Report of the 2020 RSPCA/UFAW rodent and rabbit welfare meeting

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Introduction

The RSPCA/UFAW rodent and rabbit welfare group has held a one-day meeting every autumn for the last 27 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.

This year's meeting was held online for the first time and was attended by over 400 delegates from almost 40 countries. The theme was 'cumulative experiences' with sessions on 'the science of cumulative severity' and 'practical refinements to reduce severity and promote wellbeing'. The meeting opened with an introductory talk which explained why cumulative experiences are important and how both positive and negative experiences can accumulate over an animal's lifetime to have long-term impacts on welfare. Further talks discussed different ways to recognise and assess cumulative severity, the cumulative impacts of small refinements and the concept of a 'good life' and what this means for laboratory rodents. This was followed by an update from the Home Office Animals in Science Regulation Unit (ASRU), which focussed on how cumulative experiences influence the severity experienced by animals in science. The day closed with an interactive discussion session on ways to identify cumulative suffering in rodents cage side. This report summarises the meeting and ends with a list of action points for readers to consider raising at their own establishments.

Cumulative experiences – why are they important?

Penny Hawkins
RSPCA

The experiences animals have throughout their lives can influence the way they perceive later events, both positively and negatively. It is important to recognise this, from animal welfare, ethical, legal and scientific perspectives. The concept of 'cumulative severity' or 'cumulative experiences' appears in various laws regulating animal experiments, and in guidance on implementing these. However, cumulative experiences can be difficult to predict and it is unclear how they can best be detected and assessed.

A wide range of factors may influence a laboratory animal's cumulative experiences, including the species of animal, the individual's personality, the procedures involved, housing and husbandry practices, the empathy of handlers and any prior training the animal has experienced. An animal's cumulative experiences may also be affected by both habituation (which may reduce the negative impacts of an experience) or sensitisation (which may increase the negative impacts).[1] Taking these factors into account leads to some important questions relating to the impacts of cumulative experiences, for example:

- Might non-regulated studies involving repeated or chronic sub-threshold harms end up above threshold?
- Might some procedures go beyond their severity limits due to a lack of recognition of cumulative severity?
- How can the concept of cumulative severity be used to better care for animals and improve their lives?

Detecting and predicting cumulative suffering is not easy but is essential for understanding whether severity limits may have been approached or exceeded. Noticing if an animal has become sensitised or is showing an exaggerated response to a 'routine' procedure, or that an animal no longer appears to be coping with life in the laboratory (e.g. they may show depressive behaviours or stop using enrichment) can provide some signs of this. Animal welfare science can also provide possible practical indicators of cumulative severity, such as anhedonia (no longer taking pleasure in pleasurable stimuli), 'inactive but awake' behaviour (see last year's meeting report[2]), or nest quality in mice.[3] Any welfare assessment system should include a number of welfare indicators like these to ensure it provides an accurate picture of the animal's welfare state.

Although indicators like those mentioned above are useful, it can still be difficult to fully understand the experiences of the animals in question. The principle of 'critical anthropomorphism' must therefore be applied - combining empathy with an objective, knowledge-based consideration of what is likely to be significant to an animal. This can be informed by thinking about how animals perceive and interpret their world – for example, mice have poor eyesight but good hearing, so may be sensitive to laboratory noises; are nocturnal and so are likely to be disturbed if used during the working day without a reversed light cycle; and are prey animals so can experience stress during capture and restraint. Attempting to consider laboratory practices like marking for identification, genotyping, early maternal separation and scientific procedures from the animal's point of view can give us a better understanding of the animal's whole-life experience.

In summary, there are a number of key principles underlying approaches to better understanding of cumulative experiences. Firstly, the precautionary principle should be applied, with the assumption being that if something can affect an animal's ability to cope, that it will. Next, it must be emphasised that there is always more that can be done to improve animals' lives - and this can be helped by fostering a culture of support for people who want to address animal welfare issues. Support can also come from the Animal Welfare and Ethical Review Body (AWERB), Animal Welfare Body (AWB) or Institutional Animal Care and Use Committee (IACUC). Finally, it is important that all of those involved with the care and use of animals to engage with animal welfare science, engage in critical anthropomorphism and work together to reduce the impact of research on animals.

Neural indicators of cumulative severity

Tom V Smulders, Newcastle University

Many laws and guidelines relating to animal experiments refer to 'cumulative severity' or 'cumulative suffering' as critical in assessing animal welfare. Indeed, the cumulative experience of a number of mild events can be quite severe under some circumstances, so it is very important to be able to detect whether this is happening. Good indicators of cumulative severity should respond to the individual's experience of the event (not the objective event itself), increase or decrease in value in response to positive and negative experiences, and integrate the response to those positive and negative experiences over time (Figure. 1).[4,5] But do such indicators exist?

Some potential biomarkers of cumulative experience have already been identified – for example, telomeres, which are the caps at the ends of chromosomes, shorten in response to chronic stress. However, telomeres do not appear to lengthen in response to positive experiences, so can only be used as a biomarker of

how many negative events an animal has experienced. Alternative biomarkers, which integrate positive and negative experiences, are found in the brain, and these may be useful for understanding an animal's cumulative experience.

One brain structure that consistently varies in animals exposed to unpredictable chronic stress is the hippocampus, which plays a major role in learning and memory as well as regulating stress, anxiety and emotional responses. Unlike most of the rodent brain, it adds new neurons throughout adult life. The formation of new neurons is sensitive to both positive and negative experiences. This also occurs in humans – for example, the hippocampus reduces in size in people with major depression – and in animal 'models' of major depression. It has also been shown that rats given access to running wheels – a resource they value highly – had significantly larger hippocampal volume than unexercised controls.[6] On the other hand, rats exposed to chronic immobilisation stress showed a significant decrease in hippocampal volume.[7] These results show that hippocampal volume can respond to both positive and negative experiences and so these effects can be integrated to provide an insight into cumulative experiences.

Similar evidence exists for the formation of new neurons (neurogenesis), which is significantly lower in stressed mice than in unstressed mice, whilst the number of new neurons increases in animals provided with environmental enrichment and voluntary exercise.[8] This suggests neurogenesis in the hippocampus can indicate positive emotional (affective) states over time. It has also been observed that some anti-depressant drugs that recover animals from a depressive state to a non-depressive state will also increase hippocampal neurogenesis over the same time course. That is, it takes just as long for hippocampal neurogenesis to recover as it does for behaviours to return to normal, suggesting that there may be a link between these new neurons and behavioural indicators of better welfare.

In conclusion, it seems possible that 'biomarkers' such as the volume of the hippocampus, and the formation of new neurons, could both have potential as ways of assessing cumulative experience. However, there are some limitations: estimation of hippocampal volume can be performed repeatedly in vivo, but only with the use of MRI scans. This process is expensive and involves repeatedly anaesthetising animals which is stressful (and would, ironically, add to cumulative severity). However, it could be done experimentally to help validate potential behavioural indicators of cumulative severity or to help understand the welfare impact of a particular procedure. Neurogenesis can only be assessed at the end of life, so cannot be used as a monitoring tool over time but could be used for experimentally comparing different treatments to allow users to make more informed decisions about the procedures they use with respect to the animal's welfare, or to help validate estimates of actual severity. Hence, these kinds of tools can help inform better prediction and assessment of cumulative severity.

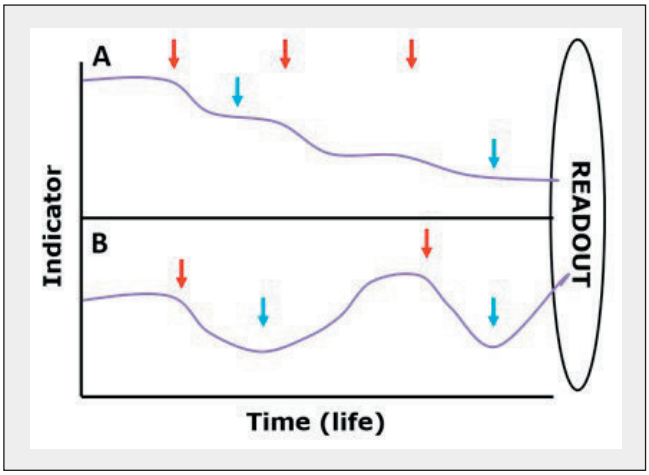

Figure 1. Two patterns of what potential indicators of cumulative experience could look like. Pattern A shows a cumulative indicator that integrates all negative experiences (red arrows) over a lifetime. Pattern B shows an indicator that integrates both negative and positive (blue arrows) experiences. The advantage of pattern A is that it allows one to measure the total negative experiences. However, positive experiences are not recorded. One indicator that might follow this pattern is the changes in telomere length. The advantage of pattern B is that it takes into account the total net experience, but it cannot distinguish between a life with barely any positive or negative experiences, and one that has large negative, but also large positive experiences. Hippocampal neurogenesis may follow the latter pattern.

Can home cage behaviour be used to assess cumulative welfare in laboratory mice?

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Cumulative welfare has become a topic of concern for research animals and may be particularly important for fragile strains, animals used in long term studies or research into ageing and breeding stock. Identifying indicators of cumulative welfare, or severity, is therefore an important goal for animal welfare researchers. Although some potential biomarkers of cumulative welfare currently show promise, such as telomere length or hippocampal volume (see above), they also have limitations for day-to-day use cage-side, as they may be expensive, invasive, involve restraint and handling, and prone to false positives or false negatives e.g. Malmkvist *et al* 2012.[9]

We aimed to identify some practical potential indicators of cumulative welfare. Our first area of focus was home-cage behaviours such as stereotypic behaviour and 'inactive-but-awake' behaviour. These behaviours are considered to indicate poor welfare but might also provide simple, non-invasive markers of cumulative experiences. For example, levels of stereotypic behaviour increase with repeated negative events in some species and also may decrease with positive experiences, such as the provision of environmental enrichment.[10–12] However, stereotypic behaviours are also prone to false negatives as indicators of cumulative welfare. In part, this may be because stereotypic behaviour appears to be subject to ceiling effects e.g. Bechard *et al* 2016, that is, stereotypic behaviours may increase in frequency with increasing stressful events up to a point, but then cease to increase in frequency with additional stressors.[10] Also, not all species or strains engage in stereotypic behaviour – for example, C57BL/6 mice, rats and guinea pigs all appear to have a low likelihood of developing stereotypic behaviours.[13,14] In such animals, other behavioural indicators such as time spent 'inactive-but-awake' may be more useful (for more information on inactive-but-awake behaviour, please see last year's meeting report).

We have also explored the potential for colony morbidity and mortality data to be used as an indicator of cumulative welfare. High morbidity and mortality rates are often associated with negative emotional states, and it is possible that negative emotions play a direct or causal role in affecting morbidity, mortality and longevity by contributing to prolonged activation of physiological systems involved in responses in stressful stimuli.[15] In a range of species, including humans and laboratory rodents, higher stress levels and negative emotions are associated with increased mortality[16–18] while greater longevity is associated with exposure to positive experiences.[19,20]

To explore this link further, 165 female mice were reared to adulthood (55 C57BL/6; 55 DBA/2 and 55 Balb/c) in environmentally enriched or non-enriched cages. After being used in behavioural research, they were then allowed to live into middle age and beyond for approximately 570 days. Over time, we found that 23% of mice (38/165) had died unexpectedly or prematurely by 570 days (including animals euthanised in response to health issues).[1*] This was predicted by housing conditions: of the mice that were still alive at 570 days, less than 65% were from non-enriched cages, compared with over 80% of the enriched mice. We also found that stereotypic behaviour predicted early death. However we found no link between inactive-but-awake behaviour and early death. We concluded that all-cause morbidity and mortality data can therefore be used as a potential indicator of cumulative welfare. However, again these results should be cautiously interpreted. Morbidity and mortality rates can be prone to false positives (e.g. species and strain differences in lifespan exist that are not necessarily correlated with welfare). There is also a risk of false negatives since mild stressors may not affect morbidity and mortality and can therefore be missed. For instance, differences might not be detected in populations not allowed to live their full lifespan since cumulative effects of stress only start affecting senescence, morbidity and mortality after middle age. It is also important to note that morbidity and mortality data can only be used as a retrospective indicator to improve future practices, rather than for current interventions.

The relationship between potential indicators of cumulative welfare is complex, variable and needs more research to help develop more useful indicators for laboratory and other settings. It is likely that there is no 'one-size-fits-all' indicator due to species, strain, sex and individual differences. However, colony 'all-cause' morbidity and mortality data does indicate cumulative stress and therefore morbidity and mortality data that is already collected in facilities can be used by colony managers as an assessment tool and means of improvement, with the principal aim of minimising preventable deaths. Increases in stereotypic behaviour are also a warning indicator of cumulative stress and increases in inactivity may also indicate cumulative stress. It should also be noted that some indicators may have opposing patterns, e.g., stereotypic behaviour may 'protect' against cumulative welfare biomarkers like shortened telomeres and decreased hippocampal volume. Therefore, consideration of multiple indicators and recognition of potentially opposing patterns is key when monitoring cumulative welfare.

Using welfare science to understand animal's experiences and needs

Lars Lewejohann, Freie Universität Berlin

The vast majority of laboratory animals in Europe are mice, with millions used or housed as stock animals and many more humanely killed because they are considered 'surplus' animals.[21] Mice are usually housed in small cages which do not offer much variety, despite the fact that laboratory mice are capable of a wide behavioural

[1*] **Note:** This was not a 'severe' study and death was not used as an endpoint. The mice were simply allowed to live out their lives into late middle age (as happens with pets or zoo animals) and sick animals were always treated and/or euthanised. Findings indicate that senescence (as indicated by a fall in survivorship) began earlier in conventionally housed than enriched animals. Under UK and EU legislation regulating the use of animals for scientific purposes, actual severity is presumed to be 'severe' if an animal is found dead, unless there is evidence otherwise. Death as an endpoint must be avoided as far as possible. In the UK, causes of death must be noted and mortality reported to the Secretary of State if severity limits have been exceeded as a result.

repertoire similar to that seen in their wild counterparts. Housing conditions for laboratory mice have been improved over time, so that items considered 'enriching' twenty years ago, such as nesting material and mouse houses, are now part of a 'standard' cage. One of the key ways to make the lives of laboratory mice better is to aim to continually improve their housing and living conditions.

Observing laboratory mice during the working day in a facility with a 'standard' light cycle may give the impression that the mice are not experiencing problems but observations conducted during the dark phase at night – when mice are most active – are more likely to pick up signs of poor welfare such as stereotypic behaviour. This kind of behaviour may be reduced with the provision of environmental enrichment and lots of items are now commercially available, such as different types of mouse houses, climbing structures and platforms to increase available space. Enrichment aimed at providing cognitive stimulation can also help to alleviate boredom – these kinds of items usually require the mice to interact with an object in order to obtain a reward. In our laboratory, we have introduced boxes with lids which the mice must lift to access millet seeds, hollow balls stuffed with nesting material and millet seeds for the mice to remove, tunnels containing pebbles which the mice can dig out and balls containing millet seeds which will fall out through a small hole if the ball is rolled around the cage. We have also noted that mice like to engage with running wheels or discs and that running discs seem preferable as the mice can run without having their spine bent as it would be in a running wheel.

To establish which of these types of enrichment items are best for promoting good welfare, we compared different housing types: a conventional cage containing a mouse house and nesting material; an enriched cage containing platforms: different types of housing, a running disc and different cognitive enrichment items, regularly exchanged to provide novelty. We found that behaviours associated with poor welfare, such as inactive behaviour and stereotypies, were significantly reduced in enriched cages compared to controls. We were also interested in rating the different enrichment items from the mouse's perspective, so we conducted a large number of preference tests. To do this, we tagged mice in the neck region with radio-frequency identification (RFID) tags so their locations in a cage could be tracked using our newly-developed surveillance system.[22] We then presented mice with different combinations of enrichment items in order to develop a rank order of preference for these items (Lewejohann and Talbot, in prep).

We found that mice showed the greatest preference for a plastic floor house on which they could climb in comparison with other types of mouse house and a ball-shaped house was least preferred. Structural items with a flat surface on top were the most preferred of all the climbing elements we presented with a plastic suspended tube the least preferred. Finally, we found that the most preferred form of cognitive enrichment was the latticed ball containing removable nesting material and millet seeds and a puzzle box which required mice to slide open a lid to access millet seeds was least preferred. Our next step will be to conduct consumer demand tests, which are tests which can be used to assess how hard mice are willing to work for access to a reward or condition.[23] We have previously shown that mice will work harder (press a lever more times) to access an enriched cage than an additional non-enriched cage, suggesting that the enriched cage is more highly valued by mice.

Beyond the forms of enrichment described here, we have found other ways that help to improve the welfare of our mice include provision of treats like millet seeds and providing opportunities for exercise by adding running discs to cages (Figure 2.). We have noticed that aged mice provided with running discs looked healthier and more active after two weeks, suggesting that this provision may be important for limiting the welfare impacts of aging. Continuing to trial and evaluate these kinds of interventions are important ways to keep improving the welfare of research animals, even outside of an experimental context.[21]

Figure 2. A mouse using a running disc. *Credit: Lars Lewejohann.*

Small refinements to improve lifetime welfare

Paulin Jirkof, University of Zurich

Refining experimental procedures to reduce pain, stress or other negative emotional (affective) states is a crucial tool to improve experimental animal welfare. However, laboratory rodents spend much of their lives in their cages, outside the experiment and many are

not even used for experiments but maintained for breeding. To ensure the lifetime welfare of all animals bred and housed for scientific purposes, all aspects of husbandry, breeding, housing and research procedures must be considered.

Mice account for the majority of research animals globally and are usually housed in groups as they are social animals. However, inter-male aggression in group-housed mice is very common, and can lead to stress, severe injuries and death – especially as fighting wounds may not be noticed until it is too late.[24] This means that the severity of fighting in male mice is often under-estimated. A potential solution to this problem is to house male mice singly, but this intervention is not ideal as it deprives mice of their social needs and also makes mice more vulnerable to cold stress as they cannot huddle together with others for warmth. In general, male mice prefer to be group housed so it is important to seek a better solution than individual housing.[25] Although there is some ambiguity in the literature regarding alternative interventions, some show consistent and promising results.

For example, some laboratory mouse strains are less prone to aggression than others, which can provide a useful starting point; grouping siblings together, grouping mice when young and keeping these groups stable once established can also help reduce aggression. The ideal group size for male mice has not yet been agreed upon, as some research has suggested that smaller groups may be better than larger groups, nevertheless recent research resulted in ambiguous results.[25–28] Aggression also tends to be lower when steps are taken to reduce stress – for example, moving used (but not soiled) nest material (not litter) into a new cage when cages are changed and choosing less stressful handling techniques such as tunnel handling and predictable handling.[28] If none of these interventions work and aggression persists, mice may have to be housed singly, but extra nesting material should be provided in order to reduce the risk of cold stress.

Another area which can be refined to improve the experience of animals and may contribute to improving an animal's overall experience is drug administration. Typically, this procedure is stressful for rodents as it may involve restraint and unpleasant or aversive experiences like injection or oral gavage. With well-trained personnel and habituation, stress can be reduced somewhat but this process is still likely to be stressful. However, it is possible to train rodents to ingest substances, either directly from a syringe or by mixing with preferred foods. Some restraint may be initially necessary, but if the carrier substance is palatable, and as the animal habituates to the experience, less restraint will be needed, possibly to the point where no restraint is needed at all.[29] This technique works with both rats and mice – and could even become a positive experience for the animal. As another alternative, drugs can be mixed with palatable substances and provided in the animal's home cage so that no handling is required. Nutella®, honey, strawberry jam, baby food and condensed milk are all good options to try and sterile or calorie-free jellies are commercially available if they are needed, as are emulsifiers which may be needed to mix the substance with the carrier. However, note that methods based on uncontrolled voluntary ingestion (e.g. via drinking water or *ad libitum* food) may not be suitable for protocols which require the animals to ingest a controlled amount, especially as eating or drinking events may vary greatly in frequency between the light and the dark phase.[30]

There is great potential for improving the lives of laboratory animals both in and outside of experiments. If you wish to apply these, or any other refinements in your facility, consider putting together an action plan which takes into account the latest advances in the field, challenges the status quo and aims to find creative ways to solve any problems that may arise. Train collaborators in the refinement procedures you wish to use and test options systematically, with alternative methods in place in case they are needed. Finally, share your experiences with internal and external colleagues – letting others know what works and what does not, is key for promoting better welfare for a greater number of laboratory animals.

Development of a visual approach to severity assessment

Jackie Boxall and Helen Murphy, GSK

Guidance on severity assessment, such as the EU Severity Assessment Framework,[31] states that the duration of adverse effects should be considered when assessing harms to animals resulting from procedures – but how do we decide when a transient effect becomes persistent? Do we all think the same way? Good communication between all stakeholders is key when making decisions about animals used in a study, but it can be challenging to ensure a consistent approach between research projects. Our Animal Welfare and Ethical Review Body (AWERB) set a 2020 objective to review internal guidance on severity assessment, clarify the transitions from mild, to moderate, to severe for commonly observed clinical signs and consider cumulative severity.

Our approach was to develop a 'heat map' for each individual clinical sign, with the descriptor of the sign along one axis and the duration of the sign along the other axis. This would allow a colour-coded severity classification to be assigned for each clinical sign that takes both factors into account, so that a sign which appears fairly mild, but lasts for a long time, may

actually be considered a sign of moderate severity, and a long-lasting moderate sign may be considered severe. The guidance we currently use leaves decisions about how the duration of a sign should be interpreted up to the observer, whereas this heat map approach can help to remove some of the ambiguity.

To develop our guidance, we formed a working group, which included animal care staff, researchers, veterinary surgeons and a statistician. The group members came from diverse areas of our animal research community and worked on a range of different species to provide a broad basis of knowledge and help achieve consensus regarding how to classify the different clinical signs. We started by forming a list of the clinical signs we wanted to develop guidance for, and discussed each sign and how to interpret it in detail. We agreed upon the basic structure of the map for each sign by deciding on what the descriptors and timelines were going to be. Next, each group member was asked to fill in the heat map independently from other Working Group members although they could consult other colleagues if they wished. Data from this was then collated and visualised using mosaic plots. The mosaic plots showed where there was a strong consensus over how a sign should be interpreted and this information was used to begin assigning colours to the boxes within the heat map. For areas without a strong consensus, we used the current guidance to help inform our decisions and engaged in further discussions within the group to better understand each other's viewpoints. This has resulted in usable heat maps for several generalised clinical signs including hunched posture, subdued behaviour and piloerection and are working on maps for body weight changes (Figure. 3).

When considering how individual clinical signs may affect cumulative severity, we must consider the total number of clinical signs as well as the magnitude and duration (Figure. 4). To use the heat maps for multiple clinical signs, we assign the severity level for each sign individually, then take the highest severity level as the minimum actual severity experienced by that animal. Where all the signs fall into one band, we can look at how close the signs are to the threshold for the next band and consider whether a higher overall severity needs to be assigned to take into account the cumulative experience of the animals.

Although the development of our heat maps has been a positive step forward, there are some limitations to our approach and some next steps we are taking to develop the guidance further. On the positive side, the effects of duration are now well-defined, severity can be assessed on a continuous spectrum and the heat maps are based on a wide consensus with transparency over all stages of the decision-making process which should mean there will be good consistency between users. However, assessment of animals still tends to be based on professional experience and opinions, rather than animal welfare or behaviour science, especially as some of the descriptors are still open to interpretation. We also have yet to define how to interpret intermittently displayed clinical signs. Our next steps will be to develop guidance for further clinical signs and procedural effects, further develop our method for interpreting multiple clinical signs and to seek further consensus and feedback to continue to improve our approach.

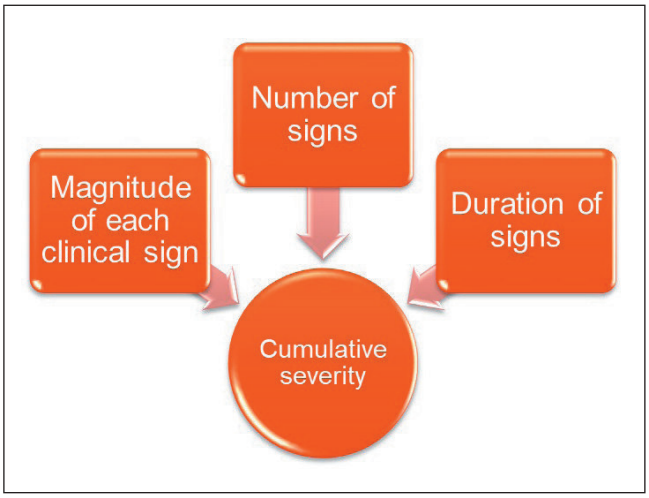

Figure 4. Factors affecting cumulative severity considered for the 'heat map' approach to actual severity assessment.

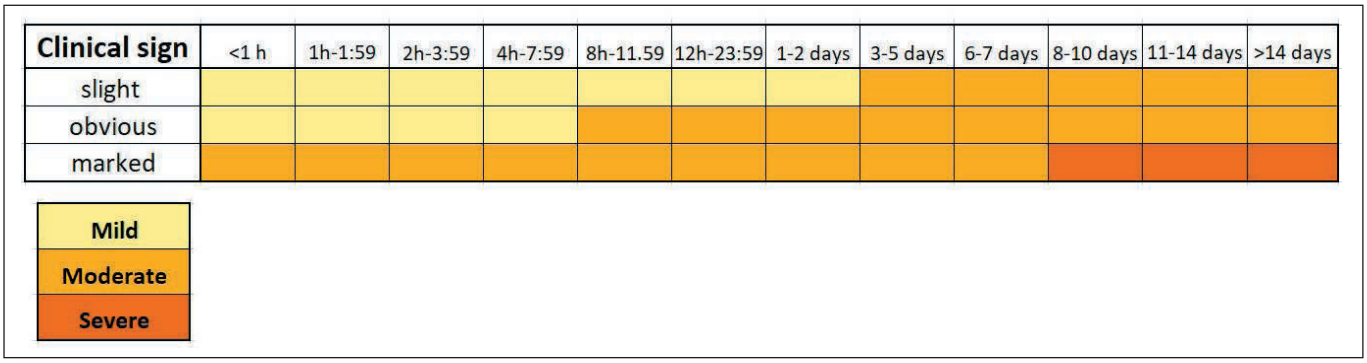

Figure 3. An illustration of a heatmap. A similar table for each clinical sign covered by the new guidance will be shared once completed. Severity bands, descriptors and timelines would be specific for each clinical sign.

Establishing trust with laboratory rats: how long does it really take?

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Laboratory rats and mice are handled frequently in research settings for example, during cage change, for physical examination and for various study procedures. Suboptimal handling and restraint procedures can cause prolonged stress responses caused by negative reactions to people, which may have further negative effects such as delayed wound healing, reduced learning and cognitive abilities and reduced animal health and wellbeing. On the other hand, the use of low-stress handling techniques like cup and tunnel capture and handling to improve human-animal interactions has a number of benefits.[32,33] Reduced fear in laboratory rodents reduces the risk of injuries to both animals and staff, makes the interactions more enjoyable which can benefit animal welfare, staff job satisfaction and the overall human-animal bond and helps to minimise bias in the study data. Despite these highly publicised benefits, there is still poor uptake of low-stress handling practices, and a common reason given is that habituation takes too long. We therefore conducted a study to investigate how long it takes to improve rat-human interactions.

The aim was to evaluate whether short periods of habituation and counter-conditioning would reduce measures of fear, stress and anxiety in handled rats. Habituation (the gradual exposure of an animal to a stimulus) and counter-conditioning (where a negatively-perceived event is paired with a positive stimulus to reduce the negative effects) are both training techniques that could be incorporated into the regular husbandry of animals to reduce their negative responses to people, procedures or the general laboratory environment. We carried out the study over a two-week period and included three groups of male Sprague-Dawley rats. The control group received no handling over the study period, the 'low handling' group received 15 seconds of gentle handling three times a week and the 'moderate handling' group received 45 seconds of gentle handling three times a week. The handling consisted of gentle body restraint and stroking of the head, body, tail and limbs on a soft handling mat with Cheerios given as treats.

At the end of the study period, we found that rats in both handling groups urinated and defecated less during cage change than control rats, were quicker to voluntarily approach the hand of an unknown person, suggesting lower fear of humans, and also eliminated less when restrained for blood collection. However there were no differences between groups in glucose levels or in behaviour when the rats were tested in an elevated plus maze, suggesting that while fear of humans had been reduced, there was still some level of handling stress. We also noted that there were no differences between the low-handled and moderately-handled groups, suggesting that only 15 seconds of handling three times a week is sufficient to reduce negative responses.

The results of this initial study are promising, as they suggest that relatively little time needs to be invested to improve the experience of laboratory rats which has important implications for the overall cumulative experience of these animals. We plan to further investigate these effects, firstly by seeing if our result can be replicated and then by carrying out this study with female rats to see if their responses differ from males. We also hope to carry out a longer study to examine how long the effects of this simple handling protocol will last.

A good life for laboratory rodents?

I Joanna Makowska,
University of British Columbia

A 'good life' requires that animals be able to express a rich behavioural repertoire, use their abilities and fulfil their potential through active engagement with their environment. Although some types of research may not always be compatible with providing laboratory animals with a good life, it is possible to consider what the minimum day-to-day living conditions would be that contribute to a good life for laboratory rodents. There are three major aspects of animals' lives which play a major role in having a good life: the animal's life outside the research context, the interactions that animal has with humans and the animal's physical environment. Here we focus on the physical environment but the importance of the animal's life outside research and human-animal interactions are discussed in Makowska and Weary (2020).[34]

A 'standard' cage for laboratory rodents has two main physical features – litter and shelter. The types of these features which are chosen can have a significant impact on welfare – for example, in North America, corncob bedding is popular for its high absorbency, but has been found to be avoided in preference tests. An alternative is paper-based material which has fewer impacts on animal health but has lower absorbency. A simple way to improve welfare for rodents is therefore to provide paper material but use a deeper layer – this is preferred by mice, and also was found to lead to lower corticosterone levels, higher body temperature, lower food intake and lower ammonia levels in mouse cages, meaning that any higher cost of using more litter may be offset by lower food costs.[35] With respect to shelters, open-ended PVC pipes are often used in rat cages, even though rats prefer hut-type shelters with

only one entrance. Mice tend to prefer nests as shelters and will choose soft paper towels or tissue paper as their main building material, even though they produce better-quality nests with crinkle paper. If both are provided, mice will use crinkle paper as the structural outer layer and paper towels as the inner layer, resulting in a comfortable and high-quality shelter.

Another important aspect of the physical environment for laboratory rodents is the level of environmental complexity. Creating more complexity, either by adding structures which increase the amount of usable space, or by adding cage dividers, is preferred and leads to lower stress levels in both rats and mice. Increased complexity can also allow rodents to use separate areas for different activities – mice housed in three interconnected cages were found to build a nest in one cage and use another as a latrine,[36] and – in general – mice provided with a demarcated area in their cage spontaneously use this area as a latrine.[37] Cage designs should therefore promote this segregation of space, for example, a litter pan containing absorbent bedding can be placed near the food and water, as mice and rats prefer to eliminate close to food and water. Doing this would also allow the rest of the cage to be disturbed less frequently, as only the litter pan would need regular changing and would also allow more comfortable bedding to be used in the rest of the cage.

Alternatives to the typical 'shoebox' cage, such as cages more similar to those used for pet rats, can provide an even greater level of environmental complexity and are associated with better welfare and a more complex behavioural repertoire. For example, rats housed in large cages containing soil were less stressed and performed behaviours not possible in a standard cage, such as burrowing, climbing and upright stretching, while mice housed in large, complex enclosures had less fat and stronger immune systems.[38] When it is not possible to provide home cages with this level of complexity, animals will still benefit from access to a 'playpen' – repurposed rabbit cages (for rats) or rat cages (for mice) that animals have regular access to can promote better welfare and a wider range of behaviours in rodents.

The refinements presented here, along with other refinements such as less restrictive handling and good socialisation protocols, are simple ways to immediately improve the welfare of laboratory rodents and contribute to them having a better lifetime experience. Over a longer time-frame, there are even more potential avenues to explore, many of which should be the ultimate goal for how animals are used in future, for example, providing options which allow animals to free-range, training animals to voluntarily participate in procedures and using pets which naturally develop conditions for studying diseases rather than created models. Taking steps like these to give animals a good life is not only our duty but should be considered a prerequisite for their use, and a starting point around which we build our research programmes.

Home Office update

Charlotte Inman, Animals in Science Regulation Unit (ASRU)

Under the Animals (Scientific Procedures) Act in the UK, any application to use animals in research is subject to a harm-benefit analysis, to ensure that any harm that may be caused to the animals is justified by the expected benefits for humans, animals or the environment. The experience of animals used under ASPA can be influenced by project-related effects (effects which are specific to the regulated procedures undertaken) and contingent effects (those which inherently arise from the experimental or scientific use of an animal). The net impact of these two groups of effects determine the cumulative severity of an animal's experience over the course of its use. The use of severity classification is required by law and qualifies the likely (prospective), ongoing (during procedures) and actual nature of the experience of an animal. Understanding the cumulative nature of animal experiences presents multiple opportunities to influence the likely and actual severity experiences of animals in science.

When considering cumulative experience, there is often a focus on the project-related effects, for example, in the case of administration of a substance, factors such as the route, the nature of the substance and the frequency can all have an impact, as will the application of good practices such as single-use needles and the use of anaesthesia and analgesia. However, contingent effects can also have a significant impact, especially as they may affect animals prior to their use and between and after procedures. Contingent effects may be broader than project-related effects, such as provision of food and water, including refinements such as the provision of wet mash post-surgery, handling, enrichment and housing conditions.

Animal Technologists can have a major impact on how both project-related and contingent effects impact animals, as they can provide highly valuable expert input due to their specific qualifications and exposure to continuing professional development. Animal Technologists will also see a broad range of studies involving a range of species and so are well-placed to identify opportunities for translational refinements or changes in practice across different studies or species. Finally, they are involved across the lifetime of an animal, not just when the animal is being used for an experiment and so are able to consider how to make incremental improvements to an animal's lifetime experience.

Often, improvements to animals' lives can be made that are not necessarily written into project licenses and Animal Technologists can be key in identifying these. For example, re-using needles for procedures can cause animals unnecessary pain and tissue damage, and can have a significant impact on cumulative experience. A survey by ASRU in 2019 found that 73% of establishments were aware of this issue and that needle re-use was occurring in 35% of establishments and establishment culture was a major reason for this. Another example of the importance of the impact of animal care staff on cumulative experience is the use of refined handling methods for mice. An ASRU themed inspection in 2019 found that 59% of establishments were only using these non-aversive methods of handling and that the primary factor for the success of these methods was engagement of Animal Technologists and agreement over the need for change. The best motivation for that change therefore came from within the technologist community. These examples demonstrate how important the role of the Animal Technologist is in helping to identify and implement positive change for animals.

Animal Technologists have the relevant professional background, interact with animals across their lifetimes and are likely to interact with animals more frequently than researchers. They are also closely involved in the care of experimental animals before, during, between and after their use, and so have the opportunity to make a really positive impact on animal cumulative experiences through their input on both the project-specific and procedural effects. It is therefore important that animal technologists are empowered to make this positive contribution.

Interactive discussion

The final session of the day was an interactive discussion around the topic 'how do we know if cumulative suffering is present in rodents cage-side?'. A brief survey of audience members at the start of the session showed that over 85% of the audience felt that cumulative severity was an issue for at least some, if not all their animals, but only 42% felt that they would be confident in identifying indicators of cumulative severity and just 38% said their establishment's welfare assessment systems included indicators that detect cumulative effects. The discussion therefore focussed on indicators that can be used to identify cumulative effects.

Some of the possible signs that may indicate issues with cumulative welfare which were suggested by participants included body condition, weight, posture and activity and it was agreed that activity levels, as well as particular activities or behaviours like nest building, can be used as indicators of cumulative welfare. This may especially apply to abnormal behaviours such as stereotypic behaviours, barbering or aggression. Another suggestion was that behavioural diversity and circadian rhythms, can be disrupted in response to stress or chronic stress, so noticing these changes can help identify poor welfare. Participants also discussed how an animal's response to handling or other human interactions may change in response to a cumulative welfare issue, although these changes are difficult to quantify and capture but are usually recognised by technologists who have the experience to recognise when an animal is 'just not right'.

Given the difficulty of quantifying some of these indicators, it was suggested that a way to help monitor some of them on welfare assessment score sheets would be free-text boxes, so that signs which do not appear on the lists of indicators but are recognised by technologists can be recorded. It was also suggested that score sheets should include a list of procedures done so that those interacting with an animal can see what the animal has previously experienced and that procedures which are not necessarily part of an experiment and may be thought of as 'routine', such as biopsies and marking for identification, may still affect animals and therefore should be included on such a list.

The discussion also covered how animals can be monitored to identify cumulative welfare indicators. For example, it was suggested that refined handling methods can be a useful tool, as some indicators of poor welfare are likely to be easier to notice when using these low-stress techniques. Another point was the importance of the timing of monitoring: it was noted that animals are often looked at for only short periods and sometimes during the day when they are asleep. We therefore may need to think more about observing animals for longer periods of time or increasing the number of observations and using up-to-date home cage monitoring technologies and methods, as well as using reversed light-cycles if not already doing so (although this does not remove all issues (see Hawkins and Golledge[39]). It was agreed that it is generally more relevant to look at animals during their active phase and sometimes 100% of certain behaviours can be missed if animals are only monitored during the light phase. However, if there is some reason where animals cannot be monitored in the dark phase or if animals are being monitored in the light phase immediately after a procedure, placing animals in a playpen can be helpful. This is because animals are generally very active in the playpen even during the light phase, so lack of activity in a playpen can help to identify issues.

One final question that was raised by the participants was how the effects of ageing and cumulative welfare can be separated. On the one hand, it was felt that the ageing process is part of an animal's lifetime experiences and so its effects cannot be separated

from other aspects of cumulative welfare. On the other hand, it was considered important to compare amongst animals of the same age group to ensure that indicators which would not be accepted in a younger animal are not ignored simply because the animal is older. One participant also added that the beneficial effects of exercise for older animals are under-estimated and that these may help limit some of the negative effects of ageing.

The discussion session closed with a general agreement that of all the possible refinements and interventions presented over the course of the day, the use of heat maps to assess cumulative severity was the one that most participants wished to try and implement in their own establishments.

Action Points

- Ask how your establishment keeps up to speed with new developments in animal welfare science. Does the Named Information Officer (NIO) have the resources they need; how does the AWERB access information; are there any researchers who work in related fields?
- Recognise the importance of considering how an animal's cumulative experiences might affect that animal's response to further procedures or experiences. You may like to raise the issue at your establishment, e.g, via the AWERB.
- Consider whether an animal's prior or cumulative experiences may cause some procedures or projects to exceed their severity limit.
- Assessing the severity of procedures, consider:
  - how many clinical signs is the animal displaying?
  - how long has each sign been present?
  - how close is each sign to the humane endpoint?
  - what is the combined effect on the actual severity?
- Review colony 'all-cause' morbidity and mortality data to see if there are any indicators that animals may be experiencing poor cumulative welfare which can be addressed but take mortality very seriously and prioritise preventing this.
- Stereotypic behaviours and 'inactive-but-awake' behaviours indicate poor welfare and staff should keep an eye out for these indicators.
- Keep a lookout for signs of sensitisation, such as exaggerated response to a 'routine procedure' or depression, such as inactivity or no longer using enrichment, which may suggest an animal is no longer coping with life in the laboratory.
- Monitor group-housed male mice for signs of inter-male aggression and remember that aggression is frequently under-estimated. If aggressive male mice must be housed singly, provide individuals with extra bedding to help avoid cold stress.
- Try and find foods which your animals enjoy eating to help train animals to voluntarily ingest substances for experiments - or to use as treats.
- Include enrichment items which provide cognitive stimulation and allow animals to exercise.
- Try to incorporate low-stress handling into your interactions with laboratory rats and mice to improve human-animal interactions. Challenge assumptions that habituation and training (for both animals and humans) will take too long.
- Provide preferred forms of litter, nesting material and enrichment to your animals, such as shredded paper over corncob bedding for mice and a mix of nest-building materials, and hut-type shelters for rats. You can research preferences in the literature or you should be supported to do your own trials.
- Try giving rodents a dish or other demarcated area to use as a latrine to keep the cage clean and minimise how often animals have to be disturbed for cage change.
- If animals cannot be housed in larger, more enriched cages, repurpose old cages and toys to create a playpen and give your animals regular access to this.

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