Report of the 2019 RSPCA/UFAW Rodent Welfare meeting

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Introduction

The RSPCA/UFAW Rodent Welfare Group has held a one-day meeting every autumn for the last 26 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. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

The 26th meeting was held in November 2019 at the University of Birmingham and was attended by over 60 delegates from across the UK. The theme of this year's meeting was 'sentience, positive welfare and psychological well being'. The meeting opened with a talk on animal sentience and what it means for animal welfare, and this topic was returned to in the discussion session at the end of the day with delegates offering their own views on animal sentience. Some talks related to various refinements including enrichment, refined handling methods and clicker training and how these refinements affect behaviour and the affective (emotional) state of animals. Other talks discussed new ways of monitoring animals for better welfare assessment, addressing neonatal mortality in laboratory mice and how small changes can have a cumulative positive effect on the lives of laboratory animals. The day ended with an update from the Home Office Animals in Science Regulation Unit (ASRU) on their themed inspections relating to lack of food and water, refined handling of mice and needle re-use. This report summarises the meeting, and ends with a list of action points for readers to consider raising at their own establishments.

Sentience… what does it mean?

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Universities Federation for Animal Welfare

Sentience has been a topic of much debate recently. It can roughly be defined as the capacity to have feelings, in other words to have positive and negative experiences such as pleasure, pain or distress. However, definitions vary: some argue that sentience simply means an ability to experience the world, whereas others suggest that it means having feelings that actually matter to the animal, or being aware of one's own self-awareness.

It is difficult to know for sure whether or not an animal is sentient but there are behavioural indicators we can look for, such as avoiding painful stimuli, seeking out pleasure, and empathy. We can also look to see if an animal has the physiology to support sentience, such as a complex nervous system sophisticated enough to take in and process sensory inputs to create a conscious experience and mechanisms which would be necessary for pain sensation or which might underlie emotional states.

If some animals are sentient, there is a moral responsibility to care about their welfare – which is why so many people are keen to have sentience enshrined in law. Although the Animals (Scientific Procedures) Act 1986 (ASPA) regulates the care and use of sentient animals, it does not explicitly mention sentience. ASPA does however, recognise that animals can be harmed and defines harm as 'the pain, suffering, distress or lasting harm likely to be experienced by animals during the course of the procedures within a project after applying all appropriate refinement techniques'. By acknowledging that protected animals may have negative experiences like pain, suffering and distress, this definition implies that the animals protected by ASPA are sentient.

So, what does this mean for rodents? Even though ASPA does not explicitly say so, it seems highly likely that rodents are sentient. They have the necessary neural structures, there is more extensive evidence for pain perception in rats and mice than in any other non-human species thanks to their extensive use in pharmaceutical research to identify analgesics, they display complex learning, memory and other sophisticated cognitive abilities, and may experience affective (emotional) states and empathy.[1] This is why it is so important to avoid or minimise pain, suffering, distress and lasting harm to rodents and it shows that their use should be regulated by ASPA. However, sentience in rodents gives rise to two important questions regarding their use in research.

The first is: are some animals 'more sentient' than others? By only regulating vertebrate and cephalopod use, ASPA tacitly assumes that all other animals are not sentient, although emerging research into the sentience of animals such as decapod crustaceans (such as crabs) may eventually lead to the addition of new species. ASPA also assumes that some animals can suffer more than others, as it requires that regulated procedures should 'involve animals with the lowest degree of neurophysiological sensitivity'. This has important implications for the use of animals – for example, should we assume that mice are less sentient than rats, or rats less sentient than primates?[2] Do some animals suffer more, or does a more sophisticated brain allow some animals to cope better with challenges than others? It is unclear at the moment whether these are reasonable assumptions but we might gain a better understanding of how best to choose species for research by going beyond neurophysiological complexity alone. Gaining a scientific understanding of sentience and to what degree it varies between species, might allow us to better predict which species are likely to suffer the least in various situations.

The second question is: when does sentience develop? Early stage fetuses are widely thought not to be sentient and some mammals, such as rats and mice, are thought to be born developmentally immature and therefore also insentient.[3] Understanding the development of sentience might help us to understand at what stage of development animal use should be regulated for each species. However, in the meantime we can apply the precautionary principle, which suggests that we should treat animals as though they are sentient until proven otherwise. As Marion Dawkins puts it: "animal welfare is far too important to be made to wait until the hard problem of consciousness has been solved".[4]

In summary, although not explicitly acknowledged by ASPA, almost everyone assumes that rodents are sentient, and strives to improve their welfare accordingly. As science continues to provide evidence that animals can have feelings that matter to them, and as sentience science develops further, we may be able to make more informed decisions about which animal 'models' we should use. However, we shouldn't wait for hard proof of sentience to reduce suffering and improve welfare in those species we reasonably think are sentient.

Inactive but awake behaviour as a potential indicator of depression

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Assessing an animal's affective (emotional) state is a challenging, but important goal in animal welfare science. Humans can experience chronic negative affective states, such as clinical depression, which can be associated with reduced activity and a lack of engagement with the environment. In animals, 'inactive but awake' (IBA) behaviour has been studied as a potential indicator of depression but its link to affective state is yet to be validated.[5,6] However, the link between these behaviours, termed 'inactive but awake' (IBA) behaviour and affective state in animals has not yet been validated.

We investigated the link between IBA behaviour and affective state in mice by identifying whether comparatively more stressful housing conditions are a risk factor for IBA behaviour, and examining the effects of changing housing conditions. We predicted that IBA behaviour would be observed more in non-enriched cages than in enriched cages, would increase if the amount of enrichment was reduced, and would reduce if enrichment was added. Based on the results of previous research,[7] we also predicted that different strains of mice would show different amounts of IBA behaviour.

To test our hypotheses we housed 31 C57BL/6J mice and 31 DBA/2J mice in mixed-strain pairs in either standard laboratory cages or large, highly enriched cages, for 3 weeks. After this period, half of the cages were swapped to the opposite housing condition for a further 3 weeks to examine the effects of a changed environment on IBA behaviour. Over both phases we observed mice during the dark phase (when they would usually be most active) under red light to see how much IBA behaviour was displayed. IBA behaviour was defined as the mouse being still with their eyes open for at least 15 consecutive seconds.

We found that, as expected, mice housed in non-enriched cages displayed significantly more IBA behaviour than those housed in enriched cages, suggesting that IBA behaviour is an indicator of a negative affective state. Moving mice from an enriched to a non-enriched cage caused a bigger increase in IBA behaviour than any other condition – this is in keeping with other studies which have found that losing enrichment can cause pessimism (and therefore likely a negative affective state) in several species.[8,9] However, we found that moving mice from a non-enriched cage to an enriched cage did not affect the amount of IBA behaviour, suggesting that even though the housing conditions had improved, it was not enough to reverse the negative effects of the non-enriched cage. We did find differences in mouse strains in the amount of IBA behaviour but contrary to previous studies, we found that DBA/2J mice showed more IBA behaviour than C57BL/6J mice. This may be because of individual differences in the mice, or differences in the experiences of the mice used in each study – they may have differed in age, early-life conditions, source, or a combination of these factors.

Overall, our results suggest that IBA behaviour may be a useful indicator of negative affective states in mice; however, more research is warranted to fully validate IBA behaviour as a welfare indicator.

Tail handling affects the capacity of laboratory mice to express disappointment and elation

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Mice are the most commonly used species for scientific research, with millions bred and housed in laboratories worldwide. As with all laboratory animals, making refinements to their husbandry and use in scientific procedures is important for ensuring good welfare. One important refinement has shown that the standard method of catching laboratory mice by the tail increases stress, anxiety and depression, which can be reduced by capturing them in cupped hands or with a tunnel.[10,11] However, tunnel handling for mice has not been widely implemented, perhaps because more research is needed to provide a more comprehensive evaluation of current handling practice on rodent behaviour, welfare and subsequent data collection.

> Anhedonia is an inability to experience pleasure from rewarding stimuli. It can be assessed in mice by measuring how much they consume of a rewarding substance (e.g. a pleasant-tasting sucrose solution), as well as by examining lick cluster size. Mice produce fast, rhythmic sets of licks which can be grouped into clusters with the number of licks in a cluster increasing as the palatability of the reward increases. Lower consumption of sucrose or smaller lick cluster size therefore indicates a more anhedonic state.

Box 1.

We have previously found that different handling methods alter the hedonic value of rewards (how pleasurable a reward is) for laboratory mice[11] (see Box 1), as mice handled by the tail consumed less sucrose and had smaller lick cluster sizes than tunnel-handled mice. This suggests that tail-handled mice perceived the reward as being lower in value than tunnel handled mice did, which indicates that tail-handled mice had a more negative affective state. This difference in how valuable a reward is perceived to be is also found in human patients with negative affective states, such as depression. Negative affective states in humans can also mean that individuals experience more disappointment when rewards are lost and less elation, or joy, when rewards are gained. It is therefore possible that measuring an animal's response to losing or gaining a reward could provide an objective measure of their underlying affective state. The aim of our study was to understand whether handling method affected how laboratory mice expressed 'disappointment' and 'elation' in response to reward loss or gain, with the expectation that tail-handled mice would show greater 'disappointment' to reward loss and less 'elation' to reward gain than tunnel-handled mice.

To explore whether handling methods changed how mice responded to losing or gaining a reward, mice were handled either by their tail or using a tunnel once a day for 9 days. Tail-handled mice were found to interact less with their handler and were more anxious in the elevated plus maze and open field tests. They also showed more anhedonia, as they had lower lick cluster sizes when given sucrose than tunnel-handled mice. Next, for ten days we rewarded mice after handling with either 4% or 32% sucrose solution. After this period, the concentrations of sucrose solution were swapped, resulting in a reward loss condition (a decrease in sucrose concentration; 32% to 4%) and a reward gain condition (an increase in sucrose concentration; 4% to 32%). Control mice received the same concentration of sucrose solution throughout the study. We assessed how mice responded to this change by examining lick cluster sizes.

We found initially that all mice showed 'disappointment' when they lost the higher-value reward, as lick cluster sizes decreased when sucrose concentration decreased. Whilst this disappointment was prolonged in tail-handled mice, tunnel-handled mice recovered and lick cluster sizes increased again when tested later. This suggests that tunnel-handled mice may be more resilient to negative events than tail-handled mice. However, when the reward increased in value, mice showed 'elation', with lick cluster sizes increasing irrespective of handling method.

Taken together, our results suggest that the handling method can affect the capacity of laboratory mice to be resilient to negative life events and that tail-handling may be associated with chronic stress in mice. They also suggest that laboratory mice are capable of positive emotions such as elation, which highlights the importance of measuring both positive and negative emotions in laboratory mice to fully understand their welfare state.

To have a nest or to build a nest? Mouse environmental enrichment for security and stimulation

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Environmental enrichment can improve animal welfare in different ways: some enrichment provides positive stimulation for animals, whilst other enrichment, such as nests and shelters, can provide security or satisfy specific behavioural requirements. There is a wide choice of commercially available cage furniture for mice, so choosing enrichment can be difficult. There are also practical considerations, such as items being hygienic, safe and not overly obstructing staff viewing of the animals.

To better understand mouse requirements for security we studied the behavioural effects and practicality of different shelters. Shelters can provide mice with security as they allow mice to maintain contact with the walls (thigmotaxis), as well as providing darkness, dampening of sounds and smells, shelter from draughts, a refuge from being chased, a more natural 3D environment with something to climb on and something to gnaw. Using a tunnel to catch mice is now strongly recommended for good welfare, so tunnels which are placed in cages for handling may provide some of these benefits and thus could serve as a shelter. However, it is unclear whether a handling tunnel provides sufficient shelter. We tested whether adding cardboard tunnels or orange dome shelters to mouse cages provided more benefits to mice than a colourless handling tunnel alone. Using 12 cages of adult C57BL/6 mice (5 females and 7 males), we provided mice with a handling tunnel alone, with a cardboard tube, or with an orange dome shelter, in a randomised order over three weeks. We found that mice used the dome shelter significantly more than the handling tunnel alone or with the cardboard tube. The dome also allowed three times as many mice to simultaneously shelter and for mice to bring nesting materials inside the shelter; the cardboard tunnel offered a gnawing substrate but little shelter.

We also conducted a study looking at the effects of different types of nesting material. Nests in mouse cages can provide a more naturalistic resting area, the opportunity for nesting behaviour and darkness. Nesting materials are also important as many lab mice are housed at below-optimal temperatures, which can result in cold stress; nesting materials therefore provide animals with warmth and provide some control over their micro-environment.[12] We studied the effects on behaviour, nest quality (which we assessed using a nest-scoring system[13]) and practicality of compressed paper squares or soft paper strips as nest building materials, versus ready-made Kraft paper strip nests.

We used 12 cages of non-breeding adult mice (3 female CBA, 3 female C57BL/6 and 6 male C57BL/6) in a randomised repeated measures design over three weeks. We found no significant differences in the amount of time mice spent nesting but nests produced using the soft paper strips and the ready-made nests had higher nest quality scores than those produced using compressed paper squares, similar to previous research.[13] In terms of practicality, ready-made nests were easiest to standardise across cages and lasted the longest of all materials tested, although paper strips were less expensive. Our results therefore suggest that ready-made nests were the best option of the materials tested. However, preference tests would need to be conducted to verify whether the mice agree. Recent research on animal boredom paves the way for investigating how important cognitive, occupational or sensory enrichment is for mice. For example, there is evidence that enrichment generally enhances mouse cognition but whether this is due to increased stimulation or increased security is unclear.[14] Mice have also been found to choose to forage even when free food is available, which may suggest they are looking for 'something to do'.[15] Furthermore, mice in standard laboratory cages show more 'inactive but awake' behaviour (see above) and more stereotypies[7] – this may signify boredom, as bored animals have previously been shown to become drowsy, but also restless and sensation-seeking.[16] Types of enrichment that may be stimulating for mice might include scatter feeding and puzzle feeders, climbing apparatus, running wheels, digging materials, longer tubes and multi-level cages, novel objects, or exploration and training opportunities. However, more research is needed to assess the effectiveness of these different options.

Overnight monitoring of animals as a welfare assessment

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The MRC Harwell Institute focusses on the generation and phenotyping of genetically altered (GA) mouse models to study the relationship between genes and disease. Many of the lines created are completely novel, and some animals have progressive conditions such as neurodegenerative diseases, diabetes and diseases of ageing. Therefore much effort is directed towards monitoring mouse welfare.

Laboratory mouse welfare is conventionally monitored through daily cage side assessment where the observer examines the cage and only opens it if there is an obvious cause for concern or at cage changing. Whilst this is a very effective way of identifying obvious welfare concerns such as wounds and weight loss, subtler indicators of welfare (such as reduced activity) can be missed altogether. These checks also only provide a 'snapshot' in time which can result in subtle early indicators being missed. Furthermore, we have shown that simply moving the cage from one rack location to another within the same room is enough to disrupt mice for up to an hour, so cage-side checks may not capture what is actually happening in an undisturbed cage. It is also important to note that mice are nocturnal animals, so many behavioural phenotypes will actually be expressed in the dark phase, where they go unobserved.

In recognition of these issues, a number of technologies have been developed to study mouse behaviour over an extended period of time. However, many of these technologies monitor behaviour outside the home cage which means that current understanding of 'normal' mouse behaviour in a home cage is limited. More recently, there has been a shift towards automated welfare monitoring within the home cage.[17,18] In our system, mice have radio frequency identification (RFID) microchips inserted, then are housed in cages which are placed on top of a baseplate containing an array of RFID antennae.[19] This system allows the location of several mice to be tracked within the cage. High-definition infrared cameras are also used to take video footage of the mice.

The use of this system has allowed the detection of a number of behaviours which may be used to assess the welfare of the mice – for example, we have observed mice showing seizure-like behaviour which had not been seen in cage-side checks.[20] In another case, we found that one mouse was showing stereotypic or hyperactive behaviour during the dark phase 24 hours before this behaviour would have been detected with a cage-side check. Using this system can therefore allow us to intervene earlier and implement earlier humane endpoints – this is important for the focal mouse, but also for other mice in the cage who may become stressed in response to a hyperactive cage-mate. We have also been able to detect novel behaviours which do not currently have an activity signature – recognition of these behaviours may help us to create a clearer repository of exact terms and allow information sharing between facilities to better understand the welfare implications.

Another benefit of our tracking system is that we are able to track several mice at once which then allows us to examine social interactions between cage-mates.[19] For example, we can see which pairs of mice spend time together and for how long.

Conducting home-cage monitoring allows us to make better informed decisions about a mouse's welfare.[19] For example, we were able to better recognise differences in behaviour and time budgets between different mouse strains. This helps when differentiating between phenotypes and behaviours that may indicate welfare concerns. We can also monitor responses to new procedures and adjust our approach if we see signs of adverse effects.

Overall, we have found that home-cage monitoring of mice has highlighted how much we do not know about mice behaviour in their home cage and has given us the opportunity to delve deeper. We have been able to collect scientifically relevant data at earlier time points, supporting earlier interventions, are able to identify novel behaviours and create a repository of exact terms for these behaviours therefore being better placed to make informed decisions about mouse welfare.

The Alive Pup Project

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Preweaning pup losses are common in laboratory mouse colonies with some strains showing up to 50% mortality and a high rate of total litter loss.[21] However, little is currently known about how to ensure good mouse pup survival. Received wisdom states that neonatal mortality in mice is caused by active infanticide, is associated with maternal stress, is affected by the number of litters a mother has had and is worse in C57BL/6 and genetically altered mice. We used these ideas as starting points to explore causes of preweaning mouse pup mortality and better understand how mortality can be prevented.

To look for active infanticide, we studied behavioural interactions between pups and dams.[22] Whilst dams were seen eating dead offspring and interacting with both alive and dead pups, no female was observed killing a pup. In a later study (Brajon *et al*, in prep), we found that less than 10% of cannibalism events analysed involved infanticide. It therefore seems that whilst infanticide does happen, it is not a major cause of mortality, and that cannibalism does not necessarily indicate infanticide.

It has been suggested that mothers provided with more enrichment may be less stressed, and therefore that pup mortality should be lower in enriched environments.[23,24] However, we have not yet found effects of enrichment on mortality. Another possible source of maternal stress is inspections by laboratory staff but we have examined both experimental and historical data and found that early post-partum inspections do not affect pup survival.

Previous research has suggested that pup survival is higher in second litters than first litters,[25] but we have been unable to find evidence for this in a retrospective analysis of 344 parturitions.[26] However, we have found that successful mothers perform more nest-building and passive maternal behaviour, perform less parturition-related behaviour (in the labour position or giving birth), pay more attention to still pups and spend less time outside the nest.[27]

We also investigated the effects of social environment on pup mortality and found that housing 2 females and 1 male together did not reduce overall neonatal mortality or total litter loss compared with single housing.[21] However, the presence of an older litter significantly increased neonatal mortality, indicating that, rather than providing extra warmth and protection in the nest, the older pups were somehow outcompeting the newborns and increasing their chances of death.

So, what does this high level of neonatal mortality mean for animal welfare? It is unlikely that the pups suffer much: a lot of pup development, including brain development, happens after birth, so it is unlikely that pups who are stillborn or die within the first five days of life have gained sentience. However, mothers who lose litters are likely to experience effects on their health and wellbeing. High neonatal mortalities also lead to larger numbers of mice being used for breeding – if the average neonatal mortality rate is assumed to be 30% in laboratory mice, based on the average EU statistics for laboratory animal use, 2-3 million more mice would be needed each year to compensate.

The results of our project suggest there are a number of things we can do to reduce or prevent neonatal mortality. These include providing nesting material, avoiding pup hypothermia, avoiding litter overlap in mice housed in trios and avoiding the use of aged females for breeding. It is also important to know about and monitor any problems in facilities maintained for mouse breeding, so reviewing inspection routines and record keeping practices is key, as is awareness of social and other environmental factors which may affect neonatal mortality. More research in this area will help us better understand the mechanisms and risk factors – refer to the Alive Pup Project website for updates on our work: https://approjectweb.wordpress.com/home/the-ap-project/

Little changes, big effects

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Of the 3Rs, the one that Animal Technologists can contribute most to on an ongoing basis is refinement. Refinement of experimental protocols is vital, as we would all wish our experimental subjects to suffer as little as possible to provide the required scientific output. However, most laboratory animals spend the majority of their lives in cages as stock or breeding animals, so small changes for the better with respect to housing and care can have a major positive effect on an animal's lifetime experience.

Within our unit, we have made a number of practical changes over the years to address areas where we felt improvements could be made. None of these changes are revolutionary but we have found that these simple changes can have a positive impact on animal welfare. With all of these changes, we have found that input from technicians is vital, as is good communication between scientists and unit staff. We have also found that having clear protocol plans is crucial, as these provide a compliance check, highlight welfare requirements and help to make staff aware of upcoming studies.

One area in which we have made changes is in animal housing. Most laboratory species are social and do not like being on their own but researchers may be motivated to house their animals individually particularly in the case of animals who have had surgery, telemetered animals, or those bearing head mounts. In one example from our facility, we wanted to attempt to group-house rats with jugular cannulas with dorsal ports. We needed to find a balance between allowing sufficient surgical recovery and reducing the period of single housing but at five days post-surgery, grouping rats led to increased levels of seroma formation under the dorsal port. However, by extending the recovery period to 7 days, we found seroma rates fell to 1% and rats could be housed in groups. This suggests there is considerable scope to introduce group housing in many models. Indeed, it is not only rodents that need social housing – zebrafish are social animals but are often individually housed for a period for genotyping. We have successfully group housed fish in these circumstances by adding them to tanks containing different-looking strains so that the focal individual is still easy to identify.

Another area in which we have been able to make improvements is in monitoring high-risk animals or animals with special requirements, such as aged animals. We have found that it is effective to keep cages that need extra checks together – for example, high-risk animals can be grouped together on the same rack for ease and efficiency, which helps stop any extra checks being missed by accident. Other strategies include arranging cages in order of age and using a system of colour-coded paperclips to indicate the frequency of checks as an easy visual reminder to staff.

The last main area in which we have introduced changes is for animals which require special nursing regimes. For example, MediGel® (Clear H_2O®) is used in our facility for post-operative animals. It is vital that the MediGel® is introduced into cages at least 24 hours before surgery to allow the animal to become used to it, as post-procedure animals are often unwilling to investigate novel objects or substances. This practice can also apply to HydroGel® (Clear H_2O®) or DietGel® (Clear H_2O®). Once again, this highlights the importance of good communication between Animal Technologists and scientists as animals must therefore be identified well before surgery takes place. We have also found that post-operative animals are less able to utilise nesting materials to provide insulation and are often less active. Raising the temperature of post-surgical rodent recovery accommodation therefore has a positive effect on recovery and reduces morbidity and mortality.

In summary, we have found that relatively small changes can make a big difference to the lives of our experimental animals. There is a clear imperative to continually reassess the daily lives of our animals and to share knowledge when we find ways of improving.

Positive reinforcement training with Balb/C mice: how quickly can they learn?

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GSK

Positive reinforcement training (PRT) has been used extensively in large animal species with success; however, the majority of animals used in research are smaller species such as rats and mice. At GSK, we are investigating PRT to ensure that the benefits for the animals and the science outweigh the time and effort PRT requires. We trained Balb/C mice to learn simple tasks that are relevant for research animals, such as entering handling tunnels. All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Following the methods of Leidinger *et al*,[28] we began by identifying treats which would work best as rewards for our mice. We placed a choice of four certified treats (Mini Yogurt Drops, Bacon Yummies™, Fruit-Topia Treats™ (all Bio-Serv) and Fruit Crunchies™ (Datesand)) into the home cages of six female and six male Balb/C mice. We found that only the yoghurt drops were eaten in every cage, so intended to use these to train mice to enter tunnels. To follow the natural behaviour of a mouse, tunnels were placed next to the cage wall. Once a mouse entered the tunnel, we pressed a clicker and offered the mouse the yoghurt drop. However, mice would not nibble the yoghurt drop, indicating that yoghurt drops did not present a high enough reward to produce the desired training effect in our mice. We therefore repeated our treat trial with different, uncertified treats. We found that Nestlé white chocolate buttons were of high value to the mice and using these, mice were successfully trained to enter the tunnels within 4 days.

Next we moved on to training our mice to enter a blood restrainer. We started by placing the tube from a restrainer into the home cages to allow the mice to acclimatise to the restrainer. Subsequent training sessions then took place using the restrainer from each mouse's home cage. In each 5-minute training session, mice would enter the restrainer and a treat was dropped through the hole in the top of the tube. While a mouse was in the restrainer, we gently handled their tails to familiarise them with the process of blood sampling. Using this approach, the mice were trained to enter the restrainer within 7 days.

Our results so far are promising and we have a number of next steps which we intend to address. These include determining whether non-certified treats can be used for experimental studies within GSK and other establishments as well as sourcing effective certified treats. We intend to address whether it is realistic to train mice within their acclimatisation period. Most importantly, we want to delve deeper into the question of whether this approach will benefit the animals and the science.

In conclusion, we believe that PRT could refine animal studies and increase reproducibility as it may remove differences caused by anxiety in mice. This approach should also help reduce the risk of incidents within the blood restrainer tube and is likely to make blood sampling easier by having a mouse that will stay in the required position.

Applying the 3Rs: how much evidence is enough? A case study on rat cage height in the UK

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The Home Office Code of Practice allows laboratory rats to be housed in cages 20cm high, even though adult rats can rear up to 30cm if they have the headroom. Evidence suggests that being able to stand upright is important for rat welfare, as rats housed in higher cages frequently perform this behaviour and rats housed in 20 cm cages appear to compensate for their inability to stretch upright by performing more lateral stretches.[29] However, even though the Code of Practice makes clear that it defines *minimum* standards which can, and should, be improved upon some animal research establishments continue to use 20cm cages. We conducted a study to better understand the perceived challenges of introducing higher rat cages and identify ways to overcome these challenges.[30]

We visited eight establishments across the UK of which some used 20-23 cm rat cages, one used only 30cm cages and others used a number of different heights. Several people were interviewed at each, including Establishment Licence Holders, Animal Technologists, Animal Welfare Ethical Review Body (AWERB) members, researchers and people with responsibilities for budgets and for staff health and safety. Participants at establishments using minimum height cages cited a number of concerns over switching to higher cages, including health and safety implications of lifting heavier cages, lack of resources, a belief that larger cages cause increased aggression among rats and negative effects on scientific data.

However, establishments which were already using larger cages had been able to address these problems – for example, one establishment housed fewer rats per cage and removed water bottles before moving cages to reduce risks to staff. Another establishment had implemented a budget for replacing rat caging over a defined time period.

One striking result of our survey was that many participants appeared to accept anecdotal evidence to support maintaining the status quo but wanted hard evidence to support change. Some suggested that there was a lack of evidence for higher caging, with clear views on the kind of evidence they would accept such as increased physiological indicators of stress in rats who are unable to stand. However, we found that papers demonstrating these findings have already been published. We propose that there is now a stronger body of evidence for higher rat cages and that both animal welfare and science would benefit if these were provided (for example, by repurposing rabbit cages).

This shows that there is an important debate to be had around how much evidence is 'enough' to consider changing practices. We also noted that some participants had knowledge gaps relating to the evidence in favour of higher rat cages; the AWERB, Named Information Officer and Named Training and Competency Officer can play pivotal roles in helping to address these gaps and issues of perception. We encourage anyone at an establishment which houses rats in cages that do not allow them to stand up to ask why this is the case and to challenge this practice.[1]

For the full results of this study, see our open access paper: https://www.mdpi.com/2076-2615/9/12/1104

Update from the Animals in Science Regulation Unit

Charlotte Inman
Home Office

A major initiative for ASRU in 2018/19 has been the completion of themed inspections. These are targeted inspections to address key areas where 3Rs' gains can be made and risk of non-compliance (and associated animal welfare implications) can be reduced. The areas for the themed inspections are provision of food and water, refined handling and needle re-use.

Failure to provide food and water for experimental animals has been a significant cause of non-compliance for several years, representing around 20% of non-compliance cases. We asked inspectors to review the strategies used at establishments to ensure food and water are provided. Some common factors associated with the lack of food and water have been identified, including changes in housing, poor communication between PILs and Animal Technologists, confusion over who is responsible for animals, especially after procedures and weekends due to reduced staffing. Addressing these problems involves reducing risk – for example, within the animal unit, risky areas such as isolators or rooms outside the main facility should be identified and addressed. Other approaches include reviewing the system of checks to ensure that it is effective and ensuring staff are appropriately trained. Data collection and review is ongoing and will help us to develop further potential strategies to reduce the incidence of failure to provide food and water.

The second area of our themed inspections is refined handling. Despite resources being readily available, uptake of these techniques has been patchy. Our strategy (in collaboration with the NC3Rs) has been to engage with establishments in order to emphasise that this is a priority, obtain baseline data on current use, signpost resources and assist with establishment strategy development. So far, we have found that 59% of establishments (out of 110 respondents) are using only non-aversive methods, with 5% not attempting to use non-aversive methods. The primary factor for success appears to be whether Animal Technologists are engaged with the process, with motivation to try these methods coming from within the technologist community. Establishments which have not made changes are often affected by the opinions of influential researchers and animal staff who dispute the evidence. We are continuing to collect and analyse data and are developing strategies for further implementation.

Our final area of themed inspections focusses on needle re-use. Re-using needles can cause unnecessary pain and tissue damage and can reduce data quality by inducing stress in animals and contributing to infection spread. Whether an establishment re-uses needles is also indicative of its culture of care. We have used questionnaires to help establish our position and understand the impacts of our approach. We found that 73% of establishments (out of 86 respondents) have considered the issue of needle re-use. Thirty-four establishments reported currently re-using needles with reasons cited including costs, convenience, staff health and safety or the re-use of needles for euthanasia. We also found that around 25 establishments had no policy on needle re-use and, in the absence of a policy, the relative level of needle re-use was higher in these establishments. Presence of a policy may be indicative of more general awareness of the issue and may also indicate a generally better culture of care. Further analysis and a follow up themed inspection, as well as working with stakeholders is planned in order to further develop policy.

Interactive discussion session on sentience

At the start of the day, delegates were given an activity asking them to indicate which of 16 organisms were sentient. Some choices were uncontroversial – most participants thought that dogs are sentient, while many agreed that trees are probably not sentient. However, there was more disagreement over invertebrate species – only some thought that honey bees are sentient and fewer still thought that crabs and spiders are sentient. Delegates also disagreed about the onset of sentience – whilst many thought that adult zebrafish are likely to be sentient, many were unsure whether a zebrafish larva would have gained sentience at three days post-fertilisation.

Later in the day, there was a discussion session about sentience and what it means for animal welfare. Some delegates pointed out that whilst discussions of sentience are important, they are not always practical for Animal Technologists, as a certain level of detachment may be needed for staff who must carry out procedures or humane killing. There was also discussion about how the topic of sentience might be addressed at participants' own institutions – the importance of the need for strong support from management was highlighted. Delegates also expressed that these may be particularly difficult discussions to have with scientists, especially if they do not see the animals every day, or may be more inclined to consider discussions of sentience as anthropomorphic. However, it was agreed that it is often part of the responsibility of an Animal Technologist to find the right skills or language to communicate concerns to researchers in the right way and several delegates suggested ways in which accusations of anthropomorphism can be countered.

List of action points based on all presentations and discussions:

– Aim to start discussions about animal sentience at your establishment – for example, with respect to welfare assessment, the harm-benefit analysis or out-of-scope animals.
– Look out for mice displaying inactive but awake

behaviour and see whether this correlates with other indicators of distress.

- If not doing so already, consider using refined handling methods such as tunnel handling rather than tail handling.
- Know that not all enrichment is created equal – where possible, evaluate different forms of enrichment (e.g. nest building materials) to see which benefits the animals most.
- Be aware that different forms of enrichment have different purposes – some may provide stimulation, some may provide security – and try and provide animals with a range of enrichment to meet these different needs.
- Cage-side checks during the light phase may not be enough to identify welfare issues in nocturnal animals – consider reviewing cage-checking procedures to see whether rats and mice can be monitored during the dark phase.
- Disturbances and moving animals from their home cages can affect behaviour and lead to welfare issues being masked – consider if there are possibilities for remote monitoring of animals.
- Review the recommendations of the Alive Pup project and see whether there is scope to further implement these.
- Review single housing of social animals and discuss possible strategies for allowing animals to be group-housed with your NACWO, NVS and AWERB.
- Review inspection routines for animals who may need extra checks, such as high-risk or aged animals. Can cages which need extra checks be placed together or some other clear visual cue be used?
- Discuss with the NVS the possibility of increasing the temperature of housing for rodents who are recovering from surgery and monitor whether this helps reduce morbidity and mortality.
- Positive reinforcement training can work for rodents and rabbits as well as larger animals – consider trying this to help refine procedures or when introducing animals to new and potentially stressful situations.
- Review practices around making sure all animals are provided with food and water – especially after cage changes or when identifying who has responsibility for animals.
- If not already in place, encourage your establishment to develop a policy on needle re-use.

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[1] Since the meeting was held, the Canadian Council on Animal Care has published new guidelines on rat housing including a requirement for caging to accommodate upright rearing (ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Rats.pdf).