

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 will help animal technicians working in research establishments to 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 take a look at the welfare benefits of tickling rats, outline recent publications on using grimace scales to assess pain, and highlight how our Regional Programme Managers can help you advance the 3Rs.



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.



Megan LaFollette

Tickling rats for improved welfare

Megan LaFollette and colleagues from Purdue University describe how tickling can benefit you and your rats.

Tickling rats isn't just for fun – it also makes handling easier and improves rat welfare. If you wish there were a better, faster way to handle rats that didn't induce fear or require restraint devices, tickling can help. In this article, we explore the benefits of rat tickling and give you tips for when and how to use this unique technique.

Rat tickling is a positive handling technique that was developed by a team of neuroscientists studying positive emotions and ultrasonic vocalisations in rats. They noticed that when rats are young, they play by running around and wrestling, similar to dogs. Like playing with dogs, so too can we play with rats.

"Rat tickling is a positive experience for the animal and the person. It creates a bond between them and reduces fear", says Dr Sylvie Cloutier, who worked with the developer of rat tickling, Dr Jaak

Panksepp, at Washington State University. Dr Cloutier conducted research showing that tickling rats before injections reduces fearfulness and increases behaviours associated with positive welfare. Tickling can also speed up restraint procedures, and is particularly beneficial for rats who are singly-housed so can't play or socialise with other rats.

But I hate being tickled; shouldn't rats hate it too? This is a common concern, and it is true that some rats enjoy it more than others. However, rat tickling is not equivalent to human tickling, especially in adults – instead, it mimics aspects of wrestling and play behaviour. To avoid confusion, some researchers call it "heterospecific rough-and-tumble play" or "playful handling" rather than "tickling".

Rat play has two parts that we try to mimic during tickling. In natural rat play,

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Tickling rats for improved welfare

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one rat jumps on the back of the neck of another, who flips over in order to pin its playmate. When you are tickling your rat, make light, quick movements on the back of the neck for 2-4 seconds, then pick the rat up for the flip. Megan LaFollette, graduate researcher at Purdue University, recommends placing your index

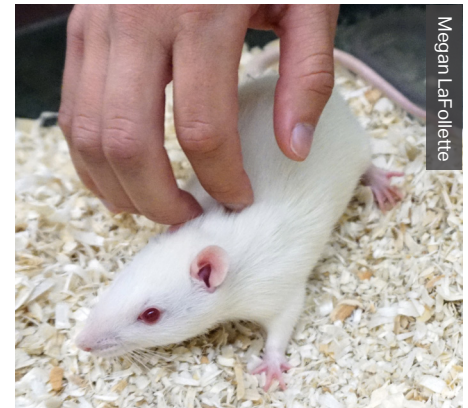
finger in front of the rat's collar bone and your thumb and middle finger under its "armpits". Then "give a flick of the wrist" – once the rat is on its back, "tickle" its belly for 3-4 seconds. Repeat three times for 15 seconds per rat. After this, you're done for the day, but ensure you repeat this process for at least the next two days before starting any procedures.

“

A simple tickling session is a great form of enrichment to make the rats happy.”

Carol Dowell, Purdue University

Some people worry that tickling will take a lot of time, is too rough, or will negatively affect experimental outcomes. In fact, tickling can be quick, purposefully mimics rough-and-tumble play, and generally improves research models by reducing anxiety. Explaining to others how this technique can replace traditional habituation procedures (and sometimes using the term "heterospecific play") can help get others on board, as more and more researchers and laboratory technicians are doing. By tickling your rats,



Megan LaFollette

you can play an important role in improving rat welfare and scientific research.

For further information on rat tickling, see the papers below. Megan will be presenting on this topic at the 2019 NC3Rs/IAT Animal Technicians' Symposium (see page 8 for details).

Top tips for rat tickling

Play like a rat. Remember that tickling mimics rat rough-and-tumble play. Use online training materials and consult with or request supervision by colleagues with experience of the technique. Use one hand to make quick, light, but assertive contacts with the rat right over the shoulder area. Make sure not to tickle the rump of the rat, as this is where aggression is directed. Carol Dowell, Training Coordinator at Purdue University, recommends picking the rat up to flip their legs forward instead of turning the rat sideways. It may take you a little while to get the hang of the technique – always ask if you're not sure.

Tickle first, manipulate later. Tickle rats for three days before any procedures commence. On the day of procedures, tickle them before rather than after, when they may not be in the mood to

play. Carol says, "if there's a study for which they're getting in younger rats, I recommend incorporating tickling into acclimating the rats and getting to know them. If you do tickling then, it makes the rats excited to see the glove."

Tickle often, not long. The minimum recommended tickling period is 15 seconds for three days. Research shows there is no difference between rats tickled for 15, 30 or 60 seconds, so even brief sessions are still effective. According to Carol, once rats have been tickled for a few days, "even if you don't tickle them for two to three weeks and come back to it, they still enjoy it."

Read your rat. Try tickling rats for three days before deciding it's not for them. Some rats just don't like tickling as much, so make sure to read your rat. Use a

bat detector or ultrasonic microphone to listen for positive or negative calls. Positive calls occur at 35-75kHz, while negative calls occur around 22kHz. You can also use the behaviour of the animal as an indicator. According to Dr Cloutier, positive indicators include "following the hand, licking the handler's fingers, and not hiding or trying to avoid the hand."

Consider your rat and the research model. Tickling is not best for every rat. Experts generally recommend not tickling breeder males or extremely stressed rats. Dr Cloutier warns that technicians should talk first to the researchers to make sure it's OK to include tickling, particularly if the research requires animals to experience anxiety or fearfulness. However, in general, tickling will improve research validity by reducing rats' stress levels.

Cloutier S et al. (2018) Tickling, a technique for inducing positive affect when handling rats. *Journal of Visualized Experiments* 135: 57190. doi: [10.3791/57190](https://doi.org/10.3791/57190)

LaFollette MR et al. (2017) Rat tickling: a systematic review of applications, outcomes, and moderators. *PLoS ONE* 12(4):e0175320. doi: [10.1371/journal.pone.0175320](https://doi.org/10.1371/journal.pone.0175320)

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 the development and use of grimace scales for assessing pain in research animals. For more information on grimace scales, and to order copies of our laboratory posters on the mouse, rat and rabbit grimace scales, please visit www.nc3rs.org.uk/grimacescales.



McLennan K et al. (2019) Conceptual and methodological issues relating to pain assessment in mammals: The development and utilisation of pain facial expression scales. *Applied Animal Behaviour Science* 217:1-15. doi: [10.1016/j.applanim.2019.06.001](https://doi.org/10.1016/j.applanim.2019.06.001)

- Grimace scales are available for many species, including mice, rats, rabbits, ferrets, cats, sheep, pigs and horses.
- Scoring of facial expressions has been shown to be effective for identifying animals experiencing pain and assessing its severity.
- However, there remain limitations and challenges to the use of grimace scales for assessing welfare.
- In their paper, Dr Krista McLennan and colleagues discuss the practical and theoretical issues associated with assessing pain and highlight the advantages of grimace scales over other pain assessment methods.
- The paper also gives guidance on developing reliable scales for new species and the best approaches for their use in practice.

These include:

- Training staff with detailed protocols.
- Placing protocols in key locations where pain assessment is required.
- Continued training and inter- and intra-observer testing to ensure a grimace scale is used consistently.
- Repeating observations over time alongside other behavioural and physiological measures.
- Displaying recorded grimace scores near animals for easier monitoring.
- Using grimace scales in conjunction with other validated indices of pain.
- Not using grimace scales for animals with head injuries/trauma.

Zhang EG et al. (2019) Influence of rater training on inter- and intra-rater reliability when using the rat grimace scale. *JAALAS* 58(2):178-183. doi: [10.30802/JAALAS-JAALAS-18-000044](https://doi.org/10.30802/JAALAS-JAALAS-18-000044)

- Before grimace scales are deployed as an assessment tool, technicians should be properly trained in how to use them.
- This ensures that pain assessment is carried out effectively and consistently across each facility.
- Emily Zhang and colleagues investigated the effect of a structured training programme on the reliability of grimace scale scores between different trainees.
- Four trainees were evaluated as they progressed through two rounds of training in rat grimace scale assessment.
- In each round, the trainees scored images of rats taken across several models of acute pain.
- These scores were then compared to those of an experienced grimace scale evaluator.
- Following each round of training, it was found that both the reliability of each trainee's scores and the consistency of scoring across the group improved.
- When the individuals were re-evaluated four years later, they were found to have retained these improvements in rating ability.
- This study demonstrates the necessity and lasting benefits of a robust training and evaluation programme for staff using grimace scales for pain assessment.

McLennan K, Mahmoud M (2019) Development of an automated pain facial expression detection system for sheep (*Ovis aries*). *Animals* 9(4):196. doi: [10.3390/ani9040196](https://doi.org/10.3390/ani9040196)

- Like many prey species, sheep make an effort to hide any signs that they are in discomfort, making it harder for those who care for these animals to detect pain.
- Sheep are increasingly used to model disease and physiology as well as in agriculture, so it is vital that efficient methods for assessing their welfare are available.
- The Sheep Pain Facial Expression Scale, described in a recent paper by Dr Krista McLennan and Dr Marwa Mahmoud, provides a reliable method for livestock owners and animal care staff to identify pain in this species.
- However, the application of this scale can be a time-consuming process, especially when caring for a large flock of sheep.
- Researchers at the University of Cambridge are developing a video-based system to automatically detect changes in the facial expressions of multiple sheep simultaneously.
- The system can send updates on a sheep's pain state directly to animal care staff.
- This software is based on the same technology used for human facial recognition and is currently in the testing phase.
- When complete, the system could significantly improve the welfare of sheep, both on farms and in research institutions, by allowing the rapid identification and treatment of injured animals and helping to control the spread of contagious diseases.

Regional Programme Managers

Supporting technicians locally

The NC3Rs has Regional Programme Managers whose positions are co-funded by universities and other research organisations. They support 3Rs activities within these organisations, interacting with research, veterinary and technical staff at all levels and advising on opportunities to implement the 3Rs, research funding and training resources. Projects they have collaborated on with technicians include:

- Encouraging the use of exercise and play pens for rats
- Sharing approaches for social housing of rodents with implanted devices
- Supporting the use of randomisation and blinding to minimise bias in animal studies
- Collecting data on aggression in group-housed male mice
- Providing practical training on refined methods of picking up mice
- Identifying 3Rs champions to feature in our Tech3Rs newsletter

To learn more about these projects and find more resources, visit our animal technician hub at www.nc3rs.org.uk/animaltech.

If you have ideas for advancing the 3Rs at your organisation, please get in touch with your Regional Programme Manager.



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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.

Zoe Windsor is a Research Support Technician at the Institute of Neurology, University College London. Here she describes her efforts to identify the most suitable nesting material for mice with head implants.

What 3Rs idea are you developing?

Mice fitted with exterior devices often face restrictions on key enrichment items that should be provided, such as nesting material, due to the risk of injury caused by entanglement. I conducted a study to investigate the safety of four different nesting materials (Pure Comfort White, Rodent Roll, Short Paper Shavings and Facial Tissue) as alternatives to shredded paper. I wanted to ascertain whether it was possible for implanted mice to safely construct nests with these materials without getting tangled and identify which nesting material was safest while allowing the mice to construct a high quality nest.

How did you develop your idea?

During my work, I frequently observed mice with shredded paper wrapped about their implants. This increased the animal's overall welfare burden, posing the risk of injury to the mouse as well as damage to the implant. On several occasions, mice were found so severely tangled that they were completely immobile. As a result, the material causing the entanglement had to be removed and an alternative nesting material identified.



Facial tissue tended to shred into long strips as the mice manipulated it.

What were the results of your study?

We found Pure Comfort White to be the safest and most suitable nesting material for head plated mice as they produced nests of high quality with minimal risk of entanglement. The study results have recently been published in the journal *Heliyon* (see below).

What are your future plans?

My findings have been used to improve the enrichment regime of the unit where the problems were occurring. I aim to continue to share this information with other mouse facilities through posters, talks and publications. I will also continue to be an active member of UCL's Welfare Group and investigate refinements that can be made within the animal unit for both implanted and non-implanted animals.

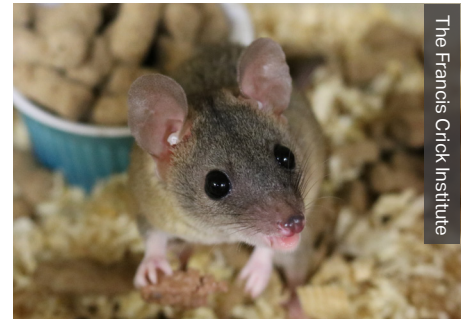
Windsor Z, Bate ST (2019) Assessing the safety and suitability of nesting material for singly housed mice with surgically fitted head plates. *Heliyon* 5(7):e02097. doi: [10.1016/j.heliyon.2019.e02097](https://doi.org/10.1016/j.heliyon.2019.e02097)

Please email z.windsor@ucl.ac.uk if you would like to get in touch with Zoe about this initiative.

Jamie Barrett, Jamie Delicata and Matt Legate are technicians at the Francis Crick Institute, which houses the only laboratory colony of opossums in northern Europe.

What is special about opossums?

Grey short-tailed opossums (*Monodelphis domestica*) may look like rodents, but they are quite different! They are marsupials from South America that give birth to up to 13 joeys after 13 days gestation. The mother has no pouch, so the tiny young latch onto her teats for their first month. Opossums are aggressive towards one another and come together only to mate. Our colony is used to study the biology of sex chromosomes and their role in health and disease, but this species is also used to study melanoma – they are the only animals other than humans to develop skin cancer caused solely by UV light.



The Francis Crick Institute

What 3Rs ideas have you developed?

We have worked hard to improve the husbandry of our opossums. They are housed singly in double-decker rat cages to give good floor space and height, and we remove the hopper for females with litters, so the joeys aren't damaged when their mother climbs.

Wild opossums have a varied, omnivorous diet (they will even eat smaller members of their own species). In addition to a pelleted insectivore diet and fruit we feed live mealworms, which the animals forage for in their wood shavings, and also dog food for females with litters – this has reduced cannibalisation. The opossums will voluntarily come out of their nests for mealworms, which aids health inspection.

Recently we have taken on overseeing the breeding of the opossums, to maximise genetic diversity in our closed colony and reduce production of animals not suitable for the research. We decide which animals are mated based on pedigree information held electronically. This has meant we don't need to set up as many matings, which reduces injury and mortality as the mating male and female can fight during the ten days they spend housed together. The grey short-tailed opossum was the first marsupial to have its genome sequenced – in future, the Institute would like to create genetically modified animals.

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.

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 highlighted a new tool for assessing zebrafish welfare, as well as one organisation's approach to establishing single use of needles as standard practice.

A new automated behaviour monitoring tool for zebrafish

The Fish Behaviour Index (FBI) is a free automated tool to assess zebrafish welfare, developed by NC3Rs grant holder Dr Lynne Sneddon and colleagues at the University of Liverpool. The system uses a camera and tracking software to record the trajectories of singly-housed fish and monitor how these change following invasive procedures such as fin clipping and in response to analgesia.



Alan Bannister and Dr Lynne Sneddon

By detecting subtle changes in behaviour, the FBI can allow quicker welfare assessment, interventions and administration of analgesia, representing a significant refinement of zebrafish welfare.

Further information:
www.nc3rs.org.uk/zebrafishFBI

Dr Sneddon will be presenting her work on refinements in zebrafish research at the 2019 NC3Rs/IAT Animal Technicians' Symposium – see page 8 for more details.

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The Fish Behaviour Index represents a major step forward in our ability to assess welfare in zebrafish.”

Dr Lynne Sneddon, University of Liverpool

Single use needles: putting refinement into practice

Since the NC3Rs' first blog post on needle reuse in December 2017, there has been a lot of discussion across establishments about the evidence base supporting the single use of needles. The UK Home Office has adopted this as a themed inspection area for 2019.

In this blog post, Dr Sally Robinson, Director of Animal Sciences and Technologies at AstraZeneca (Alderley Park), describes how the company has implemented the single use of needles as a refinement across its sites.

Further information:
www.nc3rs.org.uk/AZsingleuseneedles

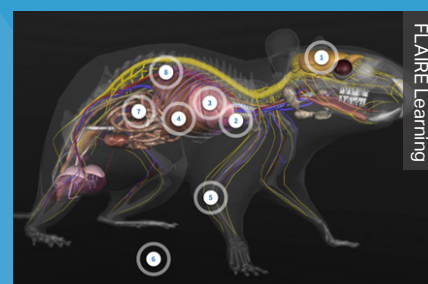
We will shortly be launching a new poster on why reusing needles should be avoided. To pre-order your free hard copies and find other relevant resources, visit www.nc3rs.org.uk/needlereuse.

Spotlight on laboratory animal anaesthesia

Two new e-learning modules funded by the NC3Rs have been launched, focusing on pre-anaesthetic preparations and choosing an anaesthetic. These modules are the first in a series on anaesthesia and analgesia for laboratory animals to be produced by FLAIRE Consultants and Newcastle University.

The modules are suitable for incorporation into Home Office Personal Licensee (PIL) Category C training for research workers.

To view these and other e-learning modules, visit our hub:
www.nc3rs.org.uk/elearning



Upcoming events



NC3Rs/IAT Animal Technicians' Symposium Wednesday 2 October, central London

The full programme for our joint symposium with the Institute of Animal Technology (IAT) is now available online. This event, which is aimed primarily at junior technicians, will give you the chance to hear about the latest approaches to improving the welfare of laboratory animals and refinement opportunities, as well as network with fellow technicians.

With the deadline to register fast approaching, don't miss out on booking your place at this event. Attendees will receive five IAT CPD credits.

To view the programme and register by 13 September, please visit www.nc3rs.org.uk/ATS2019.



NC3Rs Primate Welfare Meeting Friday 8 November, central London

Our Primate Welfare Meeting is a unique annual event for sharing best practice in the use and care of non-human primates internationally. The 2019 meeting will have a workshop format and will focus on harmonising welfare indicators for macaques and common marmosets.

We encourage all staff working directly with non-human primates to register for this event and consider submitting a poster relevant to primate welfare. Please submit your abstract by **30 August** via our online registration form.

To view the programme and register by 18 October, please visit www.nc3rs.org.uk/PWM2019.

Burrow into our new citizen science project

We have collaborated with MRC Harwell to create *Secret Lives of Mice*, a new citizen science project hosted by Zooniverse that enables members of the public to contribute to improving laboratory mouse welfare. Participants watch short videos of laboratory mice and label their behaviours, which will teach an algorithm how to recognise different mouse behaviours and track them automatically. The project uses the Home Cage Analysis system, developed with NC3Rs CRACK IT funding.

We want you to contribute to this project, and encourage your friends and family to join in too! Create an account on the Zooniverse website and start discovering what mice get up to when we're not watching.

Get involved: bit.ly/secretlivesofmice

