Report of the 2015 RSPCA/UFAW rodent and rabbit 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 21 years, so that its members can discuss current welfare research, exchange views on rodent 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.

A UFAW/RSPCA rabbit behaviour and welfare group with equivalent aims also used to meet annually, but this was disbanded due to a lack of ongoing research into laboratory rabbit welfare at that time. The last initiative by this group was the UFAW/RSPCA report on Refining Rabbit Care.[1] However, the Rodent Welfare Group decided to include rabbits within this year's meeting, which proved popular with delegates so the meetings will now cover both rabbits and rodents.

The 22nd meeting was held at Newcastle University on 23rd October 2015 and was attended by 75 delegates from universities and pharmaceutical companies throughout the UK. Presentation topics included rodent and rabbit biology and behaviour, refining laboratory rabbit housing and care, pain assessment in rabbits, olfactory effects on mouse welfare, questioning the necessity of sham operations, refining analgesia in mice, a review of refinement in Germany and reducing the use of ageing mice. The Home Office Animals in Science Regulation Unit also provided an update of its guidance and annual statistics on animal use. This report summarises the meeting and ends with a list of action points for readers to raise at their own establishments.

Rabbits and rodents: an introduction to the 'what' and the 'why'

Anne McBride, University of Southampton

The 'what' of this section is a very brief introduction to Small Prey Mammals (SPM) used in research, the rabbit and the rodents. The 'why' aims to increase our understanding of rodent and rabbit cognition (how they mentally process information acquired through the senses) and emotional states, thereby engendering a *critically* anthropomorphic attitude to them. This in turn can help improve both SPM welfare and the science that currently depends upon them.

Rodents and rabbits have been domesticated primarily in order to use their bodies for fur, meat and in laboratory experiments. Unlike more traditional 'companion' animals such as the domestic dog, these SPMs have not been strongly selected for their behaviour, other than tameness. There has been little fundamental change to their physiology or behaviour, as demonstrated by a viewing of the 'Ratlife' video (**ratlife.org**). In fact, it would be better to consider both rabbits and rodents as captive wild species when defining their housing, husbandry and care.

The rabbits and most of the rodents used in the laboratory originate from arid or semi-arid environments, areas with large temperature fluctuations including hot days and cold nights. They do not tolerate very high or low temperatures, nor damp conditions. They maintain a stable body temperature through behaviour, being most active between dusk and dawn and during the night, avoiding the heat of day in their wild habitats. This also means that their eyes are designed for low light levels.

All of the SPMs living in the wild inhabit complex worlds, where they encounter many different challenges and have to make a lot of decisions. Different species have different cognitive skills and some, especially mice and rats, are very adaptive and flexible, occupying a wide range of environmental niches – including those created by humans. Broadly speaking, all species have three main rules for life: (i) get enough to eat, (ii) do not get eaten and (iii) reproduce successfully. This section just addresses (i) and (ii).

Rule 1: get enough to eat

Rodents and rabbits naturally spend some 70% of their active time foraging and eating, i.e. 5 hours or more every day. Rabbits, degus, chinchillas and guinea-pigs are 'fibrevores', selecting a variety of herbs and grasses to provide them with a balanced diet and taste variation. They need to move around within large home ranges to find their food which takes time to chew and process. Other rodents, such as rats and mice, are omnivores. Their diet includes seeds, fruit, nuts, grasses and meat protein such as insects, grubs, birds' eggs and carcasses. This diet also requires time and movement to find and eat and also provides variety.

These diets and behaviours are very different from taking lab chow from a hopper, which is monotonous in comparison with natural foraging. However, food can be made more interesting for all rodents and rabbits by providing the diet in several locations to encourage foraging, e.g. scattering it amongst hay, litter or nesting material. Puzzle feeders are also a good idea, either commercial or homemade (e.g. search for 'bunny boredom busters' in YouTube), and it would be good to see some differently flavoured lab chows, providing a 'lab chow mix'.

Foraging and feeding in the wild can be energetically costly and risky, as you have to be out and about for much of your active time – meaning that you could end up being dinner, not the diner! This brings us to ...

Rule 2: do not get eaten

Wild rodents and rabbits are eaten by mammals, birds and snakes. They are attacked from the ground, the air and when underground in their burrows. As SPMs, they have highly acute senses of hearing and smell and a wide visual field, with their eyes located on the side of the head so that they can see behind and above them. When not foraging, they spend much of their time in hiding, for example in underground burrows, rock crevices and tunnels in tall grass (guinea-pigs). This does not equal inactivity, as life goes on below ground and homes need to be built and maintained. Burrows can be complex and surprisingly large; for example, a golden hamster burrows are naturally some 2 m long and 65 cm deep with several branching tunnels and chambers.

Another way to reduce the risk of predation is to live in groups. Most of the SPMs kept by humans are social, and group size in the wild is partly determined by species-specific behaviour and the availability of resources. A major advantage to group living is the presence of 'lookouts', meaning more time for eating, socialising, digging, playing, relaxing and grooming and a better quality of life. SPMs have emotional lives and experience pleasure (e.g. expressed by laughter in rats) as well as anxiety and fear. Social living involves learning from one another, caring (research has shown that mice and rats are capable of empathy) and, depending on the species, working together to find food, make and defend a home and protect young.

Effective communication is required for all of the above, but as SPMs live primarily in the dark and are heavily predated, their communication signals are very

subtle – and difficult or impossible for humans to detect or interpret. These include visual signals, auditory communication (much is ultrasonic, beyond our hearing range), touch and scent including pheromones. We humans need to work much harder at identifying, understanding and observing the (positive and negative) signals of each species, although some good research and initiatives are ongoing such as the Animal Welfare Indicators Network and Grimace Scales (see Leach and Roughan, this report).

What are the implications of Rules 1 and 2 above for life in captivity, welfare and the science? Behaviour and welfare will both be affected by poor physical health, inappropriate diet, environmental discomfort (e.g. uncomfortable temperature or surfaces) and constant isolation or constant unavoidable exposure to other animals in one's cage group or in other cages. All these factors are stressful and lead to anxiety, frustration and/or depression, which will negatively affect data across a range of disciplines; for example, think about the impact of stress on both the immune system and ability to learn.

How can we make this better? *Critical anthropomorphism* is the key – considering the sensory and cognitive capabilities of the animal, and striving to empathise with individuals, while recognising that they are <u>not</u> human. Here are just two examples:

Safe homes: SPMs must perceive their homes to be safe, so they will benefit from being kept in a quiet area and given a warning if there is about to be an 'earthquake', i.e. if their cage is going to be moved. Gently tapping the cage twice before removing it from the rack will become a classically associated cue very quickly and reduce stress. Refuges or nest boxes and shelves are very important for prey animals. They act both as bolt-holes and look out posts and there should be more than one available for group housed animals.

Empathetic handling: being lifted and restrained can be frightening and for prey animals the way in which they are held can be an innate fear stimulus. For example, the back and neck are kill bite areas, so being scruffed could be highly stressful. A recent study has shown that rats struggle, defaecate and vocalise less when a modified form of restraint is used that provides good support of the body while avoiding scruffing the neck area.[2] This is hardly surprising when you consider it from the rat's point of view. Giving rabbits a verbal cue (e.g. by saying "up") will perform the same 'warning' function and reduce handling stress.

So, for each species we should know and respect their physical needs, species-specific behaviour (including both activities and communications), sentience, cognition and emotions. For each individual animal, we would ideally be able to know their own history and personality (both of which may affect data), including preferred diet, enrichment items and cage/penmates, and normal wider environment i.e. their cage location, e.g. top or bottom of a rack. Historically, this was not the approach but the above concepts are now more widely recognised (although the high level of familiarity with individuals still may not be achievable if there is a large animal:carer ratio). Research has provided evidence that has widened the circle of species accepted as sentient, or able to experience a range of pleasant and unpleasant emotions and feel pain[3,4] – and to recognise emotional states in other animals;[5] these include the rodents and rabbits.

A critically anthropomorphic view of the individual animal is therefore not only scientifically valid but also an imperative in the laboratory. Critical anthropomorphism recognises the animal's sentience/emotions *and* empathically relates to the animal within the context of its 'animalness'.[6,7] Such an approach to management, handling and use can improve animal welfare. It will also improve the robustness of data, by enabling control for and consideration of the effects of individuality when choosing subjects, designing methodology and in data interpretation, especially that of outliers – which may itself led to further interesting research questions. To conclude, being SIA (Sentient Individual Aware) will improve animal welfare, make your job more rewarding and improve science – thereby improving the welfare of future human and animal generations.

Refining rabbit breeding in a Specific Pathogen Free (SPF) barrier

Rachael Birt, Envigo

Working within a Specific Pathogen Free (SPF) barrier provides certain challenges with respect to being able to provide good quality housing, including environmental enrichment, while maintaining an adequate level of biosecurity. We are working to find ways of overcoming these challenges and implementing new husbandry refinements for the rabbits in our care.

It is essential to keep SPF animals free from specific diseases, which means protecting them from contamination from the outside world. The SPF rabbit unit is therefore a closed colony with regular health screening to check and maintain the animals' SPF status. Any contamination entering the barrier unit would end the barrier and result in all the animals inside having to be humanely killed, which would be a waste of their lives and distressing for staff. Comprehensive biosecurity measures are in place, including requirements for staff to sign a declaration

that they have no rodents as pets and to shower in to a clean area where they stay all day. Equipment is sterilised using fume chambers, dunk tanks and autoclaves. We ensure that all staff understand the precautions and the reasons for them.

The stringent requirements for biosecurity can pose problems when selecting environmental enrichment, as not all items can survive the sterilisation processes. However, we are in the process of implementing a new enrichment plan (Figure 1) including hay briquettes, which have been successfully trialled at another site. We are also replacing our caging so that more compatible animals can be group housed.

Figure 1. Enrichment for breeding rabbits in an SPF barrier.

An important ethical requirement for us is to optimise breeding rates, matching supply with demand to minimise wastage. The saying "mate like bunnies" can certainly be said for our own colony, as the girls and boys are always willing to participate (!), but we also mate rabbits to accommodate specific requirements of our clients which can be more difficult. Recognising this, we have recently conducted a review of our mating procedures for the time-mated work we carry out for our customers, which will help us to further ensure that supply and demand are well matched.

Over the years we have made many changes to benefit the welfare of both our animals and our staff. These include not only refinements to housing and care but also better cleaning equipment and regimes and better documentation for breeding records and tracing animals. We believe that better staff welfare and good management systems, will lead to knock-on benefits for the animals in our care.

Housing preferences of laboratory rabbits

Jo Cruden, GlaxoSmithKline,
Jonathan Cooper, University of Lincoln,
Oliver Burman, University of Lincoln
and Greg Whelan, GlaxoSmithKline

At GlaxoSmithKline (GSK) we believe we have a moral responsibility to ensure good welfare and treatment of the animals in our care. This includes refining housing, husbandry and care and also evaluating any changes we are thinking of making to see whether and how much, animals will benefit from them. We have an ongoing programme to review rabbit housing, involving collaborations with external animal behaviour scientists to ensure that our evaluation studies are correctly conducted and interpreted. This section provides an overview of two projects, looking at floor pens and cage flooring respectively.

Floor pens

This study has been published previously in *Animal Technology and Welfare* April 2015.[8] Male Chinchilla rabbits were routinely housed individually in cages (70 x 85 cm) at GSK Stevenage, to prevent aggression, and animals were given with twice weekly access to floor pens measuring 2.5 m^2 for supplementary exercise. The decision was made to permanently move all of our male Chinchilla rabbits to floor pens (308 x 160 cm, 4.9 m^2) and the behaviour of four randomly selected individuals was monitored by video and analysed in order to compare the two housing systems and evaluate any effects on the welfare of the animals.

Analysis of activity, posture and position in the cage or pen every 15 minutes over a 24 hour period, taken from the video footage of the animals' behaviour indicated that rabbits were using the increased space in the floor pens. Important behaviours such as exploring, lying fully stretched and rearing all increased in the floor pen, while eating, grooming and sitting behaviours all decreased. The rabbits also spent more time interacting with the environmental enrichment in the floor pen and were more active during the dark phase, which is in keeping with rabbit behaviour in the wild. All of this suggests that the floor pens provided a number of benefits for rabbit behaviour and welfare, although the results of this particular study were not significant, possibly due to the small sample size.[8] Our rabbits are now routinely housed in floor pens at GSK wherever this is practical.

Cage base preferences

The rest of our rabbits are routinely housed in cages with perforated bases. This represents an improvement over 'old style' caging but a solid floor is generally regarded as the ideal, although there is little published work on the floor preferences of rabbits.[9]

In a second study we investigated preferences of New Zealand White (NZW) rabbits for solid floors with litter, which may be expected to be enriching for rabbits. Four NZW rabbits, two male and two female, were individually housed for the purpose of the study, with access to both a standard cage with a solid floor covered with 2cm of wood flakes (sawdust) and a cage with a perforated polycarbonate floor. The cages were connected by a tunnel (Figure 2). The time spent in each cage was recorded over 24 hours during both the light and dark phases (07:00 to 19:00 and 19:00 to 07:00 respectively).

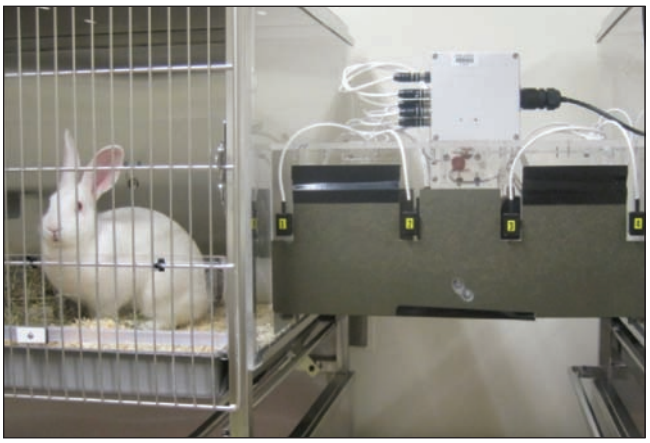


Figure 2. A preference test for different flooring materials. Legend: The rabbit had a free choice between the two test conditions and an LED array was used to record movement between the cages.

Rabbits spent more time on the perforated base in both light (70% of scans) and dark periods (69%), although this was only significant during the light phase (Paired t-test, p = 0.015). This result was unexpected, as it was assumed that rabbits would spend more time on the sawdust, because this should provide greater opportunity for environmental interaction. However, they may have found the texture underfoot aversive, or they may have avoided the sawdust because they used it as a latrine area. Furthermore, lower preference does not necessarily mean that they disliked the sawdust, as they may still value the limited time that they spent on the resource.[10,11] Interestingly, the rabbits also moved enrichment items from the perforated bases to the sawdust areas, indicating that they were making choices about the way in which they used the different areas but their motivation was unknown.

This was only a small study and we would need to use a larger population of rabbits to obtain a more definitive answer. We have planned a study using 12 male NZWs, which will also evaluate the animals' motivation for different flooring types by making them 'work' to access wood shavings, hay, sawdust or another, empty cage. The study was planned with animal behaviour scientists and will involve increasing the weight of the entry door in the connecting tunnel as shown in Figure 2. We are hoping to gain useful information that will help us to further refine rabbit housing and care. In the meantime, our studies so far suggest that it may be advisable to avoid having only deep sawdust in a floor pen or cage, as rabbits need to be able to make choices and may value the opportunity to avoid sawdust in part of the enclosure. Another important conclusion is that although rabbits may doze for a similar amount of time in a cage or pen, they have a better quality of life in an enriched floor pen.

Note: all animal studies were ethically reviewed and carried out in accordance with Lincoln University Ethical Review Procedure and the GSK Policy on the Care, Welfare and Treatment of Animals, which determined that the work did not constitute regulated procedures under the Animals (Scientific Procedures) Act, 1986 (ASPA).

Assessing post-neutering pain in rabbits using facial expression

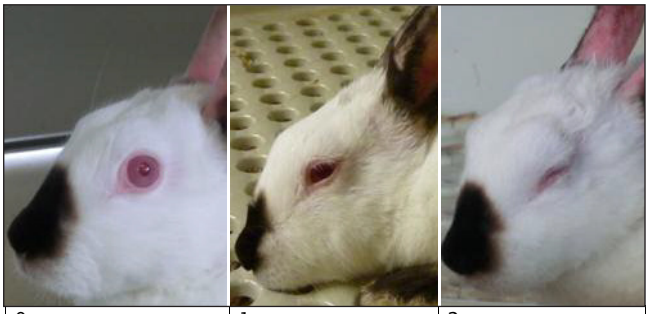
Matt Leach, Newcastle University

Rabbits are widely kept as pets (around 1.2 million in the UK alone), laboratory animals (11, 500 used in the UK annually) and farmed animals (some 326 million in the EU). Individuals will potentially undergo at least one painful procedure in their lifetime, e.g. neutering in pets, scientific procedures and tattoo marking for farmed rabbits.

The effective recognition and treatment of pain in rabbits is therefore critically important, as without adequate assessment techniques pain cannot be identified when it occurs, nor can the effectiveness of pain-relieving drugs be properly assessed – and a very large number of animals could be suffering avoidably. The work described in this section aimed to assess pain following castration, which is among the most common surgical procedures in both 'pet' and laboratory populations and is potentially painful unless appropriate analgesia is provided.

Facial expressions are widely used to assess pain in humans who cannot verbally express their pain to those caring for them and recent studies have explored the potential to use animals' facial expressions in a similar way. This approach was prompted by work which showed that human observers have a strong tendency to focus on animals' faces when assessing pain.[12] 'Grimace scales' have now been developed for mice (MGS),[13] rats (RGS),[14] rabbits (RbtGS)[15] and horses (HGS)[16]. In each of these, 'facial action units' (individual components that combine to make a facial expression) associated with the presence of pain are

compared and scored on a 3-point scale (Figure 3), then added together to give a total grimace sore.



0	1	2
Not present	Moderate	Obvious

Figure 3. A rabbit facial action unit: orbital tightening Credit: From the Rabbit Grimace Scale Manual by Dr M Leach, see www.nc3rs.org.uk/rabbit-grimace-scale. Orbital tightening is evidenced by a closing of the eyelid (narrowing of the orbital area) and a wrinkle may be visible around the eye.

Behavioural and facial expression-based indicators of pain in rabbits have been developed[15,17] but these indices have only been tested and validated for the assessment of pain in response to a limited number of potentially painful procedures (i.e. ovariohysterectomy and ear tattooing).

We aimed to determine (i) whether the existing behavioural and facial expression-based indices can be effectively used to assess pain following routine castration and (ii) the effectiveness of a Non-Steroidal Anti-Inflammatory Drug (NSAID) Meloxicam (0.2mg/kg), which is commonly used in rabbits, and of a more novel approach using a multi-modal regimen (0.6mg/kg Meloxicam with 0.5% Ligonocaine/ Bupivocaine local block) for pain prevention. These results have been submitted for publication in *PLoS ONE*, so a brief summary will be set out in this report.

Video sequences were analysed from 16 Dutch belted rabbits before and at a range of time points post-castration surgery. The footage was scored using the RbtGS and other validated pain behaviours including twitching, shuffling and wincing. Scorers were 'blinded' regarding the treatment each rabbit had received and the time point at which the animal was filmed.

The results showed that the behavioural and facial expression indices of pain significantly increased from pre-surgery to 1h and 5h post-surgery in both the NSAID and multimodal analgesia groups, before returning to baseline levels at 24 and 48 hours post-surgery. There was no difference in the behavioural or facial expression values between the two analgesic treatment groups at any of the time points tested.

These findings suggest that the existing behavioural and facial expression indices of pain in rabbits appear to generalise to the assessment of post-castration pain as well as ovariohysterectomy and ear tattooing. In addition, results indicated that the multimodal analgesia administered appeared to be no more effective at treating post-castration pain than the Meloxicam alone. Rabbits were displaying more behaviours and facial expressions associated with pain than would be desirable under both analgesic regimens, indicating that further studies are needed to develop more potent analgesic regimes for rabbits.

There were some limitations to the assessment methodologies, however. Behavioural assessments of pain can be time-consuming to develop and implement and are likely to differ between procedures. The RbtGS can lead to false positives if animals are not fully awake, so sedation and anaesthesia can have confounding effects. Further research is needed to evaluate the RbtGS before it can be applied clinically, which means that it cannot be used in isolation – although it can be used in an integrated assessment with other, validated indices.

Olfactory effects on mouse welfare

Noelia Lopez-Salesansky, Nur Hidayu Mazlan, Dominic Wells, Lucy Whitfield (Royal Veterinary College London); Cathy Fernandes (Institute of Psychiatry, Psychology & Neuroscience) and Charlotte Burn (Royal Veterinary College Hawkshead)

Mice rely heavily on olfaction, their sense of smell, in contrast to humans in which vision is the dominant sense. Olfaction is used to communicate with other mice through pheromones, for example the Major Urinary Proteins which occur in secretions such as urine. These provide information about each individual including genetic relationships, reproductive status or in the case of alarm pheromones, whether there is a danger in the environment. Mice are also born with the ability to detect kairomones, which are smells that are produced by and specific to, predators.

Mouse physiology and behaviour is therefore strongly influenced by the scents of other animals (both conspecifics and other species) and by chemicals found in the environment.[18-21] We wanted to evaluate whether common chemicals that mice may encounter in a laboratory setting may affect their welfare. For example, toluene is a widely used solvent that produced 'fear-like' responses in mice.[22]

A survey was sent to research institutions in the UK to enquire about husbandry practices that could have an impact on the olfactory environment of the mouse.[23] There were 80 usable responses originating from 51 different institutions. Most (70%) respondents reported always wearing gloves for handling mice, with Nitrile being the most common glove material (94%), followed by Latex (23%) and Vinyl (14%). Six different products were listed for cleaning surfaces and floors, and for sanitising anaesthesia and euthanasia chambers and behavioural apparatus between each mouse and at the end of the day.

In all cases Trigene™ (now called Anistel™) was the most common cleaning product used. This is available in apple, lavender, eucalyptus and citrus scents, which may have an effect on animal behaviour and welfare – and data quality but this has not been evaluated. A further potential source of confounds was the practice among 21 % of respondents of wiping behavioural chambers with water only between animals, whereas 4% rarely washed behavioural equipment at all. Euthanasia chambers were wiped with water only by 30 % of respondents and rarely washed by 8%, which could be a welfare issue if animals are distressed by traces of pheromones associated with fear or anxiety.

However, there was quite a low level of awareness of the potential for olfactory effects on behaviour, welfare or the science. Depending on the attribute considered, between 4 and 14% of the respondents thought that cleaning products definitely or were likely to, have strong effects on standardisation, mouse health, physiology or behaviour.

Following up on the results of the survey, we designed various behavioural experiments to determine the extent to which husbandry practices affected mouse behaviour. These experiments included evaluating the effect of different types of gloves and the impact of sanitising gloves with alcohol before handling mice. We have used behavioural tests such as choice chambers, open field tests and radial mazes to evaluate how mice respond to cleaning products and glove types and we are currently analysing our data.

Our survey revealed that the olfactory environment of the laboratory mouse is highly variable between institutions. Understanding whether and how, these smells affect mouse welfare will help to refine mouse husbandry and experimental procedures, enabling us to make practical recommendations to improve both the quality of life of laboratory animals and the experimental data obtained.

A critical review of applied refinement methods in Germany

Kathrin Herrmann, Free University Berlin and Paul Flecknell, Newcastle University

Mice and rats are frequently used in procedures involving recovery surgery, which can cause avoidable discomfort, pain and suffering if pain management protocols and humane endpoints are not appropriately refined. We carried out the first large-scale assessment of refinement within research applications involving recovery surgical procedures with rats and mice in Germany, to evaluate recent practices in anaesthesia, analgesia, post-operative monitoring, humane endpoints and killing methods. Over 500 applications were reviewed. The main results also have implications for research in the UK and can be used to inform best practice approaches more widely.

The most common surgical procedures in German applications to conduct regulated procedures on rats and mice are laparotomy and craniotomy (both species) and thoracotomy (mice). Injectable anaesthetics are most commonly proposed, as opposed to volatiles or a combination of the two. Our study suggested that perioperative analgesia could be improved for these surgeries. For example, intraoperative analgesics were not included for approximately 25% of mice and 28% of rats when isoflurane was used as an anaesthetic. In the cases where intraoperative analgesics were provided, the time of administration – an important welfare factor – varied greatly, with 13% of the mice and 18% of the rats only receiving pain relief at the end of surgery, which is too late. Timely pain relief is crucial in order to provide pre-emptive analgesia and to minimise side effects from avoidably high doses of anaesthetics.

Postoperative analgesic regimens were described for 67% of mice, compared with 71% of rats. In about 30% of cases, pain relief was stated as ‘not given’ and in 10%, pain relief was ‘given at the discretion of the researcher’. It was especially concerning to see that 19% of animals undergoing severe procedures apparently would not receive postoperative analgesia. As postoperative pain is to be expected after all levels of surgical intervention, considerably more justification is required for not providing pain relief as the default.

Humane endpoints were not specified in 57% of the research applications. The frequency of monitoring the animals’ wellbeing after surgery was indicated in only 33% of applications and in the majority of these cases the frequency was only once daily. When clinical score sheets were used (in only 13% of applications), only a small proportion (12%) included information about monitoring intervals. Critical times when extra monitoring and care should be given were rarely specified. The quality of the score sheets varied; for

example, important information concerning the animals, such as general potential negative effects of the procedure, were often not included and few gave clear instructions on how to intervene and treat and when to apply humane endpoints. There was no mention of recently developed and useful assessment methods such as nest-building, burrowing behaviour or grimace scales.

At the end of animals' lives, carbon dioxide is still widely used to kill rodents (about 28% of applications involving mice and about 40% for rats) although there is evidence for it causing distress, even when administered in a rising concentration. Ether, which is known to be inhumane, was proposed as a killing agent in 5% of applications.

In conclusion, the study showed a significant demand for greater awareness of the need for refinement in Germany with respect to all the techniques we assessed, especially regarding effective pain assessment methods. In fact, the majority of the proposals did not meet the legal requirements set out in Directive 2010/63/EU with respect to implementing the 3Rs. Although it is important to note that the proposals may not have included all the refinements that were actually implemented in practice, as set out they are the only materials available to use in the ethical review of the procedure and they may also be the only available material for animal technologists and the attending vet.

Much more information should be supplied on effective perioperative analgesia; comprehensive welfare assessment protocols and monitoring systems; and resources for adequate monitoring and care. This will also require more guidance and instructions with respect to essential information that should be provided in applications. There is also a need for better education about species-specific behaviour and refinement methods, to help applicants to better identify and describe potential refinements. Results of retrospective assessments, undertaken once projects have ended, should also be used to further refine procedures and reduce or avoid animal use, with replacement being the principal objective.

Are sham operations necessary?

John Atkinson, UCB Celltech

Chronic Kidney Disease (CKD) currently consumes around 5% of the NHS budget in the UK and is set to rise with increasing numbers of patients requiring renal replacement therapy. In addition to the financial burdens imposed, CKD can be severely debilitating, greatly reducing the quality of life for the affected patient. Most forms of CKD progress to End Stage Kidney Failure (ESKF) through progressive fibrosis of the organ. This fibrosis is characterised by an expansion of both the glomerular and tubular basement membranes, leading to glomerulosclerosis and tubulointerstitial fibrosis respectively. There is also increasing clinical evidence to identify CKD as an independent risk factor for coronary heart disease and cardiovascular events.

The two most commonly used animal 'models' of renal fibrosis are the subtotal nephrectomy (SNx) model and the unilateral ureteral obstruction (UUO) model. Both of these are surgical models which involve manipulating the kidney or ureter, e.g. the left ureter is tied off in the UUO model. Sham operations are used for control purposes, to control for the effect of surgery in itself on the animal. In the sham surgery, the animal is anaesthetised with isoflurane and given Buprenorphine and Carprofen to provide perioperative analgesia. One kidney is removed and re-inserted and the wound is closed.

However, there is no logical reason to expect that the act of surgery would cause renal fibrosis, nor has this been reported in any publications that I have seen. Sham surgery causes pain and distress to the animals, even with adequate anaesthesia and pain management, so we questioned whether this was really necessary.

We did this by making functional and histological comparisons between sham animals and non-operated animals and comparisons between these and the unaffected right kidney in the UUO model. For the SNx model, we found no significant difference between sham-operated animals and non-operated animals in terms of important readouts of function (serum creatinine, creatinine clearance, albuminuria, blood pressure) or histology (Masson's Trichrome or Sirius Red staining). In the UUO model we also found no difference in histology between sham-operated animals and non-operated animals.

We feel, therefore, that in the interests of animal welfare and the 3Rs, exposing animals to the stress of sham surgery when using either the SNx or UUO model is unnecessary.

Inflammation imaging and analgesic dose rate refinement in mice

Johnny Roughan, Henri Bertrand and Hannah Isles, Newcastle University

Recognising pain in mice presents some unique and difficult challenges. Although they are thought to be

able to 'hide' behavioural indicators of pain, whether they actually do this is unknown, and it could simply be the case that human observers are poor at recognising the relevant signs. In addition, the main causes of pain in mice are unclear, and they can respond very differently from other rodents to both painful stimuli and to analgesics. Nevertheless, given the very large number of mice undergoing potentially painful procedures as part of biomedical research applications, it is essential to try to increase our understanding of the mechanisms involved and how pain can be alleviated.

Inflammation is caused by increased production of COX-2* and is considered to be a major contributor to post-surgical pain, so anti-inflammatory drugs (NSAID analgesics) are commonly used to try to prevent it. One such drug, meloxicam, is effective in rats but not in mice unless very large (potentially toxic) doses are given. Several methods were used to try to detect pain following laparotomy including monitoring body weights, automated behaviour analysis (HomeCageScan; HCS) and the Mouse Grimace Scale (MGS). We attempted to determine whether the anti-inflammatory (COX-2 inhibitory) drug meloxicam would prevent changes to these parameters, so providing evidence of how effectively it relieved pain. This was the first study to use a probe to tag a fluorophore to available COX-2, which allowed imaging of the extent of the inflammatory response to surgery and a comparison of the pain-preventative versus anti-inflammatory effects of meloxicam.

Groups of 8 to 9 BALB/c mice were subcutaneously injected with saline (0.3ml) or meloxicam (at 1, 5 or 20mg/kg) 1 hour before undergoing a 1.5cm midline laparotomy under isoflurane anaesthesia. Either the COX-2 probe or a control dye (2mg/kg) was injected intravenously 3 hours following surgery and the mice were imaged 4 hours later. Behaviour data and MGS scores were respectively obtained from video recordings and photographs taken before and at 24 and 48 hours after surgery. The MGS scorers were completely naïve volunteers with no prior experience of laboratory animal pain assessment.

The intense inflammation seen in non-analgesic mice (Figure 4a) was almost completely prevented in those given meloxicam at 5 or 20mg/kg (Figure 4b). However, none of the meloxicam treatments appeared to prevent pain since all mice lost weight and became behaviourally less active and the mean MGS following surgery was increased in all groups for at least 24 hours. Post-surgical pain in mice may therefore have several causes; although inflammation is important, it is not the only consideration. Using multi-modal analgesia (perhaps combining an NSAID with an opioid) could be a more appropriate in preventing pain.

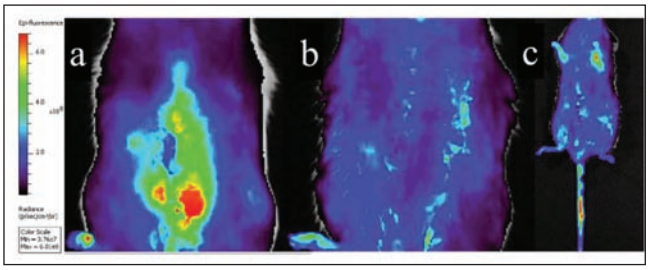


Figure 4a-c. Imaging of post-surgical COX-2 (inflammation) *in vivo* in mice. Credit: JV Roughan.[24] The results of COX-2 imaging in mice following laparotomy (Epi-fluorescent signals; photons/sec/cm^2). Mice underwent surgery following treatment with saline (a) or the non-steroidal anti-inflammatory agent Meloxicam at 5 or 20mg/kg (b). Note the comparatively severe inflammation caused by laparotomy (a), which was significantly reduced by analgesic treatment (b), but was not accompanied by significant pain relief. Panel c is a whole body image illustrating inflammation caused by tail (i/v) injection of the COX-2 probe and also inflammation on the elbows, paws and some flank regions.

A further potentially important finding was that inflammation occurred at regions remote from the surgery site. Tail damage was obviously due to the i/v injections but inflammation was also detected on the limbs and flank (Figure 4c).[24] What caused this is uncertain but some refinements to avoid unnecessary inflammation could include preparing animals for surgery even more carefully (e.g. when handling and shaving) and padding isoflurane induction chambers. Also, tail handled mice lost more weight following surgery. Thus, as shown by Hurst and West,[25] lifting mice by the tail may be generally aversive and should be avoided, especially if animals have recently received an i/v tail injection.

The MGS (also known as 'Pain Face' scoring) was found to be useful and could be applied by inexperienced staff. However, the average post-surgery MGS increase was only 0.35 points out of a possible 10. Forty five mice were needed to detect significance and with such a fine error margin normal mice could be scored as painful or a worse outcome could be false negatives in which pain would be missed. When scored 'live' at the cage-side, the MGS is highly variable within groups and between sexes and strains,[26] and even anaesthetising mice can provide false positives (Miller, pers. comm.). In this study we used the more usual approach of asking volunteers to blindly score from photographs but randomly compiling these took at least as long as is usually required for manual behaviour analysis.

*COX-2 is an enzyme which is produced in response to tissue injury and other diseases and infections. It converts arachidonic acid, which is released by damaged cells, to prostaglandins and thromboxane amongst other molecules that cause inflammation and give rise to pain.

Thus, contrary to the claim that the MGS is a rapid cage-side method surpassing other forms of manual behaviour analysis,[13] it is probably more appropriate for use within research projects aiming to evaluate responses to potentially painful procedures than for routine, cage-side pain assessment. If the MGS is used as part of routine assessment, outcomes in individual animals should be interpreted cautiously and regarded as only one component of a comprehensive welfare assessment protocol that includes other established indicators such as food/water consumption, body weight, activity, nest quality, coat condition, social interaction and demeanour when handled. If possible, manual scoring of pain-specific behaviours should be included, since these correlate with more sophisticated automated behaviour analyses.[27] Such a multi-faceted approach to welfare assessment is likely to be the only way to effectively detect poor welfare or pain so that appropriate action can be taken promptly.

ShARM – improving the welfare of and access to aged mouse models

Adele Duran, ShARM – Shared Ageing Research Models

ShARM is a not for profit organisation, funded by the Wellcome Trust. It was created to facilitate the sharing of tissues and information from aged mice in order to reduce animal numbers, improve the welfare of aged animals and accelerate research into ageing without creating the need for additional mice.

ShARM was initiated because people are living longer and also unfortunately spending more years in ill health. Research is urgently needed to gain a better understanding of the biology of ageing and to identify new interventions to manage the increases in age-related diseases. Mice are currently regarded as important 'models' of ageing, however, ageing animals need specialist care and the time and cost needed to rear ageing colonies can limit research outputs.

There are thus ethical, scientific and practical reasons to ensure that maximum research capacity is achieved from each aged mouse, without increasing the welfare impact on individual animals. To help achieve this, ShARM collects surplus tissues that are either flash frozen or formalin fixed and paraffin embedded. Tissues are stored in a biorepository, and information available online allows researchers to select appropriate samples to purchase (at cost) and use in their own studies. For bespoke collections, an online database provides details of living colonies which can be accessed by researchers upon request.

Since launching in July 2012 ShARM has attracted more than 200 members, collected over 20,000 tissues and has in excess of 1,000 mice registered in live ageing colonies. ShARM has supplied more than 500 tissues to investigators who have used data from these samples in publications such as *Aging Cell*[28] and to generate preliminary data for grant applications.

ShARM also provides MICEspace; an online, collaborative environment, for discussion and knowledge exchange on research subjects and animal welfare. Information on the 'top 10 welfare concerns' is available and forum topics currently include how to reduce fighting in males and excess scratching. MICEspace will facilitate the production of guidelines on good practice, aiming to ensuring high standards of welfare for aged animals.

By bringing together the collective resources, knowledge and experience of individuals, we can reduce the number of animals used in research as well as improving animal welfare. Please see **www.ShARMUK.org** for information about all of our services and to register your colony.

Update from the Animals in Science Regulation Unit

Giles Paiba, Home Office

The use of animals for scientific purposes is regulated under the Animals (Scientific Procedures) Act 1986 (ASPA). The Animals in Science Regulation Unit (ASRU) is responsible for overseeing the rigorous and proper regulation of this work, which it achieves through the provision of impartial licensing procedures and evidence-based advice and by encouraging the development and use of the 3Rs.

ASPA was amended in 2012 when EU Directive 2010/63/EU was transposed into UK legislation. Since then, ASRU has produced both *Guidance on the Operation of the ASPA*[29] and an updated *Code of Practice for the Care and Accommodation of Animals.*[30] ASRU also reviews and publishes its policies and guidance on the use of animals and issues advice notes on particular topics to supplement the Guidance and Code of Practice. All of these are freely available for download at **www.gov.uk/guidance/research-and-testing-using-animals**

The new Code of Practice was launched in December 2014 and includes 3 sections, each of which is stand-alone and colour coded. These are (i) minimum standards applicable now, (ii) minimum standards applicable from 2017 (Table 1) and (iii) additional advice. The third section aims to encourage establishments to promulgate high quality animal welfare and high quality science, which may go beyond the minimum requirements.

Rodents	Rabbits
Post-weaned mice and rats may be kept at higher densities for a short period, with provisos including a larger enclosure with adequate enrichment, and housing conditions that do not cause any behavioural or welfare deficits	Raised area must be provided; new standards for optimum dimensions for raised areas
Increased cage areas for mice, rats, gerbils, hamsters and guinea pigs	Some increased enclosure dimensions for larger animals
Part of the shelf area for guinea pigs may be included in the floor area, where there is adequate height above and below	Simplification in minimum cage sizes
	Part of the raised area may be included in the floor area for use animals

Table 1. Some examples of the changes within the Code of Practice (CoP) relating to rodents and rabbits which will come into force on 1st January 2017. This list is not exhaustive; it is important to carefully check the CoP.

The Code of Practice uses both 'engineering standards' (for parameters such as enclosure size, temperature range, photoperiod and perch length) and outcome-based 'performance standards' (e.g. '*noise levels, including ultrasound, shall not adversely affect animal welfare*'). The engineering standards are used because they set out clear expectations, providing a bottom line that can act as a 'welfare safety net' and compliance is easy to verify. However, performance standards are more appropriate for some parameters because every establishment (and experiment) is different and both science and our understanding of animal welfare needs are constantly advancing. For these reasons, it is not always possible to prescribe exactly how outcomes should be achieved.

Changes to the structure of the annual returns of procedures, including the recording of actual severity of procedures, have also come into force recently. The new annual statistics include downloadable data tables that can be used to explore procedures by species, purpose, genetic status and severity (see **www.gov.uk/government/statistics/statistics-of-scientific-procedures-on-living-animals-great-britain-2014**), with the intention of better informing stakeholders of the use of animals and guiding future policy. Table 2 lists a breakdown of the first actual severity data for rodents and rabbits and the online data tables also enable the severity data to be broken down into experimental procedures and the creation and breeding of genetically altered (GA) animals (Figure 5).

Severity	Mouse		Rat		Guinea pig		Other rodents		Rabbits	
	Number	%	Number	%	Number	%	Number	%	Number	%
Sub threshold	99,891	9	21,754	9	68	0	90	1	0	0
Non-recovery	81,903	7	27,111	12	17,678	65	115	2	2,375	17
Mild	488,596	42	112,754	48	3,999	15	1,982	31	9,598	69
Moderate	368,631	32	66,035	28	2,538	9	3,726	59	1,831	13
Severe	119,834	10	5,892	3	2,744	10	388	6	72	0.5
Total	**1,158,855**		**233,546**		**27,027**		**6,301**		**13,876**	

Table 2. Actual severity of procedures using rodents and rabbits in 2014. Legend: Source: Data tables in Home Office Annual Statistics of Scientific Procedures on Living Animals, Great Britain 2014. These are the data for experimental procedures only, excluding data for the creation and breeding of genetically altered animals.

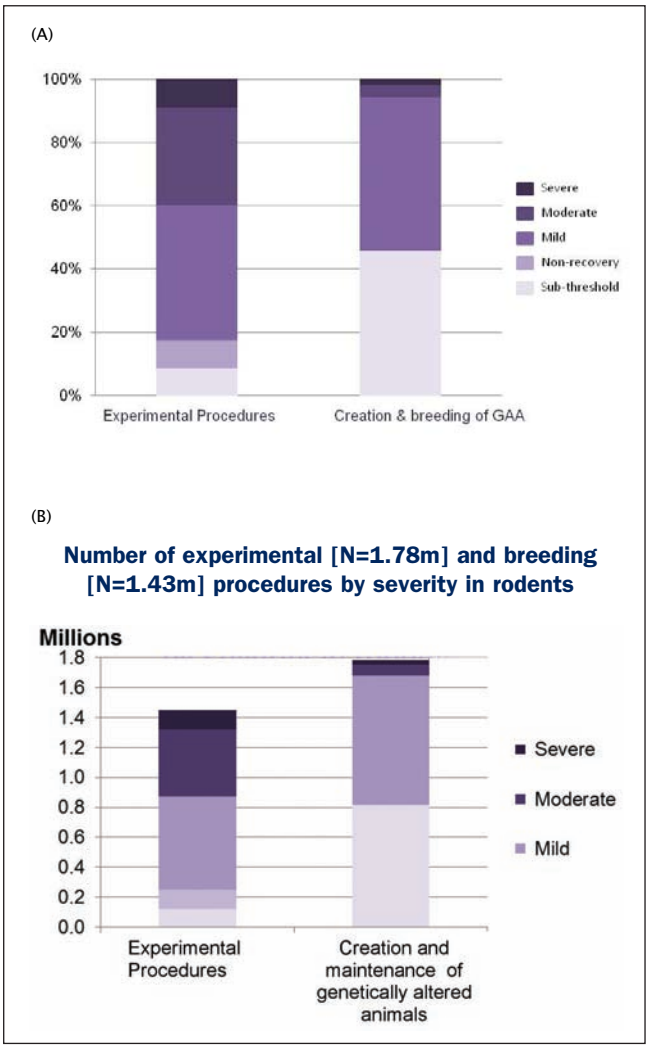


Figure 5. Severity of procedures by proportion (A) and numbers (B) of animals for rodents, broken down into (i) experimental procedures and (ii) the creation and breeding of genetically altered animals.

Other updates from ASRU related to some forthcoming Advice Notes on: Keeping Alive and Re-use; Re-homing and Setting Free; and Work with Wild Animals, all of which were expected to be published before the end of 2015. These will all be available at **www.gov.uk/guidance/research-and-testing-using-animals**

Finally, the Home Office is producing an outcome-focussed assessment tool on the Efficient Breeding of Genetically Altered Animals. This aims to help ASRU Inspectors to assess whether establishments are optimising their creation and maintenance of GA animals, minimising wastage and sharing and archiving lines. It is also intended to help establishments to self-assess their practice and stakeholders have been consulted during the development of the tool to help ensure that it is meaningful and user-friendly. The tool should be piloted early in 2016.

List of action points based on all of the presentations and discussions

- Think about how well you know the natural habitat, behaviour, biology and sensory and cognitive capabilities of the species you work with or care for. Would you like to learn more and what opportunities would you have to do so*?
- If there are gaps in the training on the above topics at your establishment, raise this with the AWERB or a Named Person such as the Named Training and Competency Officer.
- Consider whether you could suggest any refinements to housing, husbandry and care on the basis of the animals' natural environment, behaviour, senses or cognition, e.g. tapping the cage before removing it from the rack.
- If you work behind an SPF barrier, review the level and nature of enrichment provided for the animals. Could husbandry be further refined by thinking creatively and sharing experiences with other barrier units?
- If you work with rabbits housed in cages, explore whether your establishment could move to floor pens.
- Ensure that rabbits and rodents are able to exercise a degree of choice for different areas and surfaces, whether they are housed in cages or pens.
- Seek advice from animal behaviour scientists when devising and interpreting enrichment evaluation studies.
- Review pain assessment and management for rabbits and rodents post-surgery, to ensure that current knowledge and good practice are implemented.
- Use Grimace Scales for all species with caution; they can be potentially useful additions to a comprehensive welfare assessment protocol that uses several indicators but should not be used in isolation and may not be appropriate for all situations.
- Be aware of the potential for the scents of gloves and cleaning products to have an impact on laboratory mouse (and maybe other rodent and rabbit) behaviour and welfare and on the science.
- Check and review protocols for cleaning euthanasia chambers and behavioural testing apparatus between animals; the risk of animals smelling previous occupants should be minimised.
- Ensure that there is adequate discussion of pain management at the project planning stage within your establishment, e.g. via the AWERB or between the research team and Named Persons.
- If you currently conduct or are involved with, a project that uses sham operated animals, consider whether they add value to the experiment over non-

*_A Guide to the Behavior & Enrichment of Laboratory Rodents_ is a useful, free resource; **see http://www.criver.com/customer-service/resources/companion-guides#widgetTab2**

operated controls. If necessary, conduct a study to evaluate this and act on the results.
- If there is scientific justification for using sham animals, research the potential to use 'historical controls' which may allow you to only use one or two sham animals per experiment if the expected results are generally the same.
- If you sit on an Animal Welfare and Ethical Review Body (AWERB), ask for any requests for sham animals to be discussed and insist that adequate justification be provided.
- If your facility captures mice by the tail, ask for this to be reviewed on the basis that it is not only aversive, but can also cause more pain if mice have undergone surgery, and may be especially painful in those having received intravenous injections or who are more prone to inflammation.
- If your establishment uses aged mice, or their tissues, sign up to ShARM – and encourage other facilities to do the same.
- Be aware of forthcoming changes to the Code of Practice for the Care and Accommodation of Animals, recognising that although some may be improvements on the current standards, they will still be the minimum required and should be improved upon.

Look out for new Home Office Advice Notes and guidance in the areas listed above and give the Home Office feedback on these.

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