LASA 3Rs Section / UFAW Meeting

19th Sept 2023, GSK Stevenage

"Home Improvements: Looking at Evidence based Refinements"

Programme

09:20-10:20	Arrival, coffee, and registration
10:20 -10:30	**Welcome & introduction** *Session 1- Chair, Joanna Moore, GSK*
10:30-11:00	**Confidence is key: Evaluating enrichment for better animal welfare** *Khia Dobbinson, NC3Rs*
11:00-11:30	**The use of rat playpens to provide social and physical enrichment.** *Claire Gibson, University of Nottingham*
11:30-12:30	First time speaker session
	Minimising aggression in cd-1 and cd-1 background male mice with different enrichment types *Amy Veness, Sainsbury Wellcome Centre*
	An investigative study in male rats to validate the FLIR E95 Thermographic Camera for measuring body temperature *Katy Mistretta, GSK*
	Assessing the use of enrichment items by cattle in a high containment facility. *Rosanna Smith-Langridge, Animal and Plant Health Agency*
12:30-13:45	Lunch
	Session 2- Chair, Emma Stringer
13:45-14:15	**Validating the use of box training as a refinement to rabbit handling** *Alicia Kinally, University of Leicester*
14:15-14:45	**Home-cage-based testing of laboratory mice** *Lars Lewjohann, Freie Universität*
14:45- 15:15	**Assessment of cage conditions to determine the interval required between cage changes in Individually Ventilated Cages using C57/BL6 male and female mice** *Joanna Moore, GSK*
15:15- 15:55	**(Keynote) Light and The Laboratory Mouse: From Vision to Circadian Rhythms** *Prof Stuart Peirson, University of Oxford*
15:55-16:00	Final thoughts – Chairpersons. Close of Meeting

We are pleased to announce that LASA has awarded this meeting 5 CPD points

Abstracts

CONFIDENCE IS KEY: EVALUATING ENRICHMENT FOR BETTER ANIMAL WELFARE.

Khia Dobbinson NC3Rs

When trying a new form of environmental enrichment, or reviewing what is currently provided, assessing whether it improves animal welfare is a vital part of the process. Evaluations of environmental enrichment allow us to make confident, welfare-focused decisions. However, overcoming the challenges of assessing enrichment within a research setting, and knowing where to start, can be intimidating. To address this, the NC3Rs has worked with the RSPCA, the Institute of Animal Technology (IAT) and animal technicians to create an online resource to support technicians who want to evaluate environmental enrichment. The resource is filled with practical support and example study protocols that can be adapted for different species and individual requirements. This presentation will outline an example evaluation to give a brief overview of how you can use the resource (www.nc3rs.org.uk/EEE) to confidently evaluate environmental enrichment for better animal welfare.

THE USE OF RAT PLAYPENS TO PROVIDE SOCIAL AND PHYSICAL ENRICHMENT

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Environmental enrichment, defined as exposure to a combination of complex inanimate and social stimulation, aims to enhance animal welfare by providing beneficial stimuli. Enrichment via playpen access is widely used in non-human primate, dog and rabbit research facilities but use is limited and validation lacking for rodent facilities. Conventional housing for rodents, including recently introduced double-decker housing for rats, is criticised for inducing abnormal behaviours (e.g. stereotypies or compulsive behaviours evoking stress/anxiety) and reactions of stress, distress and aggression which all contribute to poor animal well-being potentially impacting experimental validity. Rodents used in biomedical research tend to be sedentary, obese and even glucose-intolerant but are often interpreted as the 'control' state potentially confounding data interpretation and study conclusions. Housing and handling techniques can directly influence the wellbeing of animals, induce a chronic low-grade inflammatory state, alter hormonal signalling and affect the outcome of behavioural experiments particularly those involving spontaneous and exploratory behaviours. Such impact on animal wellbeing, physiology and behaviours may directly contribute to increased morbidity and mortality seen in rodents housed under conventional conditions. We recently trialed the provision of social and physical enrichment to rats being housed on medium/long term behavioural studies through a playpen incorporating numerous cages of rats at once. We initially introduced a large, single level arena which resulted in the rats displaying sedentary-like behaviours. Thus, we then trialed the use of a multi-level, large cage space with various forms of cognitive and physical enrichment which enabled rats to be given regular and frequent playpen access on a rota system and provided with social enrichment due to rats from multiple cages occupying the playpen simultaneously. The playpen was placed within a larger ventilated unit which had opaque (i.e. colour tinted) screening which may reduce perception of risk and anxiety associated with large, open spaces. Our original aim for introducing the playpen system was to reduce aggression, stress and body weight gain we had observed previously in ageing rats. In this playpen trial animals gained weight with age over the expected, healthy range and anecdotally, members of the group and animal care technicians reported that animals appeared less stressed/aggressive and naturalistic behaviours emerged. However, whilst the use of playpens to provide enrichment is becoming more common practice there is a need to validate enrichment approaches to demonstrate their benefit to welfare along with examining their potential impact on (motor and cognitive) behaviours related to anxiety.

MINIMISING AGGRESSION IN CD-1 AND CD-1 BACKGROUND MALE MICE WITH DIFFERENT ENRICHMENT TYPES

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It is well known that male mice can show aggressive tendencies towards their cage mates resulting in the mice being harmed, kept in a stressful situation or having to be singly housed. All these can lead to a decrease in the animal's welfare, so it is important to find ways in which to minimise cage mate aggression and avoid singly housing animals.

This presentation will explore different types of enrichment that we used to try and minimise fighting/aggression in our CD1 and CD1 derived male mice. Between April 2020 and April 2021, we saw a high number of CD1 and CD1 derived male mice held in the facility sustaining fight wounds or being singly housed. Different enrichment products were trialled to see if this would minimise aggression, we found that adding sizzle nest and a nestlet to our standard cage enrichment showed the biggest reduction in aggression. After implementing the different enrichment types with our CD1 and CD1 derived strains we saw a significant drop in fight wounds and singly housed mice due to aggression.

After seeing another increase in aggression in early 2023 we are further refining our enrichment and husbandry routines for CD1 derived strains as well as looking into other strains held in the facility.

ASSESSING THE USE OF ENRICHMENT ITEMS BY CATTLE IN A HIGH CONTAINMENT FACILITY

Rosanna Smith-Langridge
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High containment facilities provide limited environmental stimuli for cattle. Adding environmental enrichment can reduce aggression and stereotyping, improving overall animal welfare and benefiting scientific data output. Current research into environmental enrichment for cattle in high containment units is limited despite such facilities facing unique challenges to maintaining high welfare standards. This study compared four different enrichment items in the aim to help inform high containment facilities on the most effective enrichment items for cattle. Five pens holding four 18-month-old male Hereford-Holstein cattle were equipped with control enrichment (broom head and salt lick) and one trial enrichment item. The test items were a hay net filled with hay, a knotted rope, an empty chemical drum, and a kong ball, all of which were hung from a post using rope. Items were rotated weekly for 3 weeks in total. Interactions between cattle and enrichment were recorded daily via CCTV, scored using instantaneous scan sampling and analysed using Kruskal-Wallis tests. The hay net elicited the highest interactive frequency and duration. It was also the least affected by habituation, possibly due to the nutritional incentive and novelty created when refilled each morning. The ball and drum elicited similar interactions, and both interacted with more than the control items - the cattle interacted with them as frequently but for shorter time periods than the hay net. The rope was interacted with less than the control items. Although the hay net was the most effective, all items declined in popularity over time indicating that several different items rotated sporadically may maximise the benefit of enrichment by maintaining the cattle’s interest.

AN INVESTIGATIVE STUDY IN MALE RATS TO VALIDATE THE FLIR E95 THERMOGRAPHIC CAMERA FOR MEASURING BODY TEMPERATURE.

Katy Mistretta
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Body temperature is frequently measured during in vivo studies, as it can provide important information around the health and welfare of an animal. Current methods of body temperature measurement used within GSK are regulated procedures and invasive. The Home Office requires research establishments to find and implement more refined ways of working. Therefore, there is a desire to find new methods to measure body temperature which can be used in research laboratories that are non-regulated and refined, but still reliable and accurate.
This study investigated whether the FLIR E95 Thermographic Camera could be used as an alternative non-invasive and non-regulated method to measure body temperature in rats and whether it is as accurate as invasive and regulated methods such as the rectal probe and subcutaneous temperature transponder. Measurement of body temperature investigations were split into three phases and used the thermographic camera compared to these two methods in Crl:WI(Han) rats. Phase 1 investigated which site on the animal provided the most accurate and appropriate temperature reading using the thermographic camera. Phase 2 investigated whether the thermographic camera could detect a change in body temperature, while Phase 3 investigated whether the thermographic camera was practical in a research scenario.
The data shows that the FLIR E95 Thermographic Camera on the eye and ear were the best comparators in terms of accuracy to the rectal probe and Plexx microchip. The FLIR E95 Thermographic Camera did not detect a change in temperature as well as the Plexx microchip or rectal probe, however the most aligned methods were the Plexx microchip and camera (ear). The FLIR E95 Thermographic Camera was easy to use, however the Plexx microchip remains the most practical temperature collection method. The FLIR E95 Thermographic Camera should be used alongside the Plexx microchip or rectal probe on a future study in order to further assess whether the camera can detect a change in temperature.
All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

VALIDATING THE USE OF BOX TRAINING AS A REFINEMENT TO RABBIT HANDLING

Alicia Kinally, Univerty of Leicester

In the wild rabbits are prey animals and so the act of being picked up and handled can be extremely stressful for them. Previous studies in pet rabbits have shown that during the act of lifting, rabbits show signs of struggling and aggression due to fear. This Is relevant to the rabbits we house in our facility and can cause unnecessary stress, which potentially will negatively affect the research they are being used for. In our case, the rabbits are cardiovascular models of Myocardial Infarction and so keeping stress levels down where possible is important. We investigated a new method of handling our rabbits which involved training the rabbits to jump into an animal carrier to be transported to their destination; either a playpen or scales multiple times a week.
The carrier was lined with either a vetbed that was shared amongst their group, or a vetbed that was not shared and was only given to the individual rabbit. We investigated different positive incentives but found the vetbed was the most effective for them to jump into the box. Out of the two groups we had, the shared vetbed was preferred due to the desire to investigate the different smells left by the other rabbits when it was presented to them. The rabbits were timed how long they took to jump into the box with a limit of 3 minutes. Since the initial trial, we have now also tried this method with female rabbits and have received positive results from them too. We also measured the effect of surgery on the rabbits who were our MI/SHAM models and found that during the time that they were monitored on post-op checks, they were less likely to jump into the box but quickly returned to jumping in as normal afterwards.

HOME-CAGE-BASED TESTING OF LABORATORY MICE

Lars Lewejohann, Freie Universität Berlin, German Federal Institute for Risk Assessment (BfR), German Center for the Protection of Laboratory Animals (Bf3R)

In nature, mice are the epitome of a prey animal. 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 a large number of the 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. 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.

ASSESSMENT OF CAGE CONDITIONS TO DETERMINE THE INTERVAL REQUIRED BETWEEN CAGE CHANGES IN INDIVIDUALLY VENTILATED CAGES USING C57/BL6 MALE AND FEMALE MICE

Joanna Moore, Global Laboratory Animal Medicine, GSK

The aim of this study was to determine if we could extend the Cage Change Interval (CCI) from one week to a two-week interval. We included twenty cages of 3 males and twenty cages of 4 female C57/BL6 mice housed for a 10 week period in Individually Ventilated Cages with 75 air changes per hour (ACH) with 110gms Lignocel BK8/15 bedding, and a nesting combination of 20gms Enrich n Nest and 8gms BednNest. All cages included a mouse igloo and a cardboard tunnel which were only changed when they were considered dirty and not at each CCI. The study was made up of three phases, (1) cages of 3 males and 4 females with a two-week cage change interval (CCI), (2) cages of 2 males and 3 females for a three-week CCI (3) cages of pairs of females for a three -week CCI. During each phase cages a Drager gas monitor was used to test NH3, O2 and CO2 levels in the cage. The nests were scored at the end of each CCI and mice were weighed weekly. All mice were housed in a Digitally Ventilated Cage to determine if activity around latrine area reduced over time. At the end of each phase five male and five female mice were randomly selected for histology examination of the respiratory tract. We found that NH3 levels remained below 50ppm for the majority of NH_3 and CO_2 levels remained below 0.3%. There was no chance in O_2 levels. There were no observations of fighting with male mice and no histopathological findings were reported. The majority of nest scores were 3 and the weight of the mice remained in line with the expected growth curve. In conclusion we showed that within the parameters of this study a two-week cage change internal is possible and may help with longer term male mouse housing.

LIGHT AND THE LABORATORY MOUSE: FROM VISION TO CIRCADIAN RHYTHMS

Prof Stuart Peirson
Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford

Life on Earth has evolved under a predictably changing cycle of light and darkness. Detection of light by the eye forms the first step in vision, allowing animals to create an internal representation of the world around them. But light also regulates many other non-visual effects on physiology and behaviour, including regulating circadian rhythms, hormone production, sleep/arousal, mood, learning/memory and even pain. Much of our understanding of these non-visual responses to light has originated from studies performed in laboratory mice. This includes the identification of a new photoreceptor system in the eye, comprised of a subset of photosensitive retinal ganglion cells expressing the blue-light sensitive protein melanopsin. In this talk, I will describe the role of light in regulating both visual and non-visual responses, with a focus on circadian rhythms and sleep. I will then go on to explain why current approaches used to measure light for mice are inappropriate, due to differences in human and mouse sensitivity, and provide a new approach to measuring light that accounts for the animal's experience. Finally, I will describe how we can use home cage monitoring to assess the effects of light on circadian rhythms and sleep and provide some examples of how these may influence experimental results.