Report of the 2022 RSPCA/UFAW Rodent Welfare Group Meeting

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Introduction

The RSPCA/UFAW Rodent Welfare Group has held a one-day meeting every autumn for the last 29 years, so that its members can discuss current welfare research, exchange views on welfare issues and share experiences of the implementation of the 3Rs for Replacement, Reduction and Refinement with respect to rodent use.

This meeting, held at Newcastle University in November 2022, was the first meeting that had taken place in-person after two years of online meetings due to the COVID-19 pandemic. It allowed participants the opportunity to engage in face-to-face discussions throughout the day and as part of a group discussion session at the end of the day. The talks covered topics relating to positive welfare for laboratory animals and ways to refine procedures. This report summarises the meeting and ends with a list of action points for readers to consider raising at their own establishments.

Positive welfare

The importance of providing captive animals with positive experiences to ensure a good standard of welfare has received increased attention in recent years, with recognition that to have a 'life worth living', animals need to have more positive experiences than negative ones. This means that those working with captive animals need to understand how to provide animals with positive experiences and how to assess whether animals are experiencing positive welfare.

Methods of inducing and assessing positive affective states and positive animal welfare in rats

Tayla Hammond, SRUC/University of Edinburgh

Play behaviour has previously been proposed as a potential indicator of positive welfare as it occurs when primary survival needs are met, is easily recognisable and readily seen in juvenile mammals and is associated with positive emotions.[1] There is also evidence that play is suppressed by negative experiences. However some authors have argued that there is insufficient evidence to draw clear conclusions about some aspects of the relationship between positive emotions and play.[2] This means there is a need to develop methods which can induce positive emotions in animals so that the effect of these positive emotions on play behaviour can be understood.

Rat tickling has been widely promoted as a way to induce positive emotions in rats[3] but this may not be the only way to play with a rat, as it focusses on three specific elements of play despite the fact that play in rats can incorporate many different types of behaviour. We developed a new method of play, which we termed 'playful handling' which incorporates more of these types of behaviour.

To assess whether our playful handling approach can induce positive emotions in rats, we measured the frequency of ultrasonic vocalisations (USVs) the rats produced. USVs are an objective tool for studying emotions in rats, as they are produced in different ranges according to whether rats are experiencing positive or negative emotions. We found that playful handling induced more positive USVs than control handling, both when individual rats were handled in an arena and when rats were handled in their home cage with their cage mate present. We also found that rats engaged in more solitary play behaviours before being playfully handled than before experiencing control handling, suggesting that playful handling can induce positive emotions and that this leads to more play behaviour.

We also wanted to see if we could find ways to induce positive states in our rats without relying on the interactions between humans and animals. Past research has explored how rats respond to hearing USVs, as these sounds are used for communication. Rats exposed to positive USVs in a radial arm maze tend to approach the speaker and show more exploratory behaviour while, those played negative USVs tend to reduce activity and show more freezing behaviour but it is not clear what the full behavioural response of rats would be outside of the radial arm maze paradigm. To better understand how rats respond to hearing positive USVs and whether this can improve their welfare, we conducted playback experiments in the home cage and measured the amount of USVs the rats produced as a result. We found that rats who were played recordings of positive USVs or white noise that was in a similar auditory range (50kHz) to positive USVs, produced more of their own positive USVs than when they were exposed to background noise. We also found that when rats were exposed to these stimuli over several days, the effect was maintained in rats played positive USVs but not those played white noise. Similarly, in our experiments with playful handling, we found that exposure to positive USVs also induced an increase in play behaviour before the exposure.

In conclusion, we found that both playful handling and exposure to positive USVs can induce positive emotions in rats and that these positive emotions lead to an increase in play behaviour. This suggests that play behaviour itself is an indicator of positive Animal Welfare. Finally this highlights the shared responsibility of all those involved in the care and use of experimental animals to provide their animals with positive experiences as a prerequisite for their use.

The use of ball pits and playpens in laboratory Lister Hooded male rats induces ultrasonic vocalisations indicating a more positive affective state and can reduce the welfare impacts of aversive procedures

Justyna Hinchcliffe, University of Bristol

It is well known that various factors in experimental animals can affect outcomes, such as strain, age, sex, development, social factors and affective state (Box 1). Several of these are affected by housing and husbandry. Given the current reproducibility crisis in preclinical research, there is a need to understand how we can improve research quality and reduce data variability.

> Affective state refers to the underlying emotional state of an animal. It can include both short-term emotional responses to specific positive or negative stimuli and longer, more diffuse moods.[4]

Box 1. Affective state.

Improving Animal Welfare, implementing the 3Rs and reducing cumulative suffering are all important goals to help achieve this. Inducing positive affective states in animals is an important component of welfare and may help mitigate the negative effects of housing, handling and habituation.

Refinements aimed at improving the affective states of laboratory rodents include pairing handling with rewards, tickling rats, providing enrichment and giving rodents access to ball pits and playpens – but how do we know these are working? Traditional welfare assessment approaches, such as behavioural observations or physiological changes have limited usefulness, as they may indicate arousal or motivation rather than affective state. We have therefore developed two methods to assess the affective state of rats: an affective bias test and the use of ultrasonic vocalisations (USVs).

Our affective bias test was translated from human work which has shown that patients with depression attribute less value to positive experiences. In our task, we trained rats to dig in two different but equally valued substrates. The rats experienced one substrate under control conditions and the other after experiencing some manipulation of their affective state (e.g. through a drug or environmental intervention). Each rat was then placed in a choice test to see which substrate they preferred. The rats' choices indicated which substrate they had associated with the better experience and we validated this data by testing rats after the use of antidepressant drugs or depressant drugs to ensure their choices were reflective of their mood ('affective state').

Our second method, the use of USVs, is based upon data that shows that rats vocalise differently depending on their affective state – USVs in the 50kHz range are associated with positive affective states, while those in the 22kHz range are associated with negative affective states (see Hammond). The integration of USV recordings with the affective bias test enables us to directly measure the relationship between call types and an animal's emotional experience. This means that rat USVs can provide a simple, quantifiable and graded measure of positive effect that accurately reflects the overall emotional state induced.

We used these methods to assess the efficacy of ball pits and playpens at improving rat welfare. Ball pits (Figure 1) and playpens (Figure 2) are designed to provide greater environmental complexity, which can improve welfare, give rats the space to express a wider behavioural repertoire, provide social enrichment and improve human-animal interactions.

We found that rats exposed to ball pits and playpens emitted significantly more 50 kHz USVs than those exposed to the control condition (an empty playpen), suggesting that access to these areas had a positive impact on rat welfare. We also found that this effect did not change over time, suggesting that the positive experience did not diminish with repeated exposures.

We also tested the effect of playpen access on rats who had been administered a drug to induce a negative affective state using our affective bias test. We found that access to the playpen on the same day as receiving a dose of the anxiogenic drug attenuated the drug's effects with rats that were put in the playpen for an hour showing a reduced negative state compared with rats that experienced the drug in their home cages. This shows how powerful playpens can be at improving welfare even when an animal is exposed to negative experiences.

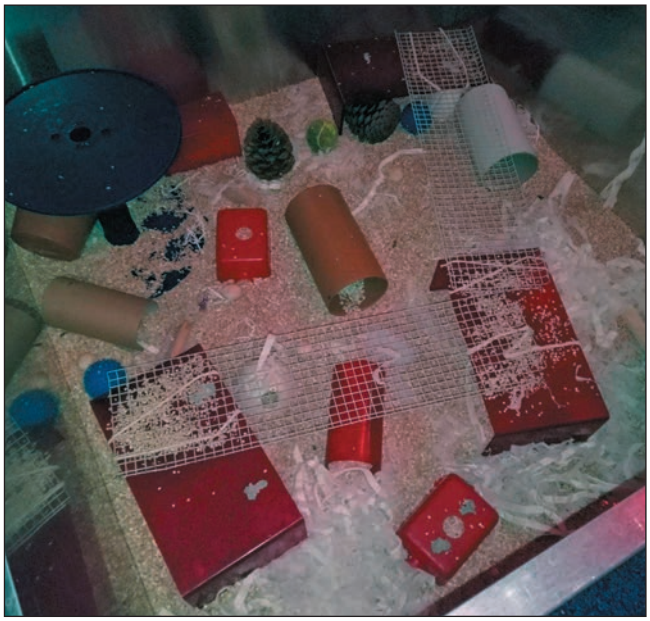

Figure 2. A rat playpen.

In summary, the '3Hs' of housing, handling and habituation can have a major impact on an animal's affective state. Refining the 3Hs can lead to better Animal Welfare which in turn improves data quality, reduces variability, increases reliability and translational value and, may be able to lead to a reduction in the number of animals used. There are opportunities to refine the experience of laboratory rodents within all these areas, including access to ball pits and playpens to improve housing, tickling to reduce stress associated with handling and restraint and, refinement of mild but repetitive procedures.

Figure 1. Rats in a ball pit.

The practicality and functionality of the rat playroom

Joanne Mains, University of Dundee

Our goal in this industry as Animal Technologists is to try and ensure that our animals have a fulfilled and enriched life regardless of the duration they remain in our care. This includes allowing the animals the ability to exhibit as many of their natural behaviours as possible. All the standardised cages available on the market today, regardless of how advanced or large they are, just do not permit a full range of natural behaviours in rats. A rat playroom is a way of providing rats with access to a space where they can explore, experience social enrichment and express a wider range of natural behaviours however issues such as cost, space and time can be restrictive. We have been lucky enough to overcome most of the constraints which are present in many other facilities to create a rat playroom.

The rat playroom came into being due to staffing changes which led to the implementation of new ideas within the unit. These ideas included floor pens for Guinea pigs, adding more enrichment for mouse and rat home cages, making home cages larger, tickling and clicker training, rehoming where possible, and eventually, the playroom.[5] (see also https://www.nc3rs.org.uk/tech3rs-issue-16-december-2022)

Our playroom is furnished with items designed to promote natural behaviours. We have used a wide variety of enrichment including red Perspex tubes, cardboard tubes, laboratory coat sleeves, old cages, igloos, bubble wrap, crepe paper and a plastic box filled with autoclaved soil, mealworms and sunflower seeds to encourage digging and foraging behaviour. We focussed on using items we already had in the unit or were free samples to keep the costs down, although we did have some small costs associated with buying a few additional items like cat toys, malt paste and mealworms.

Some facilities may struggle to find the spare space to create a playroom. In some cases where we had this issue, we instead created playpens – these are still bigger than the rats' home cages (although we use large cages as standard) and can be filled with enrichment to give rats access to different items from those in their home cages. We also noted that commercially bought playpens do not have much space for rats to explore vertically, so we created a climbing frame from an old cage rack, ropes, cable ties and some cage tops which we bent to create ramps and even some old uniform 'scrubs' bottoms!

We operate on the basis that stock rats, rats on long-term studies and those on non-behavioural studies can all go into the playroom. Our animals generally have access once a week for 45-60 minutes, usually on the day their cages are changed but no firm rota is in place. We have noted some male rats do not like being in the playroom after others, due to the scents left behind, so we give these males access earlier in the day. Cleaning is straightforward with the room only requiring a quick vacuuming and mopping which does not take more than 15 minutes.

We have found that the playroom is great for training new users and students, helping them to build confidence and improve handling skills. It is also a great site to introduce rats to each other before they are rehomed as the rats can get used to each other and to handling in neutral territory. The improvement in human-animal interactions is particularly noticeable with the Lister Hooded rats, who are more interested in human contact and playing even when exposed to novel enrichment whereas our Sprague-Dawley rats tend to prefer to explore and engage with the enrichment.

An additional benefit we have seen from the rat playroom is an improvement in staff morale. There are many emotionally challenging aspects to an Animal Technologists job and there is high potential for compassion fatigue. Since the introduction of the playroom we have seen many of our technologists (and some other staff) in the room playing with the rats during their break times. This can make it harder to say goodbye when the rats are rehomed but it is worth it to know that the rats have had lots of enrichment, human contact and positive experiences whilst in our facility.

Housing refinements to improve reproductive success in captive grey squirrels

Amanda Bulmer, FERA

The grey squirrel is native to North America but was introduced to the UK in the 19th Century. Since then, the UK grey squirrel population has grown to an estimated size of 2.5 million. This has contributed to the decline of the native red squirrel, which is thought to be due to several factors including grey squirrels being stronger and larger than reds and that grey squirrels can carry the squirrel pox virus (SQPV), which causes a serious infection in reds. As a result, research has focussed on ways to control grey squirrel populations, such as through fertility control.

Fertility control studies require reproductively active individuals of the target species. Unlike laboratory rats and mice, we cannot source proven breeders from a registered breeding facility, so we have been developing and maintaining a captive grey squirrel breeding colony with founder individuals sourced from the wild. We frequently review husbandry and care practices to promote high standards of welfare, which has led to refinements in three main areas: diet, housing and environmental enrichment. As well as increasing animal

welfare, reducing stress and promoting natural behaviours, these refinements have also had impacts on breeding success within the colony.

Grey squirrels are omnivorous and the main diet we provide is parrot mix, which contains nuts, seeds and dried fruit. We have a feeding plan in place to determine individual likes and dislikes and we do see the squirrels discarding items like chillies which are in the parrot mix. We further enrich the diet by providing fresh fruit and vegetables, particularly strawberries, carrots and oranges and sometimes make them fresh fruit kebabs. We add a range of food options on the ground or on platforms of different heights – these different feeding stations can also act as further enrichment and stimulation.

We have trialled multiple ways of housing our breeding animals. Originally, we housed squirrels in trios (1M:2Fs) which had some breeding success. We switched to mixed pairs the following year. This led to an initial drop in breeding success, possibly due to the squirrels taking time to settle to their new pens and mates but eventually resulted in an overall increase in breeding productivity, with less dominance behaviour seen. We also reviewed our practices to reduce the number of times a technician went into the pen to limit disturbances and decided to visit at the same time each day. This further improved breeding success among the colony.

More recently, we have trialled group housing by joining 4 pens together with tunnels (4M:4F). This has increased the competition between the males and increased the amount of breeding behaviour seen, resulting in greatly improved colony breeding performance. The use of tunnels allows the option to easily block pens off again if needed – for example, if we are doing a 'soft release' of a new squirrel – and we are trialling the use of a run-through microchip reader to give data on which squirrels are moving and where, and reduce the need for technicians to enter the pen if a squirrel has not been seen on CCTV that day.

We provide plenty of enrichment for our squirrels including trees, ropes, tyres, wood chews and wood chips on the floor of the pens. We also provide nest boxes. These are designed with a viewing hatch at the front and are modified so that the base can be pushed up to lift a squirrel into a trap if they need to be recaptured. We add materials like wool, moss, horsehair, leaves and pieces of turf for nesting and, also place bales of hay in the pens during the littering season when the squirrels like to forage for their own nesting materials. In cold weather, we often find cached pebbles in the nest boxes and have wondered whether these act like hot water bottles to help retain heat. We also try to encourage natural foraging behaviour by hiding treats around the pen or in milk bottles and have tried toys like a hay ball for horses which we stuffed with hay and monkey (peanuts in their shell) nuts.

2022 was our most successful breeding year so far. We hope that these refinements, particularly the introduction of group housing, will contribute to an even better breeding success in 2023.

Setting the standard; an AWERB's proactive approach to improving Animal Welfare

Claire Robinson, Newcastle University

In 2010 Professor Jane Hurst first published her paper on the negative impact of tail capture on laboratory mice and how tunnel handling or cupping can reduce stress levels during this routine experience.[6] Now, more than ten years on, low-stress handling has still not been fully adopted by the research community – so what are we doing at our establishments to make this the norm for our research animals? At Newcastle University, the Animal Welfare Ethical Review Body (AWERB) has taken a proactive approach to implementing Animal Welfare standards.

In 2019, low-stress handling was not standard across all sites at Newcastle University. To encourage uptake among technicians, we invited John Waters from the University of Liverpool to host a training workshop for technical team members. Moving forward, as some of the technicians who had attended the workshop became more involved in training new licence holders, they refused to teach anything other than low-stress handling methods to new staff.

The technical team then decided to take matters further and made a presentation to the AWERB to request a low-stress handling standard, during which they argued that low-stress handling should be a refinement adopted in licences unless there is sound justification not to. Key points raised included: that low-stress methods are essential to an establishment's Culture of Care; that handling for routine husbandry is probably the most common stressor that animals experience and so, the use of habituation and non-aversive handling would enormously refine the use of animals and that there were significant training benefits, as tunnel handling improves confidence and skills in handling mice and creates a more positive human-animal connection.

Despite the benefits listed by the technical team, some members of the AWERB wanted more evidence that low-stress handling is effective which led to a more extensive ethical debate. As a side note, we found this a useful opportunity to put the 'ethics' back into the AWERB and following this presentation the AWERB regularly has debates on topics like training and recruitment of researchers and staff, openness initiatives, colony management practices, the use of positive reinforcement training, administration using non-invasive methods and conditions of care and standards for non-regulated

animal work. Now, we have a standard low-stress handling technique which is registered across the entire university and significant justification is needed for an exemption.

Our experience with implementing this medthod led us to wonder how widespread adoption of this method is across the country. To assess this, we sent a training video and survey to attendees at a trainers' day, which was focussed on training and competency delivery methods, implementation of this way of handling and the consistency of approach at establishments.

A key finding from our survey was that only 67% said that low-stress handling was mandatory at their sites with 10% reporting that low-stress handling was not used at all. Some of the reasons given for this included that mice were being housed in isolators or in containment levels 3 and 4 – despite the fact that this way of handling can be used in these situations. We also asked participants about the length of time it would take staff to achieve handling skills to a local level, or the level shown in the training video and found a great deal of variation, with some reporting that it would take a day and others reporting that it would take two to three months.

What does all this mean? Our own experiences and our survey results raise a number of important questions – including why isn't low-stress handling the standard across the country? Why are teaching bodies not making it a standard? Why are we not all expecting the same minimum standard for a licence holder? What would be the response from the general public if they knew we were knowingly allowing establishments not to use low-stress handling? We have been able to implement this through a standard set by our AWERB – so those working in facilities without such a standard may wish to ask their AWERB why this is – and most importantly, remember the phrase 'just because we can, does not mean we should'.

Refining procedures

Towards humane deaths for laboratory mice: hypobaric hypoxia is a potential alternative to Carbon dioxide exposure

Jasmine Clarkson, University of Glasgow

Millions of mice are used annually for biomedical research. A prerequisite to their use in the UK is that they must be humanely killed upon completion of the scientific work. Approved killing methods under the Animals (Scientific Procedures) Act 1986 are assumed to be humane; however, there is debate over the appropriateness of some methods due to Animal Welfare concerns. For mice and other laboratory rodents, exposure to Carbon dioxide (CO_2) remains one of the most commonly-used methods but CO_2 exposure has the ability to induce anxiety, dyspnoea (shortness of breath) and pain at high concentrations.[7,8] There is also growing evidence that people responsible for killing laboratory rodents are susceptible to compassion fatigue.[9] Therefore finding a humane alternative to CO_2 is a key priority for the research community.

To better understand the use of CO_2 across the UK, we conducted a survey of UK establishments which received 219 responses.[10] CO_2 use was widespread with most respondents (78.5%) stating it was available at their establishment. However we were surprised to find that most respondents reported introducing CO_2 to the bottom of the chamber despite good-practice guidelines recommending top-filling to ensure a more even dissipation of gas throughout the chamber and. less than 20% used the recommended flow rate. We also found that many respondents were unaware of the fill method or flow rate used which was concerning as CO_2 can cause pain at higher concentrations and understanding any killing method is important for proper application. Most respondents ranked humaneness as the most important factor behind choosing CO_2 as a killing method, as well as its ability to be easy to use and provide a non-contact approach. However respondents also considered that minimal training was needed to use CO_2 – which was concerning given our findings regarding good practice not being followed. Overall, our results suggest that CO_2 continues to be widely used but is often employed incorrectly and currently there is a lack of knowledge and consistency surrounding its use across the UK.

As an alternative to CO_2, we are investigating the use of hypobaric hypoxia via gradual decompression for humane killing. Gradual decompression results in hypoxia (low oxygen levels) due to low pressure, thus simulating a process similar to ascending to high altitude. Hypoxia is insidious in humans with most people being unaware they are becoming hypoxic before they lose consciousness. So this may offer a higher-welfare alternative to the use of CO_2.

We demonstrated proof of principle using terminally anaesthetised mice by showing that hypobaric hypoxia can achieve 100% kill success in viable time frames and with minimal gross pathological damage. Faster rates of decompression did result in faster times to hypoxia and death but were associated with some middle ear congestion and haemorrhage, therefore slower changes in pressure are recommended for application in conscious mice.[11]

To explore the welfare impacts of this method, we looked at the behavioural responses of mice undergoing decompression and compared these to mice exposed to CO_2 and a sham treatment (placing mice in a chamber

for 6 minutes).[12] We also explored the additional use of pain relief (analgesic) and anti-anxiety drug treatments. Although gradual decompression took longer to kill the mice, they displayed a 'normal' behavioural repertoire, similar to sham-exposed mice (e.g. exploring, grooming, digging). We also found no difference in the number of mice performing behaviours that could reflect potentially negative sensations (e.g., head flicking or ear scratching) between mice undergoing gradual decompression compared to mice placed in the chamber alone (sham treatment). Analgesic and anti-anxiety drugs had no impact on the expression of 'normal' behaviours or behaviours that might have been associated with negative sensations, suggesting that the mice were unlikely to be showing or altering any behaviours due to pain or anxiety. We found that mice exposed to CO_2 gasped more frequently and made a greater number of escape attempts than those exposed to gradual decompression or the sham treatment. We also found that CO_2 exposed mice treated with analgesia gasped less, suggesting that gasping may be painful.

To support our interpretation of spontaneous behaviour, we are currently exploring electrical brain activity via electroencephalogram (EEG) recordings to independently define the conscious phase of concern during induction of decompression or exposure to CO_2. However our findings provide encouraging evidence that gradual decompression may offer a potential alternative to CO_2 with better welfare outcomes and therefore could offer a major refinement for the way that we kill millions of laboratory mice worldwide.

Mouse MApp – automated scoring of the mouse grimace and body condition scales

Matt Leach, Newcastle University

The use of mice in scientific research carries the risk of pain and distress due to scientific procedures, husbandry procedures such as ear notching and genetic modification. However there are moral, legal and scientific reasons to try and prevent pain and distress. As a result, we need to be able to accurately identify and assess pain and distress when they occur.

Two of the most commonly-used techniques that have been developed for assessing pain and distress in mice are the mouse grimace scale (MGS) – a three-point scale based on components of a mouse's facial expression[13] – and the body condition score (BCS), which involves palpating a mouse to see how fat or thin they are and assigning them a score (several different scoring scales are available e.g. Burkholder T. *et al.* (2012).[14] Each technique is well-validated and generally considered accurate, reliable and easy and quick to learn and implement. However there has not been widespread adoption, possibly because they involve manual scoring which may be perceived as too time-consuming and labour intensive for routine use and because of a belief that highly trained personnel are needed to implement them.

A proposed solution to this problem is to use machine-learning algorithms to automatically score pain and distress. We have embarked upon an NC3Rs CRACK IT Challenge to try and achieve this solution. The project had three aims: to develop an automated MGS system, to develop an automated BCS system and to integrate these systems into a multi-platform mobile application (app).

To meet the first aim, we developed a computer system that can accurately predict the intensity of a grimace score on a 0 (high confidence of no pain) to 10 (high confidence of severe pain) scale. This was based on an existing automated system which was trained on a database of around 6,000 images of white mice which had also been manually scored.[15] However the accuracy of this system is slightly lower than a human and only determines the presence or absence of pain rather than individual scores. To improve the accuracy and allow the system to be used on a range of coat colours, the updated system has so far been trained on over 10,000 images of white mice and over 3,000 dark coated (C57) mice.

To automate the BCS, we gave people access to a small mobile phone adapter that could produce an infra-red image, giving an accurate outline of the body. This image can then be compared to a body condition score on a 1-5 scale obtained by palpating that same mouse. Currently, we have more examples of mice that scored a 4 or 3, which is due to the fact that under the UK legislation, a mouse scoring a 2 or a 5 would probably have to be euthanised and a mouse scoring 1 would have to be euthanised rapidly. However so far, our system has an overall accuracy of 78.9% and the accuracy will increase as more images are used to train the system.

To meet our final aim, we are currently developing a mobile app that will integrate these systems. This app will give a simple user-defined pre-determined threshold for action using a traffic light system with green meaning no further action is required, yellow meaning that further action or monitoring is required and red, meaning that immediate action is required. The app will also be able to provide interested users with the detailed underlying data linked to the existing scales.

In summary, we are currently halfway through this project, and are focussing on training and validation of our systems using data provided by our sponsors. The accuracy and reliability of our systems are increasing constantly and we are on-course to deliver a functional system that has the potential for high impact around the world and will be able to help improve the welfare of all mice used in research.

Strategic approaches to ending severe suffering – how can Animal Technologists contribute?

Chloe Stevens, RSPCA

Around 3% of the 3 million animals used in research and testing in the UK each year experience 'severe' procedures. Procedures may be classed as 'severe' when animals used in science are likely to experience severe pain, suffering or distress or long-lasting moderate pain, suffering and distress or, severe impairment to their wellbeing or general condition. Severe suffering may be caused in models or studies of diseases or conditions that are severe in themselves, because of the cumulative impact of multiple less-severe factors or where animals die unexpectedly or, death is used as the endpoint of the study. Although all laboratory animal suffering is a concern and therefore reducing and avoiding severe suffering should be of top priority.

Since 2012, the RSPCA has been working collaboratively with the scientific community in the UK, European Union and internationally, to initiate and promote a range of activities aimed at identifying and promoting practical steps which will help researchers to reduce or, ideally avoid severe suffering. The project has two key objectives – the first is that we try and help establishments refine the models they are using to bring them to a lower severity limit where possible and the second is to ensure that there has been robust discussion and a clear rationale that justifies the need for severe models and procedures where they still exist.

One of the major resources that forms part of this project is the Roadmap to Reducing Severe Suffering. The 'Roadmap' is a practical exercise that can help you focus on procedures in your institution that could cause severe suffering, or you can apply its approach to reduce suffering within any severity category. It involves an audit of procedures, carried out by an appropriate team of people with different expertise and perspectives. All those involved in the care, regulation and use of animals in science have a part to play in helping to reduce and avoid suffering and such a team might include scientists, vets and Animal Technologists. Animal Technologists can often make particularly valuable contributions to this exercise, as they are likely to be heavily involved in the day-to-day care of animals and will have invaluable knowledge of the biology, behaviour and welfare needs of the animals. Technologists will also be familiar with conducting cage side welfare assessment and so are well-placed to identify opportunities for reducing suffering and understanding and alleviating harms to animals.

The best starting point is a collective agreement within an institution that ending severe suffering is desirable, possible and deserving of the necessary time and resources, followed by the setting of some clear objectives for what you would like the 'Roadmap' to achieve. This could be a particular model or procedure or it could be more ambitious – for example, reviewing all the severe work in the institution and aiming to reduce the number of animals experiencing severe procedures by a set amount. This can be viewed as part of the local Culture of Care.

The exercise consists of four stages: analysis which involves establishing the group, setting objectives and gathering background information; evaluation which examines the potential sources of severe suffering; identifying issues during which any mitigation strategies or refinements are implemented; and overcoming obstacles where you review your work and decide the next steps.

As mentioned above, severe suffering may be caused through the study of severe disease models, in cases where animal deaths occur or as a result of cumulative severity. In the case of severe suffering caused by cumulative severity, it is very important to think about the animal's lifetime experience. Animal Technologists are well-placed to identify and understand harms caused throughout the lifetime of the animal and to suggest and develop new refinements that can reduce those harms and further contribute to reducing severity. Some examples of relevant lifetime experiences are available on the focusonseveresuffering.co.uk website. On the website you can also find worksheets designed to help identify potential factors contributing to severity, consider the animal's experience, the potential welfare issues and possible ways to mitigate the effects.

The principle of 'marginal gains' that underlies the 'Roadmap' provides a feasible route to improving the welfare of animals that suffer severely and also those that experience moderate or even mild suffering. By involving a variety of participants and working towards reduced suffering, we can all contribute to a better life for laboratory animals.

Taking the adversity out of suffering

Claire Pearce, King's College London

Within animal research facilities, there will inevitably be experiments that lead to expected adverse effects but unexpected adverse effects can also occur. Often adverse effects result in animal suffering but should we accept suffering as an inevitable part of using animals? At King's College London (KCL), we have implemented a system of study plans to provide an opportunity for us to learn from individual experiments, reduce protocol severity and reduce suffering for the animals involved.

Study plans were initially introduced at KCL following the easing of the first COVID lockdown in July 2020. These include details of the project licence (PPL), the protocols to be carried out, the known adverse effects listed in

the PPL and the humane endpoints. The form initially consisted of a Word document, which would be completed by the project licence holder or personal licence holder, before being submitted to the interim facility director who would cross-check the plan against the PPL. More recently, we have moved to a new system, where the Named Animal Care and Welfare Officers (NACWOs) are responsible for checking and approving the plan and the competency of the individuals involved. Plans are widely accessible by all staff and further discussion of the plans can occur before they are approved, such as with the AWERB, the research community and with the interim director.

Before the implementation of study plans, NACWOs would be notified of unexpected deaths by researchers or staff and a standard condition 18 report would be submitted but it was not clear how far information about the lessons learned would spread through the department. Now with study plans in place, there have been benefits for both individual experiments and across the wider department. For example, listing the expected adverse effects in the plan allows unexpected adverse effects to be identified more easily – this then allows further discussion with all those involved in the work to see why the adverse effects have occurred, how they might be addressed and what needs to be done to allow the study to move forward. This has also enabled us to more effectively share the lessons learned from previous studies and share knowledge to positively impact future work.

The use of study plans has allowed us to reduce suffering in a range of different studies and procedures. For example, sepsis studies previously lasted for 48 hours after animals received a sub-lethal dose of lipopolysaccharide, resulting in moderate but prolonged suffering. After the study plan was reviewed, a discussion about the existing data and the scientific requirements took place which allowed the time course to be modified to 12 hours and a better monitoring regime was implemented to further reduce suffering. Similarly myocardial infarction studies previously had a severity limit of 'severe' and the protocol only allowed for recovery. During discussions of the study plan, the scientists and the Home Office Inspector found a series of early time points that could be investigated with a non-recovery model, which meant that severe endpoints could be avoided.

It was important to us to gather feedback from the facility management team on how the use of study plans has helped to avoid unnecessary suffering. Managers have reported that more detail is available on adverse effects which has led to improved monitoring regimes to ensure that adverse effects are avoided or picked up at early stages. Other feedback has mentioned that facility staff have better awareness of study start dates which allows monitoring to start when the study begins, that drug doses have been modified and that studies can be refined before they start.

In the short time that study plans have been in use, they have reduced animal suffering and highlighted non-compliance issues and the need for individuals to receive further training. As we move forward, we intend to develop an adverse effect repository so that other research groups can have awareness of adverse effects and potential refinements. It is important to us at KCL to continue to reduce suffering and we will continue to review PPLs alongside study plans to improve practice and refine the whole animal experience.

Action point

- Consider how to introduce positive experiences into laboratory animals' lives, rather than just reducing negative experiences – for example, can you introduce rat tickling or playful handling?
- Give rodents access to 'playrooms' or areas with enhanced levels of enrichment – and remember that playrooms do not need to cost lots of money to set up.
- If CO_2 is used for humane killing of rodents in your facility, check that good-practice guidance is being followed (e.g. for top-filling of chambers and flow rates).
- Ensure low-stress handling is the standard handling method used for rodents at your establishment if it is not already.
- Take the 'Roadmap' to an AWERB meeting for discussion or discuss it in project preplanning or wash-up meetings. Remember that it can be applied to all levels of severity.
- When thinking about severity, make sure you consider the full lifetime experience of an animal and the potential cumulative impacts of stressful or painful experiences or procedures.
- Consider the introduction of individual study plans if they are not already being used in your institution.

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