Report of the 2018 RSPCA/UFAW Rodent and Rabbit Welfare Group meeting

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Introduction

The RSPCA/UFAW Rodent (and now Rabbit) Working Group has held a one-day meeting every autumn for the last 25 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 and rabbit use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents and rabbits ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

This year's meeting was held at The Francis Crick Institute in London on 30th October 2018 and was attended by over 80 delegates from the UK and overseas. To mark 25 years of Rodent and Rabbit meetings, the day opened with a retrospective look at how animal technology has developed over the past 25 years, and how these developments have impacted laboratory rodent and rabbit welfare. This was followed by a look to the future, with a talk that discussed how animal welfare science and practices might change over the next 25 years. Other presentations covered ways to encourage laboratory rats to nest-build by giving them appropriate building materials, tips on designing new rabbit facilities to best promote rabbit welfare and a discussion of how imaging techniques can be used to refine experimental procedures and reduce the number of animals used in studies. The day ended with a presentation from the Home Office Animals in Science Regulation Unit and an interactive discussion session, both on the topic of ensuring that laboratory rodents and rabbits never go without food or water. This report summarises the meeting and ends with a list of action points for readers to consider raising at their own establishments.

Advances in animal welfare and technology over the last 25 years

Robin Lovell-Badge, The Francis Crick Institute

A lot has happened since 1993. There have been

significant, sometimes dramatic, advances in technology covering aspects such as animal husbandry, surgical practice, methods of cryopreservation, imaging and, of course, methods of genetic alteration. Many of these have led to better welfare, to more precise ways to test hypotheses and to reduced animal usage; others have led to substantial increases in the numbers of animals used for certain types of experiment, or to altered practices that may not always be beneficial to the animals or to scientific understanding.

One of the most notable developments of the last 25 years is the advance in methods of genome editing. Genome editing allows us to alter DNA sequences very precisely and probably gives us the ability to modify any living organism which, along with other techniques such as directed differentiation of stem cells and development of organoids, may continue to help replace animals and yield better 'models' of human disease.

Another significant area of development has been in the generation and archiving of mouse lines which has greatly improved. This means that fewer animals need to be maintained in the laboratory. For example, there are now better methods of cryopreservation for embryos, sperm, oocytes and ovaries. New methods have arisen in reproductive biology, such as somatic cell nuclear transfer (SCNT), the method which was used to produce Dolly the sheep in 1997.[1,2]

A further area of development is in the use of fluorescent protein markers,[3] or other types of reporter such as luciferase. These allow us to follow cells *in vitro* or *in vivo* and give us the ability to do live imaging studies. Imaging modalities such as MRI and ultrasound have also developed, allowing us to reduce laboratory animal numbers by carrying out longitudinal studies.

The drivers of these advances in technology are widespread. They include curiosity about how genes are expressed during development, a greater understanding of the roles of specific genes, increasing knowledge of stem cell biology and cancer biology and better ways to follow cell fate decisions during development. Additionally, there has been interest in practical applications of this technology in animals – for example, how to make them more productive, grow faster, have disease resistance or how to use them as 'bioreactors' to produce valuable human proteins. However, these possible applications come with their own suite of ethical and animal welfare issues to address.

The advances in Animal Technology over the last 25 years have also impacted on animal welfare, husbandry and the 3Rs, though not always with positive results. Cages have largely switched from open-topped cages to individually ventilated cages (IVCs) with in-built watering systems, which may help maintain the health status of the animals but may not be good for the animals' natural behaviour or normal physiology as they cannot smell neighbours or interact with each other if singly housed. The increase in Specific-Pathogen-Free (SPF) facilities is good for the animals' health but also leads to animals having an underdeveloped immune system and simpler gut microbiota which does not accurately reflect animals in the wild or humans. There has also been an increase in the general understanding of environmental enrichment needs and animal handling techniques which cause less stress and promote better welfare.

Alongside the technological advances of the last 25 years, we have seen better training programmes and career structures becoming available for Animal Technologists. This means that higher skilled individuals are looking after the animals and are able to carry out procedures but may also mean that scientists are less likely to visit animal facilities and as a result may have unrealistic expectations or develop less empathy for their animals. Similarly, improved databases and animal management systems may appear to be beneficial as they offer more streamlining and centralised control but may further contribute to keeping scientists out of the animal facility.

Two encouraging trends underlie all the developments discussed above. The first trend is one of much greater transparency and openness in science which can be seen in initiatives such as the Concordat on Openness and the rise of open access publishing. The second is an increase in genuine concern about animal welfare by all those involved in research and, reflecting this, there has been widespread adoption of the 3Rs, greatly supported and assisted by Animal Technologists.

Rodent and rabbit welfare – what might the next 25 years hold?

Robert Hubrecht and Huw Golledge, UFAW

Laboratory animal science has come a long way in the past 25 years. The genetic revolution has transformed the way mice are used in research, bringing both challenges and opportunities. We have also made great strides in the way we care for rodents in the laboratory, both by better understanding their needs and by spreading that knowledge through training and education. What might the next 25 years bring?

There are a variety of opportunities for replacing animal models with non-animal alternatives, such as organoids, 'organ-on-a-chip' technology, tissue culture, imaging in humans, the use of data acquired from animals undergoing routine clinical veterinary

treatment, risk or hazard assessment and modelling. Some of these techniques are already established, although it is not easy to predict how they may develop.

There are also opportunities which may lead to reductions in animal numbers, although it is difficult to estimate how many animals might be used in 25 years (see Table 1 for the number of procedures conducted on rodents and rabbits in 2017). For example, initiatives like ShARM (Shared Ageing Research Models) allow researchers to share resources such as surplus tissues, reducing the total number of animals needed to generate samples.[4] Sharing of data is also likely to provide a major opportunity, through creation of repositories of animal studies, or the use of 'big' epidemiological data from companion animals to study drug efficacy in naturally occurring disease models. Data sharing might also allow 'read-across' approaches – where similar chemical substances are grouped into 'families' for chemical safety assessments, so that information on the toxicology of a well-understood substance can be used to make inferences about similar substances for which less information is available without having to repeat animal studies.

Species	Experimental procedures
Mouse (*Mus musculus*)	1,094,867
Rat (*Rattus norvegicus*)	233,676
Guinea-pig (*Cavia porcellus*)	22,560
Hamster (Syrian) (*Mesocricetus auratus*)	1,126
Hamster (Chinese) (*Cricetulus griseus*)	0
Mongolian Gerbil (*Meriones unguiculatus*)	311
Other rodent	2,105
Rabbit (*Oryctolagus cuniculus*)	10,362

Table 1. Numbers of rodents and rabbits used for experimental procedures in the UK in 2017 according to UK Home Office annual statistics.[5]

A further way to reduce the numbers of research animals is through better experimental design. Evidence suggests that a large proportion of studies that use animals fail to adhere to several principles of good experimental design, such as randomisation or blinding.[6] Addressing this problem would also help improve the quality of science.

There will also be opportunities to further apply refinements in animal research. Progress has already been made, from the improved training of technologists to better housing conditions (e.g. double-decker cages for rats). However, progress can be slow – for example, although providing nesting material for mice is now standard in the UK, this is not necessarily the case elsewhere. To make further progress in refinement, an overall better understanding of long-term welfare impacts is needed, including insights into 'cumulative suffering'. By better understanding the welfare impacts of both scientific procedures and life in the laboratory on animals, we can gain a better understanding of where refinement or replacement is most important.

The rise of automation in animal facilities offers opportunities to improve welfare, as monitoring or phenotyping of animals can be less invasive and yield better data. Automation can also help avoid human error – for example, by avoiding the misidentification of animals. However, there is also the possibility that losing human input could lead to problems being missed – thus, a mixture of automation and human attendance is necessary.

The techniques used for euthanasia in laboratory animals offer yet another opportunity for refinement. Carbon dioxide is now widely understood to be aversive[7] but, subject to proper evaluation, new technologies could offer the possibility of humane and practical alternatives, such as Low Atmospheric Pressure Stunning (LAPS) or focussed microwave irradiation.

As with the other areas of the 3Rs, there are threats to the future of refinement. For example, new animal models developed in the future may be found to have significant or unpredictable welfare implications. Threats to refinement also exist where there is still a lack of knowledge about animals' needs – for example, relatively little is known about the social needs of rabbits, or how best to keep male mice.

There may in future, also be occasions where reduction and refinement conflict, for instance where fewer animals can be used in longitudinal studies which require repeated imaging under anaesthesia as an alternative to using more animals which must be killed to obtain samples. In such cases the cumulative impact on individual animals may be greater (since anaesthesia has an impact on welfare) but far fewer animals will need to be used. In such circumstances many prioritise the harm caused to individual animals but the harm-benefit analysis will need to carefully weigh Reduction against Refinement.

To conclude, it is possible that we will no longer be using mammalian models in 25 years – although if we are, we can expect them to be more valid. However, the best prediction we can make is that we will see significant further advances in the 3Rs, although we must not forget that threats and challenges are also likely to arise. Animal Technologists will continue to be essential advocates for animals in the future.

Building refinements for rabbits into a new facility

Anna Slaviero, University of Surrey

Recent years have seen many new refinements of various aspects of housing and husbandry of rabbits. This has also been reflected in the new 'Code of Practice for the housing and care of animals bred, supplied or used for scientific purposes' which sets out the standard of care and accommodation for animals under the Animals (Scientific Procedures) Act 1986 (ASPA).[8]

At the University of Surrey we have aimed to improve every aspect of our research work with rabbits over the past three years. This has been beneficial for both animals and research, as it is widely recognised that a high standard of animal welfare makes sense from ethical, legal, economic and scientific points of view. The opening of our new animal facility was the chance to apply and implement refinements on housing from the early stage of facility design. This consequently increased our opportunities to apply refinements in other areas, such as husbandry and enrichment, to improve animal welfare. It also allowed us to have a state-of-the-art facility to further develop our work to challenge and improve standard practices in different animal procedures carried out at the University of Surrey. This section tells the story of the implementation of our rabbit housing refinements, and how this affects refinements in husbandry and enrichment.

Prior to our redesign, our rabbit housing was typical of many facilities. Rabbits were housed in cages singly or in pairs, with the light, humidity and temperature of the room controlled centrally. Although initial attempts to improve rabbit welfare through socialisation, breeding and habituation programmes were successful, the opportunity to develop a new facility allowed us to take these improvements a step further.

In designing the new facility, planning discussions involved as many stakeholders as possible: the Establishment Licence Holder, the Home Office inspector, architects and builders, NACWOs, Veterinarians, Researchers and Animal Technologists. We also had to consider several factors: did we want to refurbish our old unit, or build a brand new unit? Where should it be located? What was our budget? And how could we meet both animal welfare and research needs? The final design for the facility divided the unit into three blocks. The first, the 'noisy' block, contains the changing room, cage washers, autoclave, necropsy suite and so on, in order to contain all areas that would involve noise and minimise disturbance throughout the rest of the facility. Animal housing and procedure rooms are contained in the second 'quiet' block. The third 'super-quiet' block, contains the surgery suite and sleep suite. Building materials were chosen to minimise noise transfer from one block to the next.

In contrast to the central control in the previous unit, temperature and humidity are controlled separately for each room. This allows us to have different temperature and humidity for mouse or rabbit rooms but also for the other rooms like the surgery suite and cage wash. Two lighting systems are in place – red lights and LED lights – to allow visual access to animals out-of-hours without causing disturbance. The health status of the animals is maintained through the existence of negative pressure and air filters, Individually Ventilated Cages (IVCs), decontamination procedures using autoclaving or Vaporised Hydrogen Peroxide (VHP).

Building on the refinements to rabbit housing and husbandry we introduced in our old facility, the rabbit rooms have various features to promote rabbit welfare. Rooms have anti-slip flooring so that rabbits can move easily when let out for play and exercise. A bespoke modular pen system allows the pens to be adapted to the needs of different rabbits. For example, new rabbits can be settled into the facility in phases, starting in a smaller, quiet space, with new areas opened as the rabbits become more settled. Enrichment to encourage play behaviour is available to the rabbits and the equipment provided can be restructured to maintain novelty. Enrichment, social experience and exercise are all available to rabbits in our class II room as well as the main rabbit room.

Furthermore refining the design of the facility, we also introduced refinements relating to staff behaviour. Technologists spend 10-15 minutes with the rabbits each day in order to allow the rabbits to habituate to their presence and to being held and checked. Researchers are now required to visit the facility at least three times over at least one week prior to starting experiments to let rabbits habituate to them as well. One outcome of this rule was that researchers showed more concern for rabbit welfare when they spent more time with the animals. We also introduced positive reinforcement training procedures, such as presenting rabbits with a basil leaf when they hop on a scale, or training rabbits to associate certain odour cues or music with positive experiences to promote better welfare when rabbits are moved to the class II room.

Although our refinements have helped improve rabbit welfare, new refinements are being planned all the time. For example, we hope to introduce burrowing pits to encourage more natural behaviours. Continually checking data, reviewing the results and putting lessons learned into practice will continue to promote and improve the welfare of our rabbits.

Exploring rabbit personalities

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Personality in animals has been an area of growing interest over the past 10-15 years.[9-11] However, attention from a variety of disciplines has led to a wide range of different methods being employed to test for and assess personality. While there have been an increasing number of attempts to explore and describe dog and cat personality in recent years, only limited studies have explored personality and individual behavioural profiles for rabbits. Validated tools to assess personalities in rabbits may have applications across a wide range of contexts, including at rehoming centres, to select suitable animals for use in animal-assisted therapy or specific research paradigms and to understand the behaviour of rabbits in a laboratory setting. Such tools may help in the selection of traits that indicate which rabbits might cope better in captivity or support the matching of individuals to particular settings. In order to develop tools to explore possible rabbit personalities and identify suitable assessment methods for this species, we aimed to answer several questions. Firstly, do rabbits show between-individual variation and within-individual consistency in behaviour? If so, what traits are important to a captive setting? And finally, which tools are suitable to measure these?

The first tool we developed to explore rabbit personality was based on a suite of behavioural tests. A sample of 52 mixed-sex adult rabbits from four land-based college training units were assessed in two trials, spaced three months apart. Trials consisted of an open field test, time taken to exit a carrier, a novel substrate test and a novel object test. Our results suggested that rabbits showed evidence of individual differences in boldness, activity and exploratory behaviour. Ten of the twenty variables studied were consistent over time, indicating that individual rabbits do show consistent differences in these personality traits.

The second tool, the Rabbit Behaviour Rating Tool (RaBRT), was derived from a literature search of rabbit behaviour articles. We identified 47 behavioural descriptors and each was rated on a 5-point scale by pet owners and people that work with rabbits, with 1172 full responses received. Only 17 items demonstrated fair to excellent inter-rater reliability. Statistical analysis identified three key behavioural indicators, which related to social interactions with humans, activity levels and antisocial interactions with humans.

In conclusion, we found that rabbits did show between individual variation in behaviour which could be detected using both tools, although the specific traits that could be measured depended on the tool being used. Behavioural tests also indicated a low-moderate level of individual consistency over time. Further validation studies are underway, including validation of these potential behaviour assessment tools and comparisons to home cage behaviour observations.

A natural approach – how to increase rat nest building behaviour in a laboratory environment

Demi Minhinnett, Durham University

Rats account for a large proportion of scientific procedures on animals every year in the UK; in 2017 rat use accounted for 6.3% of all procedures.[5] This is one reason why it is vital to focus on the welfare of laboratory rats, including allowing them to exhibit natural behaviours, such as nest building. However, it is not always easy to facilitate natural behaviours and nest building behaviour in rats (in the laboratory) is not as commonly observed as it is in mice. Whilst mice have been observed to spontaneously nest build when provided with nesting materials in laboratory settings, rats may not do so, suggesting that nest building in rats is a learned, rather than innate, behaviour.[12] We proposed to give rats the opportunity to learn to build nests, trialling different nesting materials and noting the effects on nest building behaviour.

The nesting materials included in the trial were selected by considering the kinds of materials rats would naturally encounter in the wild, such as grasses. Rats were therefore provided with one of three different kinds of material for this study: hay, paper wool, or a mix of hay and paper wool. The rats used were all breeding females from either Wistar or Lister Hooded strains, and none had exhibited nest-building behaviour before being included in the trial. The quality of the nests produced was also assessed based on a system used to score mouse nests.[13]

Several rats in the trial exhibited nest building behaviour but only when provided with hay, or a mix of hay and paper wool. This was thought to be due to the architectural properties of hay: providing hay allowed nests to be built upwards and outwards to create ball-shaped nests, which score highly when assessing nest quality. The presence of hay was also beneficial for litter production: when rats were provided with paper wool and no hay, pups tended to be found scattered throughout the nest (Figure 1) and mother rats showed more signs of disturbance when the cage was opened – scurrying around the cage and attempting to move pups under the hopper. Two litters were also

abandoned and two dead pups were found. In contrast, when hay was provided, pups tended to be found in a cluster in a nest (Figure 1), mother rats showed less disturbed behaviour and there were no litter abandonments or pup deaths.

This trial highlights the importance of looking at an animal's natural history to find ways to promote natural behaviours, which may have notable impacts on the animal's welfare. By attending to the natural history and natural behaviour of laboratory animals, we can learn more about how to best support them in a laboratory setting.

Figure 1. Images of rat pups scattered throughout the nest made from paper wool (left) and pups clustered together in nest made of hay (right).
Credit: Demi Minhinnett.

Modifying laboratory rat housing for improved welfare

Rebecca Terry, University College London

The importance of allowing rats space to stand on their hind legs is largely recognised (e.g. by the NC3Rs and RSPCA)[14–16] but most current caging does not provide the height to allow for this. Not being able to stand up limits rats' ability to express natural behaviours and is thought to lead to muscle wastage in their hind legs. Conventional cages may also not provide enough room for rats to express play behaviour, and can expose albino rats to too much light, causing retinal degradation.[17] We therefore aimed to improve the welfare of the rats kept at UCL Cruciform by modifying the cages to allow this additional height.

Various factors had to be considered in order to produce a feasible cage design. The room needed to be able to house a similar number of rats as could be housed in the conventional cages, but research equipment stored in the room could not be moved, space was limited, racks could not be made larger, and cages which would require additional equipment, such as air handling units for IVCs, were not feasible. The new cage designs had to meet several requirements within these limits: cages needed to provide adequate height for rats to stand at full height, provide a higher level of enrichment and have space to provide hides to minimise potential retinal damage. Cages also had to be a financially feasible option for other facilities and the change to new cages had to be hassle-free in order to encourage others to adopt the new design.

In order to provide more space within these limits, the total number of cages in the room was reduced from 80 to 64. This did not affect rat research as the room was rarely operating at full capacity. This revision created more vertical space in each rack, allowing a total of 14 cm extra height to be added to each cage. To add this space, a modified raised hopper based on the Tecniplast -123 series was added to the conventional cage bases already in place. Shelving was added to the room to replace the cage racks – this shelving is cost effective, adjustable and can be dismantled when not in use to maximise space in the room.

The new design of the cages resulted in a total height of 35cm, which should allow an adult rat to stand comfortably. These taller cages also allow space for deeper litter to encourage digging and a shelter to protect from light. Enrichment designed to encourage play behaviour can be suspended from the bars to increase floor space and can allow individual rats to spend time away from one another. These changes, promoting natural rat behaviours, are likely to result in data obtained from the rat, being of improved quality. Furthermore, from a practical perspective, adjusting the cage height rather than designing a larger cage base means that cages are not significantly heavier than before, making it easier for staff to move and clean them.

The changes made to the current caging are likely to be highly beneficial for rats, as well as practical and cost-

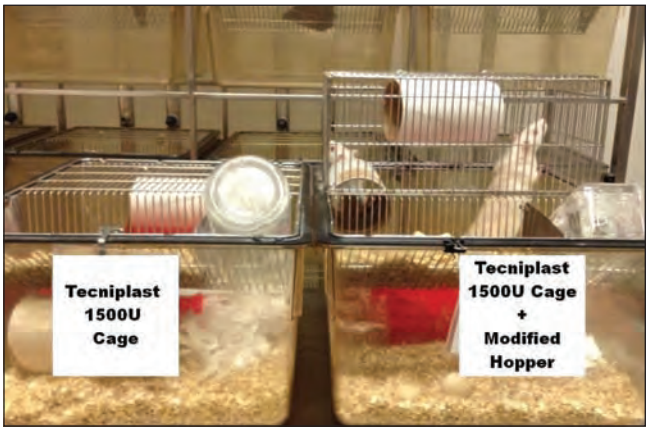

Figure 2. Conventional rat cage (left) and rat cage with modified hopper (right). The modified hopper allows space for more enrichment and for rats to rear to full height.

effective for staff. Tecniplast have therefore produced a prototype based on the changes made and further adjustments will be made to the design before they are developed. This demonstrates that relatively simple changes can be made which are likely to greatly improve the quality of life of laboratory animals.

Severity classification of repeated anaesthesia

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Within the concept of the 3Rs of Russell and Burch[18] one strategy to reduce the number of laboratory animals is the repeated use of a cohort of animals over the course of an experiment. For example, in imaging studies, animals are anaesthetised each time imaging is carried out to avoid artefacts caused by unpredictable movements. However, little is known about the effects of repeated anaesthesia, which may have a greater impact on the wellbeing of the animals than a single anaesthetic episode.[19] The 3Rs advantages of repeated animal usage are therefore only relevant if the animals involved do not experience more suffering, pain or distress than animals used for single procedures. Reflecting the lack of knowledge in this area, Directive 2010/63/EU states that the severity of general anaesthesia is mild but does not differentiate between single and repeated anaesthesia. We therefore aimed to investigate the welfare impacts of single and repeated anaesthesia on mice.

In order to examine the effects of repeated anaesthesia, we explored the effects of two common anaesthetic methods on adult C57BL/6JRj mice of both sexes: inhalation of isoflurane (induction: 4.0%; maintenance: 1.75–2.50%) in 100% O_2 for 45 minutes and injection with a combination of ketamine (80 mg/kg) and xylazine (16 mg/kg) (KX). For each method, mice were randomly allocated to either control, single anaesthesia, or repeated anaesthesia groups (anaesthesia every 3-4 days, a total of six times). Welfare was assessed after the last anaesthetic episode according to our protocol 'systemic assessment of wellbeing in mice for procedures using general anaesthesia', https://paperpile.com/c/EbWJuJ/racD[20] which includes the Mouse Grimace Scale (MGS), burrowing and nest building, the free-exploratory test for anxiety-related behaviour, home cage activity, food intake, and bodyweight, as well as the analysis of faecal corticosterone metabolites (FCM) for acute stress (24 h post-anaesthesia). In addition, hair corticosterone concentrations were measured. We found that neither single nor repeated use of isoflurane influenced nest building, home cage activity, bodyweight, FCM or hair corticosterone concentrations.[21] Isoflurane increased MGS scores in female mice 30 minutes after anaesthesia compared with controls but scores did not differ between single and repeated anaesthesia. Repeated anaesthesia reduced burrowing behaviour in both males and females and increased time before displaying exploratory behaviour in female mice, indicating greater levels of anxiety than those exposed to single anaesthesia or controls.[21]

Anaesthesia with KX did not affect nest building, home cage activity, or hair corticosterone concentrations.[22] Both single and repeated KX anaesthesia increased MGS scores 150 minutes after anaesthesia compared with controls. Repeated KX anaesthesia increased the time before displaying exploratory behaviour in female mice one day after anaesthesia, although single anaesthesia did not. However, after eight days, female mice exposed to single or repeated anaesthesia showed greater time before exploring than controls. Changes in food intake and FCM excretion indicated an increased stress response in male mice after single KX anaesthesia, although there was no effect of repeated anaesthesia.[22]

Besides the degree of pain, suffering, distress or lasting harm, an understanding of the duration of the negative effects is essential for severity classification of any procedure. Although we saw behaviours suggesting increased anxiety following repeated isoflurane anaesthesia, these would be associated with mild, rather than moderate, levels of severity and the wellbeing of the mice was affected for only a short term – mainly in the immediate post-anaesthetic period.[21] In our view, therefore, the severity of repeated isoflurane anaesthesia in C57BL/6JRj mice can be classified as mild. This also applies for other protocols using a comparable anaesthesia regime. However, severity may deviate if a different anaesthesia regime, mice of a different age, other mouse strains, or other mouse disease models are used. Within the mild severity category, repeated isoflurane anaesthesia would clearly be of higher severity than a single isoflurane anaesthesia.[20] For the final severity classification of repeated KX anaesthesia, further investigations are needed in order to specifically determine the effects on anxiety and the duration of the mild distress indicated by changes in food intake and FCM excretion.[22]

Welfare implications of different identification methods for mice

Dominic Wells, Royal Veterinary College

A variety of methods for marking mice, both permanent and temporary, are used in UK laboratories, but little is known about the animal welfare impacts of these methods. A survey of animal units showed that ear punching and notching are the most common mouse identification methods, followed by marking with ink on the tail.[23] We therefore chose these methods for further investigation into their welfare impacts on marked mice.

We initially explored the effects of ear punching and notching on male and female C57BL/6 and Balb/c mice. Mice were either i) ear punched, ii) ear notched, iii) restrained or iv) not handled, and behavioural measures of welfare and faecal samples for corticosterone measurement were collected. Mice showed an immediate head startle response to punching and notching compared with restrained mice as well as more grooming and freezing behaviour in their home cages. However, no significant differences in faecal corticosterone levels were found. Ear notched mice also ate less novel food the following day, indicating higher levels of anxiety, than unrestrained control mice.

Next, we examined whether marking mice with a marker pen or using local anaesthetic alongside ear punching would improve welfare indicators in marked mice. Male Balb/c mice were housed in pairs, with one mouse from each pair undergoing either ear punching, ear punching with the application of EMLA cream (lidocaine/prilocaine), marking with a permanent marker pen or no marking. The second mouse in each pair was unmarked. As marking mice with a pen would need to be repeated regularly, marker pen was applied weekly for the duration of the experiment, whereas ear punching was only done in week 1. The welfare of the animals during marking was assessed by counting the number of animals which defecated during the marking process. In the first week, similar numbers of mice defecated during marking, regardless of the method used. However, defaecation during marking with a marker pen significantly decreased by week 3, suggesting that mice had habituated to the method. Furthermore, mice that were ear-punched, whether or not EMLA cream was applied, were more likely to receive grooming from the unmarked mouse they were housed with, whilst those that received an ear punch and EMLA cream application were more likely to groom their ears and less likely to eat novel food.

In conclusion, we found that ear punching and notching appear to cause short-term pain and anxiety to mice but that application of a local anaesthetic cream did not help to alleviate these responses and caused greater behavioural disturbance. Our results suggest that regular use of a permanent marker pen is a reasonable option which mice appear to habituate to. This may therefore be a good refinement option for those needing to mark individual mice.

How modern imaging techniques contribute to the 3Rs

Thomas Snoeks, The Francis Crick Institute

Over the last decade, imaging has made its way into most academic animal facilities. At The Francis Crick Institute, for example, we use bioluminescence and fluorescence, ultrasound, microCT, 9.4T MRI, PET/MRI, SPECT/CT and intra-vital microscopy. The use of imaging and the wide range of techniques on offer, can make a valuable contribution to the 3Rs in a number of ways, by helping to reduce the number of animals used, refining techniques and helping in the earlier anticipation of disease.

One of the major benefits of using imaging techniques is that they allow longitudinal measurements. This means a single cohort of animals can be used over time, reducing the total number of animals used. Longitudinal measurements can also improve experimental design, as they yield paired data, meaning that studies have higher statistical power than designs which yield unpaired data. Imaging also allows more flexible time points to be used in experiments. However, there are potential welfare impacts to consider, as repeated imaging sessions will involve repeated anaesthesia (see above), which requires a careful harm-benefit assessment.

Although reducing animal use is a decided benefit of using imaging techniques, the impact of imaging on animal research stretches beyond this straightforward reduction in numbers. Imaging can also offer refinement opportunities – for instance, by reducing intra-observer variation. Various image-guided approaches can also be used, such as image-guided injection or image-guided irradiation. For example, ultrasound imaging can be used to guide injections into the pancreas, hepatic portal vein, or other organs or tissues without the need for additional surgery. Image-guided injection has been used at the FCI to produce mice with lentiviral-mediated transgenic skin, leaving the rest of the mouse unmodified and therefore avoiding certain pathologies that may be associated with that transgenic model. Image-guided irradiation can also make it easier to shield tissues that are not of interest and better target tissues that are of interest.

Other areas in which imaging techniques can offer refinement opportunities include the anticipation of disease. Early disease detection allows for refined

experimental protocols where animals are enrolled into experiments before the onset of overt clinical symptoms, which can reduce the degree of suffering the animal experiences. Researchers are also able to better identify the animals they want to include in their experiments – for example, imaging can be used to detect tumours and assess whether they are the right size to be included in a study. Imaging can also be used in the phenotyping of new models – for example, imaging allows the contraction of the heart or blood flow through the aorta to be compared in different subjects.

In summary, the use of the wide variety of imaging techniques available offers opportunities for both reduction in the number of animals used, generating paired data and lower intra-observer reliability, and refinement in procedures, through early disease detection, image-guided methods and phenotyping. Whilst the welfare impacts of repeated anaesthesia must be contrasted with the alternative of using a greater number of animals, researchers can make changes to their experiments to effectively contribute to the 3Rs by exploring and applying the imaging techniques available at their own institutions.

Home Office update: food and water

John Marshall, Animals in Science Regulation Unit, Home Office

The freedom from hunger and thirst is the first of the Five Freedoms to which all animals are entitled (Box 1). Therefore, the provision of food and water to experimental animals is a fundamental part of ensuring animal welfare. Establishment licence (PEL) standard condition 4(3) states that protected animals must be provided with food and water unless authorised by the Secretary of State (i.e. as an experimental procedure), and PIL holders are entrusted with the primary responsibility for animals on whom they have performed regulated procedures. Failure to provide food and water causes unnecessary suffering and potentially death of animals, while experimental data and therefore the benefits of animal use are lost. However, failure to provide food and water does occur and is a major concern for ASRU.

- Freedom from hunger and thirst
- Freedom from discomfort
- Freedom from pain, injury or disease
- Freedom to express normal behaviour
- Freedom from fear and distress

They were originally set out for farmed animals by the Farm Animal Welfare Council but are often applied in other contexts.

Box 1. The Five Freedoms.[24]

Failure to provide food and water as part of the normal care and husbandry of animals represents a significant cause of non-compliance, accounting for approximately 20% of non-compliance cases annually.[25] For many of these cases, the causes fit into certain themes, such as changes in housing, lack of communication, occurrences over weekends and failure to identify the problem over multiple checks.

Changes in housing, whether these are following transportation or delivery of animals, due to the use of weaning or splitting cages, or after procedures, are a common cause of failure to provide food and water. This is particularly found to be the case where both are missing. A lack of communication between facility staff and researchers and a lack of understanding of responsibility are linked to this – for example, after a procedure where food or water has been withheld there may be confusion as to which team member is responsible for returning food or water to the cage.

Another common feature of cases of non-compliance due to lack of food and water is that issues tend to occur over the weekend, perhaps due to changes in staffing or the checking schedule. Lack of food and water is often identified on a Monday, meaning that the initial incident leading to food and water not being provided has usually occurred the previous week. In these cases, where food and water have been absent for several days, the most severe consequences for animal welfare tend to occur. These cases are also particularly concerning, as this usually means that several different people have checked the animals and failed to note the lack of food and water.

There are a number of relatively easy interventions that can be done to reduce the risk of non-compliance. For example, within the animal unit, the set-up of animal housing should make it easy to observe animals, and overcrowding should be avoided. It can also be helpful to identify units that may be 'at-risk' – for example, isolators, cabinets, recovery areas or any rooms outside the main facility – so that these can be appropriately addressed. Staff should also consider what the most effective system of checks would be for their unit. Technology interventions that have automated checking of food and water may also help here but should be an addition to, not an alternative to, human checks. During the meeting, we asked participants to share their own good practice tips to help ensure animals do not go without food or water. These are listed below:

1. Have multiple checks throughout the day – usually one in the morning and one in the afternoon.

Ideally, each check should be done by a different person.
2. When checking for water, make sure that water bottles are **touched** and not just observed.
3. Make sure labelling is informative, especially if animals have special requirements. For example, weaning mice are clearly labelled at an establishment where these are uncommon.
4. Ensure the NACWO is made aware of any absence of food or water, even if this is short term.
5. Carry out random audits of rooms to check general performance across a unit, as well as to flag any specific racks or rooms that might be higher risk.
6. Ensure that the roles and responsibilities of Animal Technologists are clearly defined – for example, by assigning individual rooms or areas to different people, or by ensuring clear instructions are present as to who should return food and water (and when) if these have been restricted as part of a procedure.
7. Consider using a timer when animals are on diet or water restriction, so that when the timer goes off it serves as a reminder to return the food or water bottle.
8. Although automation may help avoid situations where there is a lack of food or water, or other undesirable situations, be aware that it is not a perfect solution, and automation should be combined with checking from staff as well.

Editors Note. The IAT Animal Welfare Group has published an advice notice on the Feeding and Watering of Laboratory Animals. and is available to download from the IAT website www.iat.org.uk/news. This document aims to provide advice with a focus on the role of Animal Technologists, on the steps to be taken to ensure compliance with the terms of ASPA standard condition 4.

Meeting action points

The following is a list of action points, based on all the presentations and discussions, which may be of use to you in your facility:

- If your facility is being refurbished, or a new facility is being built, ask the NACWO and/or NVS to ensure that all Named Persons and Animal Technologists have appropriate input into the design, so that new thinking about refinement can be fully incorporated.
- Encourage researchers to visit the facility regularly (if they do not already), to encourage greater awareness of the animals' welfare needs.
- Be aware that (like many other species) rabbits can have different personalities, which may have implications for welfare assessment, evaluating refinement, and day-to-day care.
- Ask for a review of the amount and type of nesting material provided for rodents at your establishment, especially rats. If nests have not been of good quality to date, consider trying different materials or offering a combination of materials.
- If rats are housed in cages that do not allow them to stand up at your facility, ask for this to be discussed at the AWERB (using the paper by Makowska & Weary, reference [16] below). Could a plan be drawn up to change to taller caging?
- If you are caring for animals undergoing repeated general anaesthesia using gaseous agents, discuss the potential for increased anxiety with the researcher and include indicators of anxiety, such as reduced exploratory behaviours, in welfare assessment protocols.
- Ask for a discussion and review of the methods used to mark mice at your facility, using the section on '*Welfare implications of different identification methods for mice*', above.
- If imaging is used within scientific protocols at your establishment, ask the researcher(s) to give a presentation to animal unit staff, so that you can learn more about different imaging techniques and have a discussion on further opportunities to implement the 3Rs.
- Initiate a review of protocols in place to ensure animals do not go without food or water at your establishment – and watch out for forthcoming guidance from the Institute of Animal Technology on this topic.

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