Report of the 2024 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 31 years. These meetings provide an opportunity for members to discuss current welfare research, exchange views on welfare issues and share their experiences of the implementation of the 3Rs with respect to laboratory rodent use.

This meeting was held in-person at the University of Bristol on 28 November 2024 and was co-hosted by the 3Hs Initiative. Eight excellent presentations were given on various aspects relating to the 3Rs, with a particular focus on refining the lifetime experiences of rodents through housing, handling and habituation. The day ended with an interactive discussion session in which attendees discussed the opportunities and challenges related to implementing further refinement. This report summarises the meeting and ends with a list of action points for readers to consider implementing at their own establishments.

Introduction to the 3Hs framework

Emma Robinson, University of Bristol

Refinement encompasses the lifetime experience of laboratory animals and improving welfare needs to extend beyond consideration of the most refined methods for specific procedures. Factors which may particularly contribute to cumulative suffering and therefore have a significant impact on an animal's whole lifetime experience include laboratory caging systems. These often restrict natural behaviour and limit opportunities for enrichment; the impact of repeated physical restraint; and negative associations with humans, which may be due to a combination of a natural fear response and prior aversive experiences. Identifying these factors led to the development of the 3Hs: housing, handling and habituation.

The 3Hs initiative aims to raise awareness about the positive welfare benefits which can be achieved through some simple changes in management approaches.

It has two key objectives:

- Reduce the use of physical restraint through habituation to handling and refined methods for substance administration.

- Increase the opportunities for animals to experience positive wellbeing through positive reinforcement and access to sensory enrichment.

In addition, it aims to provide evidence for the welfare benefits that can be achieved using these methods using objective measures of affective state (or mood).

There is a need to refine methods that require physical restraint because restraint is used for almost all methods of substance administration in both rats and mice even though it causes them distress. For example, most mouse restraint involves aversive methods such as tail-handling, despite the increased knowledge of more refined mouse-handling techniques in recent years, such as cupping and tunnel handling. The impact of these aversive experiences is often underestimated. For example, acute restraint has been shown to increase levels of stress hormones[1] and induce a negative affective bias.[2] The stress caused by this can affect control data, data variability and reproducibility which is a significant problem for Animal Welfare and for scientific quality.

Positive reinforcement can provide an opportunity to refine these types of aversive experience because pairing specific experiences with rewards can lead animals to anticipate a positive experience and create a more positive affective state (or mood). This can lead to animals having a more positive association with humans which could in turn lead to a reduction in long-term harms and cumulative severity and potentially a situation where routine management promotes positive wellbeing.

Assessing Animal Welfare can help us continue to identify factors that will most benefit animals but some commonly used welfare assessment methods (e.g. grimace scales, measurement of stress hormones like corticosterone and score sheets for specific procedures) lack sensitivity and may be subject to the biases of the assessor. Objective measures of affective state are valuable tools for testing interventions and can help lead to evidence-based changes in 3Rs guidance and expectations. For example our objective measures of welfare have shown that refined handling techniques reduce the stress caused by drug administration in rats with the refined method leading to a 40% reduction in corticosterone levels and a more positive affective state. Other objective measures such as the number of ultrasonic vocalisations (a sign of positive welfare) have provided evidence of the welfare benefits of rat play-pens while development of a reward-induced bias has been used to provide evidence that male mice prefer to be singly-housed but do not like being housed in individually ventilated cages (IVCs).

The 3Hs framework, although developed for laboratory rats and mice is relevant to all management systems and has the potential to improve welfare for a wide range of species. The use of positive reinforcement, habituation to handling and reducing the amount of physical restraint used can be considered for many management systems. Although implementing the 3Hs may increase the amount of time spent with the animals this can have major benefits for both human and animal wellbeing and the time invested can pay its own rewards as, for example habituated animals who associate human interactions with positive events are quicker and easier to manage. It is well known that better welfare can support better science and the 3Hs can help to achieve this.

Practical applications of the 3Hs

Megan Jackson, University of Bristol

Objective measures of welfare developed by the 3Hs initiative have been used to provide evidence that changes in housing or scientific procedures can benefit Animal Welfare.

But how do we implement these changes as part of our scientific practice? Implementing refinements requires finding a reasonable balance as animal wellbeing needs to be considered alongside the need to achieve our scientific objectives as well as practical considerations such as the amount of space available in the animal unit.

Housing

The issue of whether to house male mice alone or in groups has been a subject of much debate with concerns over male-male aggression on the one hand and concerns over the isolation of social animals on the other. The 3Hs initiative argues that male mice should be housed alone but with enrichment. Enrichment items such as a shelter, nesting material, a chew block and tubes (both hanging and on the cage floor for exploration, shelter and handling) can promote natural behaviours. While access to a playpen can allow for exposure to a more complex sensory environment. Adding these types of enrichments to a group-housed cage of male mice does not necessarily solve the issue of fighting and could even exacerbate it due to an increase in territorial behaviour.

The current standard housing conditions for rats limit animals' ability to express their natural behaviours. Adding cage furniture such as tubes and platforms can increase the amount of usable space available which can benefit welfare. As with mice, the use of playpens can provide rats with access to a more interesting and complex sensory environment which allows the expression of more natural behaviour. This can be particularly beneficial for singly housed rats.

Habituation

Providing animals with rewards after potentially aversive experiences can increase the positive associations the animals have with the experience and lots of types of reward are available. Laboratory diet suppliers offer a variety of treat options that are suitable for different species and management systems. Or mealworms can be crushed or liquidised to create a crumb. Human food like biscuit crumbs or bacon sprinkles, or liquids like milkshake or peanut butter can also be used. Treats are often high in calories so liquid options can be diluted to reduce their nutritional value while measuring spoons are useful to help manage portion sizes.

- With both mice and rats, a gentle stepwise approach is necessary to habituate animals to handling as it minimises initial stress and the formation of negative associations. An example approach for mice is:
- Day 1: gently use tube handling to move the mouse onto the palm of the hand and allow the mouse to move off.
- Days 2 to 3: gently use tube handling to move the mouse onto the palm and let the mouse move from palm to palm.
- Days 4 to 5: gently guide the mouse onto the palm from the home cage.

Treats should be provided at every stage. After this process, the mouse will sit on the open palm. For rats, an example approach may be:

- Day 1: gentle lifting from the cage and back.
- Day 2: gentle lifting and transfer to travel box.
- Day 3: wait for the rat to approach before handling.
- Day 4: introduce dosing positions.

As with mice, treats should be provided at every stage.

Handling

The impact of restraint on rodent wellbeing can be mitigated by creating a positive context for handling. This can be achieved by habituating animals to associate being handled on a Vetbed with a food reward. This positively associated environment minimises the aversive impacts of the restraint.

Similarly, a positive approach to handling can be used during procedures such as intraperitoneal injection in rats. While conventional methods can be stressful for both animal and handler, a positive handling approach with reduced physical restraint reduces overt signs of distress, causes less pain for the rat as the abdominal muscles are relaxed and reduces the risk that the rat will bite the handler.

A demonstration of this approach and of other refined handling approaches, can be seen at the 3Hs handling web page.[3]

Refined oral dosing methods

Julia Bartlett, University of Bristol

Oral dosing is one of the most common routes of administration of drugs in human medicine as it is the simplest, safest and most convenient route for long-term treatment. Ideally translational studies should use the same route of administration; however in animals oral dosing is made more complicated as they are unlikely to voluntarily swallow tablets or capsules. This results in most oral dosing studies requiring gavage dosing which generally involves firm restraint. This can be a stressful procedure which is negative both for Animal Welfare and for scientific outcomes. Oral gavage also carries a risk of adverse events including oesophageal trauma and tracheal dosing. There is the potential for handlers to experience stress and receive bite and scratch injuries too.

A potential way to refine oral dosing studies is to replace oral gavage with voluntary ingestion. Voluntary ingestion methods tend to fall into one of three categories –

- In-cage, where the drug is added to a palatable food like jelly or a wafer biscuit and left in the cage to be consumed.
- Modified diet or water, where the drug is added to the animals' regular food or water supply.
- Syringe or micropipette dosing, where the drug is mixed with a palatable solution that the animal will drink when it is presented.

Unlike the other methods the use of a syringe or micropipette allows control over the timing of dosing and volume of drug ingested. This also allows flexibility over the choice of dosing vehicle. However there are some potential cons or challenges associated with using this method, namely the need for habituation and the need for the dosing vehicle to be palatable.

As discussed above, habituation can reduce the negative welfare impacts of interactions on animals (see Figure 1 for an example habituation protocol). Rats and mice are naturally neophobic and cautious around novel food sources and attempting to dose animals with an unfamiliar vehicle may slow or prevent ingestion.

Familiarising animals with the dosing process can speed up the interaction and encourage the animals to consume the full dose. Habituation can also help build a positive association between the syringe and the vehicle, which further encourages ingestion and can help prevent conditioned aversion if the drug has unpleasant side effects. However it is important to remember that different strains or individuals may be more hesitant than others and that animals should be habituated to the exact vehicle to be used during the study. These habituation protocols can be combined with handling habituation as a positive reward following human interaction or can be continued on non-dosing days to help further prevent conditioned avoidance.

Box 1. Example habituation protocol for voluntary ingestion for syringe or micropipette

Day 1:

- Place 0.3/0.5mL per animal of chosen palatable solution in Petri dish and place in cage where it can be accessed by all animals in cage.

Day 2:

- Remove all but one animal from the cage and place in travel bucket or temporary cage.
- Present 0.3/0.5mL of palatable solution in syringe/micropipette and wait for animal to approach syringe. Tap the syringe/micropipette against the bars of the cage to alert the rat to its presence.
- When animal starts to lick the end of the syringe/micropipette, steadily dispense the solution ensuring that you are not going so fast that any of the solution drips on the floor.
- Repeat for all animals in cage.

Day 3-5:

- Repeat day 2

Figure 1. Box 1.

The palatability of the dosing vehicle is another key element of voluntary ingestion methods. Many drugs have an unpleasant taste which can affect voluntary consumption which is an issue that is not unique to animal research but has been shown to be a factor amongst paediatric and geriatric patient groups. Being creative in the choice of drug vehicle and volume can improve the degree to which animals comply with voluntary ingestion. An example is that fruit flavours can mask bitter tastes more effectively and a larger volume at a lower concentration can prevent taste aversion. Administering a flush immediately after dosing can also minimise any aftertaste.

Approaches like this can make real improvements to the lifetime experience of laboratory rats and mice and reduce their cumulative suffering while supporting the achievement of scientific outcomes. Guidance documents and videos to support anyone wishing to trial these in their facility are available on the 3Hs initiative website.[4]

Optimising welfare through reproducibility

Michelle Taylor, University of Bristol

The reproducibility crisis is a well-known issue in the life sciences. It manifests as a lack of translation of results into meaningful progress because results cannot be directly verified or replicated. This irreproducibility has substantial impacts on animals due to the wastage of lives and repeating of procedures or experiments, often with marginal gains in benefits. From an ethical point of view non-reproducible science is hard to justify.

Several well understood factors contribute to non-reproducible results including flawed experimental design and misuse of statistical techniques. Understanding these factors can help avoid common mistakes and increase data quality and cumulative knowledge per study. For example, the three commonly employed experimental approaches (pilot studies, exploratory work and confirmatory direct hypothesis testing) each have advantages but reproducibility problems often arise from a failure to recognise their limitations and the practice of using any data to generate *p*-values for publication. For instance, pilot studies (typically initial runs with poorly defined outcomes, small samples, low power and high risk of bias) are best suited for estimating effect sizes, assessing variance and refining protocols. Conversely, confirmatory testing tends to have clearer hypotheses, larger samples, higher power and lower false positive rates, yielding data suitable for effect size calculation and significance testing. The quality of the data generated in each of these situations depends on the degree of certainty about the outcome and many non-reproducible results arise from presenting weak pilot or exploratory data as strong findings from confirmatory hypothesis testing.

Effective experimental design also requires objectivity and the elimination of bias to ensure that conclusions can be generalised to the population of interest. Appropriate collection of random, representative samples and careful use of randomisation, controls and blinding can help to ensure that the data collected answers the question of interest and is robust to scrutiny.

Alongside reproducibility is replication. Currently the most reliable way we have of synthesising evidence is through meta-analysis which is the statistical analysis of effect sizes. The effect size represents the magnitude of difference between treatment groups on a standardised scale so that results can be compared across different studies. When we replicate similar effect sizes across studies we accumulate evidence that a particular treatment or intervention produces a particular effect. Conversely when effect sizes differ in magnitude or direction across different studies we can examine whether this is due to biological heterogeneity or experimental artefacts. Meta-analysis can also help us to see how much evidence has been produced to date and whether we might continue to explore a particular question. Cumulative meta-analysis of clinical interventions has shown that many trials have been conducted well beyond the optimum point at which evidence for the true effect size had been accrued making further clinical trials both a waste of resources but also potentially dangerous for subjects involving severe interventions. Reviewing existing data early, including the data already available in repositories and unpublished

data across similar research groups, before undertaking any new study can help to assess our cumulative knowledge and avoid using animals for further work that yields marginal returns.

An alternative to meta-analysis is to pre-plan replication studies across multiple labs with a known set of variables that differ across time and space. These collaborations can increase the known heterogeneity across labs producing results that are closer to the truth for the same number of animals.

When we optimise data quality and replication we increase the benefits of research and decrease the impacts on animals compared to the benefits gained. These processes work in tandem to optimise reproducible science which in turn can help to optimise welfare. By investing more in the earlier stages of research we can avoid wasted effort much earlier, which can only be a good thing for the welfare of animals used in research.

Evaluating environmental enrichment for better Animal Welfare

Jessica Eddy – NC3Rs

Environmental enrichment can refer to any objects or general practices that enhance the level of physical, mental or social stimulation for captive animals. It can improve animals' quality of life by enabling them to carry out natural species-specific behaviours, providing cognitive challenges and opportunities to make choices and have a degree of control over their environment. However laboratory animals in most cases spend most of their lives in their home cages with relatively little enrichment which can result in animals that are stressed, bored and psychologically and physiologically unhealthy. Providing more enrichment therefore can both improve Animal Welfare and the lifetime experience of animals and promote normal, healthy behaviour and physiology for high-quality data collection.

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-focussed 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 those who want to evaluate environmental enrichment.[5] The resource is aimed primarily at technicians but will be of use to anyone wanting to evaluate enrichment and the principles can also be applied to other types of welfare trial to support evidence-based refinements.

The evaluating environmental enrichment resource guides users through the whole process of evaluation from gathering information and designing a study to collecting, interpreting and sharing the data. The resource is extremely comprehensive and includes important questions to ask yourself when planning and support on minimising bias[6] and otherwise maximising the quality of your data.[7] It also includes example protocols depending on the reason that you may be looking to perform an evaluation (for example, I am aiming to address a behaviour of concern or I would like to generally improve welfare by introducing enrichment). The resource then presents users with guidance and options on how you might achieve your aim, including the groups that you might be comparing to answer your question. Following a step-by-step protocol allows you to plan to improve the chances of a successful evaluation, assign roles to team members, collect data in a consistent way and remember what you have done and why you have done it, either to report on or repeat your evaluation.

When it comes to collecting your data the resource includes various additional documents to support you including example ethograms for mice, rats, rabbits and zebrafish. Data collection sheets can be adapted to better meet your needs. There is also guidance on looking at the data you have collected to help you interpret your findings from inputting and sorting the data in a spreadsheet to visualising the data using charts and graphs. The final section of the resource covers ways to implement your findings, depending on the results you have found and provides suggestions for ways to share your findings within your institution or more widely.

The NC3Rs can provide free training[8] on how to use the evaluating environmental enrichment resource, as well as other forms of training[9] in different aspects of the 3Rs. Information about the 3Rs for technicians can also be found in the Tech3Rs newsletter[10] which contains information useful to technicians interested in putting the 3Rs into practice in their facilities. For more information, the training and engagement team can be contacted at training.engagement@nc3rs.org.uk.

What constitutes healthy lighting for rodents?

Rob Lucas, University of Manchester

Healthy lighting is crucial for laboratory rodents and other animals including humans. Light is essential for vision and helps regulate our sense of time via circadian rhythms. These rhythms do not just dictate sleeping and waking cycles. Almost every system in the body has its own circadian rhythm which is reset to the local time by light. Departure from this 24 hour cycle can have negative effects for example mice housed under a non 24 hour

light cycle (4L:4D) accumulated bodyweight more slowly and tended to die sooner than those housed under a 12L:12D cycle.[11] While hamsters exposed to dim light at night showed increased depressive-like responses and a decrease in their general activity levels.[12] Light intensity has been shown to vary substantially across cages in different positions in an IVC rack and exposure to dim light during the day as a result of these different positions can affect activity patterns in mice.[13] Light has been shown to impact experimental outcomes such as novel object recognition[14] and fear conditioning.[15]

The cumulative message from studies such as these is that having the right light is important for animal health and welfare, for reproducibility and for statistical power. This includes having a regular daily light:dark cycle as a disrupted cycle impairs health and alters an animal's behavioural and physiological state as well as having light of the right intensity both at night and during the day. However measuring and quantifying light to ensure that the right light is being provided presents a significant challenge.

Quantifying light presents a problem. Lights vary in their total amount of energy (e.g. the variation in light levels in a room lit by sunlight on a clear day compared to a cloudy day or under fluorescent lights at night) and how that energy is distributed across the spectrum (e.g. some light contains more energy in what humans perceive as the blue part of the spectrum). Additionally, various species exhibit differing sensitivities to specific wavelengths of light. This means that although two lights which are matched in lux may appear identical in brightness to a human observer, a rodent might perceive them as looking different to one another which is different to how a human would perceive them. Metrics for measuring light such as candelas, lux and lumens are based upon the apparent brightness of light for a human observer. Electric lighting is designed according to the human experience, we may not be aware of for example, differences in lighting between different rodent facilities. This is a potential problem for both welfare and reproducibility of studies. To normalise the animal experience we need another way of measuring light.

A workshop on light measurement, co-convened by Rob Lucas and Stuart Peirson, was convened in 2023 to try and address some of these issues. Two papers were published as a result with one presenting a consensus view on how to quantify light for laboratory mammals[16] and one providing practical recommendations for husbandry.[17] One of the key recommendations from these papers was that we need a measurement for light that works across species. Melanopic equivalent daylight illuminance (Mel EDI, unit = lux) is a suitable metric for this as it is now an established way of measuring light that works for humans and for rodents, allowing a better understanding of different animals' experiences of light.

The practical conclusions for housing laboratory rodents are that healthy lighting requires a stable 24-hour light:dark cycle and a maximum at-night light exposure of less than 0.1 lux Mel EDI which is equivalent to a moonlit night. During the day irradiance should be higher than 10 lux Mel EDI. Animals should also have access to a place to escape from light exposure, such as a nest box or nesting material. For animal studies, light intensity should be recorded in Mel EDI during data collection to allow this information to be reported. These guidelines can support better welfare for laboratory rodents and can promote increased standardisation between facilities to help increase reproducibility of animal research studies.

Imaging and the 3Rs

Katie Dexter, University of Southampton

A wide range of *in vivo* imaging techniques are available for use in animal research, including X-ray CT, optical bioluminescence and fluorescence imaging, PET and SPECT scans, MRI and ultrasound, to name a few. These powerful, non-invasive techniques hold great potential for improving Animal Welfare in research through reduction and refinement, as well as improving data quality (Figure 1).

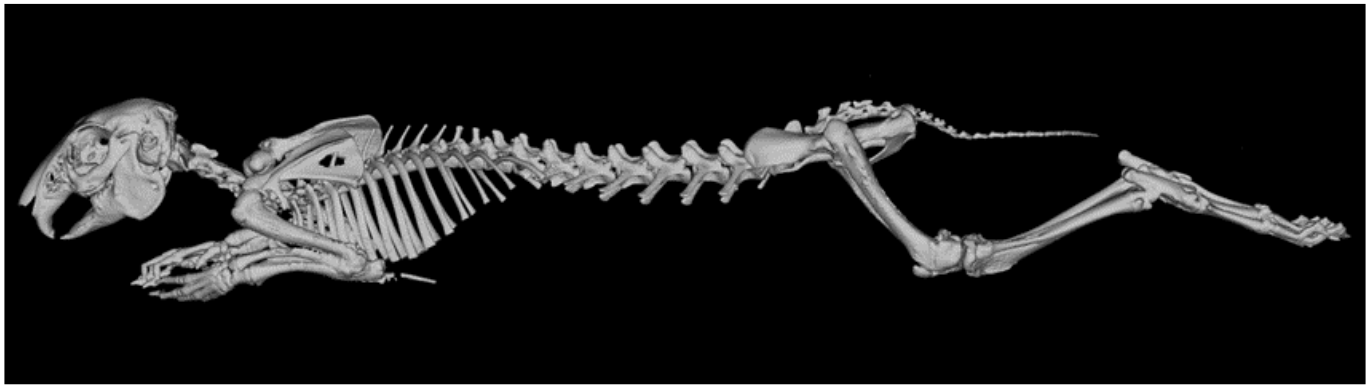

Figure 1. Image: 3D render of a rabbit CT scan focusing on the skeleton (image by Dr Katie Dexter with thanks to Dr Karen Marshall and Dr Orestis Katsamenis).

One of the major benefits of imaging techniques is that they allow for repeated measurements in longitudinal studies. This approach rather than culling cohorts at each study time point allows a significant reduction in the number of animals required. The use of imaging as a reduction tool goes further than this because imaging the same animal each time removes sources of statistical variation (such as natural variation) between different individuals. In essence, each animal acts as its own control producing higher-quality data supporting better statistical analysis and further reducing the number of animals required.

Other benefits of imaging techniques include the ability to use imaging to inform when a treatment or intervention should start. This is more effective than a traditional approach of simply waiting for a set amount of time post-surgery or injection, as each animal can be monitored and the intervention or treatment started at the most appropriate time (e.g. when a tumour reaches a certain size). Imaging can also be used to improve injection accuracy and for diagnostics or investigations into colony health.

The Biomedical Imaging Unit (BIU) is a multi-user facility at the University of Southampton serving the Faculty of Medicine as well as the wider University and the University Hospital Southampton NHS Foundation Trust. Our state-of-the-art equipment includes a wide range of instruments and scanners for different applications and with different advantages. For whole-animal imaging we currently have a MILabs microCT and optical imaging scanner. This scanner has the capability to image animals using X-ray CT, fluorescence, and bioluminescence, as well as a combination of these in quick succession. We have the capabilities to image mice, rats and larger animals such as rabbits.

Advanced hardware and software facilitate lower X-ray doses, improved image acquisition and reconstruction protocols within the microCT sphere. These advances help to minimise the time animals are under anaesthesia through shorter and more targeted scans. To date, the MILabs scanner has been optimised for several disease applications including bone regeneration and remodelling, dementia, obesity and cancer. For example ongoing research has used these techniques to explore how soluble proteins drain from mouse brains to help model dementia. This type of study would not be possible without the use of such imaging techniques.

Maintaining Animal Welfare is of high importance in our laboratory. We use several sophisticated monitoring approaches, including ECG for cardiac monitoring, respiratory monitoring and temperature monitoring to track animal wellbeing throughout the imaging process. We use this information to edit the temperature of the bed or the anaesthetic levels as per each animal's needs.

Downstream imaging and analysis are realised at the BIU with our incredible range of imaging equipment, including multiple light microscopes, transmission and scanning electron microscopy, spatial biology systems and ex vivo X-ray imaging systems. We continue to innovate by expanding the capabilities of our existing techniques, routinely upgrade and purchase new equipment to support the needs of our research community.

Why you should participate in a technician exchange programme

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When people are asked to imagine how something (an object, a scenario, an activity – could be different) they tend to imagine how it could be better.[18] This finding appears to hold true even when the subject imagines something that they already consider to be good. We can learn some useful lessons from this work, firstly that a different and fresh perspective can provide different suggestions about how things can be better and secondly that being given advice on how to improve does not mean the person providing the advice thought the original situation was bad. They may simply have thought of ways things could be better. Recognising this can be important for adopting a mindset that is open to continuous improvement.

Continuous improvement and refinement are a core principle of good animal research. Within animal facilities staff are likely to frequently see how things around them can be improved. For example refinement ideas are frequently developed but implementation or dissemination of these ideas and gaining practical experience may be challenging and potentially frustrating. One approach that can help with this is to participate in a technician exchange programme.

Exchange programmes challenge us to step outside our comfort zone, give us exposure to different techniques and provide an opportunity for us to offer advice. This can be hugely beneficial for animals under our care through exchanging knowledge about Animal Welfare improvements, can support the professional development of staff through providing training opportunities in a judgement-free scenario where staff are separated from their usual day-to-day job and also personal development by allowing staff to develop support networks.

Examples of projects, equipment and processes that technicians may wish to learn about include the 3Hs initiative (see above), the use of the FishGun feed dispenser and the housing and study of pigs at the Translational Biomedical Research Centre. Participants in an exchange programme can learn about how these things work but importantly exchanges provide opportunities to gain experience about things that do

not work so well. For example, if any pieces of equipment are prone to breaking, while the opportunity to work with different species can provide ideas of how to improve your own set-up. The University has a compassion fatigue and emotional resilience programme which technicians may find useful to learn about.

So, how can technicians get involved? The University of Bristol participates in setting up exchanges within the GW4 group. Please get in touch for more details. the Institute for Animal Technology can help to facilitate conversations between facilities to set up exchanges. More information can be found on this web page www.iat.org.uk/techexchange or by emailing techexchange@iat.org.uk.

Meeting action points

The following is a list of action points based on all the presentations and discussions that you may find useful in your facility:

- Visit the 3Hs initiative website to learn more about ways to reduce cumulative severity and improve the overall lifetime experience of laboratory rodents.
- Consider adding additional enrichments such as tubes and platforms to increase the available floorspace, shelters, nesting materials and chew blocks to encourage natural behaviours, especially for singly housed animals.
- Enrichment items or practices should be chosen based on scientific evidence that they benefit welfare. If you cannot find evidence for a new enrichment approach, use the evaluating environmental enrichment resource to assess the welfare impacts on animals before wider use.
- Use playpens to give animals access to a more interesting and complex sensory environment and promote more natural behaviours.
- Provide animals with rewards after potentially aversive experiences, such as handling or dosing.
- Introduce gentle, stepwise approaches to habituating rodents to being handled or to voluntary ingestion via syringe or micropipette as a refined method of oral dosing (see above for example approaches).
- Consult with a biostatistician when planning studies to maximise the quality of data gained from animal studies.
- Measure the lighting levels in rooms in which animals are housed. Light should be in a stable 24-hour cycle with a maximum at-night light exposure of less than 0.1 lux Mel EDI.
- Ensure that all animals have access to a place to escape from light exposure, such as a nest box.
- Record light intensity in Mel EDI during experiments to allow this information to be reported in publications.
- Consider participating in a technician exchange programme to promote knowledge sharing, network building and continuous improvement.

Acknowledgements

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5 Evaluating environmental enrichment | NC3Rs

6 Planning an enrichment evaluation | NC3Rs

7 Improving the scientific quality of your enrichment study | NC3Rs

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