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 hear from animal care staff at MRC Harwell who have used the Home Cage Analyser, an NC3Rs-funded technology for non-invasive continuous monitoring of mice. We also highlight research on environmental enrichment for zebrafish and invite you to join us in celebrating the contributions of animal care staff to the 3Rs during Animal Technologist Month in March.

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.

When the techs are away, the mice will play

Animal care staff share how they use the Home Cage Analyser to discover what their mice do when they are not looking.

This year the NC3Rs celebrates 10 years of CRACK IT Challenges, our open innovation funding competition to deliver marketable products that benefit the 3Rs (visit **www.nc3rs.org.uk/crackit** to find out more). Rodent Little Brother was one of the first Challenges the NC3Rs launched, in collaboration with MRC Harwell. The aim was to develop an automated and minimally-invasive system for recording and analysing the activity, behaviours and interactions of individual mice 24/7 when group-housed in the home cage. The end product, the Home Cage Analyser (HCA) developed by Actual Analytics Ltd., can be used with existing racks and cages (IVC or non-IVC) and contains video, illumination and tracking systems to record from multiple microchipped animals 24/7 for an extended period of time.

Jackie Harrison and Marie Hutchison, animal technicians and NACWOs at the Mary Lyon Centre (MRC Harwell), have used the HCA and found it has improved animal welfare and science in their facility.

The continuous monitoring system has allowed them to better understand welfare issues such as aggression among group-housed mice. Watching video recordings from cages where aggressive episodes had taken place revealed that there were early signs of aggression, such as cornering of cage mates or stealing food, that had not been spotted during routine cage change. The team has now been able to use these signs to manage aggression before serious injuries occur.

Data collected using the HCA has also helped the Mary Lyon Centre better understand how to house mice based on their phenotype. Staff were surprised to see how different groupings could affect behaviour. When they performed a controlled experiment observing two background strains with very different circadian rhythms, FVB and C57BL/6, they noticed that the different strains began to influence each other's activity patterns. This is an important observation **(continued on page 2)**

Jackie Harrison

Marie Hutchison

(continued from front page)

as the scientific goal of many studies is to compare behaviours and researchers need to understand how co-housing can affect the data they collect.

The HCA technology has also allowed the Mary Lyon Centre to establish new humane endpoints or bring forward existing ones to minimise suffering. Mice are prey animals and often conceal signs of suffering. This combined with their nocturnal nature can make assessing welfare during the working day difficult. However, the HCA recordings can reveal even subtle changes in body condition or behaviour (e.g. staying distanced from the social group) that indicate compromised welfare. This has been the case with one of the Centre's genetically altered lines where some animals were occasionally observed, during cage change, to have unexpected abnormal behaviour,

An award-winning citizen science project

The HCA generates large datasets of video recordings, which are labour intensive for a researcher or team to analyse manually. To address this problem and develop a machine learning algorithm for faster analysis, MRC Harwell and the NC3Rs launched the citizen science project 'Secret Lives of Mice' , asking volunteers to watch short clips of laboratory mice recorded by the HCA and label their behaviours. Over 6,000 people have taken part so far. The projects achievements in involving the public in mouse behaviour research were recognised at the 7th Annual Openness Awards organised by Understanding Animal Research.

resembling epileptic seizures. When this line was studied with the HCA for just 12 hours overnight, it was revealed that some of these animals were actually having episodes as often as every 30 minutes. Realising that the problem was greater than anticipated led Jackie, Marie and their colleagues to the decision to minimise animal suffering by humanely euthanising these mice.

Similarly, the fact that the HCA collects data 24/7 means that some studies can be completed in a shorter time period, further minimising the potential for animal suffering. An example of this is a neurological mutant with a progressive motor phenotype. This strain starts to show clinical signs of interest from ten weeks of age but is usually maintained for longer periods to allow researchers to complete their observations. However, as the use of the HCA allows for a more efficient and detailed collection of behavioural data in the home cage, studies can be completed faster and the animals humanely euthanised before their condition worsens unnecessarily.

Jackie and Marie have also been using the HCA to observe wild type mice and their behavioural profiles before they are used to produce transgenic lines. A clear understanding of the wild type behaviour is essential as it allows strains with the most appropriate background to be selected. An example of behavioural differences between background strains has been revealed by observations made at the Mary Lyon Centre using the HCA, which showed that three commonly used strains (C57BL/6J,

C57BL/6Ntac and C3H/HeH) display a distinct pattern of activity during the night, with the C57BL/6J mice being the most active ones. C57BL/6J mice were also found to remain unsettled for longer after an external disturbance (e.g. moving the cage to the HCA testing rack), highlighting the differences in temperament between different strains. These findings have also been published in a peer-reviewed journal (Bains *et al.* 2016).

The benefits of using the HCA are not limited to animal welfare and research. Marie and Jackie talked about how their day-to-day job has also been affected: "knowing that we can have a quick check on the animals we care for without any disturbance, any time of the day (or even during the night if we needed to), gives us great reassurance when we have mice that have undergone surgery or if there is any other health concern".
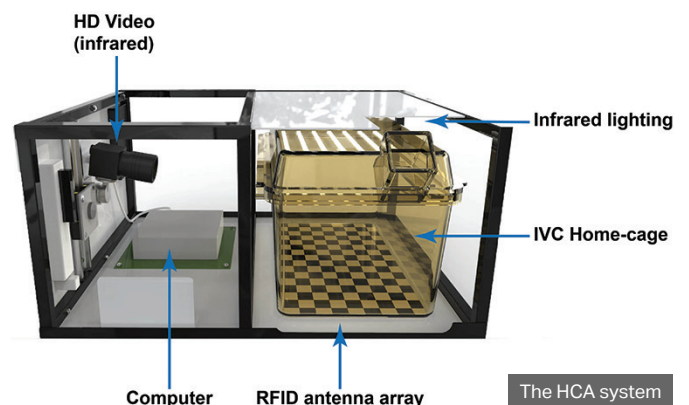
Jackie and Marie now want to use the HCA to look at more aspects of their animals' lives, such as environmental enrichment preferences or regrouping of unfamiliar female mice after weaning.

Learn more about the HCA at www.nc3rs.org.uk/hca.

Bains RS *et al.* (2016) Analysis of individual mouse activity in group housed animals of different inbred strains using a novel automated home cage analysis system. *Frontiers in Behavioural Neuroscience* 10: e106. doi: 10.3389/fnbeh.2016.00106

HD Video (infrared)

Infrared lighting

IVC Home-cage

Computer

RFID antenna array

The HCA system

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 look at how different types of environmental enrichment and different exposure patterns can affect zebrafish welfare.

- The authors of this paper investigated whether the colour of the environment in which zebrafish are housed influences anxiety-like behaviour and whole-body cortisol levels, a physiological biomarker of stress.

- Adult zebrafish of both sexes were randomly allocated into different groups and housed for 15 days in either open (uncovered) transparent tanks or in similar tanks covered with opaque white, black, yellow, blue or red plastic tops.

- The zebrafish individually underwent the novel tank test, where the fish are placed in an unfamiliar tank and the time spent bottom-dwelling instead of exploring is used as a marker of anxiety-like behaviour.

- At the end of the experiment they were culled and their whole-body cortisol was extracted.

- Overall, zebrafish housed in black and blue tanks demonstrated lower anxiety-like behaviour than fish housed in transparent or white tanks. Whole-body cortisol was lower in fish housed in blue tanks compared to white tanks.

- These findings highlight the importance of considering the colour of housing environments for zebrafish, and how even simple changes such as the colour of the tank lid can affect the welfare of the fish.

- This study examined the novelty-seeking behaviour of zebrafish housed in enriched or non-enriched environments.

- Adult zebrafish of both sexes were housed in an enriched environment (with stones and artificial green plants) for either 7 days, 14 days, or for 14 days with the enrichment element changed on day 8 (to PVC tubes and different artificial plants). The non-enriched control condition consisted of a tank with only recirculating water.

- The fish were then placed in an unfamiliar tank and their novelty-seeking (exploratory) behaviour was assessed, i.e. how long the fish spent exploring their new environment.

- Exposure to enrichment for 7 days, and for 14 days with change of enrichment, led to a reduction in exploration (total distance travelled) in the novel tank compared to non-enriched controls. This was not due to increased anxiety.

- Exposure to the same enrichment for 14 days did not have the same effect; fish in this group were keener to explore the novel environment.

- Based on these observations, the authors suggest that providing the same environment for long periods may result in fish becoming "bored".

- This paper evaluated the enrichment preferences of singly-housed female zebrafish, focusing on animate enrichment (visual access to singly- or group-housed fish in adjacent tanks) and inanimate enrichment (e.g. marbles, mirrored paper, photographs of fish).

- The test tank was divided into two and the inanimate enrichment items were placed in one side. The test fish could move freely between the two sides. Similarly, an empty tank and a tank containing one or more zebrafish were placed on either side of the test tank. In both cases, the location of the fish inside the test tank was used as a marker of their preference for the different enrichment.

- The authors observed that the test fish preferred to be on the social side of the tank when a single neighbour was present. Enrichment items that promote social behaviours, such as mirrored paper, were highly preferred.

- Static photographs of zebrafish, scaled to size, did not elicit a preference response, highlighting the importance of simulated movement.

- Therefore, items such as mirrored paper or housing next to another fish should be considered as enrichment strategies for singly-housed zebrafish.

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3Rs champions

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

Rosie Payne is an animal technician and NACWO at the University of Surrey. She spoke to us about evaluating enrichment items to improve the welfare of ageing mice.

What 3Rs idea have you developed?

We have a colony of ageing mice, including C57BL/6 and various NOX transgenic strains. Older mice are less active and more prone to weight gain, so we wanted to explore enrichment options to get them moving around the cage. We tested various items in our ageing colony using a simple preference test and a scoring system.

We found that, as they age, C57BL/6 mice require a greater variety of enrichment to keep them interested and therefore active. Foraging items were preferred, but as obesity is one of the main problems for older mice, we had to think carefully about what enrichment was appropriate.

How did you develop your idea?

I worked closely with Anna Slaviero (our Named Veterinary Surgeon) to design a study and carry out data collection for evaluating enrichment preferences in

ageing animals and the effect of repeated exposure to the same enrichment. We collected the data over an 11-month period, in three different age groups. We evaluated items to stimulate nesting (nestlets, hay), foraging (chocolate cheerios) and manipulatory behaviour (tinker toy, Aspen chew blocks). We also developed some new enrichment tools specifically for our ageing animals.

We found that certain types of scented Aspen chew block (a new enrichment tool developed by Anna) encouraged gnawing activity, without increasing calorie intake. The mice showed strong preferences for different scents; there was little interest in apple, but almond was very popular.

I tried to keep costs down by using items we already had; for example, the hay was left over from keeping rabbits. We also created 'piñatas' ourselves, by wrapping treats in paper. The treats elicit initial interest, while the paper wrapping encourages a greater level of activity than simply providing a treat alone. The mice unwrap the treat and then use the paper as nesting material. Creating the piñatas was quite time consuming, but thankfully we are a close-knit team and everyone was happy to lend a hand!

What are your future plans?

I would like to streamline the piñata-making process to save us some time! I will also continue to rotate and seek out new enrichment items to account for the loss of interest that we observed in older C57BL/6 mice when they are repeatedly exposed to the same items. You can tell that the mice are happy as, amongst other things, they make beautifully constructed nests out of the different materials we provide.

When observing interactions with environmental enrichment in different NOX transgenic strains, we made an unexpected finding in Cybb mice, which develop signs of cognitive impairment with ageing. The interaction scores of middle-aged Cybb mice were significantly lower than those of younger adults. Anna and I are continuing

Mice enjoy unwrapping treats from the piñatas created by Rosie's team, then using the paper as nesting material.

to investigate this. We believe their interactions with enrichment could be used as a refined method for assessing signs of early cognitive impairment, which are normally detected at standard health checks at later stages of their life.

I have been able to put my ideas into action to benefit our mice, and I have continued working with Anna to share our findings, including creating a poster and presenting at two Institute of Animal Technology meetings. I am also in touch with technicians at other facilities and it has been so useful discussing what types of enrichment work for different strains and ages across different laboratories. Overall, I have gained so much from this experience and I have lots of ideas for the future!

To learn more about this project, you can view Rosie and Anna's poster at www.nc3rs.org.uk/ageingmiceposter.

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.

Help us celebrate Animal Technologist Month!

March is Animal Technologist Month and an opportunity to recognise the important roles of technologists in animal research. The NC3Rs will be celebrating the contributions of animal care staff to the 3Rs, including through the difficult circumstances of the past year. We will be posting regularly throughout the month via our LinkedIn page, and we will give all of you the opportunity to share your stories, acknowledge the achievements of your colleagues and connect with others in the field. Don't miss out – find our page at **www.nc3rs.org.uk/linkedin**.

# Highlights from the NC3Rs website

Outstanding Technician of the Year awarded to John Waters

John Waters, until recently Chief Animal Technician at the University of Liverpool, won Outstanding Technician of the Year at the 2020 Times Higher Education (THE) awards, widely recognised as the 'Oscars of the higher education sector'.

It was John's commitment to supporting a culture of care at the University of Liverpool and beyond that impressed the judging panel, alongside his involvement in the development of non-aversive mouse handling methods. His passion, practical insight and support for other animal care staff have been key in achieving widespread uptake of non-aversive mouse handling across the technician community.

John is a tireless champion for laboratory animal welfare and has shown enormous commitment and leadership in improving the lives of laboratory rodents and the people that look after them. You can read the inspirational story of John's career path in the May 2020 issue of Tech3Rs (**www.nc3rs.org.uk/tech3rsissue7**).

To find out more about non-aversive mouse handling, visit our website hub at www.nc3rs.org.uk/mousehandling.
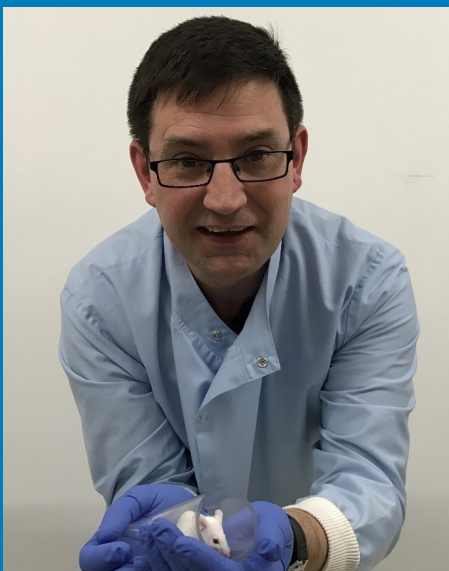
Analysing mouse movement in 3D could bring 3Rs benefits to behavioural tests

A recent paper by NC3Rs David Sainsbury Fellow Dr Riccardo Storchi describes a computational approach to automatically track and record the behaviour of freely-moving mice in 3D, which could be widely applied in fields reliant on behavioural analysis.

In the paper, Riccardo and his colleagues describe how this approach allows detailed study of the way mice respond to a range of visual and auditory stimuli. For example, a shadow passing above the arena mimics an overhead predator, to which the mice respond with specific behaviours.

This approach represents a significant refinement compared with other commonly-used behavioural tests. For example, swimming-based behavioural tasks used to test visual acuity in mice can be highly distressing, particularly for mice with impaired vision.

In addition to improved animal welfare, the computational method provides highly detailed analyses of mouse behaviour, while also reducing procedure time, variability and failure rates. The software, algorithms and full 3D dataset of mouse poses used in this study are publicly available.

To find out more, including how to contact Riccardo to discuss applying this approach in your research, visit www.nc3rs.org.uk/3Dmouseanalysis.
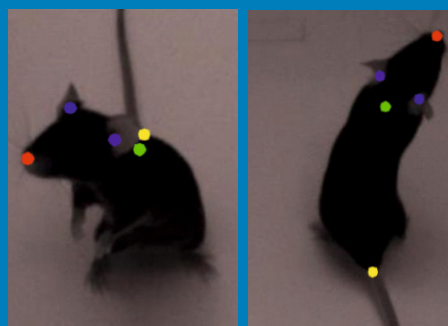
Webinar recording: tickling rats for improved welfare

Last month, rat tickling expert Dr Megan LaFollette of the North American 3Rs Collaborative (NA3RsC) presented a webinar on rat tickling as a positive handling technique, providing guidance on putting this refinement into practice for laboratory care staff and researchers.

If you missed the live webinar but are interested in learning more about how human-animal interactions can improve laboratory animal welfare, a recording of Megan's presentation is now available to catch up on.

Visit www.nc3rs.org.uk/webinars to view this recording, as well as recordings from other NC3Rs webinars and details of upcoming webinars. You can also learn more about rat tickling at www.nc3rs.org.uk/rat-tickling and www.na3rsc.org/rodent-handling.

Credit: Megan LaFollette

Credit: Megan LaFollette

Upcoming virtual events

FOCUS ON FISH
Practical refinements for fishes in research and testing

Focus on fish: practical refinements for fishes in research and testing
Tuesday 23 February

As fishes are increasingly used in research and testing worldwide, the importance of improving fish welfare and minimising harm is being recognised more widely. This RSPCA-led meeting will bring together experts from academia and industry to present on a range of topics, including the use of analgesia, opportunities to refine procedures and the implementation of humane endpoints. There will also be a virtual poster session and a panel Q&A.

This meeting is appropriate for all those involved in the care, use and regulation of fishes in research and testing, including animal technologists and care staff.

To learn more and register, visit www.focusonfish.co.uk.
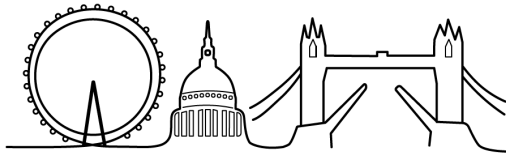
Pan-London 3Rs Symposium

Pan-London 3Rs Symposium
Wednesday 3 – Thursday 4 March

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.

Visit london3rs2021.eventbrite.co.uk to register by Friday 26 February. Registration is open to employees and research students of London universities and research institutes.

Efficient management of genetically altered mouse colonies
Monday 1 March

This joint webinar from the NC3Rs and the Mary Lyon Centre (MRC Harwell) will explore how to apply best practice to address common challenges in the management of genetically altered mouse colonies. Dr Sara Wells and Dr Michelle Stewart from the Mary Lyon Centre will introduce key themes from the NC3Rs Breeding and Colony Management resource, including strategies for obtaining and maintaining colonies and breeding genetically altered strains for experiments. This webinar will be relevant to anyone who manages mouse colonies or provides training in breeding and colony management.

Visit www.nc3rs.org.uk/GAcolonywebinar to register.

IAT Congress 2021
Tuesday 9 – Thursday 25 March

Throughout March, the Institute of Animal Technology (IAT) is holding a virtual congress with a varied selection of presentations, posters and workshops.

On Wednesday 10 March at 4pm, the NC3Rs is offering a workshop on "Managing aggression in laboratory animal species". Aggression can cause stress and painful injuries, or lead to single housing, negatively affecting animal welfare. It is therefore crucial to be able to detect aggressive behaviour early on, understand the causes, and take steps to minimise it.

In this interactive workshop we will discuss the reasons why aggression may occur within the animal facility, how this can affect animal wellbeing and science, and strategies and tips for preventing and managing aggression in a range of mammalian species. Participants will be able to share their experiences of reducing aggression and will receive practical recommendations and handouts with sources of further advice.

Further details, including how to register for this year's event, can be found at eu.eventscloud.com/website/2712/.