National Centre for the Replacement Refinement & Reduction of Animals in Research

Issue 6, February 2020

Tech3Rs

Providing animal technicians with the latest news from the NC3Rs

Welcome to the latest edition of Tech3Rs. In each issue, we share updates on recent advances in the 3Rs and highlight new resources, research and events.

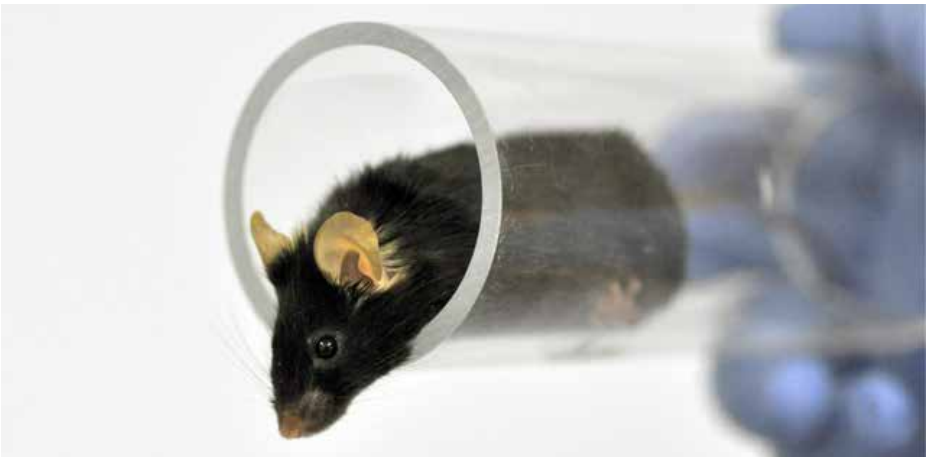
This newsletter is for animal technicians working in research establishments to help with identifying opportunities to embed the 3Rs in practice and ensure high standards of animal welfare. If you have any ideas for future issues or are working on a 3Rs approach you would like us to feature, please get in touch – we would love to hear from you! You can email us at **tech3rs@nc3rs.org.uk**.

In this issue we focus on mice and rats, which accounted for 77% of the procedures performed on animals in Great Britain in 2018. As well as our regular features, including 3Rs champions refining mouse and rat care in their facilities, we highlight new evidence supporting refined mouse handling.

Don't miss the next issue!

UK institutes can request free hard copies of Tech3Rs by visiting **www.nc3rs.org.uk/tech3rs**.

If your institute is based outside the UK, you can stay up-to-date on the latest issue of Tech3Rs and more via the NC3Rs e-newsletter. Visit **www.nc3rs.org.uk/register** to subscribe to our monthly updates.



More reasons to tunnel handle your mice

New research shows immobilisation and injection do not reverse the positive effects of tunnel handling mice.

Picking up mice by the tail is a major source of stress that induces strong aversion to the handler. In contrast, mice handled with a tunnel or cupped hands exhibit low stress and anxiety and actively seek interaction with their handlers.

It has been suggested that the stress associated with experimental procedures may outweigh the welfare benefits of refined handling. However, new NC3Rs-funded research shows that tunnel-handled mice continue to experience lower anxiety and assessment of threat compared to tail-handled mice even when subjected to repeated immobilisation and subcutaneous injection. This work, recently published in *Scientific Reports*, indicates that the method used to pick up mice in the first place has a greater influence on their welfare than experience of the experimental procedure itself.

The authors also found that brief handling, as required to transfer mice between cages (~2 seconds), is sufficient to familiarise mice with tunnel handling. On the other hand, tail-handled mice show strong aversion and anxiety even when handled only briefly, regardless of the frequency of handling. This work reaffirms that tunnel handling is a highly feasible method for minimising aversion and anxiety, as mice can quickly become familiarised with it in the course of standard cage cleaning protocols.

For support with implementing refined mouse handling methods, visit our 'How to pick up a mouse' hub at **www.nc3rs.org.uk/mousehandling**.

Gouveia K, Hurst JL (2019). Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports* 9: e20305. doi:10.1038/s41598-019-56860-7

3Rs champions

We would like to hear from you and help you share your ideas with others. In every issue of Tech3Rs we highlight animal technicians who are championing the 3Rs at their establishments.

Anna Morgunowicz and Chelsea Cavanagh, animal technologists at King's College London, share their approach for decreasing the number of spontaneous seizures in mice through early detection of affected animals and outbreeding.

What 3Rs idea have you developed?

In September 2017 all our mice (mostly genetically altered strains on a C57BL/6 background) were transferred from open top cages to IVCs. We noticed that this coincided with an apparent increase in the incidence of spontaneous seizures, which can involve jerks, shaking, convulsions and unconsciousness. Due to concerns about the welfare of animals experiencing seizures and the need to repeat experimental studies due to epileptic activity, we decided to investigate possible contributing factors.

How did you develop your idea?

With support from our NTCO, we conducted a study whereby every animal technologist in our unit helped to record the incidence and severity of epileptic fits over an eight-month period. Since the majority of seizures were observed when the cage was opened in a change station, we also recorded the levels of various ambient noises. Coat condition, an indicator of overall health in mice, was recorded as well.

Sound frequencies measured during our study were too low for the mice to hear, suggesting other factors might be causing seizures. The offspring of fitting mice also showed seizures, suggesting a genetic cause. By only selecting breeding stock that had not exhibited seizures in any consecutive generations, we eliminated 99% of seizure attacks throughout the unit.

What are your future plans?

We presented our findings at the 2019 IAT Congress, for which Chelsea won a First Time Presenter award, and we also published a paper in the IAT Journal. Our aim is to raise awareness of how spontaneous seizures impact animal welfare and can increase the number of animals used, and how outbreeding can help solve these problems. Training technicians and researchers to identify seizures early and adapt breeding regimes could lead to significant improvements in animal welfare.

Mouse experiencing a spontaneous seizure, observed by Anna and Chelsea.

Morgunowicz A *et al.* (2019). Increased incidence of spontaneous seizures in laboratory mice in an IVC environment. *Animal Technology and Welfare*, 18(3): 159-166. Available at journal.atwjournal.com/atwdecember2019#page=12.

Diane Fleary-Jones and Jim Hall are animal technicians at the University of Birmingham. Here they describe how refining peri-operative care for rats with implanted catheters has allowed group housing of the animals during subsequent experiments.

What 3Rs idea have you developed?

Rats implanted with catheters or ports in the dorsal aorta or jugular vein can experience large seromas (fluid build-up) around the implant following surgery, leading to them being single-housed for the entirety of the study. This is particularly true for certain thin-skinned strains, such as the Lister Hooded rats. We decided to take action to reduce seroma occurrence and the duration of single housing by refining our surgical technique and peri-operative care.

How did you develop your idea?

We took a team approach to developing a new protocol for catheter surgery. We temporarily single-house the rats for seven days post-surgery to allow their wounds to heal and implants to bed in. The rats can hear and smell each other during this period and are given additional environmental enrichment. We remove the food hoppers and instead provide food on the floor, as the catheters can occasionally be caught on the hopper as the rats walk past, which hinders the implant healing process. We've also introduced an enhanced peri-operative analgesia protocol to ensure pain is continuously managed; if the rats are in discomfort, they are more likely to interfere with their wounds. Aseptic technique, sterilising the implant with ethylene oxide, and considering the biocompatibility and shape of implant materials are equally crucial.

After seven days, healing is assessed, and the rats are regrouped along with extra enrichment. Small seromas may form around the dorsal port, but these often resolve by themselves without intervention and do not cause any distress to the animal.

What are your future plans?

As with any procedure, we try to make sure we offer the best animal welfare possible. We found that this protocol worked very well for us, but its success was due to combined effort and good communication between researchers, technicians, NACWOs, the NVS and Facility Director. Moving forward, we will continue our close communication network to ensure all new ideas to refine and improve procedures are explored.

Would you like to be featured in our next issue, or find out more about the refinements featured above? Please email tech3rs@nc3rs.org.uk.

3Rs papers of interest

Each issue we summarise recent 3Rs publications, providing links to the full articles for you to discuss with your colleagues (for example, in your next team meeting). This issue we focus on mouse husbandry.


King's College London

Brajon S *et al.* (2019). Social environment as a cause of litter loss in laboratory mouse: A behavioural study. *Applied Animal Behaviour Science* 218: 104827. doi:10.1016/j.applanim.2019.06.008

- Perinatal mortality, often manifested by the loss of the entire litter within the first days of life, can be a widespread issue for some facilities and some mouse strains (e.g. C57Bl/6).
- The underlying causes of perinatal mortality are poorly understood and it is often considered "normal".
- This study investigated the effect of social environment on parental behaviour and pup survival in C57BL/6 mice.
- Multiparous females (i.e. females that have given birth multiple times) were allocated to one of three groups: single housing, social housing in trios of two females and a male without the presence of another litter, or social housing with an older litter.
- The highest mortality rates were found in socially-housed mice with an older litter, where half of the younger litters were lost entirely.
- The presence of adult cage mates did not affect litter survival.
- The study concluded that being born in a cage where there is already an older litter is a major risk factor for litter loss, and strategies should be developed to avoid housing litters of different ages together.

Azkona G, Caballero JM (2019). Implementing strategies to reduce singly housed male mice. *Laboratory Animals* 53(5): 508–510. doi:10.1177/0023677219845028

- The maintenance of genetically altered mice or aggressive strains within the laboratory can sometimes lead to the single housing of males, which is known to have a negative impact on their welfare.
- The authors implemented two strategies in their facility to reduce the number of male mice being single-housed.
- The 'companion mouse' strategy for genetically modified mice involved keeping a 'companion' littermate of unwanted phenotype, to avoid social isolation of a single male with the genotype of interest.
- The 'post-weaning grouping' strategy consisted of incorporating the single male into a cage of males with less than a week's age difference between them.
- Both strategies proved to be suitable husbandry practices to reduce the number of single-housed males in a number of strains.
- The facility achieved a progressive reduction in single housing (42% in three years) without experiencing an increase in aggression.

Barabas AJ *et al.* (2019). Proteome characterization of used nesting material and potential protein sources from group housed male mice, *Mus musculus*. *Scientific Reports* 9: 17524. doi:10.1038/s41598-019-53903-x

- Mice rely heavily on scent cues to communicate social messages.
- It has been suggested that these odours are carried in their nesting material, which has led to an increasing number of recommendations to transfer used nests at cage cleaning to carry familiar odours over to the new cage and thus maintain a stable hierarchy.
- The authors performed a detailed analysis of the different proteins found in nesting material from cages of group-housed eight-week-old C57BL/6NCrl male mice in order to understand what specific odours are found in mouse nests.
- They detected a variety of proteins that primarily originate from saliva, urine and sweat from plantar glands in the sole of the foot. A large proportion belonged to the major urinary protein (MUP) and odorant binding protein (OBP) families, which send messages about individual identity.
- These data support current recommendations to preserve used nesting material at cage cleaning to maintain odour familiarity and thereby reduce aggression.

OPEN ACCESS

Highlights from our news and blog

The NC3Rs blog is a platform to talk about the research we champion and the issues we care about.

We have recently featured two guest blog posts that highlight the work being done by staff at the Universities of Glasgow and Dundee to promote a culture of care and improve the welfare of their animals.

Changing mouse handling practice at a university establishment: an NTCO's perspective

Lesley Gilmour, the University of Glasgow's NTCO, shares her experiences of implementing refined mouse handling across the institution and the challenges she overcame. Lesley shares her top tips for promoting uptake of the refined handling methods at your institution. Her blog post also features videos of mice before, during and after habituation to refined handling.

Read Lesley's inspiring blog post at www.nc3rs.org.uk/lesleygilmour.

Making refinements a reality – why we can be proud

Animal care staff at the University of Dundee's Medical School Resources Unit (MSRU) have put a number of refinements into practice over the past 18 months. These have included floor pens for guinea pigs, improved environmental enrichment for mice to reduce barbering and fighting, and better conditions for breeding rats. In this blog post, animal technician Joanne King discusses the changes and how they've made a difference.

Read more about the great work being done by staff at the MSRU at www.nc3rs.org.uk/MSRUrefinement.

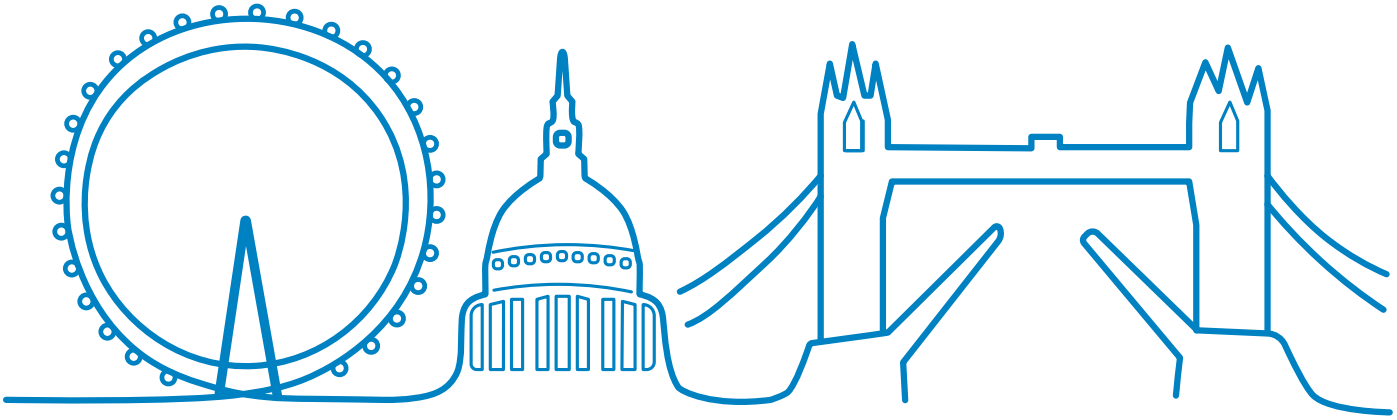
Even jumpy strains can become habituated to refined mouse handling methods.

Refining rat housing by connecting two cages with a standard red tube.

Old plastic water bottles can be recycled into mouse handling tunnels to cut down on costs and resources.

Repurposing a vacant room to create a floor pen for guinea pigs containing extra enrichment.

Upcoming events



Pan-London 3Rs Symposium
Wednesday 22 April, University College London

Co-organised by the NC3Rs, this symposium will bring together researchers and animal care staff from across London's world-class research institutions to showcase their 3Rs initiatives and share ideas to further implement the 3Rs.

The programme will cover a range of topics including how environmental enrichment can refine mouse cancer models, the development of new technologies that can reduce animal use and improve welfare, and the importance of good experimental design in developing new therapies. There will be a dedicated Technician Showcase Session in which technicians will share their 3Rs initiatives.

Submit a poster abstract: animal technicians and researchers are invited to present posters, with the opportunity to win one of two £100 prizes sponsored by the NC3Rs. Abstracts should focus on the 3Rs aspects of the work and be no longer than 300 words.

Please visit **www.bit.ly/3Rsposter** to submit an abstract for the poster session. The deadline to submit your abstract is 5pm on Thursday 12 March.

Visit london3rs2020.eventbrite.co.uk to register by Wednesday 8 April. Registration is open to employees and research students of London universities and research institutes.

Animal Science and Technology Conference (AST 2020)
Tuesday 24–Thursday 26 March, Edinburgh, UK

AST 2020 is a joint conference between the UK laboratory animal professional bodies LASA, LAVA and IAT. The NC3Rs is sponsoring a session on Wednesday 25 March, 2-4pm, entitled **New Technologies Which Advance Refinement and Science.**

This session will showcase new technologies developed by NC3Rs grant holders that are transforming the way animal research is done, bringing significant benefits to animal welfare and science. These include new tools that can analyse mouse ultrasonic vocalisations, train non-human primates in their home cage, and monitor individual behaviour or food intake in group-housed mice.

Our grant holders and staff will be available to answer your questions about the power of these technologies and the discoveries they are enabling, as well as funding opportunities for 3Rs research and development. Don't miss this opportunity to learn about the latest innovations in refinement first-hand.

Invited speakers:

- **Dr Sara Wells, MRC Harwell** – on the **Home Cage Analyser**, a sophisticated monitoring system that continuously records and analyses the behaviour of individual mice group-housed in their home cage.
- **Dr Liane Hobson, MRC Harwell** – on **AVERT**, an automated tool to record and analyse mouse ultrasonic vocalisations in order to detect welfare issues.
- **Dr Lawrence Moon, Research Devices** – on **Moshers**, a new method to accurately measure individual food intake and feeding behaviour in group-housed mice.
- **Dr Steve Kennerley, University College London** – on **Mymou**, a low-cost, home cage training system for non-human primates.

To learn more and register by Friday 6 March, visit www.ast2020.org.

Spotlight on mouse husbandry

We have updated our webpage on husbandry and housing for mice. Visit **www.nc3rs.org.co.uk/mice** to learn more about mouse behaviour, environmental enrichment and appropriate caging.

The photos on the right have kindly been provided by King's College London. If you have any photos you would like to share with us and help improve our resources, please email **enquiries@nc3rs.org.uk**.

And finally... playing hide-and seek with rats!

After learning how to drive tiny "ratmobiles"[1], rats have yet again shown how intelligent they are by learning how to play hide-and-seek with their handlers after just two weeks of training[2].

Researchers at the Humboldt University in Berlin taught rats how to both hide and seek and found that the animals fully engaged in this role-play and knew not to switch between roles when they were in the middle of a game. They also seemed to understand the rules of the game as they'd remain silent when hiding, whereas they'd "squeal with joy" when they discovered the hiding scientists.

The rats were rewarded with tickling by their handlers at the end of the game (see Tech3Rs issue 4 to learn more about the welfare benefits of rat tickling). However, the rats would often execute "joy jumps" and go hide again after been discovered, initiating a new round of hide-and-seek themselves. This could mean that they enjoyed the game itself, not just the tickling reward at the end.

To watch rats playing hide-and-seek visit www.bit.ly/hideandseekrats or scan the QR code below using your smartphone camera.

1. Crawford LE *et al.* (2020). Enriched environment exposure accelerates rodent driving skills. *Behavioural Brain Research* 378: e112309. doi:10.1016/j.bbr.2019.112309

2. Reinhold AS *et al.* (2019). Behavioral and neural correlates of hide-and-seek in rats. *Science* 365: 1180-1183. doi:10.1126/science.aax4705

NC3Rs
Gibbs Building
215 Euston Road
London NW1 2BE

T +44 (0)20 7611 2233
F +44 (0)20 7611 2260
enquiries@nc3rs.org.uk

www.nc3rs.org.uk
www.crackit.org.uk
@NC3Rs