

Providing animal technicians with the latest news from the NC3Rs

Tech3Rs

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 identify 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 share our new resource on colony management and introduce a new e-learning module on post-anaesthetic care. We also highlight research on non-aversive mouse handling, share webinar recordings you may have missed, and celebrate three years of our *3 Minute 3Rs* podcast.



Don't miss the next issue!

Tech3Rs is currently published online only – read all our past issues at www.nc3rs.org.uk/tech3rs.

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.



Colony management best practice

A new online resource to help researchers and animal care staff manage their animal colonies efficiently.

As animal care staff navigate through an ever-changing working reality as a result of COVID-19 lockdowns, being able to efficiently manage animal colonies to meet the dynamic needs of researchers is key.

Working with an expert group of researchers and colony managers, the NC3Rs has produced an online resource on breeding and colony management. This openly available tool is aimed at anyone working in animal research, trying to manage colonies during the COVID-19 pandemic. It offers guidance on specific post-lockdown scenarios (e.g. rapidly expanding a colony that was reduced during lockdown), as well as information on best practice in colony management (e.g. the importance of good record keeping).

Applying best practice to colony management avoids the creation of surplus animals, which reduces care

requirements and animal wastage. It also helps to maintain the integrity of strains (which is particularly important for scientific validity) and to prevent the loss of important genetically altered lines. Efficient colony management can also reduce costs, save space and increase facility resilience to unexpected events.

The amount of influence that technicians have over colony management practice can be variable and dependent on their facility. This variety is reflected in the resource, with valuable content for research and technical staff heavily involved in breeding and archiving, as well as those who want to learn more about the basics, such as common terminology or how to calculate breeding numbers.

To learn more about managing your colonies and breeding programmes effectively, visit the resource at www.nc3rs.org.uk/colonymanagement.

3Rs champions

We would like to help you share your ideas for putting the 3Rs into practice. In every issue of Tech3Rs we feature animal technicians who are championing the 3Rs at their establishments.

Carina Christoffersen is an Animal Welfare Technician at Ellegaard Göttingen Minipigs, Denmark. She spoke to us about her experience creating an enrichment room for pigs.

What 3Rs idea have you developed?

We raise barrier-bred minipigs and are always looking for ways to improve their environment and welfare. We provide straw for the minipigs to root around in as standard and also introduce toys for them to play with. However, I noticed that after around ten minutes the pigs would get bored and stop interacting with the toys. I was reading a lot of articles on enrichment and had the idea that we could combine lots of different enrichment items in a special playroom.

How did you develop your idea?

I spoke to Kirsten, our Head of Scientific Management. She thought it was a good idea and I was initially given two pens to turn into an enrichment room. I did a lot of research on what items would be appropriate for bringing into our barriers. Barriers are bio-secure units, so the enrichment items needed to be easy to clean and suitable for disinfection.

Despite our initial concern that novel items could potentially scare the minipigs, they actually love the playroom and really tire themselves out playing! The minipigs play in the enrichment room at least once a week and especially like the different flavoured chews. The small pigs love running through the tunnels. Play helps young pigs form stable social groups and since introducing the enrichment room we have seen less fighting. The enrichment room can easily be modified to fit the specific animals' interests: for example, older animals prefer different types of enrichment to young animals.

What are your future plans?

Since our initial playroom was such a success, we are now converting pens in other units into additional playrooms. We've found that the enrichment rooms can be used to introduce other novel



The enrichment room at Ellegaard Göttingen Minipigs, Denmark.

items, such as transport crates. This means the pigs are more confident around crates when it comes to being moved to a new facility. Kirsten and I have already presented our work at the Minipig Research Forum 2019 and hope to present it at other conferences or seminars in the future.

Patrick Mason is a Senior Technician/ NACWO at Cardiff University. Here he describes how he and his colleagues are tackling mouse aggression.

What 3Rs idea have you developed?

Aggression can be a problem in group-housed male laboratory mice, occasionally leading to serious injuries. Ideally, we want to avoid housing mice individually in response to episodes of aggression or injury, as this too has a negative welfare impact.

At our facility, we have been focusing on measures to reduce the need to singly house mice. The changes we have put in place are centred around general husbandry practices and the use of different types of enrichment for transgenic lines with a C57Bl/6 background (e.g. 3Tg and Thy1.1 mice).

How did you develop your idea?

A group of Cardiff technicians took part in the NC3Rs mouse aggression study (Lidster *et al.*, 2019), which motivated

us to think about what we could do differently with our male mice. We acted on NC3Rs recommendations, searched for further information, and monitored the changes we made.

When we make decisions about grouping mice together, we consider factors like age at weaning and whether they are littermates. Cage cleaning can be a trigger for aggressive behaviour in mice. To reduce fighting at clean-out, we scatter seeds, which seem to act as a distraction – but it's important to make sure there are plenty of seeds for everyone! We also spot-clean cages, where possible, and transfer used, scent-marked items with mice when they are moved to new cages.

In some cases, we have found cage dividers useful for creating respite areas for subordinate mice. Monitoring the introduction of enrichment devices, such as cage dividers, chew balls and swings, is particularly important as these can sometimes trigger aggressive behaviour.

What are your future plans?

I have learnt so much during my 16 years as an animal technician, but I am acutely aware that what we know about mice and rats is only the tip of the iceberg! My personal plan is to continue to develop my knowledge so that I can enhance the welfare of the animals I work with. As a NACWO I am in a great position to bring any new developments in welfare to my Director, fellow NACWOs and the research teams.

Lidster K, Owen K, Browne WJ, Prescott MJ (2019) Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project. *Scientific Reports* 9: 15211. doi:10.1038/s41598-019-51674-z

Would you like to be featured in our next issue, or learn more about the refinements featured above? Get in touch at tech3rs@nc3rs.org.uk.

3Rs papers of interest

Each issue we feature recent 3Rs publications, providing summaries and links to the full articles for further reading.

This issue we focus on new research on the welfare benefits of picking up mice using handling tunnels. For further details of these papers and more exploring non-aversive handling methods, download our summary table at www.nc3rs.org.uk/MHpapers.



Henderson LJ et al. (2020). Benefits of tunnel handling persist after repeated restraint, injection and anaesthesia. *Scientific Reports* 10: e14562. doi: 10.1038/s41598-020-71476-y

- There is a significant body of work that demonstrates the welfare benefits of non-aversive methods of picking up mice (i.e. using a tunnel or cupped hands).
- This study investigated whether repeated stressful procedures, such as injections and anaesthesia, undo the welfare benefits of non-aversive handling methods.
- The authors examined the effects of repeated restraint, repeated intraperitoneal injections and repeated short duration (5 min) isoflurane anaesthesia on BALB/c mice that were picked up either by their tail or using a handling tunnel.
- Tunnel-handled mice were significantly more interactive and exploratory than tail-handled mice, and less likely to defaecate during procedures. These positive behavioural effects persisted even after repeated aversive procedures.
- The findings show that tunnel handling provides the opportunity to improve the welfare of mice undergoing stressful procedures.
- This is the first study to demonstrate that tunnel handling can reduce the negative welfare effects associated with repeated isoflurane anaesthesia.

Clarkson JM et al. (2020). Negative mood affects the expression of negative but not positive emotions in mice. *Proceedings of the Royal Society B* 287(1933): 20201636. doi: 10.1098/rspb.2020.1636

- This study looked at how affective state (mood) can affect the way mice experience positive and negative events.
- Initially, the authors confirmed that tail handling leads to increased anxiety and depression (negative mood). In addition, larger adrenal glands were observed for the first time in tail-handled mice, indicating chronic stress.
- Therefore, tail and tunnel handling were used to create a negative and positive mood respectively in male C57BL/6 mice.
- The mice were accustomed to drinking sugar (sucrose) solution with either a low or high sugar concentration. As a negative event, the high sugar mice were provided with low sugar, and vice versa for a positive event.
- Both groups of mice were able to respond to the positive event regardless of handling method. However, tail-handled mice were more sensitive to the negative event (they showed more sustained disappointment and were less resilient) than tunnel-handled mice.
- This finding suggests tail-handled mice may be more severely affected by any aversive procedures they undergo in the laboratory.

Sensini F et al. (2020). The impact of handling technique and handling frequency on laboratory mouse welfare is sex-specific. *Scientific Reports* 10(1): 1-9. doi: 10.1038/s41598-020-74279-3

- The authors investigated the effect of handling technique and frequency on the stress response of male and female C57BL/6NCRl mice.
- The mice were tail or tunnel handled daily or weekly, and behavioural and physiological measures were used to quantify stress.
- Tunnel-handled mice showed increased voluntary interaction with their handler, and less defensive (negative) digging behaviour, compared to tail-handled mice.
- Burrowing activity was reduced in male, but not in female, mice after two weeks of daily tail handling.
- Daily handling seemed to have a positive habituation effect on female mice, reducing their stress levels compared to weekly handling. Frequent handling didn't seem to benefit male mice.
- This study replicates previous findings on the beneficial effect of non-aversive handling, while also highlighting sex-specific differences in handling associated stress responses.

Highlights from the

NC3Rs-led work reduces fish numbers in bioconcentration studies

Recommendations from an NC3Rs-industry project have been incorporated into guidance published by the US Environmental Protection Agency (EPA), potentially reducing fish use by one-third, around 240 animals per year.

Regulatory agencies require bioconcentration factor (BCF) studies to assess the potential for pollutants to accumulate in fish and enter the food chain. BCF studies have traditionally used multiple doses. However, a project led by the NC3Rs, in collaboration with scientists from Syngenta, BASF and Dow AgroSciences, found that for the majority of chemicals there is no value in testing at multiple concentrations.

The EPA has agreed to accept a one-concentration approach in certain circumstances. This paves the way for the NC3Rs to continue its work on widening regulatory acceptance of this approach elsewhere in the world.

Read the news story at www.nc3rs.org.uk/BCFReduction.



Refining DNA sampling through skin swabbing in model fish species

A 2016 survey conducted by the NC3Rs showed that 85% of the 89 worldwide zebrafish facilities that responded use fin clipping to collect DNA from live animals. However, fin clipping can negatively affect fish welfare.

New research, funded by the NC3Rs and published in *Scientific Reports*, suggests that skin swabbing provides a less invasive alternative for DNA collection. Swabbing triggered fewer behavioural and physiological changes in zebrafish, indicating that it could offer a refined way of genotyping in fish. The authors also observed that swabbing resulted in less data variability, which could decrease the number of fish required in experimental studies.

With an estimated 20,000 DNA sampling procedures occurring annually in the UK and over one million worldwide, this new data supporting skin swabbing as a refined DNA collection method could have a significant welfare impact in fish research.

Read the news story at www.nc3rs.org.uk/skinswabbing.



the NC3Rs website

New e-learning module on post-anaesthetic care (EU 21-6)

A new add-on to Research Animal Training's Advanced Anaesthesia e-learning module (EU 21) is now accessible via the NC3Rs website. Developed by FLAIRE Consultants with NC3Rs funding, Post-Anaesthetic Care (EU 21-6) guides users through the post-anaesthetic recovery process, providing information on factors that influence recovery speed, potential unexpected side effects of anaesthesia, and appropriate supportive care.

The learning outcomes focus on:

- How to ensure animals make a smooth and rapid recovery from anaesthesia.
- The consequences of anaesthesia in general, including how the choice of anaesthetic can affect recovery.
- How to avoid or manage potential problems that may arise.

Find this module and more at www.nc3rs.org.uk/elearning.



Protective cap for macaque cranial implants promotes wound healing

Neuroscience studies with macaque monkeys can require the animals to be fitted with cranial implants to stabilise the head or gain access to the brain for scientific purposes (e.g. electrophysiological recordings). However, wound management following implant surgery can be complicated due to the monkeys frequently picking at the sutured skin margins around the implant.

A collaboration between technical and research staff from the University of Oxford and Newcastle University has led to the development of a user-friendly head cap to promote wound healing. The protective cap restricts the monkey's fingers from accessing the wound margin while allowing air to circulate and promote healing.

The head cap was tested across the two UK facilities and was found to reduce wound opening and the need to re-suture surgical wounds. The study results have now been published in the *Journal of Neuroscience Methods*.

Read the news story at www.nc3rs.org.uk/macaquecap.



Catch up on our latest collaborative content

We work with a number of organisations to produce online resources. Here we highlight our most recent collaborations and how they can help you put the 3Rs into practice.



Celebrating the 3Rs across Europe

Europe is home to a strong community of organisations dedicated to replacing, refining and reducing the use of animals in research. A recent three-day virtual event saw six European 3Rs centres come together to highlight the latest 3Rs opportunities. Two speakers from each organisation presented scientific research on a range of subjects, including guidelines for marking and tagging fish, and pain management and assessment in mouse fracture models. Recordings of all of the talks are available to view individually, so you can pick and choose the topics that pique your interest.

View the webinar recordings at www.nc3rs.org.uk/europe3Rs.



Compassion fatigue: the cost of caring

In our last issue we highlighted how compassion fatigue can affect laboratory animal care staff and encouraged you to join Dr Megan LaFollette as she presented the latest research on this topic. The recording of this joint webinar from the NC3Rs and the North American 3Rs Collaborative (NA3RsC) webinar is now available online. As well as insight into the risk factors associated with compassion fatigue, the webinar provides advice on reducing the emotional toll on laboratory animal care staff.

Visit www.nc3rs.org.uk/webinars to view this and other NC3Rs webinar recordings, and visit the NA3RsC website to learn more about compassion fatigue: www.na3rsc.org/compassion-fatigue.



Happy third birthday to 3 Minute 3Rs!

For the past three years the NC3Rs has been working with *Lab Animal* and the North American 3Rs Collaborative on the 3 Minute 3Rs podcast. Every third Thursday of the month we provide a three-minute recap of the latest news and research in 3Rs science and technology. Recent topics have included managing aggression in group-housed mice and the impact of stress on zebrafish behaviour.

Visit www.nc3rs.org.uk/podcast to listen to all the past episodes, or search for "3 Minute 3Rs" in your podcast app of choice.