National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

NC3Rs update

Vicky Robinson

Pioneering Better Science

Role of the NC3Rs

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Research funding

Pioneering Better Science

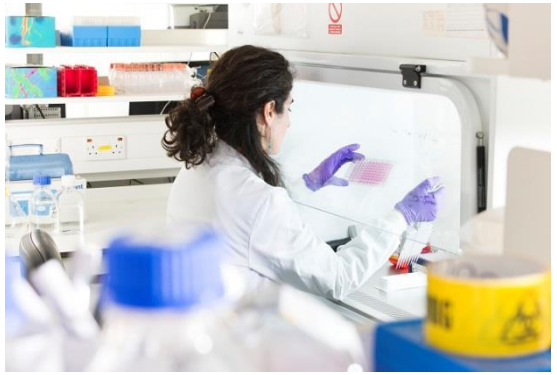
www.nc3rs.org.uk/funding

Project Grants

- Projects up to 36 months
- Typically around £350k
- Outline submission deadline: mid-January

PhD Studentships

- Minimum 5 years' postdoc experience
- £90k over 3 years
- Covers student stipend, fees & research costs
- Informal outline submission: mid-May



Training Fellowships

- 0-3 years' postdoc experience
- Salary + up to £15k p.a. for 2 years (non-FEC)
- Submission: mid-September

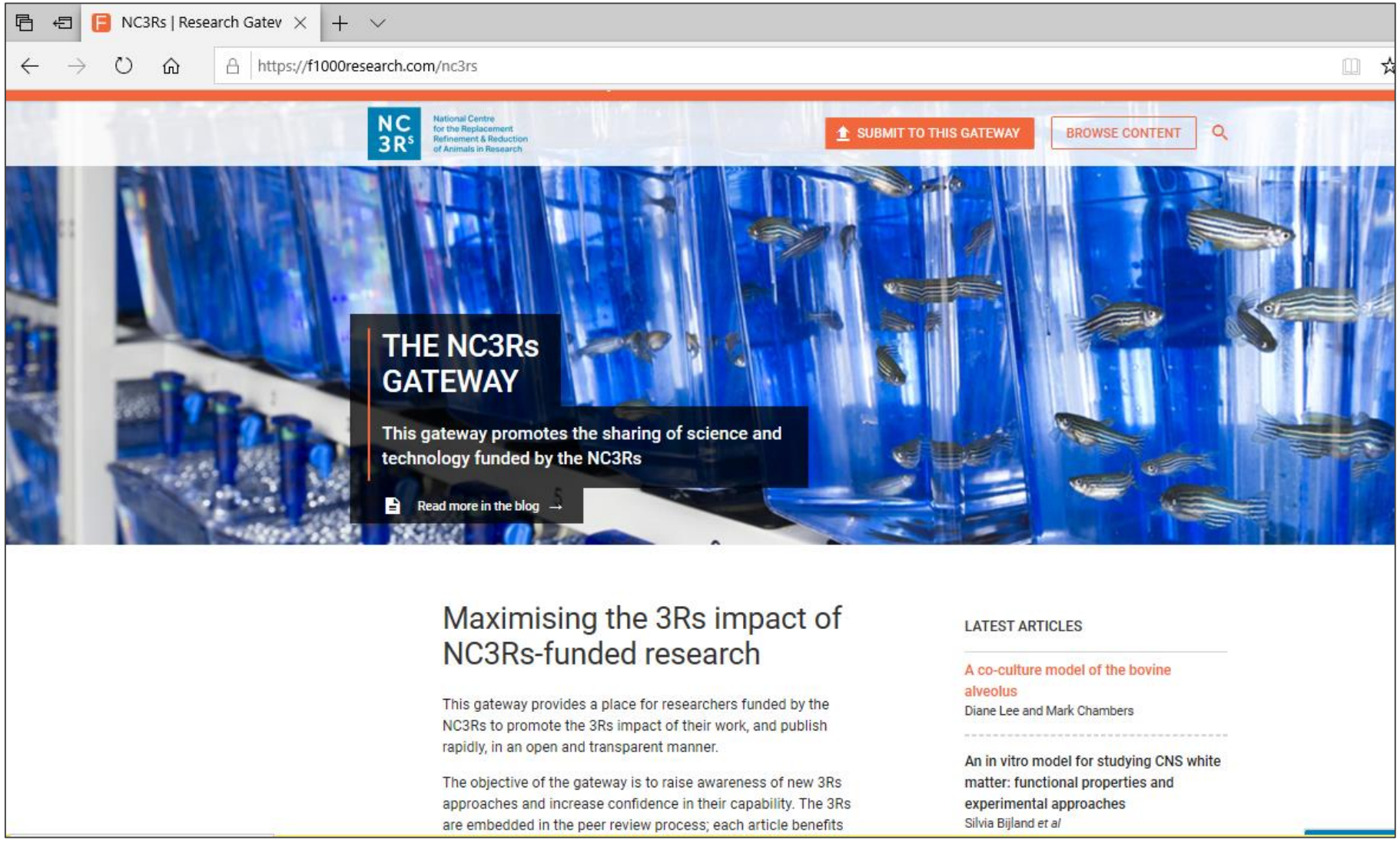
Skills & Knowledge Transfer Grants

- Projects up to 24 months
- Up to £75k
- Submission: mid-November

Reviews 2019

NC 3Rs

NC3Rs gateway – detailed methodologies



Article information						Platform		PubMed	
Title	Authors	Published	Last revised	Indexed	Citations	Views	Downloads	Views	Downloads
Using zebrafish larval models to study brain injury, locomotor and neuroinflammatory outcomes following intracerebral haemorrhage	Crilly et al	08 Oct '18	08 Nov '18	35 days	4	1383	156	242	55
A method for transplantation of human HSCs into zebrafish, to replace humanised murine transplantation models	Hamilton et al	15 May '18	23 Dec '18	35 days	3	1342	190	325	95
A bilayer tissue culture model of the bovine alveolus	Lee et al	01 Apr '19	30 Jul '19	127 days	0	509	59	-	-
Preparation of organotypic brain slice cultures for the study of Alzheimer's disease	Croft et al	15 May '18	27 Jun '18	7 days	6	2344	342	1077	243
Embryonic zebrafish xenograft assay of human cancer metastasis	Hill et al	22 Oct '18	20 Dec '18	21 days	6	944	170	458	158
A convenient protocol for establishing a human cell culture model of the outer retina.	Lynn et al	18 Jul '18	-	57 days	1	1120	179	305	110
The use of PrP transgenic Drosophila to replace and reduce vertebrate hosts in the bioassay of mammalian prion infectivity	Thackray et al	15 May '18	-	28 days	0	458	67	103	21
An in vitro model for studying CNS white matter: functional properties and experimental approaches	Bijland et al	29 Jan '19	-	21 days	1	823	126	-	-
Refinement of a mouse cardiovascular model: Development, application and dissemination	Taylor et al	15 May '18	-	10 days	0	532	67	93	25
The NC3Rs gateway: Accelerating scientific discoveries with new 3Rs models and technologies	Percie du Sert et al	15 May '18	-	-	1	419	29	66	17

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Resources

Pioneering Better Science

Reuse of needles

- Guest blogs
- Laboratory poster
- Website hub

www.nc3rs.org.uk/needlereuse

Home Office thematic review

Blog post – Reuse of needles: is this an indicator of a culture of care?

Dr Lucy Whitfield (Royal Veterinary College) and Dr Sally Robinson (AstraZeneca) explore the issues associated with reusing needles.

Blog post – Single use needles: putting refinement into practice

Dr Sally Robinson explores how AstraZeneca has implemented the single use of needles as a refinement across their sites.

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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**

NC 3Rs

How to pick up a mouse

www.nc3rs.org.uk/mousehandling

Mouse handling research papers

Below are links to the original research papers that provide the evidence-base for improved welfare and scientific outcomes with the tunnel handling and cupping methods of picking up mice. We also provide access to papers which validate or use the refined mouse handling techniques.

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In each case, a short summary of the key findings is provided, along with notes. We recommend reading the papers in full.

We update this document as new research is published. To alert the NC3Rs to further papers on mouse handling, please email enquiries@nc3rs.org.uk.

The original research

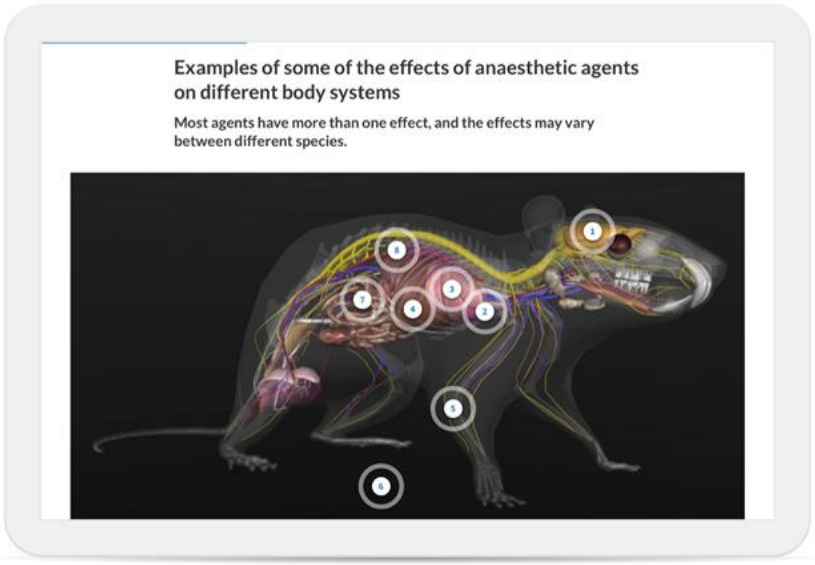
What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Hurst JL, West RS (2010) Taming anxiety in laboratory mice. *Nature Methods* 7: 825-826. doi:10.1038/nmeth.1500 (full text: bit.ly/2Jhgb.Jb) Picking up mice by the tail induces aversion and high anxiety levels (i.e. avoidance of the human gloved hand, greater urination and defecation during handling, a higher frequency of protected stretch attend postures, fewer open arm entries and less time spent on the open arms of the elevated plus maze). These responses can be minimised by instead using a tunnel or cupped hands. The positive effects of tunnel handling and cupping generalise across strains, handlers, and the light/dark phase. Mice handled by their home cage tunnel or cupping are *much* more willing to approach the handler than those picked up by the tail, even after restraint by the scruff of the neck or lifting by the tail for abdominal inspection. Scruff restraint does not reverse the taming effects of tunnel handling or cupping. Mice picked up by the tail do not habituate to tail handling.						
Tail, tunnel, cupping (Tunnel then cupping was used for one cohort of C57BL/6 mice, producing similar results to tunnel handling: Suppl. Fig. 4, Suppl. Tables 2 & 3) Tunnels were clear acrylic, familiar (home cage) tunnels and were present in all cages Measures: voluntary interaction with handling device; urination and defecation during handling; anxiety in elevated plus maze	Minimum nine daily handling sessions of 2x30s. Acclimation extended variably up to 16 sessions to address specific responses. EPM anxiety tested after seven or nine handling sessions For tail handling, the base of the tail was grasped between thumb and forefinger and the mouse gently lifted onto the opposite gloved hand or laboratory coat sleeve and held there by the tail for 30s before release back into the cage; after 90s handling was repeated Mice handled consistently by one of 11 handlers	N/A	Cages randomised into handling methods and balanced on the cage rack. Order of testing randomised but balanced across methods Blinding used, but not consistently No sample size justification N=47 cages per handling method (BALB/c N=23 cages x 3 methods; ICR N=8 cages x 3 methods; C57BL/6 N=16 cages x 3 methods; tunnel to cup method, N=8 cages of C57BL/6). 298 mice in total	BALB/c, ICR(CD-1), C57BL/6 Males and females 8-10 weeks old at start of testing; 11-15 weeks old at end Housed two per cage	Open (MB1)	ASAB, BBSRC, NC3Rs, Wellcome

techniques.

supporting refined handling techniques and practical tips

Two new e-learning modules on laboratory animal anaesthesia

- Focusing on pre-anaesthetic preparations (EU21-1) and choosing an anaesthetic (EU21-2). First in a series on EU21.
- Produced by FLAIRE Consultants and Newcastle University, and funded by the NC3Rs.
- Suitable for incorporation into Home Office Personal Licensee (PIL) Category C training.



Topics covered include:

- Why anaesthetise laboratory animals.
- Preparing for anaesthesia.
- Selecting inhalational and injectable anaesthetics.
- Balanced anaesthesia.
- Anaesthesia of animals in poor health.

https://nc3rs.org.uk/e-learning-resources

Tech3Rs: A newsletter for animal technicians

Regular features:

- 3Rs papers of interest
- A spotlight feature
- 3Rs Champions
- Pull-out A3 poster
- New 3Rs resources, research and events

Request hard copies: tech3Rs@nc3rs.org.uk

Animal technician hub: www.nc3rs.org.uk/animal-technician

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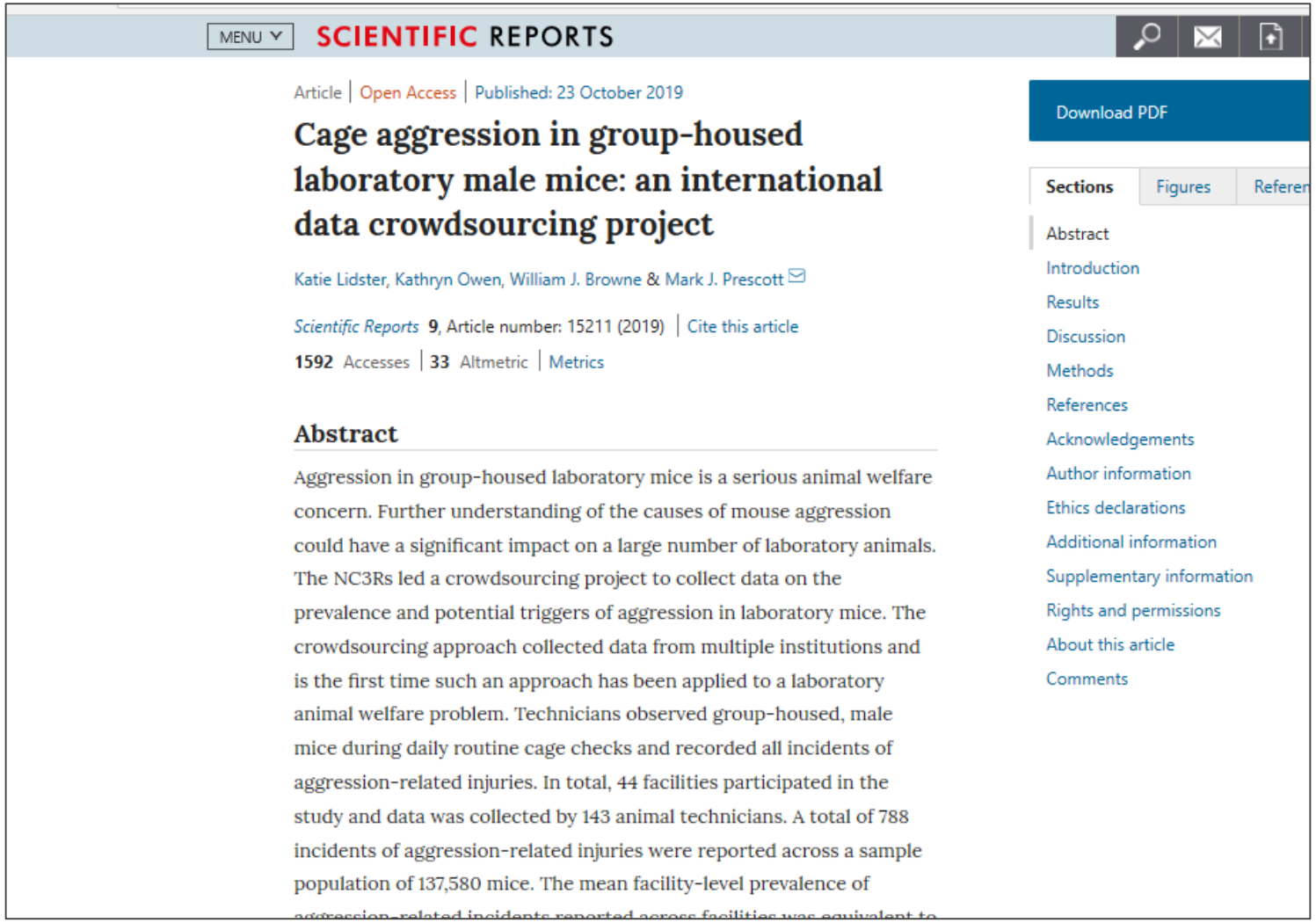
Programmes

Pioneering Better Science

Male mouse aggression – data crowdsourcing



MENU SCIENTIFIC REPORTS

Article | Open Access | Published: 23 October 2019

Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project

Katie Lidster, Kathryn Owen, William J. Browne & Mark J. Prescott

Scientific Reports **9**, Article number: 15211 (2019) | Cite this article

1592 Accesses | **33** Altmetric | Metrics

Abstract

Aggression in group-housed laboratory mice is a serious animal welfare concern. Further understanding of the causes of mouse aggression could have a significant impact on a large number of laboratory animals. The NC3Rs led a crowdsourcing project to collect data on the prevalence and potential triggers of aggression in laboratory mice. The crowdsourcing approach collected data from multiple institutions and is the first time such an approach has been applied to a laboratory animal welfare problem. Technicians observed group-housed, male mice during daily routine cage checks and recorded all incidents of aggression-related injuries. In total, 44 facilities participated in the study and data was collected by 143 animal technicians. A total of 788 incidents of aggression-related injuries were reported across a sample population of 137,580 mice. The mean facility-level prevalence of

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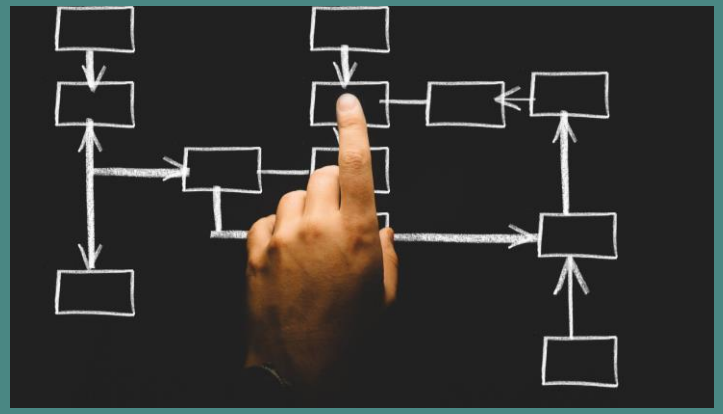
Sections | Figures | Referen

Methodology



1. **Study recruitment**
   - Study open to all licenced facilities with group-housed male mice.
   - Participation encouraged by the NC3Rs.
   - Participants invited to watch an online video tutorial, providing step-by-step instructions.
2. **Data collection**
   - Four-week period (between September and November 2017).
   - Data submitted in confidence to the NC3Rs.
3. **Data anonymised and checked.**
   - Queries cleared with participants.
4. **Data analysis**

Results

Overview of study participants

A total of **44** facilities from 9 countries. Including universities, large pharma, government labs, CROs and charities.

A total of **143** animal technicians participated in collecting data.

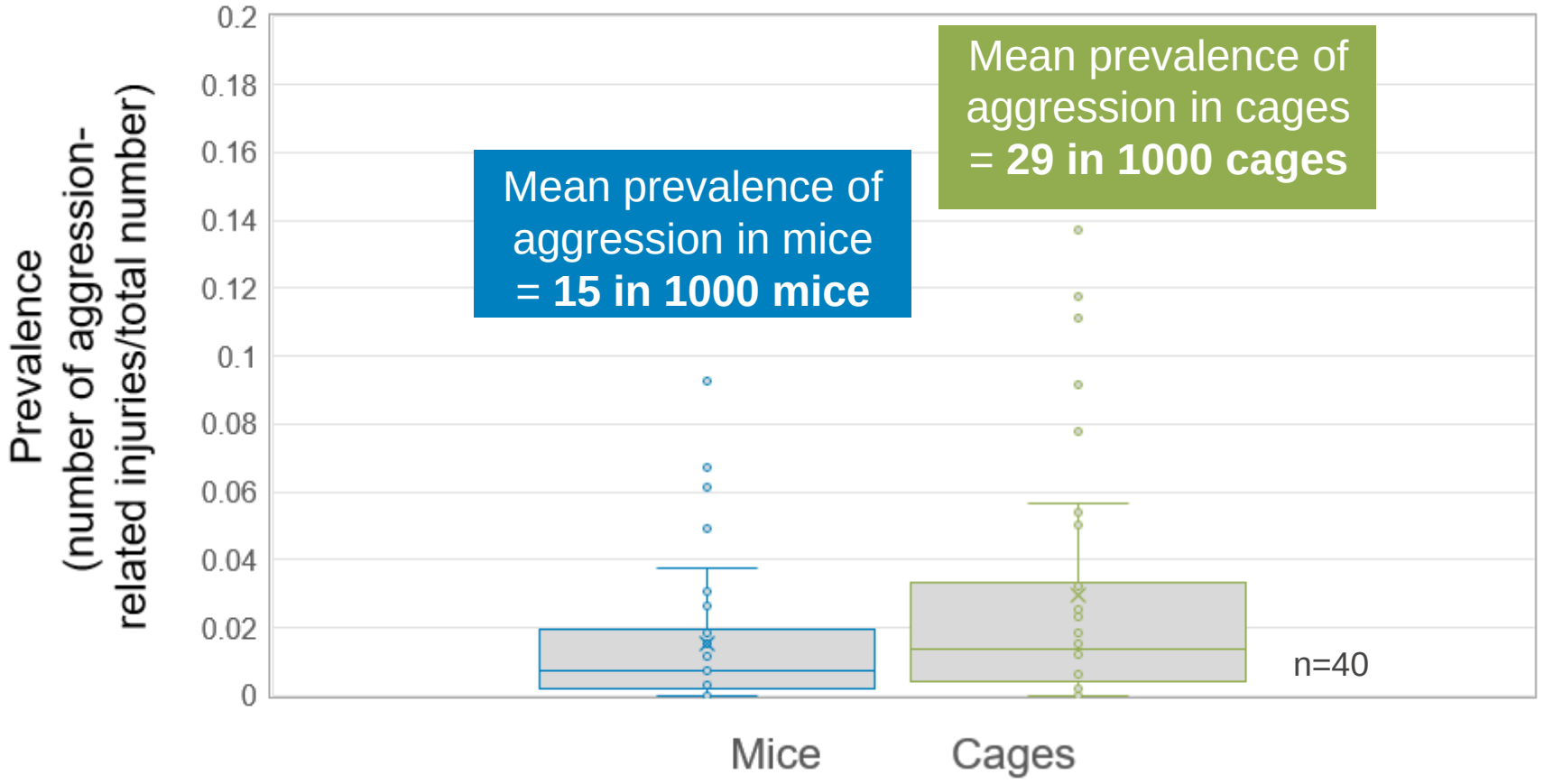
A total of **1,200** UK Institute of Animal Technology CPD credits were awarded for animal technicians participating in the study.

A total of **137,580** mice in **45,412** cages observed during the data collection period (n=40*).

*Four establishments did not submit Section A4.

Prevalence of mouse aggression

$$\text{Prevalence} = \frac{\text{Number of mice/cages with aggression-related injuries}}{\text{Total number of mice/cages held during the data collection period}}$$



A total of six facilities reported no incidents of aggression.

Standard conditions

Participants completed a ‘Standard Conditions’ questionnaire focused on standard husbandry conditions across the facility.

Data was collected on the following:

- Age at weaning
- Standard method of identification
- **Standard number of mice housed per cage**
- **How mice are selected into the cage**
- **Routine suppliers**
- Routine method of handling
- Frequency of handling
- Bedding material
- **Nesting material**
- Cage enrichment
- **Cage cleaning protocol**
- Cage type
- Diet and water
- Light cycle
- Room temperature
- Room humidity
- Number of air changes

Aggregated data from 40 facilities was combined to identify standard condition variables of interest using a multilevel logistic regression analysis.

Acknowledgements

- AstraZeneca, Sweden
- AstraZeneca, UK
- Babraham Institute, UK
- Cardiff University, UK
- Delware Valley University, US
- École Polytechnique Fédérale de Lausanne, Switzerland
- Eisai, UK
- Envigo, UK*
- Fera Science Ltd, UK
- Imperial College London, UK
- Maastricht University, Netherlands
- Max Planck Institute for Biophysical Chemistry, Germany
- Montreal Clinical Research Institute, Canada
- MRC Harwell, UK
- MRC Laboratory of Molecular Biology, UK
- National Institute for Biological Standards and Control (NIBSC), UK
- Novo Nordisk Research Center, Denmark
- Novo Nordisk Research Center, USA
- Plymouth University, UK
- The Francis Crick Institute, UK
- University College London, UK*
- University of Aberdeen, UK
- University of Dundee, UK
- University of Edinburgh, UK
- University of Edinburgh, UK*
- University of Glasgow, UK
- University of Manchester, UK
- University of Nottingham, UK
- University of Oxford, UK
- University of Sheffield, UK
- University of Warwick, UK
- Warsaw University of Life Sciences, Poland
- Wellcome Genome Campus, UK

- Sarah Wells, Marie Hutchinson, Mark Gardiner (Harwell), James Bussell, Mark Griffiths (Sanger) – help with study design.
- William Browne (Bristol) – help with statistical analysis.

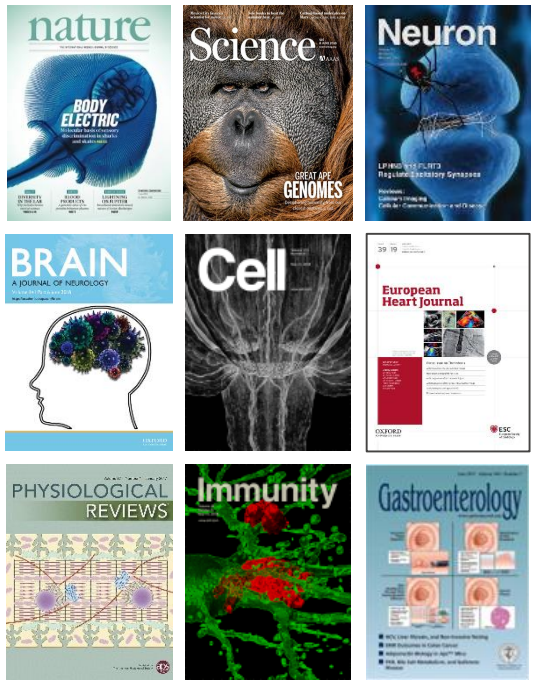
NC 3Rs

Endorsement of the ARRIVE guidelines

https://www.nc3rs.org.uk/arrive-guidelines

Over **1,000** journals and organisations recommend the ARRIVE guidelines

Journals	Funders	Universities	Learned Societies



wellcome

BBSRC

MRC Medical Research Council

CANCER RESEARCH UK

EPSRC

The Academy of Medical Sciences

amrc
ASSOCIATION OF MEDICAL RESEARCH CHARITIES

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Cold Spring Harbor Laboratory
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

New Results

Comment on this paper

The ARRIVE guidelines 2019: updated guidelines for reporting animal research

Nathalie Percie du Sert, Viki Hurst, Amrita Ahluwalia, Sabina Alam, Marc T. Avey, Monya Baker, William J. Browne, Alejandra Clark, Innes C. Cuthill, Ulrich Dirnagl, Michael Emerson, Paul Garner, Stephen T. Holgate, David W. Howells, Natasha A. Karp, Katie Lidster, Catriona J. MacCallum, Malcolm Macleod, Ole Petersen, Frances Rawle, Penny Reynolds, Kieron Rooney, Emily S. Sena, Shai D. Silberberg, Thomas Steckler, Hanno Würbel

doi: https://doi.org/10.1101/703181

Abstract

Reproducible science requires transparent reporting. The ARRIVE guidelines were originally developed in 2010 to improve the reporting of animal research. They consist of a checklist of information to include in publications describing *in vivo* experiments to enable others to scrutinise the work adequately, eva res jou inc reporting in animal research publications have not been achieved.

1. Items reorganised, prioritised in two sets
2. New items
3. Explanation and Elaboration

Cold Spring Harbor Laboratory
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

New Results

Comment on this paper

Reporting animal research: Explanation and Elaboration for the ARRIVE guidelines 2019

Nathalie Percie du Sert, Amrita Ahluwalia, Sabina Alam, Marc T. Avey, Monya Baker, William J. Browne, Alejandra Clark, Innes C. Cuthill, Ulrich Dirnagl, Michael Emerson, Paul Garner, Stephen T. Holgate, David W. Howells, Viki Hurst, Natasha A. Karp, Katie Lidster, Catriona J. MacCallum, Malcolm Macleod, Esther J Pearl, Ole Petersen, Frances Rawle, Penny Reynolds, Kieron Rooney, Emily S. Sena, Shai D. Silberberg, Thomas Steckler, Hanno Würbel

doi: https://doi.org/10.1101/703355

Abstract

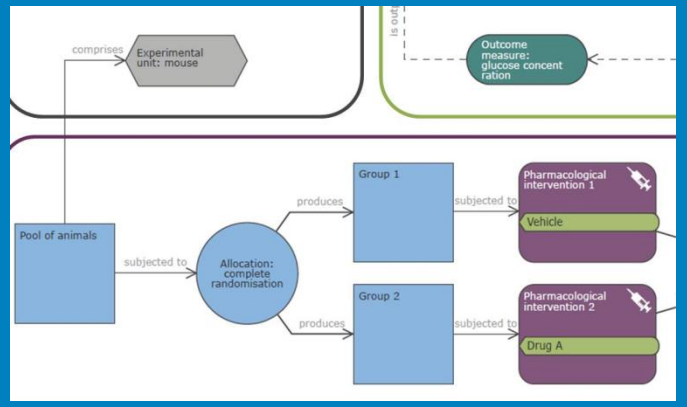
Improving the reproducibility of biomedical research is a major challenge. Transparent and accurate reporting are vital to this process; it allows readers to assess the reliability of the findings, and repeat or build upon the work of other researchers. The NC3Rs developed the ARRIVE guidelines in 2010 to help authors and s identify the minimum information necessary to report in tions describing *in vivo* experiments.

e widespread endorsement by the scientific community, the impact of the ARRIVE guidelines on the transparency of reporting in animal research publications has been limited. We have revised the

NC 3Rs

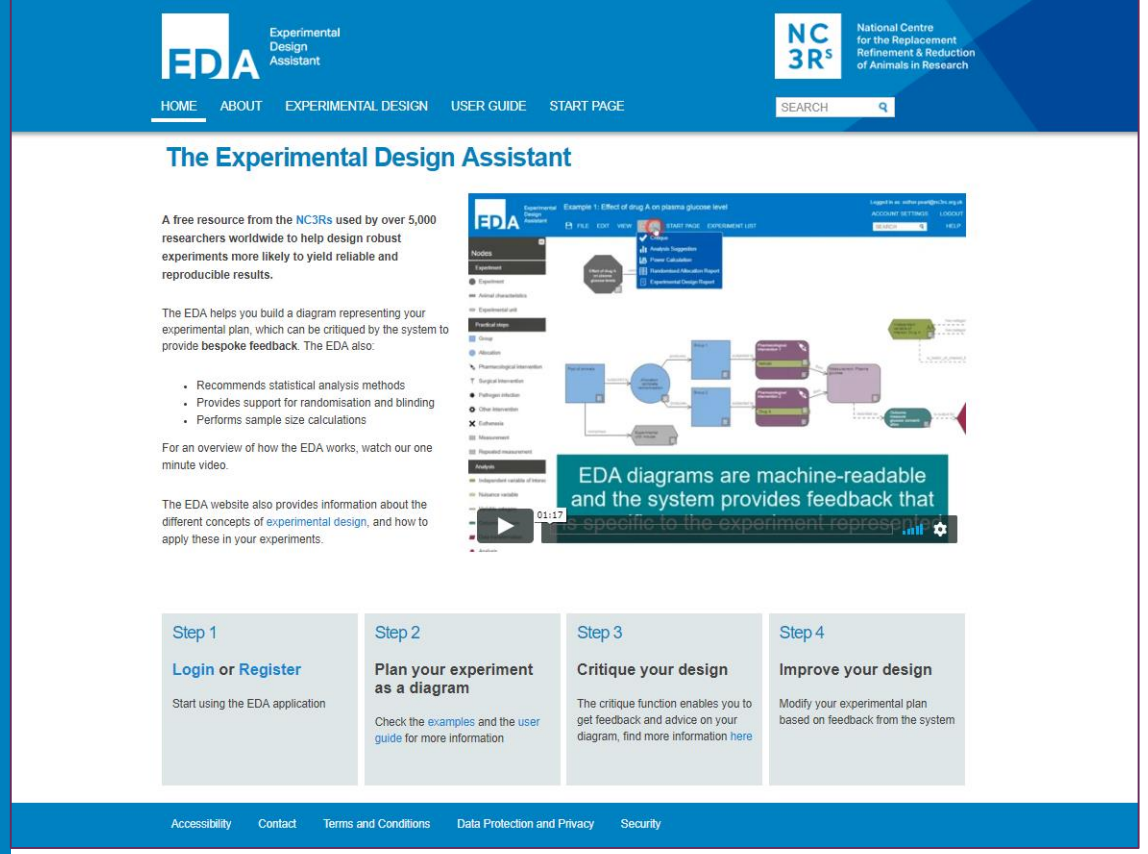
Experimental Design Assistant (EDA)

Online tool for researchers to design *in vivo* experiments



EDA can help to ensure robust study design and reliable and reproducible findings

https://eda.nc3rs.org.uk/



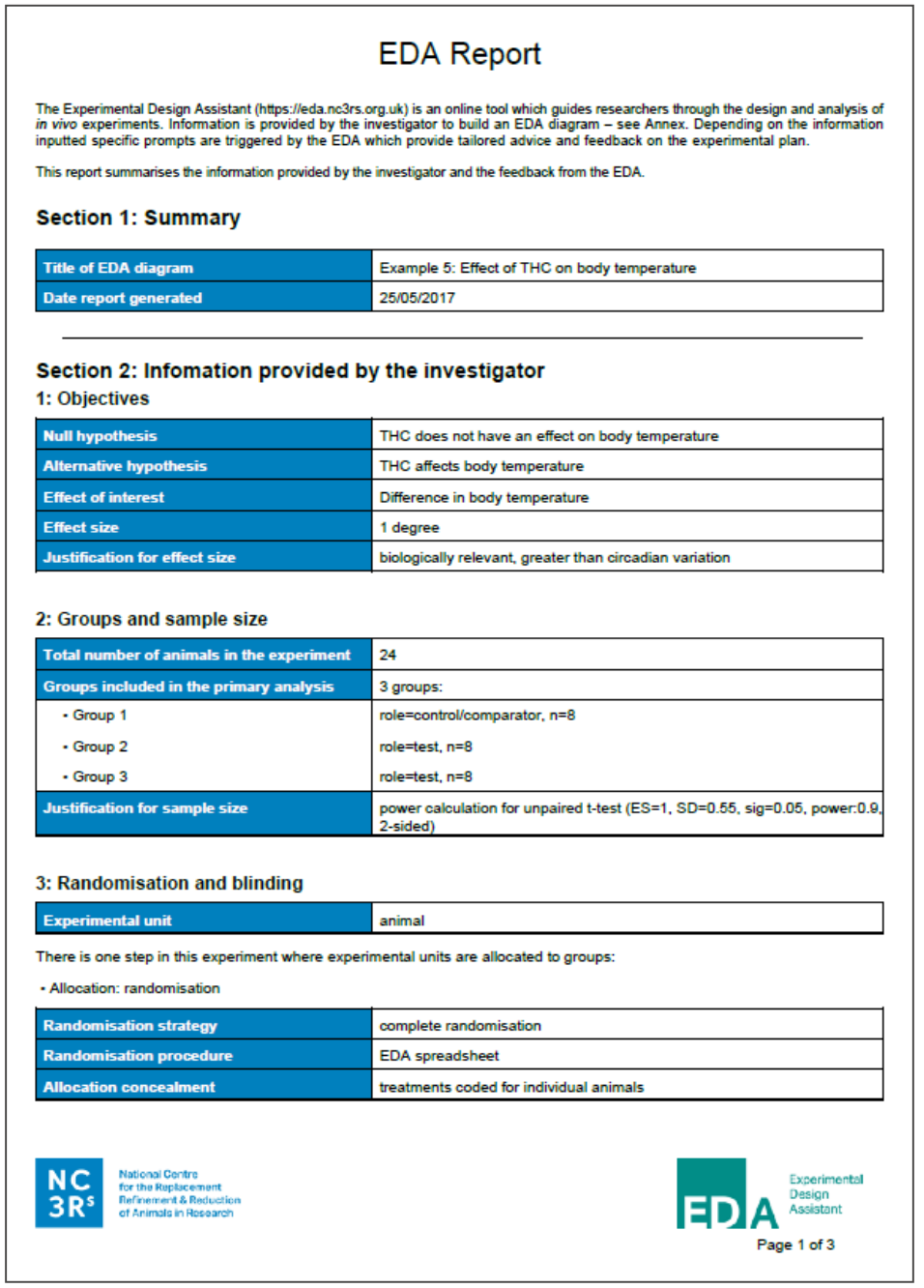
Benefits of the EDA include:

- Advice to improve the experimental plan
- Recommendations for the statistical analysis
- Power calculation
- Randomisation and blinding
- Summary report

EDA Report

Key information requested by funders:

- Objectives and hypotheses
- Animal numbers and justification for sample size
- Steps taken to minimise the effect of bias
- Primary and secondary outcome measures
- Planned statistical analysis



EDA Report

The Experimental Design Assistant (https://eda.nc3rs.org.uk) is an online tool which guides researchers through the design and analysis of *in vivo* experiments. Information is provided by the investigator to build an EDA diagram – see Annex. Depending on the information inputted specific prompts are triggered by the EDA which provide tailored advice and feedback on the experimental plan.

This report summarises the information provided by the investigator and the feedback from the EDA.

Section 1: Summary

Title of EDA diagram	Example 5: Effect of THC on body temperature
Date report generated	25/05/2017

Section 2: Infomation provided by the investigator

1: Objectives

Null hypothesis	THC does not have an effect on body temperature
Alternative hypothesis	THC affects body temperature
Effect of interest	Difference in body temperature
Effect size	1 degree
Justification for effect size	biologically relevant, greater than circadian variation

2: Groups and sample size

Total number of animals in the experiment	24
Groups included in the primary analysis	3 groups:
• Group 1	role=control/comparator, n=8
• Group 2	role=test, n=8
• Group 3	role=test, n=8
Justification for sample size	power calculation for unpaired t-test (ES=1, SD=0.55, sig=0.05, power:0.9, 2-sided)

3: Randomisation and blinding

Experimental unit	animal

There is one step in this experiment where experimental units are allocated to groups:

• Allocation: randomisation

Randomisation strategy	complete randomisation
Randomisation procedure	EDA spreadsheet
Allocation concealment	treatments coded for individual animals

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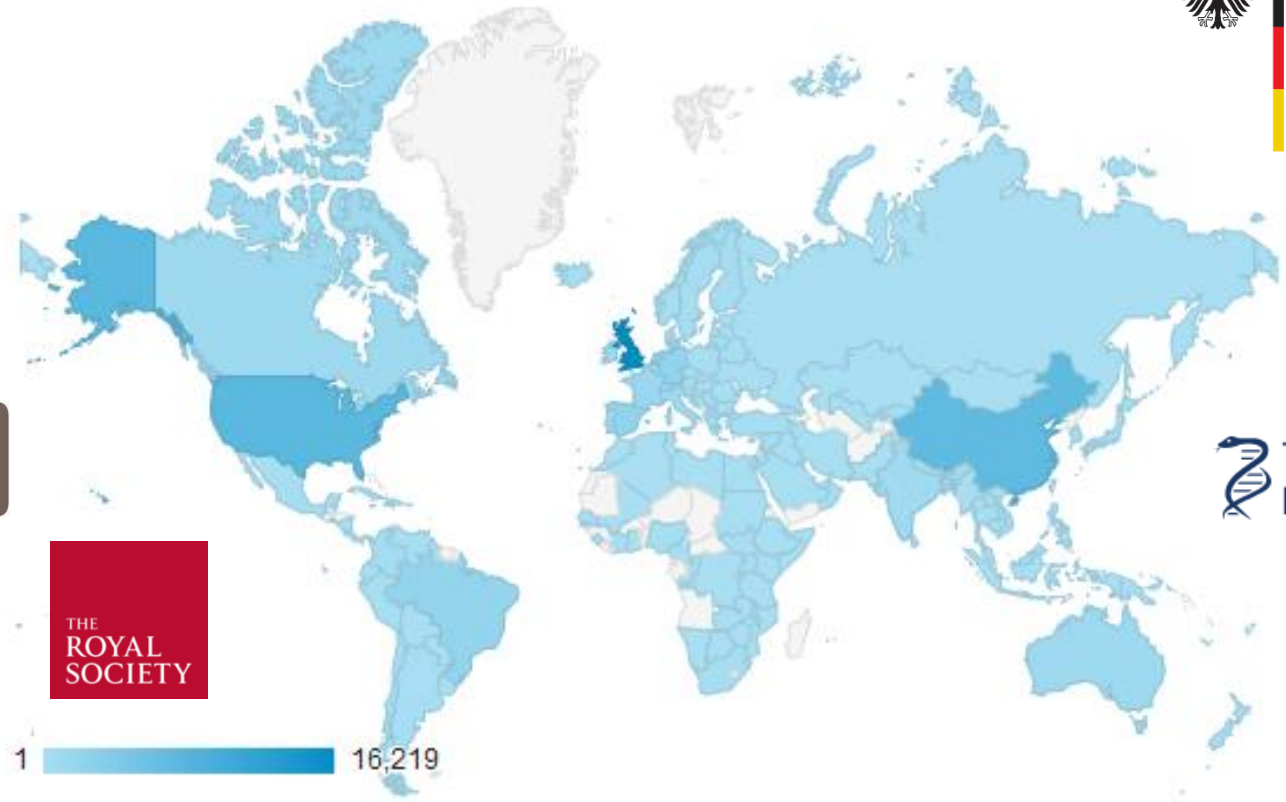
EDA Experimental Design Assistant

Page 1 of 3

Use of the EDA

7,700 accounts on the system, 3,000 website visits/month

Recommended by 79 organisations in 17 countries



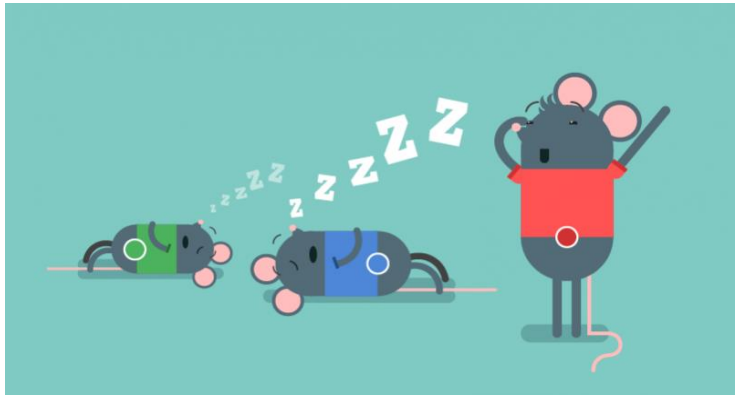
Percie du Sert *et al*. (2017) The Experimental Design Assistant. Plos Biol. 15, e2003779.

Rodent Little Brother: Secret Lives of Mice

- A **citizen science** project based on the Rodent Little Brother CRACK IT Challenge.
- Home Cage Analyser system enables continuous 24/7 recording of the behavior of individual mice, group-housed in a standard home cage:
  - Improves welfare assessment.
  - Reveals subtle consequences of genetic alterations.

However:

- Need to develop algorithms for data analysis, based on human observations.
- Volunteers watch 6s video clips.



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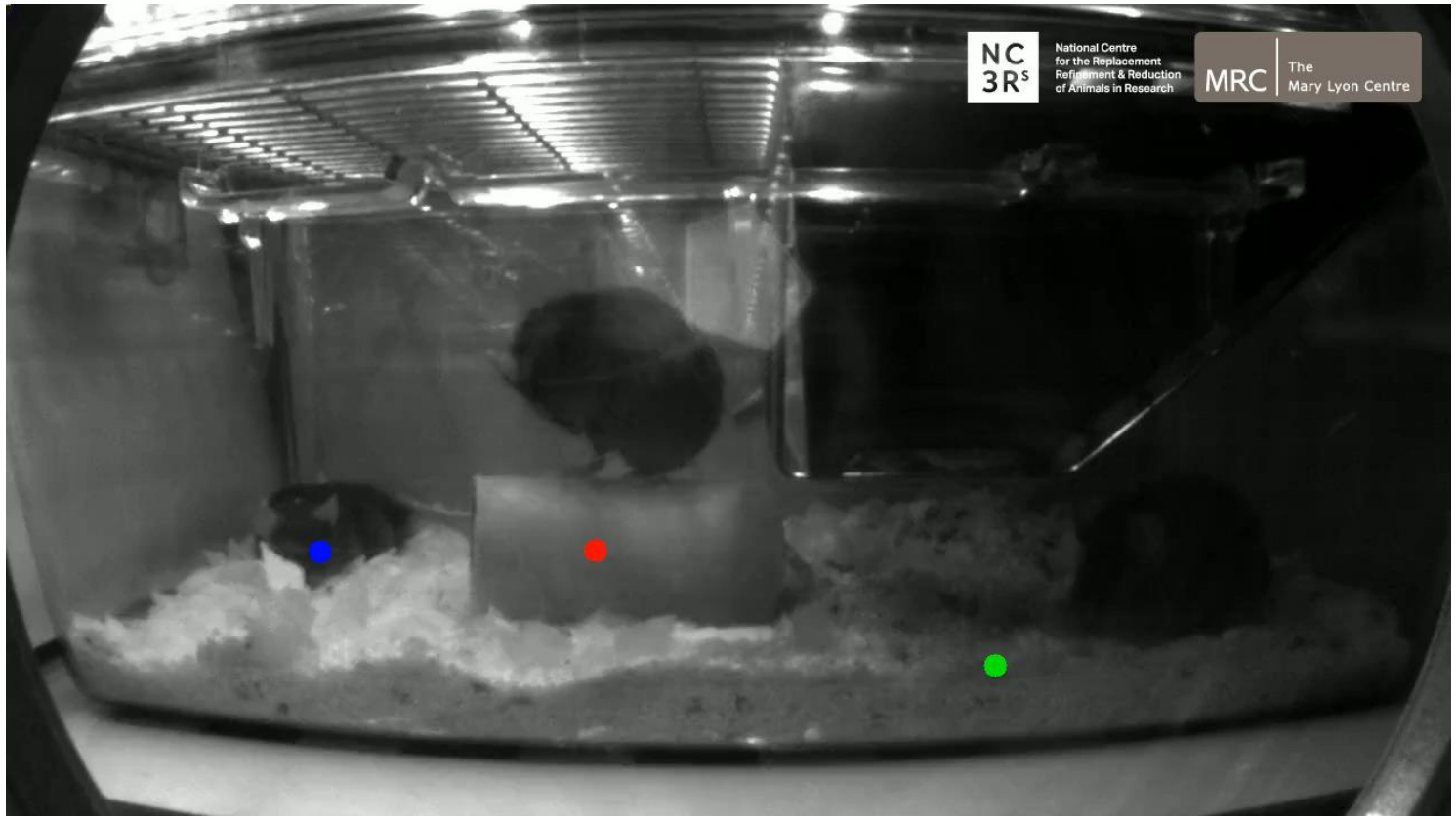
MRC The Mary Lyon Centre

NC 3Rs

https://www.zooniverse.org/projects/r-dot-bains/rodent-little-brother-secret-lives-of-mice

Secret Lives of Mice



Excellent blog on the NC3Rs website from Emma Robinson

https://www.nc3rs.org.uk/news/help-us-discover-more-about-secret-lives-mice

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Coming soon

Pioneering Better Science

3Rs self-assessment tools

- Framework which allows research organisations to benchmark their 3Rs activities and progress – comprised of six main categories
- Second, shorter, simpler framework for individual research groups
- Online tools, with the functionality to map scores and provide guidance and examples on how to improve
- Use of the tools will be voluntary and confidential

Leadership: taking a strategic approach

People: ensuring the right culture

Research and infrastructure: supporting the best science

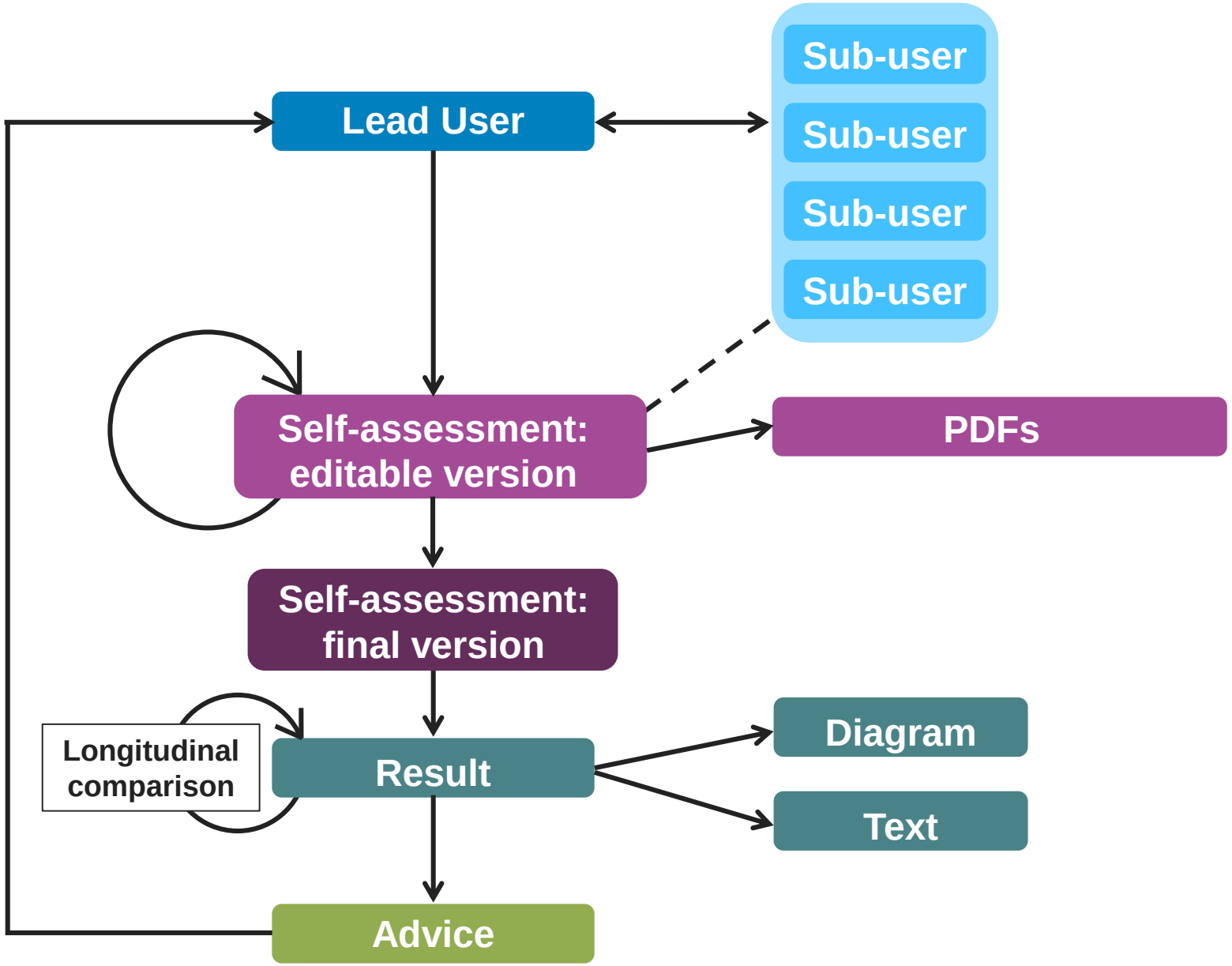
Experimental design: ensuring robust and reproducible experiments

Training: building capability

External dissemination: publications and the wider audience

NC 3Rs

Workflow


Sub-user
Sub-user
Sub-user
Sub-user
Lead User
Self-assessment: editable version
PDFs
Self-assessment: final version
Longitudinal comparison
Result
Diagram
Text
Advice

Here today ……

Mark Prescott

Director of Policy and Outreach

Nathalie Percie du Sert

Head of Experimental Design and Reporting

Emma Stringer

Regional Programme Manager – Midlands

Eleanor Humphrey

Science Manager – Technology Development