Institution:
UNIVERSITY OF LIVERPOOL

Unit of Assessment:
UOA5 – Biological Sciences

Title of case study:
Improved Methods of Handling Laboratory Mice to Increase Safety and Reduce Anxiety

1. Summary of the impact

The mouse is the most important laboratory animal used worldwide in biomedical research and for regulatory testing of products. Research at the University of Liverpool by Prof Hurst has led to a change in the methods universally recommended for routine handling of mice to minimize a well-recognized problem that handling can create high anxiety, stress and a risk of animals biting the handler. This has impacts for animal welfare, for practitioners, and for reliability in a broad range of research and testing using mice (e.g. in the pharmaceuticals industry) where responses can be confounded by uncontrolled anxiety responses. Mouse handling guidelines have been changed and are being implemented in animal research facilities.

2. Underpinning research

The mouse is the most important laboratory animal used worldwide in industrial and academic biomedical research, and for regulatory testing of products. Routine handling is an essential component of animal experiments and for maintaining animals in captivity but is widely known to have profound effects on their anxiety and stress (physiological and behavioural), which affects both animal welfare and the reliability of the data provided. Despite this, there had been little attempt to understand how to handle rodents to minimize such effects beyond the need to 'accustom' animals to being handled. By far the most common method for routine handling of laboratory mice, universally recommended in training materials and specified in standardized research protocols prior to this research, was to pick up and restrain a mouse by the base of its tail [1]. The research provided strong scientific evidence that this tail handling method induces aversion and high anxiety and that mice do not readily habituate. By contrast, use of a handling tunnel or cupping mice on the open hand without direct physical restraint leads to voluntary approach of the handler, low anxiety and acceptance of physical restraint. Using a new approach to assess voluntary interaction with the handler, in addition to well established validated measures of anxiety and stress, the research has further established that responses to these different handling methods generalize across sexes, ages and strains, across handlers with differing levels of prior experience, and regardless of the light phase in which animals are handled. On-going research by Hurst (e.g. [2]) and collaboration with large rodent facilities has now established the practicality of these new methods, and how they can be implemented in animal facilities to replace tail handling. This considerably reduces risks to handlers (including biosecurity risks) and substantially reduces anxiety in animals; anxiety is a very important confounder in research and testing where animals must be handled.

The impact derives entirely from research carried out by Professor Jane Hurst from 2006 to 2013 at the University of Liverpool (UoL), where she has held the William Prescott Chair of Animal Science since 1998. The initial research was carried out with an undergraduate vacation student (Rebecca West) under Professor Hurst's instruction to investigate effects on animal welfare and improve research results in an on-going BBSRC research programme. This was then expanded by Professor Hurst to establish how general the effects were across strains, in different contexts, with differing experience of restraint and to better understand the extent of stress and anxiety responses. Further research into the practical implementation of the new handling methods and their impact in reducing an important confounder in research using mice has been carried out under the award of one of the first NC3Rs postgraduate studentships (£120k) to Professor Hurst (student: Kelly Gouveia), which includes work in collaboration with pharmaceutical and charity animal research facilities. Demonstration that use of handling tunnels reduces anxiety regardless of animal experience [2] further improves practicality of implementation with negligible cost.

3. References to the research

Key reference

The main findings that have led to changes in animal handling guidelines were published in [1] and

an additional practical issue (that handling tunnels do not need to be present in every animal's home cage to be highly effective) was addressed in [2].

1. **Hurst JL** and **West RS**. (2010) Taming anxiety in laboratory mice. *Nature Methods* 7(10), 825-826. DOI:10.1038/NMETH.1500 Impact Factor: 23.565

2. **Gouveia K** and **Hurst JL**. (2013) Reducing mouse anxiety during handling: effect of experience with handling tunnels. PLOS ONE 8, e66401. DOI:10.1371/ journal.pone.0066401 Impact Factor: 3.730

Key Grants

2004-2008. **BBSRC** (S19816). MUPs, MHC and mate choice in house mice, £605k,000, **JL Hurst** (PI), **R Beynon**, WER Ollier and **P Stockley**

2004-2009. **BBSRC** (BBC503897), MUP knockout mice: implications for chemical communication and a generic research tool, £534,640, **JL Hurst** (PI) and **R Beynon**

2010-2014. **NC3Rs**. Taming anxiety and variation in laboratory mice, £120,000, **JL Hurst**

2006. **Wellcome Trust** 8 weeks vacation studentship (VS/06/LIV/A10), Reducing anxiety and environmentally-induced variation in laboratory mice through handling experience. (with supplement from **Association for the Study of Animal Behaviour**), **RJ West** (student) and **JL Hurst** (supervisor):.

2013-2014. **BBSRC Sparking Impact Award**, £10k, **JL Hurst**. Better animal handling improves science and the welfare of animals and handlers. Funding to create a CPD tutorial on the new handling methods for free circulation to any courses involved in training animal care staff and researchers in mouse handling, together with sample handling tunnels.

4. Details of the impact

Prior to this research, there had been no recognition that picking up mice by the tail caused problems for the welfare of the animals or for research in which animal stress is an important confounding factor. On publication, the key initial 2010 paper [1] generated considerable interest. It featured in Nature News [12], a NC3Rs press release newsletter, a Wellcome Trust press release and was recommended by Faculty 1000. It won the SGK sponsored NC3Rs prize, which is awarded to the lead author for the best original contribution to scientific and technological advances in 3Rs within the last two years [13]. Subsequent research on the practicality of using handling tunnels and impact on reduction in animal anxiety was featured in The American Scholar in August 2013 [14].

As a direct result of the research, a number of important guidelines have already been changed.

- Presentation of the findings at a Universities Federation for Animal Welfare meeting in 2009 prior to publication, led to immediate reference to the new findings in the 8th edition of the UFAW Handbook on the Care and Management of Laboratory Animals, a definitive guide across the EU [3]**.**
- Revision of the new *Procedures with Care* open-access web resource (a joint venture between the Institute of Animal Technology and NC3Rs) showing recommended practice for administration of substances to laboratory animals [4,5].
- Presentation at a joint RSPCA / UFAW meeting on animal welfare in 2010 attended by animal care personnel from industry and academia led to changes to RSPCA recommendations for handling laboratory and pet mice [6-8].

In addition to this, Professor Hurst is working with AstraZeneca [9] and with Cancer Research UK [10] to investigate practical implementation of the new handling methods in their animal facilities through collaborative studies.

Professor Hurst has given talks to many additional audiences to promote uptake, including:

- CPD training session at AstraZeneca (2011) leading to further collaborative research to investigate practical implementation in their animal facilities.
- CPD training course for the Swiss Laboratory Animal Science Association, which runs courses on animal care issues (obligatory CPD attendance for Swiss scientists using animals) in November 2011. As a result, officers of this association now teach the use of these handling methods in training sessions for mouse handling [11].
- Workshop at the Institute of Animal Technicians Congress, March 2012, attended by technicians from industry and academia.
- Training session and talk to animal care personnel at GSK in February 2012 (Stevenage and Ware, also recorded for transmission to their US sites). As a result, GSK purchased new handling tunnels to be trialled across their sites from April 2012 to give further insight into the impact of the new methods. The GSK animal welfare officer (Joanna Cruden) submitted a report of this training visit for publication in the Laboratory Animal Science Association forum newsletter to encourage wider uptake across the community
- Training session to personnel at Novartis (Basel) in November 2012.
- Talk at Tecniplast, a large cage manufacturing company in Varese, Italy (June 2011).
- Talk at a symposium organised by the Fondazione Guido Bernardini which promotes 3Rs practices in Italy (June 2011).
- Seminar at MRC Harwell, an international centre for mouse genetics and home to the Mary Lyon Centre, one of the large mouse facilities in the UK (February 2012).
- Talks given at several meetings of the National Centre for 3Rs in the UK, attended by biomedical researchers and animal care personnel in industry and academia, representatives of animal welfare charities and Home Office animal's inspectorate (April 2009, January 2011, June 2011, July 2012).

The major beneficiaries of this work are:

- all mice kept in research animal facilities and supply establishments (improved animal welfare); over 3 million mice are used annually in Home Office licensed procedures in UK alone and many more kept for unlicensed work and breeding – globally this is many times greater;
- all staff who have to handle laboratory mice (improved biosecurity, practitioner utility and satisfaction);
- the public concerned about the welfare and use of these animals, influencing public support for the continued use of animals in biomedical research (improved animal welfare and social approval of necessary research);
- scientists and those benefitting from the broad range of scientific research and testing where stress caused by handling can causes a confounding response in the animals, reducing the reliability of data (improved data reliability for industry and academic research, providing economic benefits and faster research progress);
- charities and regulators promoting refinement and reduction in the use of animals in research, or promoting better care for companion animals (improved animal welfare);
- pet mice and their owners, and veterinary surgeons involved in their treatment (improved animal welfare, biosecurity and utility).

5. Sources to corroborate the impact

Each source listed below provides evidence for the corresponding numbered claim made in section 4 (details of the impact).

3. UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, 8th Edition (Eds R Hubrecht & J Kirkwood) Wiley-Blackwell 2010.

4. *Procedures with Care* open-access web resource, a joint venture between the Institute of Animal Technology and NC3Rs showing recommended practice for administration of substances to laboratory animals (http://www.procedureswithcare.org.uk/handling-and-

restraint-of-the-animal/).

5. Letter: CEO of NC3Rs; confirming award of the NC3Rs prize, direct incorporation into guidance provided by NC3Rs, widespread and rapid uptake that is international as well as national.

6. Mice: Good practice for housing and care. Guidelines for members of local ethical review processes published by RSPCA Research Animal Department. Available at http://www.rspca.org.uk/sciencegroup/researchanimals/ethicalreview/housingandcare

7. Handle your mice carefully. RSPCA Companion Animals Pet Care Factsheet providing advice on how to handle pet mice: http://www.rspca.org.uk/allaboutanimals/pets/rodents/mice/health

8. Letter: RSPCA Research Animals Department; confirmed that the RSPCA recommends handling protocols for both laboratory and pet mice based on Professor Hurst's research. Further, these protocols are being recommended in relevant expert working groups reports, will be used at RSPCA animal homes, and protocols for catching and handling mice have been changed at several institutions as a direct result of this research.

9. Letter: AstraZeneca;Laboratory Animal Sciences UK; confirmed their interest in establishing the practical implementation of these methods at AstraZeneca's Alderley Park site. They have also confirmed that they have gained positive results from the internal pilot study that they have already carried out, that this refinement could have a big overall impact on mouse welfare as well as improving data reliability in many experiments.

10. Letter: London Research Institute, Cancer Research UK; confirmed their collaboration with Professor Hurst's team to establish whether the new handling methods improve breeding performance in their unit, and the positive behavioural responses gained so far.

11. Letter: Institute of Laboratory Science, Zurich; and the Federation of European Laboratory Animal Science Association; confirmed that he regards the new handling methods as a very important contribution to 3Rs in animal research that will substantially improve both animal welfare and the reliability of data. Individual confirms that he educates almost all researchers using animals in the German-speaking part of Switzerland (approximately 500 per year) and teaches the use of the new handling methods for mice in his courses.

12. Weaver J (2010). Getting a better grip on lab mice. Nature News. doi:10.1038/news.2010.462 (http://www.nature.com/news/2010/100912/full/news.2010.462.html)

13. NC3Rs. (2011) http://www.nc3rs.org.uk/news.asp?id=1457 (confirmation of prize)

14. The American Scholar (2013) http://theamericanscholar.org/eek-a-human/ - .UkgniGR4Z8x