Report of the 2021 RSPCA/UFAW Rodent Welfare Group Meeting

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Introduction

The RSPCA/UFAW Rodent Welfare Group has held a one-day meeting every autumn for the last 28 years, so that its members can discuss current welfare research, exchange views on welfare issues and share experiences of the implementation of the 3Rs of Replacement, Reduction and Refinement with respect to rodent use.

This year's meeting was held online for the second year running and attracted nearly 500 registrants from all over the world. The day included sessions on 'Evaluating Enrichment' and 'Better Welfare Equals Better Science'. This report summarises the meeting and ends with a list of action points for readers to consider raising at their own establishments.

Evaluating enrichment

This session began by highlighting a new, online resource to help Animal Technologists evaluate enrichment. This was followed by presentations on experimental design, Animal Welfare science methods, examples of enrichments to trial and a tool for auditing outcomes.

Introducing the Evaluating Environmental Enrichment online resource for Animal Technologists

Khia Dobbinson, NC3Rs, UK

When trying a new form of environmental enrichment, assessing whether it improves Animal Welfare is a vital part of the process. However, assessing enrichment within a research setting may not always be straightforward and it can be hard to know where to start. The NC3Rs has worked with the RSPCA, the Institute of Animal Technology (IAT) and Animal Technologists to create an online resource to support those 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.

The resource includes guidance that can be adapted for different settings in an accessible, practical and time saving format. It is designed to cover all stages of the process of evaluating enrichment, from choosing an enrichment and planning an evaluation to data

collection and implementing and sharing findings, to ensure that users are supported every step of the way.

Before starting any evaluation, investing time in planning will help make the project easier and, the resource provides guidance on key points to consider before starting, such as who should be involved, what the overall aim of the project is, the equipment needed, where to find more relevant background information and how to conduct literature searches. A project plan sheet is also provided to help with this and to promote clear communication with all colleagues involved in the project.

The resource also outlines different approaches to evaluating enrichment, such as behavioural monitoring, with guidance on creating an ethogram (list of species-appropriate behaviours) and, examples of published studies and 'walkthroughs' of protocols which show the step-by-step methodical process that should be followed. Each example protocol includes data collection sheets which can be adapted for your own use, as well as example and general ethograms and links to further resources for a range of common laboratory species, including rodents. There is also guidance on the different stages of data handling and analysis, starting with examining the raw data in Microsoft Excel, collating and summarising data, and creating informative data plots and graphs. The resource does not assume any prior knowledge, so users without experience in Microsoft Excel or with data analysis will still be able to use these resources.

A crucial part of conducting any study, including ones evaluating enrichment, is to understand the limitations of your study design and understand ways to improve the scientific quality, such as the use of randomisation and blinding. These are discussed and demonstrated in the resource and further information on this is also given in the next section of this report.

Early reviews from Animal Technologists have said the resource is useful and motivating, so why not have a look at the resource, discuss it with other staff and start planning your own enrichment evaluation today!
https://nc3rs.org.uk/evaluating-enrichment

Experimental Design: what happens when things change?

Eloisa Brook, GSK, UK

Good experimental design is a key part of robust experiments, enabling you to draw sound conclusions, make data-driven decisions and generally get the most out of the data you are collecting. The three key aspects, or pillars, of good experimental design are sample size, randomisation and blinding.

Sample size refers to the number of animals that are going to be in your study. This can be determined by thinking about your success criteria (what are you going to be measuring and how are you quantifying it?), how you are going to estimate any variability in your measurements and how much power you want your experiment to have. All of these should be thought about before setting up the study.

The next pillar is randomisation – randomly assigning animals to different groups. To do randomisation, you will need to know what your study design will actually look like - how many groups will you have and how many animals in each group? A simple approach is to randomly assign three animals to each group (although the welfare implications of randomising also need to be considered, as this can affect social hierarchies and lead to increased levels of aggression.[1] Animals should always be randomly assigned using a computer, to avoid bias – this can be done very simply using the randomisation function in Microsoft Excel or there are various useful pieces of software which can be used for this (see the end of this section).

The final pillar of good experimental design is blinding – ensuring that measurements and assessments are done without prior knowledge of what that animal has experienced. This is especially important when there is any possibility of subjectivity in measurements - if there is a chance that a measure could be interpreted differently by different people, then the person measuring that endpoint should be doing it 'blind'. This may not always be possible depending on the experimental conditions but this is the ideal situation to aim for. An example of this is for assessment of clinical signs – ideally the person assessing clinical signs should not have known what has happened to that animal. The ultimate goal is blind dosing of your animals, where dosing refers to the treatment you are trying to assess the effects of – this could be drug or other compound dosing but could also refer to the enrichment condition or the type of handling.

Unless you have the statistical expertise yourself, it is good practice to consult a statistician to help with these fundamental principles of robust experimental design. If someone with relevant expertise is not available at your institution, consultant statisticians are available, or a scientific colleague should be able to advise you. Ensuring that good practices are in place means that key decisions and inferences can be made from your data as planned.

However, although you may plan your study to fit with the ideal situations described above, sometimes things change which affect how the study is carried out. This may be because there are fewer animals available than expected, methods change, the equipment needed is unavailable or even due to unpredictable constraints

like social distancing. Understanding these changes and how they affect your study, will inform what conclusions you can draw from your data. For example in a study we designed to test the effects of three different types of enrichment, we had to change our original design of mice experiencing the enrichments in a different order to a design where all the mice experienced the enrichments in the same order. In this study design, we cannot say whether any welfare benefits were entirely due to the enrichments or whether there were other effects on a particular week that also affected welfare and confounded the results.

In conclusion, it is important to be aware that sometimes things do change which will affect your study design. When this happens, remember that it is not the end of the world, but that changes do matter, so understanding what the effects of these changes are will help to ensure your study is still robust and useful. And finally, when in doubt, consult a statistician!

Free tools for sample size and randomisation:

Sample Size and Randomisation:
NC3Rs Experimental Design Assistant

Sample Size Only:
Invivo Stat
PowerandSampleSize.com
PS Power and Sample Size
Benchmark 6ix Sigma

Randomisation Only:
Random.Org/Lists
Research Randomizer
Graphpad.com
Randomization.com
Sealed Envelope
RRApp

The appliance of Animal Welfare science

Oliver Burman, Animal Behaviour, Cognition & Welfare Research Group, School of Life Sciences, University of Lincoln, UK

Animal Welfare science gives us lots of possible approaches that we can use to evaluate enrichment. Three common behaviour-based approaches are behavioural monitoring, preference testing and motivation testing. These approaches can be used to ask different questions, so selecting the right approach for your situation is important. It is also worth spending time on designing your study to maximise the scientific quality and therefore the robustness of your results.

The first approach, behavioural monitoring, involves observing animals with and without the enrichment to see how their behaviour changes. Before starting any study like this, there are lots of things to decide. You will need to think about which enrichment(s) you want to test, how long for and what your experimental design will be – for example, you could do a between-subjects comparison (where one group is in enriched and one group is in standard conditions) or a within-subjects comparison (where all animals are observed as a baseline before being given the enrichment, then observed with the enrichment and then observed again once the enrichment is removed). You will also need to decide which behaviours you are going to observe. Using an ethogram – a table of clearly defined behaviours – can be helpful here and many are available for different species which can be modified for your study. A final consideration is who will observe the animals and when – you may need to consider when your animals are most likely to be active and will need to check that you and any other observers are consistently recording behaviour in the same way.

After conducting this kind of study, you need to interpret the meaning of the behaviours you have observed. A good starting point is to create some graphs. Choose a graph that best represents the question you are asking – for example, if you want to know whether the percentage of rats sleeping under the hopper is higher in standard conditions compared to when housed with enrichment, then you could plot this as a bar graph that allows an easy visual comparison of the same behaviour in the two different housing conditions. At this stage, it is important to stick to the predictions you initially made and to look at all your results together, rather than examining each result individually, as it is likely to be much more informative to consider changes in several different behaviours together. Remember that a behavioural change does not necessarily imply better welfare, especially if it is short-lived.

The next type of behavioural approach for evaluating enrichment is to conduct a preference or choice test - these tests are a way to 'ask' animals what conditions they prefer. As with behavioural monitoring, there are lots of things to consider before you start – which different options will you provide for them to choose between and, how many options will you have? How will you measure preference? You will need to ensure you provide all the essentials, such as food and water, in all conditions so that animals are not biased towards a particular choice. You will also need to consider what the animals have previously experienced, whether animals might initially fear a new condition (neophobic) that they actually go on to prefer once they have experienced it and how different individuals/sexes/ages/strains might differ in their preferences. There are also some limitations to what these types of tests can tell you – an animal can only choose the most preferred of the available options, so it may be that both options are actually aversive and the animal is picking the least aversive of the options available.

Motivation tests, which 'ask' animals how **much** they want something, have similar considerations as preference tests. These kinds of tests allow you to find out how hard an animal will work for access to a condition or resource, for example by using doors of different weights that the animals must push open. These sorts of tests can be quite costly and complex to carry out and measurement of 'effort' from the animal can be hard to interpret but, they can help determine which resources are valued more and allow different resources to be titrated against one another.

Although a lot can be learned from each of these approaches, it is important to emphasise that they are not mutually exclusive. Combining different approaches, such as carrying out a preference test followed by behavioural monitoring can allow you to integrate your results and make it easier to interpret your results. Even when interpretation is not straightforward, it is also important to give animals the benefit of the doubt - if you are unsure whether the enrichment conveys a benefit, as long as there are no negative effects, it is better to provide it than not.

Finally, we should note that it would be almost impossible for anyone to assess all possible enrichments for all possible combinations of strain, sex, age or group size for every species. However, we can all help to fill in the gaps, if we work together in a systematic and robustly scientific way and properly communicate our findings.

The 3Hs: Happiness, Home and Hammocks – how our programme of environmental enrichment for animals and staff is impacting on our shared environment, welfare and daily lives

John Hobbs, University College Dublin, Ireland

In UCD Biomedical Facility we always strive to improve our animals' welfare and environment. In 2019 we started a programme which involved introducing and trialling several different forms of refinement, including enrichment, refined handling and rat tickling.

A form of enrichment we tried was the use of hammocks for our rat cages. Initially we introduced hammocks to eight cages, four for each sex. We found that adult males tended to chew on and shred hammocks but the females used them for nesting. We also tried some hammocks in breeding cages and found that males would rest in them, while mothers used them to look after the young pups. Once they had been introduced into the breeding cages and the first cage after the pups had been weaned, we tended to see rats moving all nesting material into the hammock and nesting in them until the hammock needed to be replaced. As an enrichment item, these hammocks worked well, as they were relatively cheap, machine washable, autoclavable and reusable.

Our next form of refinement that we implemented was increasing the amount of habituation to handling that our rats received. This habituation involved gentle handling of the rats, touching the rat on different parts of the body, gently restraining the rat without scruffing, and touching the rat with a syringe with no needle on it to get the animals used to these procedures. We noticed several benefits, including that the animals showed less fear, less stress and more voluntary engagement with the handlers. We also found that staffs' levels of confidence in handling the animals increased, so staff felt less stress, resulting in more empathy and a more relaxed environment for all involved and better human-animal relationships. This has been an important part of our work to continuously improve and develop our Culture of Care.

The third type of refinement we trialled was rat tickling. We started with a few cages of 25 day old male and female rats and increased the numbers over time. We mimicked playfighting as the rats did when they were pre-weaned pups.[2] This was done any time the rats were removed from their cages with our aim being to improve handling and reduce stress. We observed positive responses in males after three days and in females after five days. These positive responses included signs that the rats were anticipating and waiting for the tickling, were less likely to hide or avoid handlers, would come back to the handlers' hand seeking to be tickled again and the females showed excitement through their ears twitching. This refinement increased empathy towards the animals from the staff, and was enjoyed by the staff and the research groups as well as the animals.

One of our most recent refinements has been to introduce the use of playpens. We used old hypoxic chambers for the pens and filled them with an assortment of old wooden and plastic houses, ladders to access upper levels, a sandpit and hammocks. We also buried treats in the sand and the nesting material to encourage foraging behaviour. We started by introducing three boxes of male rats to the playpen for 40-minute sessions but this quickly increased to 20 boxes of rats within a few weeks. The rats clearly enjoyed having access to the playpens and were observed cleaning and grooming themselves and had visibly cleaner coats and tails over time. After about three sessions we also noticed the rats would wait at the door of the pen when the time was up showing they had become used to this routine. We have recently sourced another playpen which we plan to begin using for female rats.

It has been noted that there can be a risk of compassion fatigue in animal care staff,[3] so we were pleased to see that these changes gave everyone a lift, especially

during lockdown. The increased interactions with the animals, the noticeable welfare benefits and the active participation and engagement of the staff and researchers with the programme has helped improve our shared environment and increased the emphasis on our Culture of Care.

Auditing environmental enrichment – designing an adaptable tool for tracking enrichment strategies

Ciara Larkin, Dublin City University, Ireland

Environmental enrichment is a key component of any Animal Welfare programme. Periodic reviews of enrichment ensure that good practice is being implemented and that new information has been considered. It also enables us to track and assess the impact of any changes that have been introduced. When our Animal Welfare Body recommended that our rats were given more enrichment, we decided to conduct such a review. We developed an auditing worksheet to help us do this, that has been successfully implemented at our institution. This gave us a clear baseline, before we implemented any new enrichment strategy.

We structured our auditing worksheet to examine each of five categories of enrichment in turn – social, physical, nutritional, sensory and occupational enrichment.[4,5] We also included sections on the worksheet for administrative information, such as the room and species, as well as some questions about standardisation and reporting. Because current practice may be different in different areas of an establishment, it may be necessary to run the audit more than once. We ran ours three times – once for rats and twice for mice housed in different areas. Although our audit focussed on rodents, the worksheet is adaptable for different species, as some enrichment strategies will be species-specific.

The first section covers social enrichment. Most laboratory animals are social and are capable of complex social interactions. This level of complexity is almost impossible to replicate in the research environment but some social enrichment should still be provided. The worksheet uses a mixture of simple questions and dropdown responses to allow the user to record information about the social environment of the animals, such as whether animals are housed in single-species rooms, group size and compatibility, the opportunity for positive species-specific social behaviours like grooming and play and group stability. There is also a section to record whether any animals have to be singly housed, what the justification is for this and, what additional strategies have been used to minimise harm, such as providing extra nesting material to assist thermoregulation.

The next section of the worksheet examines physical enrichment – these are items that are added to the cage to increase the complexity of the environment and help give animals a sense of choice and control over their environment. They may include litter and nesting material, nest boxes and shelters, and climbing structures (including ropes and hammocks). The worksheet user can record the use of these objects and is asked to consider object placement, whether the objects are regularly changed and, whether the focus is on novelty or complexity. The values of novelty and environmental complexity are the subject of some debate and may differ between (or even within) establishments, so the spreadsheet provides an easy way of measuring what works for you and your animals.

Many wild animals spend more than 50% of their time foraging but this time is greatly reduced in laboratory housing, so nutritional enrichment can help increase the amount of time performing this natural behaviour. This section of the worksheet examines what kinds of food are provided (do you provide whole foods, such as seeds or nuts?), whether strategies such as hiding food in the litter are in place and whether toys or manipulanda which contain food are being used. There are also open questions about monitoring bodyweight and the placement of the enrichment within the cage.

We then looked at sensory enrichment. Sensory systems are highly specialised and have evolved to support animals in the environment to which they are adapted. Animal and human senses can be very different, so we must ensure we are examining conditions from the perspective of the animal. For example, albino rodents are highly light sensitive and may require a low light refuge. They also perceive a different auditory range to humans (e.g. rodents can hear ultrasound) and rats and mice have different auditory ranges from each other. Olfactory cues are known to be important for rodent reproduction and aggression. However in addition to needing to control adverse sensory experiences, some species also enjoy sensory stimulation, so the worksheet includes an open section for detailing any sensory enrichment.

The final type of enrichment is occupational enrichment. These are enrichments that encourage physical activity, like exercise wheels, or cognitive stimulation, such as puzzles or toys. Some items provide multiple types of enrichment, e.g. providing food in a KONG toy could be considered occupational, sensory and nutritional enrichment. Human-animal interactions such as training or tickling can also be considered occupational enrichment. These can all be recorded on the worksheet.

At the end of the worksheet is a section on standardisation and reporting. Here we look for details of relevant standard operating procedures to ensure enrichment is incorporated into the day-to-day running

of our unit. Although the effect of enrichment on experimental variation is complex, transparent reporting is important for replicability and for collaborations with others. Recording information in this way can also help minimise the potential harms associated with trial-and-error testing and, especially in establishments where staff turnover is high, ensures that the current generation learns from the positive and negative experience of their predecessors.

In conclusion, auditing enrichment ensures that good practices are in place, gives a platform for incorporating new understanding, and allows tracking of improvements over time. Our primary goals were to create a practical tool that facilitated the identification of those changes that produce real improvements and that supported lasting change, incorporating 3Rs principles and stimulating collaboration, so we have decided to make the worksheet freely available. If you would like a copy of this document, you can email me at *ciara.larkin28@mail.dcu.ie*.

Action points from Session 1:

- Think about whether there is a need to trial environmental enrichment for rodents at your facility – are you uncertain whether they are benefiting from a current refinement or would you like to gather evidence for a new enrichment?
- When designing a study to evaluate environmental enrichment, ensure you have a clear plan in place before you start.
- Use resources such as the NC3Rs' Evaluating Enrichment tool to help plan, carry out, analyse, implement and disseminate the results of your study.
- Always ensure that animal numbers are sufficient to be statistically significant, and incorporate randomisation and blinding into your study design whenever necessary and feasible.
- Discuss your study design with someone with statistical expertise if necessary, particularly if circumstances force you to change your study design.
- Consider trialling the use of refinements like hammocks, increased habituation to handling, rat tickling and the use of playpens in your facility to help improve the wellbeing of both animals and staff.
- Review the use of enrichment in your facility and keep records of what has been trialled, what has worked and what has not.

Better welfare equals better science

This session began by looking at aggression in male mice and its impact on welfare and science; first taking an epidemiological approach and then focussing on aggression within oncology studies. The third presentation provided an update on new research into the effects of light on mouse behaviour and welfare.

Triggered: the epidemiology of male mouse aggression

Brianna Gaskill, Novartis, USA

Mice are a social species and guidelines for laboratory animal housing in the UK, EU and USA all recommend social housing for mice. However, excessive aggression in laboratory mice is widespread and injuries from fighting are a common cause of premature death.[6,7] High levels of aggression can also destabilise dominance hierarchies, induce immune suppression, cause poor overall welfare and lead to undesirable variation in data, all major issues for both animal welfare and the quality of the science.

Aggression is not a straightforward topic – there seem to be many factors that can contribute to it and understanding these factors is complex. An epidemiological approach can be a powerful way to address these complex problems. It involves using the natural variation in a population of mice to look at how different risk factors contribute to problems like aggressive behaviour, in a way that would be impossible to control for in a single experiment.

We examined controlled studies in the literature to identify potential risk factors which might trigger aggression. We then documented these variables in all of the mice and cages we observed – for example, genetic differences in aggression are widely reported, so we tested the effect of this by documenting the strain of the mice we observed.[8] Other key triggers or potential triggers include the number of mice in the cage, husbandry factors such as cage type and bedding, the type of ear identification method, other potentially painful procedures, temperature, humidity, time of year, the presence of different enrichments, row height and the orientation of the cage to the wall as a way of measuring different levels of human traffic.[9–12]

To collect our data, we surveyed mouse cages at a research institution. We selected rooms to maximise variability of the data, then randomly selected racks within the room and visually assessed each cage within that rack. We recorded data for all the variables mentioned above, examined each cage individually and then observed the entire rack for five minutes after all the cages had been assessed. We designated cages as 'fighting' if we observed bouts of fighting behaviour and also looked for characteristic lesions on the rump, tail and tail base.

Of the 2679 cages we observed, 841 contained group-housed males, which were used as our final dataset. Fighting was observed in 116 of these (13.8%).

We found that husbandry conditions had the single biggest effect: IVC cages containing corncob bedding had significantly higher levels of aggression than static

cages containing woodchip bedding. Unfortunately, we were unable to tease apart these effects further. Genetics also had an effect, with C57BL/6 mice showing higher levels than Swiss mice or other background types. C57BL/6 mice had much higher levels of aggression than expected, as they were similar to FVB mice, a strain which is generally considered highly aggressive. Another factor that affected aggression was cage location, we found that aggression was higher in cages at the top of the rack than at the bottom and, those housed in racks parallel to the wall over those that were perpendicular. Finally, aggression was found to be seasonal, peaking in the summer months.

Despite having been chosen due to reported studies suggesting these factors can affect aggression, we did not find any evidence that aggression was affected by temperature, humidity, the number of mice in the cage, enrichment presence or type, surgery or the use of ear punches over tail tattooing for identification.

Surprisingly, the presence of fight wounds was not a good indicator of fighting, wounds were observed in only 16 of the 116 'fighting' cages (13.8%) which suggests that staff might need to undertake more direct observation to get a better understanding of the prevalence of fighting. Other ways in which staff can implement changes based on these results may be limited - we cannot do much about genetics, row height or the time of year. However, knowing which cages are more likely to be at risk can help staff monitor aggression more effectively and housing systems, bedding and rack orientation may be changeable. Overall, the single most important predictor of aggression was housing (IVC+Corncob). Controlled studies will be needed in future to further tease out these effects and to follow up on the other results of this study but this highlights why changes in husbandry and housing must be done carefully with appropriate monitoring and should be backed up by evidence.

Recognising male mouse aggression and reducing the incidence and impact of fighting on oncology studies

Kate Shenton, AstraZeneca, UK

Male mouse aggression is a common problem in laboratory environments. Aggression can cause injury, stress and anxiety and, can mean individuals are prevented from accessing resources like food and water, leading to reduced bodyweight and dehydration. This can lead to significant effects on data variability, as a stressed mouse is not a normal mouse and this variability may be wrongly attributed to study effects if the aggression is not identified. Fighting can also lead to mice being killed (by other mice) or euthanised (for welfare reasons due to fight injuries) and thus the data from these mice can be lost. Aggression can also be stressful and distressing for staff working with male mice, especially as it may be difficult to make decisions over whether to separate mice, who to separate and when to separate them. Managing aggressive mice also takes time, which can increase the pressure on staff.

To help minimise the incidence and impact of fighting, on our mice and on the studies they are used in, we have developed guidelines for working with male mice. These are based on recognised actions that help avoid known triggers for fighting, based on our pooled experiences and the published literature. They include having male-only housing rooms and procedure areas, placing food on the cage floor to minimise food guarding, additional nesting materials, cleaning gloves between cages and using refined handling techniques like tunnel handling. We have noted some observations which may reduce aggression but need to be investigated further, such as aggression being lower in quieter rooms. We've also developed a host of resources to assist staff making decisions on housing, such as posters and booklets which cover both physical and behavioural indicators and consider strain differences and possible study effects.

We have found that using our single-housing record to record the incidence of aggression is invaluable for monitoring aggression. If a mouse is singly housed for any reason, the reason for separation and any further details like sex and strain are added to the sheet. This allows patterns to be seen and the extent of single housing in our facility to be easily accessed, including information on injuries and behaviours observed. The information is also accessible to scientists so they can see what has happened to the mice in their studies.

Another approach we have used to reduce aggression was to make changes to one of our standard protocols for oncology studies. In this protocol, mice are implanted with tumour cells and the tumours are allowed to grow for a time (referred to as the select phase). The mice are then randomly allocated to study groups according to tumour volume to avoid bias. This randomisation process meant that mice were being mixed across cages which led to more fighting due to the disruption of stable social hierarchies. This was sometimes made worse because some mice were in the select phase for quite a long time and so were more mature when they were allocated to studies and more prone to fighting. With this design, it was not unusual to have to separate out 30-50% of the cages involved in a study. This observation of an increase in fighting was supported by our use of the single housing record, so we knew this was not just based on a perception.

To address this issue, we took a whole-establishment approach, working with scientists, personal and project licence holders, our Named Veterinary Surgeon (NVS) and a statistician. Our discussions led us to trial a

new approach to randomisation, in which mice are still randomised on a statistical basis by tumour volume, but are recruited to groups within their home cages, keeping the hierarchy stable. Although some fighting leading to separation remained, this was vastly reduced (down to 0-5% of cages) and was almost always where one mouse in a cage of three was not recruited to a study, so the number of mice in the cage went from three to two, disrupting the hierarchy. We found that this approach was much less stressful for the mice and the change still allowed the scientific aims of the study to be achieved.

In conclusion, our experience is that you are almost certainly going to see aggression if you work with male mice, so strategies need to be put in place to address this issue. With this in mind, we have further work planned which includes the development of a new app tool to make the process of box randomisation easier, accessible to all and applicable to all study types. We also have recruited a global male aggression team to keep driving progress, collect data and share information. Continuing to record as much data as possible and communicating about this data, are both vital elements of our ongoing work to assess, learn and improve the management of male mice.

Effects of light on home cage activity and mouse behaviour

Stuart N. Peirson

Sleep and Circadian Neuroscience Institute (SCNi), Nuffield Department of Clinical Neuroscience (NDCN), University of Oxford, UK

Mice are widely used in vision research and as a result we know a huge amount about the mouse visual system. For example, whilst the human retina is dominated by cones (photoreceptive cells responsible for colour vision) and has a cone-rich central region called the fovea, the mouse retina is dominated by rods (photoreceptive cells which detect low light) and has no fovea. This means that mice have much lower visual acuity than humans (in human terms they would be considered legally blind) and are more sensitive to low light. In addition, as mice lack a long-wavelength sensitive cone (L-cone) they have different sensitivity to colour and are much less sensitive to red light than humans (although this does not mean that mice cannot see red light). Research on the mouse visual system has also led to many advances in our understanding of the roles of the eye.

The eye performs two general functions, it allows us to form images of the world around us which gives us our overall sense of sight and it allows us to detect the brightness of our environment. This latter function is very important for the regulation of circadian rhythms – the rhythmic changes in behaviour and physiology which happen over a roughly 24-hour period.[13] These rhythms enable animals to anticipate and exploit regular changes in their environment. Circadian rhythms persist even in constant darkness, so we know that they are generated by an internal biological 'clock' but this biological clock is not exactly 24 hours, it has to be 'set' (entrained) to the environment by light. However even mice genetically altered to have no circadian clock show changes in activity under a light-dark cycle. This means that measuring activity under a light-dark cycle is not the same as measuring circadian rhythms because changes in activity can also be directly induced or suppressed by the action of light.

Much of what we know about circadian rhythms and how they are affected by light, has been learned from studying mice. This is typically done by looking at wheel-running behaviour in the home cage. For example, if mice are kept in constant darkness, the onset of their wheel-running activity will drift earlier because the clock is no longer entrained. However exposing the mice to just 15 minutes of light at the beginning of the night is enough to delay the circadian clock and the onset of activity. By contrast, light exposure later in the night can advance the onset of activity.[14] These effects are called phase shifts and we can use these responses to measure how the circadian clock responds to different light conditions. The size of the phase shift depends on both light intensity (brighter means a bigger phase shift) and wavelength (colour) with mice still showing circadian responses to red light, although they are less sensitive to it than other wavelengths of light. Even mice without 'classical' photoreceptors (rods and cones) show responses to light in this way. an observation that led to the discovery of another photoreceptor in the eye. These are intrinsically photosensitive retinal ganglion cells - cells which express a blue-light sensitive pigment called melanopsin and form a photoreceptive network across the retina, detecting light to entrain circadian rhythms.[15] However mice which do not express melanopsin also show phase-shift responses which shows that under normal conditions circadian rhythms are regulated by a combination of light inputs from rods and cones into melanopsin-expressing cells.[16]

We have recently been working in collaboration with Tecniplast on the effects of cage position and cage filtering on light and activity. We have measured the light levels in both standard green line Individually Ventilated Cages (IVCs) and red cages across the rack, and found that light levels are much lower in the red cages and are lower in cages at the bottom of the rack than those at the top.[17] We have also measured activity over seven days in different positions in the IVC rack. Our results show that mice in red cages are still clearly detecting the light as they are still showing nocturnal behaviour patterns, but their activity levels are blunted. We have also found that the onset of activity is much earlier in

a red cage than in a standard IVC cage and that mice in red cages show more activity during the light phase when animals are normally inactive or asleep.

A final piece of work we have recently carried out has been to look at whether mice want access to light. Mice have typically been regarded as photophobic so we tested this using an operant sensation seeking task. In this task, mice were placed in a test arena containing a lever which, when pressed, would turn on a light (Tam et al, in preparation). We found that mice placed in the arena would quickly learn this task, even though there was no reward associated with pressing the lever. This shows that mice do value having some access to light and find it rewarding and in future it may be possible to train mice using this kind of stimulus as the reward.

Action Points from Session 2

- If working with male mice, familiarise yourself with known triggers for fighting so that these can be minimised and be aware of indicators for fighting or unrest in a cage.
- Consider the use of a recording system which is accessible to all staff and researchers for keeping track of mice that have to be separated so that you can look for patterns and all staff and researchers are aware of what has happened to each mouse.
- If you suspect male mouse aggression in your facility, you may need to undertake some direct observation, as fight wounds may be a poor indicator of fighting prevalence.
- Housing and husbandry conditions can be a trigger for male mouse aggression, so any changes to these should be done carefully with appropriate monitoring over the course of the change.
- Be aware that mice can see in red light, contrary to some misconceptions, that light exposure can affect activity patterns in mice, and that mice are not entirely photophobic – in fact, they will work for access to light.

References

1 **Lidster K., Owen K., Browne W.J. and Prescott M.J.** (2019). Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project. *Scientific Reports*, Vol. 9, 15211.

2 **Cloutier S., LaFollette M.R., Gaskill B.N., Panksepp J. & Newberry R.C.** (2018). Tickling, a Technique for Inducing Positive Affect When Handling Rats. *Journal of Visualized Experiments*, Vol. 135, e57190.

3 **LaFollette M.R., Riley M.C., Cloutier S. *et al.*** (2020). Laboratory Animal Welfare Meets Human Welfare: A Cross-Sectional Study of Professional Quality of Life, Including Compassion Fatigue in Laboratory Animal Personnel. *Frontiers in Veterinary Science*, Vol. 7, 114.

4 **Bloomsmith M.A., Brent L.Y. and Schapiro S.J.** (1991). Guidelines for developing and managing an environmental enrichment program for nonhuman primates. *Laboratory Animal Science*, Vol. 41, 372–377.

5 **Coleman K., Weed J.L. and Schapiro S.J.** (2017). Psychological Environmental Enrichment of Animals in Research. In *Animal Models for the Study of Human Disease (Second Edition)* (Conn P.M., ed.), pp. 47–69. Academic Press.

6 **Weber E.M., Dallaire J.A., Gaskill B.N., Pritchett-Corning K.R. and Garner J.P.** (2017). Aggression in group-housed laboratory mice: why can't we solve the problem? *Lab animal*, Vol. 46, 157–161.

7 **Theil J.H., Ahloy-Dallaire J., Weber E.M. *et al.*** (2020). The epidemiology of fighting in group-housed laboratory mice. *Scientific Reports*, Vol. 10, 16649.

8 **Gaskill B.N., Stottler A.M., Garner J.P. *et al.*** (2017). The effect of early life experience, environment, and genetic factors on spontaneous home-cage aggression-related wounding in male C57BL/6 mice. *Lab animal*, Vol. 46, 176–184.

9 **Greenberg, G**. (1972). The effects of ambient temperature and population density on aggression in two inbred strains of mice, Mus musculus. *Behaviour*, Vol. 42, 119–130.

10 **Van Loo P.L.P., Kruitwagen C.L.J.J., Van Zutphen L.F.M., Koolhaas J.M. and Baumans V.** (2000). Modulation of Aggression in Male Mice: Influence of Cage Cleaning Regime and Scent Marks. *Animal Welfare*, Vol. 9, 281–295.

11 **Baumans V., Schlingmann F., Vonck M. and van Lith H.A.** (2002). Individually ventilated cages: beneficial for mice and men? *Contemporary topics in laboratory animal science / American Association for Laboratory Animal Science*, Vol. 41, 13–19.

12 **Howerton C.L., Garner J.P. and Mench J.A.** (2008). Effects of a running wheel-igloo enrichment on aggression, hierarchy linearity, and stereotypy in group-housed male CD-1 (ICR) mice. *Applied animal behaviour science*, Vol. 115, 90–103.

13 **Bell-Pedersen D., Cassone V.M., Earnest D.J. *et al.*** (2005). Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nature reviews. Genetics*, Vol. 6, 544–556.

14 **Yoshimura T. and Ebihara S.** (1996). Spectral sensitivity of photoreceptors mediating phase-shifts of circadian rhythms in retinally degenerate CBA/J (rd/rd) and normal CBA/N (+/+) mice. *Journal of Comparative Physiology A*, Vol. 178, 797–802.

15 **Hattar S., Lucas R.J., Mrosovsky N. *et al.*** (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*, Vol. 424, 76–81.

16 **Tam S.K.E., Brown L.A., Wilson T.S. *et al.*** (2021). Dim light in the evening causes coordinated realignment of circadian rhythms, sleep, and short-term memory. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 118.

17 **Steel L.C.E., Tir S., Tam S.K.E. *et al.*** (2022). Effects of Cage Position and Light Transmission on Home Cage Activity and Circadian Entrainment in Mice. *Frontiers in Neuroscience*, Vol. 15.