

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 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 take a look at our mouse aggression study and highlight recent publications in pig enrichment. We have also included a pull-out copy of our new poster on avoiding the reuse of needles – to order more copies, visit www.nc3rs.org.uk/needlereuse.



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.



Minimising mouse aggression

The results and recommendations from our study of aggression in group-housed male mice are now available.

Aggression in group-housed laboratory mice is a serious animal welfare issue. In 2017 the NC3Rs launched a data crowdsourcing study to determine the prevalence and triggers of cage aggression in male mice, and to provide an evidence base to inform and support best practice for minimising aggressive behaviour while avoiding the use of single housing. 143 animal technicians at 44 facilities participated, collecting data on aggression-related injuries over a one-month period from a total population of 137,580 mice. UK technicians received 10 IAT CPD credits for their involvement.

The results and recommendations from the study are now published in *Scientific Reports*. The mean prevalence of aggression across facilities and strains was 15 in 1,000 mice. Based on the study findings, we make the following

recommendations for preventing or reducing aggression:

- Consider whether strains exhibiting a low prevalence of aggression (e.g. 129S, C57BL/6, BALB/c) could be used in studies.
- Spot clean dirty bedding as needed, rather than changing the full cage weekly or fortnightly.
- Transfer clean and dry nesting material during cage change.
- Establish stable groups with littermates wherever possible.
- Discuss with suppliers the steps they could take to reduce aggression.

We'd like to say a big thank you to all the technicians who provided data for this study. For further information, please read the full paper.

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](https://doi.org/10.1038/s41598-019-51674-z).

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.

Emma Filby is Senior and Surgery Technician at the University of Cambridge. Here Emma describes how she has used an automated system to reduce how frequently mouse cage bedding is changed without compromising cleanliness.

What 3Rs idea have you developed?

Our mouse unit is equipped with a digital rack system from Techniplast, which is fitted with sensors that can collect information on bedding conditions and animal activity from individual cages. We decided to use this system to automatically identify cages requiring a change of bedding, instead of routinely changing all cages at the same time. To do this, we had to "teach" the system what constituted a dirty cage based on parameters like air quality, what proportion of the bedding was wet, and whether the nest was free of faeces.

Switching to this automated system has dramatically decreased the frequency of cage changing without compromising cleanliness. We were surprised to find that cages containing one pair of mice continued to look clean and dry for up to six weeks. Ammonia levels also remained low (0-1 ppm).

How did you develop your idea?

This idea came from the need to decrease animal stress within the unit. Gerdin *et al.* (2012) compared the effect of tail tip blood sampling and cage change on heart rate, a measure of acute stress. Although blood collection involved nicking the tail of the mouse, simple cage change increased heart rate for a longer period, suggesting that it too can be a stressful experience. Allowing the mice to be exposed to olfactory cues by reducing the frequency of cage changing has notably decreased their stress levels. They perform more complex, naturalistic behaviours such as building high-quality nests, which is an indicator of improved welfare. This approach has financial and environmental benefits as well – we've seen a reduction in money spent on consumables and a decrease in waste.

What are your future plans?

The next step is to test the cages for bacterial contamination, in collaboration with Charles River. We plan to take routine bioburden samples to ensure bacterial contamination does not exceed safety levels, even when cages appear clean.

Gerdin AK *et al.* (2012). Experimental and husbandry procedures as potential modifiers of the results of phenotyping tests. *Physiology and Behaviour* 106-20(5): 602–611.

[doi:10.1016/j.physbeh.2012.03.026](https://doi.org/10.1016/j.physbeh.2012.03.026).

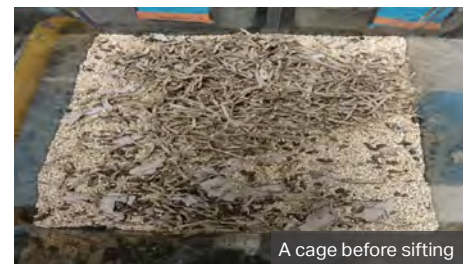
Seonagh Henderson is an animal technician and NACWO at the University of Glasgow. Here Seonagh shares a new cage change method developed to help rats settle back into their cage faster after cleaning.

What 3Rs idea have you developed?

I've developed a "sifting" cage change method for rats which maintains more of their scent in the cage, whilst allowing the removal of faeces and soiled nesting material. This new method has had a noticeable positive effect on the behaviour of the rats and how quickly they settle into their "clean" cage. The rats are far less jumpy and much calmer, and as a result both staff and researchers feel more confident handling them.

How did you develop your idea?

After seeing how skittish and stressed the rats in my unit were, I wanted to do something to improve how they interacted with my colleagues and me. Normally when a cage is changed, a small amount of dirty bedding is put into the clean cage to retain olfactory cues for the animals; however, I felt this was not enough. Initially, I trialled a cat litter scoop but this could not remove all the dirt from the cage bedding. I then tried a sieve pan, which allowed me to sift all of the dirty bedding to remove faeces, chewed cardboard and dirty nesting material, without removing bedding impregnated with the rats' scent. This proved much more effective.



What are your future plans?

I recently presented this work at our University's 3Rs symposium, where it was awarded the animal care staff prize. After the meeting, I received a lot of positive feedback from other technicians and research staff. I hope to encourage other units and institutes to try this method. It can be adopted easily, and the cost is minimal. In fact, this new "sifting" method helps to save on consumables and is good for the environment. However, the biggest and most noticeable impact is on the rats themselves. They are so calm and naturally inquisitive, instead of hiding for cover whenever I am in the room.

Want to be featured in our next issue, or nominate someone else championing the 3Rs at their facility? We would love to hear from you! Tell us all about it by emailing 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 environmental enrichment for pigs – in particular, what type of enrichment pigs prefer, how to keep them engaged for longer, and how to identify a positive response to enrichment objects. The studies used large, farm pigs but the findings are likely to be relevant to laboratory pigs, including minipigs.



Beaudoin J et al. (2019). Growing pigs' interest in enrichment objects with different characteristics and cleanliness. *Animals* 9(3): 85. doi:10.3390/ani9030085.

- Rearing spaces for pigs should include environmental enrichment to allow them to fully express natural behaviours, such as rooting and chewing, and also deter abnormal or injurious behaviours (e.g. tail biting).
- Choosing the most suitable enrichment will satisfy the pigs' needs and interests and keep them engaged for longer.
- This study explored how the characteristics of an object, such as its malleability and cleanliness, may affect its attractiveness to pigs.
- The authors tested how interested pigs were in eight different objects by measuring how often they interacted with them and for how long. The objects included suspended rubber sticks, fixed polyurethane balls, and a dried wooden beam.
- The pigs interacted more with the most chewable objects, specifically the wood and the polyurethane balls, and showed no preference for clean over dirty objects.
- Therefore, destructible and malleable objects seem to provide the best enrichment material for pigs, without requiring regular cleaning and maintenance to retain their interest.

Blackie N, de Sousa M (2019). The use of garlic oil for olfactory enrichment increases the use of ropes in weaned pigs. *Animals* 9(4): 148. doi:10.3390/ani9040148.

- Pigs tend to rapidly lose interest in standard enrichment objects.
- This study looked at the efficacy of an enrichment object with both olfactory and tactile characteristics: a garlic-scented rope.
- The authors investigated whether garlic, with its strong olfactory properties, could delay a pig's loss of interest in a rope and maintain its novelty for longer.
- The authors compared weaner pigs' interactions with a garlic-scented rope *versus* an unscented rope and found that the pigs interacted with the garlic-scented rope for significantly longer periods, demonstrating a strong preference for olfactory enrichment.
- The pigs did eventually start to lose interest in the garlic-scented rope, but re-spraying the rope with garlic oil renewed their level of interaction.
- This work shows how providing a combination of olfactory and tactile enrichments can positively impact pig welfare by encouraging them to interact with standard enrichment objects for longer periods.

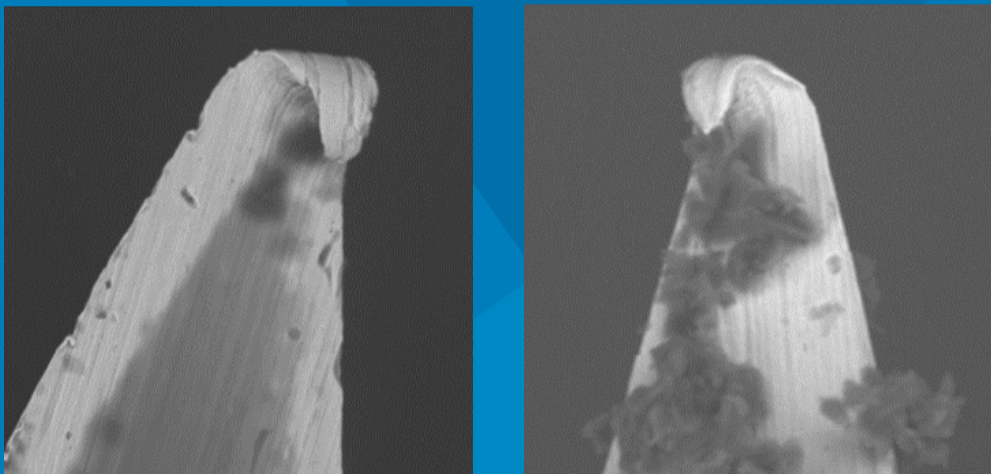
Marcet-Rius M et al. (2019) Are tail and ear movements indicators of emotions in tail-docked pigs in response to environmental enrichment? *Animals* 9(7): 449. doi: 10.3390/ani9070449.

- Currently, assessment of farm animal welfare is mainly based on physical health markers. There are no clear emotional markers that can be used to assess the welfare of farm animals.
- In this study, tail and ear movements of pigs were assessed as potential indicators of positive and negative emotions respectively.
- The frequency and duration of tail and ear movements were recorded for each pig, in the presence and absence of environmental enrichment, such as straw in a rack, wooden logs or metal chains.
- Pigs moved their tails for longer when they interacted with the enrichment.
- When enrichment was not present, ear movement did not increase.
- Increased tail movement was therefore found to be a valid and feasible indicator of positive emotions in pigs.
- However, more research is required to identify whether ear movement could be used as an indicator of negative emotions.

Why use needles only once?

Single-use needles are designed to be used once. If used again, there is a risk that the needle will dull and cause the animals pain.

You should avoid reusing them and single use should be your standard practice.



Electron micrograph images of 27G needles (1000x magnification) after injection into mice demonstrating the potential for dulling of the needle and contamination when reused.

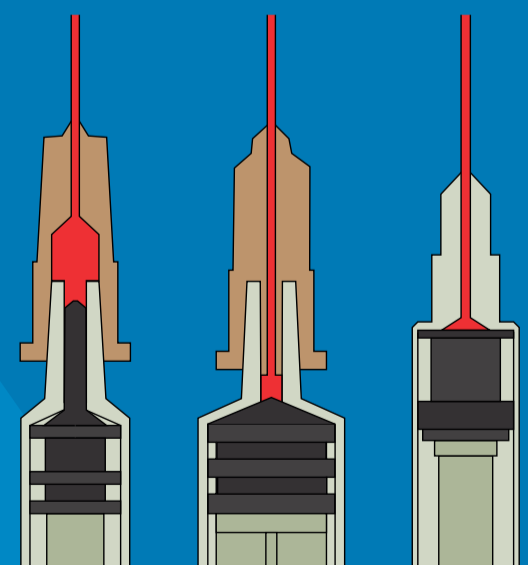
Source: AstraZeneca

The risks of reusing needles include:

- Causing unnecessary pain and suffering as well as tissue damage.
- Transferring tissue products between your animals, which could compromise your science.
- Spreading infection between your animals – even low-level infections can have an impact on your animals and therefore your science.

Practical tips:

- If your injectable substance is in limited supply, low dead-space single-use needles (commonly used in human blood sampling) can be used. Your local vet can advise on suppliers.
- If there is a justifiable scientific or practical reason why single needle use is not feasible (e.g. critical shortage of your injectable substance), check your institution's approval policy and consult your ethical committee before proceeding.



Examples of commercially available low dead-space single-use needles.

More information on single-use needles: www.nc3rs.org.uk/needlereuse

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. Recently we hosted a symposium for animal technicians in collaboration with the Institute of Animal Technology.

This year's Animal Technicians' Symposium was held in October and welcomed over 150 technicians from over 40 research organisations in the UK and abroad. The event featured talks on the latest refinement opportunities in animal research.

Speakers included Ms Marie Eriksson from Research Institutes of Sweden (RISE), Ms Megan LaFollette of Purdue University, USA and Dr Lynne Sneddon of the University of Liverpool, who all shared their expertise in improving animal welfare in the laboratory. During these three highly visual and engaging presentations, we heard about training techniques that can reduce stress in laboratory rodents, the welfare benefits of rat tickling and how zebrafish welfare can be improved through enrichment, behavioural assessment and analgesia.

The Symposium also focused on the fundamental role animal care staff can play in the refinement of

animal procedures and use, as well as highlighting their commitment to putting the 3Rs into everyday practice. 16 technicians from 13 institutions were given the opportunity to showcase their innovative 3Rs ideas during a flash poster session. The quality of the research presented this year was impressive, encompassing a broad range of laboratory animal species from mice and rats to frogs and axolotls. Speakers presented a number of new approaches that have improved animal welfare and reduced animal use in their facilities.

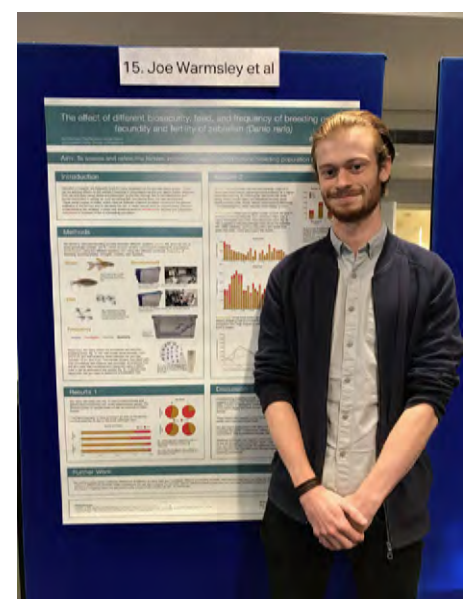
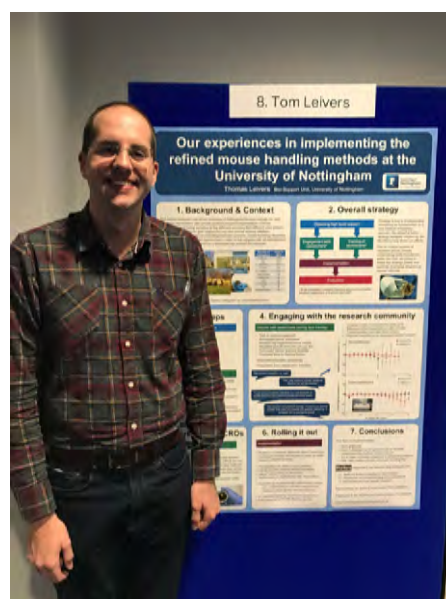
A £100 voucher prize was awarded to the best poster presented, based on the 3Rs impact, quality of the work and overall presentation. The winner of this year's prize was Nicola Cook from Fera Science, for her work on introducing refinements to improve the care and welfare of their CAX mouse colony, inspired by the NC3Rs Mouse Aggression Study. The poster judges

were impressed by the comprehensive approach Nicola had taken to reduce aggression and the success she had achieved, as well as the overall clarity of the poster.

Due to the high quality of the posters presented this year, we also had two runner-up awards. These were awarded to Tom Leivers from the University of Nottingham for his work on implementing non-aversive mouse handling methods, and Joe Warsley and colleagues from University College London for their work on improving zebrafish husbandry.

Thanks to everyone who attended this event, and to the Institute of Animal Technology for co-organising.

Read the full event summary at www.nc3rs.org.uk/ATSblog.

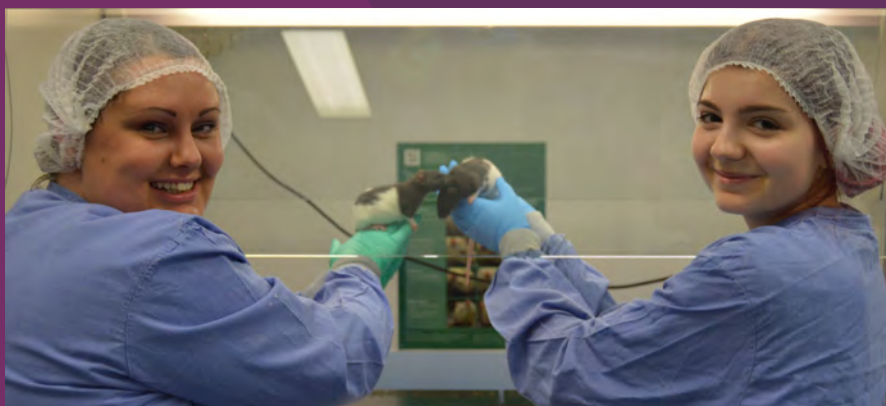


Left to right: the poster prize winners from this year's NC3Rs/IAT Symposium – first prize winner Nicola Cook (Fera Science) and runners-up Tom Leivers (University of Nottingham) and Joe Warsley (University College London).

Spotlight on rat husbandry

We have updated our webpages on husbandry and housing for rats. Visit www.nc3rs.org.uk/rats for information on rat behaviour, enclosures and environmental enrichment ideas.

The photos on this page have kindly been provided by the University of Leicester's Preclinical Research Facility. If you have any photos you would like to share with us, please email enquiries@nc3rs.org.uk.



Clockwise from top: rats calmly resting on the hands of animal care staff, Aimee (left) and Alicia (right), without the need to be restrained; a large playpen allowing rats to climb and explore; coloured balls used for enrichment.

Listen to the 3 Minute 3Rs podcast

The *3 Minute 3Rs* podcast has a new look to celebrate its second birthday!

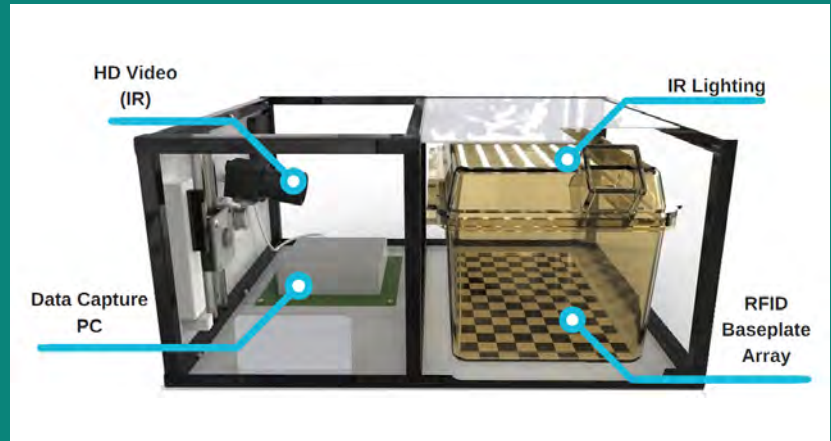
3 Minute 3Rs summarises the latest research and news in 3Rs science and technology every month. Working with *LabAnimal* and the North American 3Rs Collaborative, we cover three papers in around a minute each, helping you stay up-to-date with the latest innovations in all three 'Rs'.

December's episode will highlight the report from our mouse aggression study, including its recommendations for minimising aggression.

Visit www.nc3rs.org.uk/podcast to listen or subscribe to *3 Minute 3Rs*, as well as listen to all our past episodes. You can also search *LabAnimal* in your favourite podcast app.

3 Minute
3Rs

Upcoming events



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

AST 2020 is a joint conference between LASA, LAVA and IAT. The NC3Rs is sponsoring the following session on Wednesday 25 March, 2–4pm:

New Technologies Which Advance Refinement and Science

This refinement-themed symposium 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. Our grant holders and staff will be available to answer your questions about the power of these technologies, the discoveries they are enabling, and funding opportunities for 3Rs research and development.

Confirmed speakers include:

Dr Liane Hobson, MRC Harwell Institute

Dr Hobson was awarded an NC3Rs Training Fellowship in March 2019 to study ultrasonic vocalisations in mice and deduce links between these vocalisations and behaviours indicative of welfare issues, such as aggression or pain. Dr Hobson aims to build a searchable repository for this data, helping animal care staff intervene earlier when welfare issues arise.

Dr Steve Kennerley, University College London

Dr Kennerly's lab recently developed the Mymou system, a low-cost, home cage training system for non-human primates. The wireless device runs continuously and automatically 24 hours a day, every day, so monkeys can perform tasks in their home environment without social separation or restraint. In September 2019 Dr Kennerly was awarded an NC3Rs Skills and Knowledge Transfer grant to roll out this refinement to other neuroscience laboratories around the UK.

Dr Lawrence Moon, King's College London

Dr Moon was awarded a CRACK IT Challenge award in January 2019 to develop a new method for accurately measuring individual food intake in groups of three to five mice, in collaboration with Research Devices Ltd. This new technology will allow animal care staff to measure food intake without the need to singly house animals or remove them from their cage for weighing.

Dr Sara Wells, MRC Harwell Institute

Dr Wells will speak about Rodent Little Brother, a sophisticated home cage monitoring system that can automatically analyse the behaviour and activity of individual group-housed rodents simultaneously. Dr Wells will explain how this system, developed through our CRACK IT programme, offers animal welfare as well as scientific benefits.

To learn more and register, visit www.ast2020.org. Registration closes on 6 March 2020.