steroid hormones on direct and indirect social interaction in mice in home-cage system - Veronika Borbélyová (Institute of Molecular Biomedicine, Faculty of Medicine, Bratislava, Slovakia)

12:20 - 12:35 The impact of the atypical antidepressant on the behavioural consequences of chronic early life stress - Fiachra McEnaney (University of Leeds, MRC Harwell campus, UK)

12:35 - 12:45 Discussion

Anna Kiryk, Nencki Institute of Experimental Biology, Poland
Animal behaviors as biomarkers in disease research

The use of animal behavior as biomarkers in disease research has become increasingly valuable for understanding and treating neurological disorders. These behavioral markers provide quantifiable insights into biological processes, aiding in the detection of disease states. Neurodevelopmental and

neurodegenerative animal models, which mimic key characteristics of human diseases, are essential for early-stage biomarker discovery.
Recent advances now enable the measurement of many behavioral biomarkers in the home cage environment of mice. Automated systems, including passive infrared sensors and video tracking, allow continuous, non-invasive monitoring of behaviors such as activity, social interactions, grooming, and sleep patterns. This natural, stress-free environment enhances the accuracy and sensitivity of biomarker detection, making it ideal for tracking disease progression over time. Home cage monitoring is especially valuable in longitudinal studies, where subtle behavioral changes can provide insights into the underlying mechanisms of disease.
Machine learning has further advanced this field by analyzing complex behavioral data. Algorithms can detect early signs of diseases like Alzheimer's with high accuracy, enabling the identification of early biomarkers that might be missed by traditional methods.
Behavioral biomarkers offer a more objective and sensitive means of assessing disease states, addressing the limitations of subjective diagnostic tools. The integration of home cage monitoring and advanced analytics is expanding the potential of disease research, facilitating more personalized and effective treatments. In conclusion, the integration of behavioral characteristics into disease research, along with the use of home cage monitoring, is not only broadening our understanding of various disorders but is also paving the way for more personalized and effective treatments in human medicine.

Veronika Borbélyová, Emese Renczés, Petronela Sušienková, Nikola Dudová, Emma Skyová, Peter Celec (Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia)

Influence of sex steroid hormones on direct and indirect social interaction in mice in home-cage system

Sex differences in the brain and behavior have drawn attention due to the differing prevalence, onset, and symptoms of neuropsychiatric conditions between males and females. Autism (ASD) and attention-deficit/hyperactivity disorder (ADHD) are more common in males, suggesting a role for sex hormones in their etiology. Males with ASD and ADHD show social deficits in childhood, while females exhibit more severe symptoms in adolescence and adulthood. Given the complex mechanisms of social behavior, more research, particularly through studies in laboratory rodents, is necessary to better manage social deficits in ASD and other neuropsychiatric conditions.
Researchers often evaluate the social behavior of laboratory rodents individually in various mazes, such as the three-chambered or reciprocal social interaction test. These tests involve placing animals in novel environments that may affect their behavior. Monitoring social behavior in home cage systems can help address this issue. Experimental research on the role of estrogens is very limited compared to research on androgens, particularly regarding the role of estradiol in animal models of ASD or ADHD. Additionally, most experiments focus on males, with female animals underrepresented in research.
This project used ovariectomy and hormonal supplementation to assess the effects of testosterone and estradiol on the social behavior of female C57BL/6J mice in PhenoTyper cages. 2-months-old

female C57BL/6J mice (n=40) underwent either ovariectomy (OVX, n=20) or sham surgery (F, n=20). Two weeks following surgery, females were randomly divided into four groups. For two weeks, sham-operated female mice were treated with either an aromatase inhibitor, letrozole (1mg/kg, F+LET, n=10) or olive oil vehicle (1ml/kg, F, n=10). OVX females were treated with either 17β-estradiol (10µg/kg, OVX+E, n=10) or olive oil vehicle (1ml/kg, OVX, n=10). Treatments were administered subcutaneously once daily between 2 and 3 p.m. On the final three days, female mice underwent open field, indirect social interaction, and reciprocal social interaction tests, all conducted in PhenoTyper cages between 9 and 12 a.m.
In comparison to the F group, OVX females had a significantly lower uterus weight. Compared to the OVX group, OVX+ E females had a higher uterus weight. The uterus length of the F+LET females was longer than that of the F and OVX+E females. The ovariectomy had no effect on the locomotor activity or anxiety-like behavior of the female mice, but it led to a decrease in social disinterest compared to the F mice in the direct reciprocal social interaction test. In addition, OVX+E females showed lower social disinterest compared to F+LET females. Letrozole treatment had no effect on the behavior of mice.
Automated measurement of indirect social behavior showed no differences between female groups, though the EthoVision XT 10 software has limited detection ability. Direct reciprocal social interaction results are preliminary, based on analysis by a single blinded observer. However, our study's key strengths include the use of female mice, as well as surgical and pharmacological methods to induce estrogen deficiency and test social behavior in a home-cage system.
This study was supported by grants VEGA 1/0341/23 and APVV-20-0185.

Fiachra McEnaney (University of Leeds, MRC Harwell campus, UK)
The impact of the atypical antidepressant tianeptine on the behavioural consequences of chronic early life stress.

Major depressive disorder (MDD) is a leading global health burden, affecting around 300 million people worldwide. Faster-acting more efficacious antidepressant treatment is highly sought after due to the relatively low efficacy (~1in3 patients are unresponsive) and delayed onset of action (4-6 weeks) to the current gold standard antidepressant pharmacotherapy (selective serotonin reuptake inhibitors e.g. fluoxetine). Tianeptine is an atypical antidepressant and opioid receptor agonist that can improve clinical symptoms of depression after chronic administration for as little as 14 days. One major clinical risk factor for developing MDD are Adverse Childhood Experiences (ACEs). These experiences are any type of chronic stress during childhood such as abandonment, parental neglect and abuse (emotional, physical and/or sexual). As chronic early-life stress has been known to cause social anhedonia in humans, we examined how chronic tianeptine administration impacts the change in mouse social behaviour caused by early life adversity (ELA) using the limited bedding/nesting model in the 3-chambered sociability test.
From P2-9, C57BL6/J dams and pups were placed in cages with reduced bedding on a metal mesh platform (ELA group; n=11 dams) or in standard cages (control group; n =9 dams). Dam sortie count and pup weight during development were measured. ELA dams had significantly increased sortie numbers and ELA pups had significantly decreased weight until adulthood. At 10 weeks, male and female offspring were given subcutaneous tianeptine (30 mg/kg) or vehicle (saline) injections 2x

daily for 7 days (control + tianeptine: n=13; ELA + tianeptine: n=14; control + vehicle: n=11; ELA + vehicle: n=13). Additional ELA groups received tianeptine (n=12) or tianeptine + cyprodime (a μ-opioid receptor (μ-OR)-selective antagonist; 10 mg/kg; n=13). ELA reduced social interaction and switched the preference towards the familiar mouse, actions that were reversed by tianeptine; this effect was blocked when tianeptine was co-administered with cyprodime. As a control experiment, control groups received cyprodime (n=10) or vehicle (n=10). Surprisingly, chronic cyprodime administration recapitulated the effects of ELA (no preference for social interaction and an aversion towards social novelty). No sex differences were observed.
Multivariate ANOVAs were conducted to compare datasets with 3 or more groups with pairwise comparisons of simple main effects. Otherwise, all data was analysed using unpaired t-tests. The data was analysed manually, with the experimenter blind to all experimental conditions (rearing condition, drug, sex and the location of the novel and familiar mouse during social recognition testing).
These results indicate that tianeptine administration can ameliorate the impact of chronic early-life stress on social behaviour in mice. The μ-OR is important in this action; however, due to cyprodime having an effect by itself, this implies the potential role of an underlying endogenous μ-OR tone on social behaviour. Collectively, these findings may have implications for our understanding of the role of opioid-receptor signalling in social anhedonia and resilience in the face of environmental stressors.

METABOLISM

Chair – Baiba Svalbe

14:00 – 14:20 Knockout animal metabolism and behaviour studies using metabolic cages - Baiba Švalbe (Latvian Institute of Organic Synthesis)

14:20 - 14:40 The role of the new anti-obesity drug “semaglutide” induced side effects on the behavior using a home-cage monitoring approach – Andrej Feješ (Insitute of Molecular Biomedine, Medical faculty, Comenius Universtiy, Bratislava, Slovakia)

14:40 - 15:00 It’s damn cold in here! – A simple solution to improve mice wellbeing in metabolic cages - Philipp Villiger (University of Zurich, Switzerland)

15:00 – 15:30 Rodent models of Wolfram syndrome – Mario Plaas (University of Tartu, Estonia)

Baiba Svalbe, Ennata Viluma, Edgars Liepinsh, Liga Zvejniece
Latvian Institute of Organic Synthesis
Knockout Mice and Metabolic Cages: Insights into Gene Function, Metabolism, and Behaviour

Knockout mice are indispensable for advancing our understanding of genetics and disease, serving as powerful models for exploring gene function, disease mechanisms, and therapeutic interventions. Recently, metabolic cages have become increasingly popular tools in this field, offering a highly controlled and precise environment to study the complex relationships between genetics, metabolism, and behaviour. In this study, we used Sigma-1 receptor (S1R) and

trimethyllysine hydroxylase epsilon (TMLHE) knockout (KO) mice. The S1R is a mitochondrion-associated endoplasmic reticulum membrane protein expressed in the central nervous system and peripheral organs. TMLHE encodes the first enzyme in carnitine biosynthesis, a critical process in energy metabolism.
Using metabolic cages, we conducted a comparative analysis of metabolic and behavioural parameters between knockout (KO) and wild-type (WT) adult mice, including both male and female subjects. The **Promethion Core system** was employed to synchronize metabolic data with behavioural events, enabling a comprehensive assessment of food and water intake, energy expenditure, and activity levels. Additionally, C13-labeled glucose and lactate isotopes were measured using a Stable Isotope Analyzer.
Our findings revealed differences in food and water consumption between KO and WT mice and between genders, although energy expenditure was consistent across both male and female mice. Oxygen consumption and carbon dioxide production were similar across both genders during the four-day observation period. Interestingly, female mice exhibited significantly increased voluntary wheel-running activity, while ambulatory locomotor activity remained unchanged between S1R KO and WT mice in both genders. Despite this, energy expenditure rates remained comparable between KO and WT groups for both sexes. Notably, C13-labeled lactate and glucose metabolism showed a marked increase in male S1R KO mice but not in females. Furthermore, TMLHE-KO mice were more active than WT mice, although WT mice consumed more food and water.
In conclusion, a metabolic and behavioural phenotyping system provides valuable insights into the individual metabolic rates and physical activity levels of knockout mice. This approach is essential for understanding gene-specific metabolic and behavioural differences, enhancing our ability to study the underlying mechanisms of disease and potential therapeutic strategies.
Acknowledgements
The authors acknowledge the access to the infrastructure and expertise of the Baltic Biomaterials Centre of Excellence (BBCE) (European Union's Horizon 2020 Framework Programme under Grant Agreement No. 857287).

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The impact of a new anti-obesity drug, semaglutide, on home-cage behavior in mice

Overweight and obesity rates have increased dramatically over the last three decades. Both conditions increase the risk of cardiovascular morbidity and mortality. However, current dietary, pharmaceutical, and surgical treatment strategies available for the management of obesity are not as successful. Recent animal experiments and clinical trials have demonstrated that Glucagon-like Peptide-1 receptor agonists (GLP-1RAs) are more effective in treating or preventing obesity. Semaglutide (GLP-1RAs; Ozempic®) is precipitating a revolution in the pharmacological treatment of obesity by targeting neural circuits to reduce satiety and hunger. However, clinical trials have shown that semaglutide can induce side effects such as nausea, vomiting, and diarrhea, which are both dose- and time-dependent. Therefore, standard behavioral tests outside of the mouse home cage will not be optimal for observing the impact of these side effects on mouse behavior. Home-

cage monitoring systems offer continuous observation of the “natural” behavior of mice and could potentially address these challenges. The aim of the present study was to compare the effect of semaglutide on the behavior of mice in the standard open field test and the home-cage monitoring system (PhenoTyper cages).
In this experiment, adult male C57BL/6J mice at the age of 6 months were used. Mice in two cohorts (n=26) were divided into two groups, receiving a subcutaneous injection of either semaglutide (60 mg/kg) or a vehicle (1x PBS). First cohort of mice underwent 48 hour-long habituation in PhenoTyper cages (with sawdust, shelter, water, and food ad libitum). Subsequently, these mice underwent 24 hours of testing in PhenoTyper cages (baseline session), after which the mice were injected with semaglutide (n=4) or vehicle (n=4) 1 hour before the start of the active phase and left for another 24 hours of testing in PhenoTyper cages (experimental session). The locomotor activity, time spent in the shelter (sleeping), and time spent in the drinking and food zones were analyzed. The second cohort of animals was subjected to a standard Open field test (without sawdust, shelter, water, or food access) in PhenoTyper cages for 10 minutes, 4 hours post-injection (semaglutide: n=9; vehicle: n=9). Locomotor activity and time spent in the center zone were analyzed.
Testing the mice in PhenoTyper cages revealed lower locomotor activity, lower food and water intake in semaglutide-treated mice compared to control mice in the dark (active) phase (all: $p<0.01$). Additionally, semaglutide-treated mice spent more time inactive in the shelter (sleeping) compared to controls ($p<0.01$). In the light (passive) phase, no differences were found in any of the observed parameters between groups of mice. There were no differences in behavior of mice in the standard Open field test.
Our results suggest that testing mice in the standard Open field test fails to show any behavioral differences between semaglutide-treated and control mice. However, using PhenoTyper cages, it is possible to detect nausea-linked behavioral parameters in mice that are comparable to the side effects observed in patients treated with semaglutide.

This work was supported by the following grants: UK/1084/2024, VEGA 1/0341/23, NNF21OC0067317, Excellence Emerging Investigator Grant - Endocrinology and Metabolism 2021), and the Swedish Research Council Starting Grant 2020 (Medicine and Health; 2020-02473).

Philipp Villiger (University of Zurich, Switzerland):
It’s damn cold in here! – A simple solution to improve mice wellbeing in metabolic cages

Metabolic cages [MCs] are frequently used to collect feces and urine samples. However, MC housing is stressful for mice and thereby potentially influences parameters of interest. We compared a standard protocol for MC housing (4 days at 23°C, including 3 days of permanent acclimatisation and 24h sampling) with a) short-term intermittent acclimatisation (3 days for 3h plus 24h MC housing), b) providing a nest (4 days at 23°C) and c) MC housing at thermoneutrality (4 days at 30°C). C57BL6/N mice were implanted with telemetric transmitters acquiring ECG, blood pressure, body core temperature, and activity. Body core temperature decreased, and heart and respiratory rates increased during MC housing at 23°C. Mice housed in MCs with a nest or at 30°C showed strongly reduced heart/respiratory rates and a higher body core temperature compared to the group housed at 23°C. Mice ate more and lost more weight when housed in MCs at 23°C in combination with a

much higher brown fat activity. Torpor-like episodes were observed in male mice housed at 23°C. In addition, they had higher corticosterone levels. Our study demonstrates that MC housing at 23°C strongly influences mouse physiology and metabolism due to a substantial cold stress. Female mice seem to be less affected compared to male mice. MC housing at thermoneutrality offers a simple solution to improve animal wellbeing during MC housing. Moreover, one short-term acclimatization presumably has the same acclimatisation effect as repeated exposure to the MCs.

Mario Plaas (University of Tartu, Estonia):
Rodent models of Wolfram syndrome

Wolfram syndrome (WS) type 1 is a rare monogenic disorder predominantly caused by recessive mutations in the Wolframin 1 (WFS1) gene. The clinical manifestations of the disorder is summarized by the acronym "DIDMOAD," representing, diabetes insipidus, juvenile-onset insulin-dependent diabetes mellitus, optic atrophy and deafness. Currently, there are no approved treatments specifically for WS. We have developed a Wolfram syndrome rat in Tartu, in which coding exon 5 of the Wfs1 gene is missing. The WS rat develops almost all the pathologies associated with WS over its lifespan. Therefore, it is a valuable model for studying the progression of WS and can be successfully used for drug development purposes. After testing of several promising drug candidates, we have found that commonly used diabetes medications, such as glucagon-like peptide-1 receptor agonists (GLP1-RA), can significantly slow the progression of WS-related diabetes and neurodegeneration. Given that many individuals with Wolfram syndrome are treated with GLP1-RA, it is essential to pursue further research using WS animal models to explore combinations of GLP1-RA therapy with other supportive drugs or supplements. In my presentation, I will discuss recent discoveries in Wolfram syndrome-related drug development that may have disease-modifying effects.

DAY-2 – 24.9.2024

SLEEP

Chair – Veronika Borbelyova

09:00 - 09:20 Sleep modulation in experimental conditions: advantages and disadvantages of current methods - Dragan Hrncic (Belgrade University Faculty of Medicine, Serbia)

09:20 – 09:40 Determination of sleeping behavior using HCM in animal model of autism spectrum disorders after inhibition of aromatase - Petronela Sušienková (Institute of Molecular Biomedicine, Slovakia)

09:40 - 10:00 Sleeping and functional abnormalities in collagen-induced arthritis mouse model - Jakub Szabó (Institute of Molecular Biomedicine, Slovakia)

10:00 - 10:30 PhD chaos: Weird phenotype and new methods in a classic pharmacology lab – Davor Virag (University of Zagreb School of Medicine, Croatia

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Sleep modulation in experimental conditions: advantages and disadvantages of current methods

Abstract

Sleep is a vital physiological process in which humans spend almost one third of their lives. Despite making them vulnerable to predators, sleep has been ubiquitously preserved in mammals, birds and reptiles. However, contemporary lifestyles, shift work, social jet lag and environmental blue light pollution significantly compromise sleep quality in an increasing number of people. Poor sleep quality has been demonstrated to be a risk factor for a number of diseases, from cardiovascular to neurological including malignancies. Therefore, there is an increasing scientific interest in research on sleep quality and sleep medicine nowadays.

Sleep quality is not characterized only by total sleep duration, but also by sleep architecture and sleep latency. Sleep architecture denotes cyclic patterns of sleep stages alterations, i.e. basic structure of NON- REM stages (N1-3) and REM phase transitions, each of them being characterized by specific electroencephalographic (EEG) and behavioral features. REM sleep is characterized by rapid eye movements, desynchronized EEG pattern resembling awakening (paradoxical sleep) and skeletal muscle atonia, a feature used in many experimental techniques to modulate REM sleep in laboratory rodents.

Techniques to study sleep in experimental conditions evolved from the Jouvet's introduction of flowerpot method for selective REM sleep deprivation. Major types of sleep disruption in laboratory rodents used in contemporary literature are: total sleep deprivation, selective REM (or paradoxical) sleep deprivation and sleep fragmentation. Variety of different techniques has been developed for each type of sleep disruption. Selective REM sleep deprivation techniques are predominantly based on atonia during REM phase and includes single classical platform methods, modified multiple small platform methods and grid over water. Total sleep deprivation techniques include handling and disc over water. Sleep fragmentation could be achieved by using treadmill or handling. Protocols could include or exclude EEG registrations. Rotating discs and sleep deprivation chambers are recently introduced. All these methods have advantages and disadvantages that will be further discussed.

A significant number of these methods require social isolation of rodents and expose them to additional stress of environment in which sleep modulation occurs and development of anxiety. Therefore, home – cage based sleep modulation techniques should be further developed and investigated, beside improvements done in monitoring sleep in home cage systems.

Key words: sleep, sleep quality, deprivation, fragmentation, home cage, rodents, rats

Sušienková Petronela, Skybová Emma, Dudová Nikola, Feješ Andrej, Szabó Jakub, Borbélyová Veronika, Celec Peter (Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University in Bratislava, Slovakia)

Determination of sleeping behavior using home-cage monitoringin animal model of autism spectrumdisorders after inhibition of aromatase

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by difficulties in sociability and repetitive and stereotypic behavior. Many comorbidities such as sleeping difficulties are identified in patients with ASD. Etiopathogenesis of ASD is unclear, however, the role of genetic and environmental factors is described. In addition, higher prevalence of ASD in boys compared to girls (3:1) suggests the role of sex steroid hormones in the etiopathogenesis of ASD. Scientific research is focused more on boys or male animals than girls or female animals in context of ASD. Therefore, the aim of the present study was to explore the effect of chronic aromatase inhibition by a 21-daylongletrozoletreatmenton sleeping behavior in female mice genetically predisposed to ASD-like behavior(Shank3b-/-mice)and wild-type mice. 12-hour home-cage monitoring in the PhenoTyper cageswas used to determine the sleep duration and time spent in drinking and feeding zone during active (6-hour) and passive (6-hour) phase of day. Similarly, the food and water intake were measured. To confirm the successful inhibition of aromatase by chronic letrozole treatment, the uterus weight, an indirect measurement of estradiol deficiency, and testosterone concentrations in plasma were measured. Higher circulating testosterone concentration was observed in mice treated with letrozole. Aromatase inhibition in combination with the genetic predisposition to ASD had no effect on sleeping duration or time in drinking zone in active nor passive phase. The effect of genotype and treatment interaction was observed in time spent in feeding zone during passive phase followed by non-significant post hoc test. However, amount of consumed food or drunk water during 12 hours did not differ depending on genotype, treatment, or their interaction. In passive phase, Shank3b-/-mice spent less time sleeping compared to wild-type mice regardless the treatment. Similarly, distance moved in active and passive phases was shorter intheShank3b-/-mice than wild-type mice. The main effect of treatment with letrozole was not observed in any measured variable. Our results suggest no effect of aromatase inhibition on sleeping behavior in genetically predisposedShank3b-/-female mouse model of ASD. However, mainly genetic predisposition to ASD seems to play an important role in sleeping duration. Future research should focus on longer observation of sleeping behavior and determination of sleeping interruptions, typical for patients with ASD.
This study was supported by research grant APVV-20-0185.

Jakub Szabo
Sleeping and functional abnormalities in collagen-induced arthritis mouse model

Introduction: Rheumatoid arthritis (RA) is an autoimmune, inflammatory disease which primarily affects the musculoskeletal apparatus. The chronic nature of RA affects the overall well-being of the patient, often leading to a decline in sleep quality exacerbating extraarticular functional impairments. Animal models are essential for studying RA, however, analysis of the sleeping behavior in mice can be challenging. Home-cage monitoring (HCM) offers uninterrupted observation of natural animal behaviors, allowing a precise detection of sleep patterns.

Methods: In the collagen-induced arthritis (CIA) model, we analyzed the sleeping behavior of DBA/IJ mice of both sexes over 12 hours of HCM. In addition, traditionally used behavioral assays for locomotion, exploration, nesting, grooming, sociability and short-term memory were applied.
Results: CIA mice showed 50% less locomotor activity and 50% less sleeping time than control animals. They exhibited considerable sleep fragmentation demonstrated by a 3-fold shorter average sleeping period and twice as many sleep disruptions during the passive phase of HCM compared to the control mice. Worse short-term memory, higher anxiety-like behavior, 5-fold increased self-grooming and 80% less exploration was all found in CIA mice. Notable sex differences were observed in response to CIA treatment in anxiety (50% less time spent in the center zone for CIA males), as well as in drinking and feeding behavior (twice as much time spent in feeding zone for females).
Conclusion: CIA is one of the most widely used animal models for studying RA, but observations on the sleeping behavior are lacking. Our results show that CIA mice suffer from complex sleep disruptions alongside other functional deficits. These findings underline the translation potential of the CIA model in RA research, demonstrate the methodic advantages of HCM in rodent studies and open new avenues for exploring the role of sleep in the overall well-being of RA patients.

PHENOTYPING WITH HCM

Chair – Lars Lewejohann

10:00 - 10:30 PhD chaos: Weird phenotype and new methods in a classic pharmacology lab – Davor Virag (University of Zagreb School of Medicine, Croatia)

10:30 - 11:00 IntelliCage - Obstacles and solutions - Pia Kahnau (German Federal Institute for Risk Assessment)

11:00 - 11:20 Operant behavioral assessment in a self-built homecage environment - Patrick Reinhardt (Ruhr-University Bochum, Germany)

Davor Virag (University of Zagreb School of Medicine, Croatia)
PhD chaos: Weird phenotype and new methods in a classic pharmacology lab

Though there is a substantial amount of resources devoted to preclinical research of Alzheimer's disease (AD), very few drugs make it to clinical trials. Due to its complex pathophysiology, modelling the disease in animals is a challenge. However, many models, from flies to rats, share some behavioural traits that aren't commonly associated with AD, but somewhat resemble ADHD. The lab I work in has been researching the intracerebroventricular streptozotocin (STZ-icv) rat model for the past 30 years. I worked there over the last two years of med school and continued as a PhD student, with the goal of i) getting a wider picture of the STZ-icv model's behavioural aspects, and ii) analysing the effects of an ADHD drug on the model. The idea was to achieve this using markerless bodypart tracking (DeepLabCut/SLEAP) and DIY home cage monitoring devices - MIROSLAV (Multicage InfraRed Open Source Locomotor Activity eValuator) and VlaDiSlav (VDS-based home cage operant conditioning task). As I am entering the fourth and final year of my PhD, I'd like to discuss the

challenges of implementing (or, worse - creating) new methodology in a classic pharmacology lab over a few very short years and critically re-weigh the decisions I've made along the way.

PHENOTYPING WITH HCM

Chair – Lars Lewejohann

11:10 - 11:40 IntelliCage - Obstacles and solutions - Pia Kahnau (German Federal Institute for Risk Assessment)

11:40 - 12:00 Operant behavioral assessment in a self-built homecage environment - Patrick Reinhardt (Ruhr-University Bochum, Germany)

Pia Kahnau, PhD Laboratory Animal Science, German Centre for the Protection of Laboratory Animals, German Federal Institute for Risk Assessment

IntelliCage – Obstacles and Solutions

In the past, I have gained extensive experience with the IntelliCage system (New Behaviour, TSE GmbH, Germany) in various projects. The IntelliCage is an RFID and home cage-based test system for examining the learning behaviour of group housed mice. The IntelliCage consists of four conditioning corners. Each corner is equipped with an RFID antenna, a presence sensor (differential temperature sensor), two nosepoke sensors (light barriers), six LEDs, two doors for access to water, two water bottles and an airpuff valve for possible punishment. The mice have to learn where, in what order and at what time they have access to water. The advantage of this system is that the mice can be tested during their active (dark) phase with minimal human influence over several weeks or months. In my talk I will present the design and functionality of the IntelliCage and the IntelliCage Plus software. Both simple and very complex learning experiments can be designed with the system. When designing such an experiment, it is important to pay attention to certain settings where mistakes can easily be made. It is also important to regularly test the hardware and check its functionality. In my talk I will show how to test the different sensors and what to do if a lickometer fail. The aim of the talk is to identify possible obstacles and offer solutions. This should give a first impression of the system and ensure that future IntelliCage experiments run as efficiently as possible.

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Operant behavioral assessment in a self-built homecage environment

Operant behavioral assessment describes the training of an animal to perform a specific task using a reinforcer. It is a widely employed paradigm to investigate behavior in basic research and behavioral changes in preclinical animal models. Testing is mostly performed in operant chambers where the animal has a timeframe to obtain food rewards via correct responses on a touchscreen or by pressing a lever. Benefits include a standardized and controlled environment and the possibility to perform behavioral batteries to test a variety of behavioral domains. However, time removed from the homecage and food restriction to increase motivation can cause stress to the animals and alter behavioral outcomes. In addition, the manual placement of the animal in the chamber by the experimenter holds several influencing aspects. Aim of the presented project is therefore to connect an operant chamber to the animal's homecage via a transition tube to allow free access. Employing RFID- and weight sensors enables restricted access for one animal at a time and the collection of individual data. The transition system is based on affordable microelectronics and constructed with an open-science idea to enable easy and cheap implementation in other labs. It will be connected to an already existing, self-built, raspberry pi based operant chamber, running with an open-source code (Open Toolbox for Behavioral Research). Construction results of the setup will be shown and an overview of a proof of concept study to assess effects on animal welfare will be presented.

YOUNG RESEARCHERS IN TEATIME

Chair – Selin Çevik

12:00 - 12:15 Past, current and future opportunities for Young Researchers in TEATIME – Selin Çevik (Mersin University, Turkey)

12:15 - 12:30 TEATIME not-for-profit future – Indrek Heinla (Estonian Research Council)

12:30 – 12:45 Discussion of future activities for Young Researchers in TEATIME and beyond

Özge Selin Cevik (Mersin University, Turkey)
Past, current and future opportunities for Young Researchers in TEATIME

As a post-doctoral researcher at Mersin University, Faculty of Medicine, Department of Physiology (2022-2023), and now an Assistant Professor (2024–present), I have actively contributed to the scientific community through multiple initiatives. In recent years, active contributions have been made within the TEATIME COST Action as a member of WG2, WG3, and Young Researcher Inclusiveness (YRI) leader. Efforts in 2023 included organizing two online meetings to unite young researchers, providing a platform for discussing expectations within the scientific community. Collaborative work with other COST Actions, such as CA19117 – Researcher Mental Health (REMO) and IMPROVE, led to impactful events, including a webinar with experts in alternative methods to animal experiments, offering valuable career guidance to young researchers.
Further activites will be done during the next months. "**The Workshop for career development**" scheduled for October 23, 2024. This program offers workshops focused on academia, industry, and PhD pathways, designed to help researchers embrace challenges as opportunities for growth. Plans for an additional young researcher meeting in November are underway, aiming to encourage

knowledge exchange through short presentations. Additionally, work supported by an STSM Grant will share insights into the development of home cage systems, focusing on design and implementation challenges in the young researcher meeting.

Indrek Heinla

Regarding the future of the TEATIME community, we need to address the following questions:
1.Do we want to continue after the end of COST funding?
2.If yes, why should we continue?
3.How should we proceed moving forward?
• To comprehensively answer these questions, I propose conducting a foresight exercise during or in parallel with our next in-person meeting. This exercise will allow us to envision possible future scenarios for home-cage monitoring and animal experimentation in general, helping us focus on the most relevant and impactful actions.

WELFARE

Chair – Sophie Schober

13:45 - 14:15 Monitoring Animal Welfare in Day-to-day Behavior in the Home Cage - Lars Lewejohann (FU Berlin, BfR, Germany)

14:15 - 14:45 Why contactless monitoring matters to animal welfare and your research – Nuno Franco (i3S, Universidade do Porto, Portugal)

14:45 - 15:00 The Pathway to Automation - from Home Cages to Welfare (outcome of Brussels welfare meeting) – Jan Rozman (University of Luxembourg)

15:00 - 15:30 Discussion of animal welfare and HCM

Lars Lewejohann, Freie Universität Berlin, German Federal Institute for Risk Assessment (BfR), German Center for the Protection of Laboratory Animals (Bf3R)
Monitoring Animal Welfare in Day-to-day Behavior in the Home Cage:

In nature, mice are the epitome of a prey animal. High vigilance, digging and hiding are just some of the behaviors used to avoid predators. One should be aware that the natural behavioral repertoire is also preserved in laboratory mice. It is therefore not surprising that the animals become easily stressed when they are picked up and taken from their safe environment. However, when testing laboratory mice for scientific purposes, they are usually transferred into a test apparatus by manually picking them up. In addition, mice are nocturnal, but many traditional tests are performed at times when the mice would normally be asleep. New techniques (video tracking, RFID, bioacoustics) allow long-term observations in the home cage to be performed at increasingly high levels. Such techniques allow to monitor the wellbeing within the home cage as well to conduct behavioral research without unnecessarily stressing the animals. For other tests, we propose to include the animals' home cage as a safe refuge wherever possible. To this aim, we are investigating systems

that allow mice to enter connected test cages by themselves. From the animals' point of view, this leads to less stressful experiments. However, it must be considered that this will add to the importance of the home cage in which the animals spend almost their entire lives. Therefore, refinement of cage size and design for better animal welfare must be considered continuously.

Nuno Franco (i3S, Universidade do Porto, Portugal)
Why contactless monitoring matters to animal welfare and your research

While the scientific advantages of home-cage monitoring systems are increasingly evident, their animal welfare and 3Rs benefits have been less explored. This talk will cover how non-invasive monitoring through HCM reduces animal stress and how it can be used for detecting and signalling early signs of points-of-no-return in disease development, that can be signal humane endpoints. Concerns about the correct detection of the experimental unit, inter-individual variability, within-cge interactions, and the implications of these factors for the principle of reduction will also be covered.

Jan Rozman, University of Luxembourg
The Pathway to Automation: From Home Cages to Welfare – Outcome of the Brussels Welfare Meeting

The TEATIME meeting, held at the COST Association Office in Brussels on September 2-3, 2024, brought together 41 experts from 13 countries to discuss advancements in home cage monitoring (HCM) and its implications for improving animal welfare. The event featured alternating sessions with presentations and breakout groups, fostering intensive discussions among participants.
During the presentations members of the TEATIME Cost Action and invited external experts gave an overview on different HCM technologies, assessment of well-being and other parameters that can be monitored in addition to standard video or activity assessment, data management and analysis.
Presentations highlighted biomarkers that can be automatically monitored, such as body weight, temperature, and respiratory rate. Some systems are now capable of automating complex behavioral assessments through deep learning techniques. Insights from the automated monitoring of larger animals, such as pigs and cattle, were shared to improve laboratory animal welfare. Effective data management, adhering to FAIR principles, was emphasized as critical for ensuring data accessibility and utility.
A key theme in one of the breakout groups was the challenge of experimental design, where equipment is often procured before clear hypotheses are formulated. This frequently results in underutilization of advanced technologies due to inadequate data processing and a lack of standardization. The need for validated instruments and accessible online resources for baseline data and model validation was emphasized. The diverse expertise among users, ranging from basic to advanced, highlighted the necessity of simplifying complex systems to accommodate different user groups.

Discussions centered on animal welfare parameters such as pain, stress, and activity, underscoring the importance of precise measurement and standardization to enhance reproducibility across laboratories. Unexplored areas, including the use of ultrasound and the detection of sporadic behaviors like seizures, were identified as important topics for further research. Additionally, the scalability and cost of HCM technologies were addressed, particularly in the context of broader adoption. The meeting underscored the expanding role of automated HCM in enhancing both scientific research and animal welfare.