How the AWB can help with reproducibility

PENNY HAWKINS

RSPCA RESEARCH ANIMALS DEPARTMENT

Photo: RSPCA

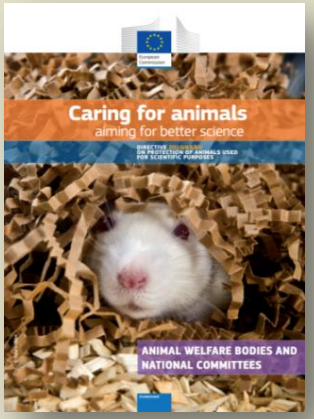
RSPCA Research Animals Department works to:

- Promote **replacement**, **reductions** in numbers and suffering, and improvements in **welfare** while animal use continues
- Ensure that **ethical issues** are raised and addressed
- Promote critical and open **harm-benefit analysis** of animal use
  - reproducibility is critically important, as it directly impacts on benefit

Four key RSPCA work areas …

- **Severe suffering** project, with LASA, LAVA and IAT (here)
- Promoting **effective regulation**, UK and internationally
- **Getting more welfare and ethics** into education and training
- Work to promote effective **ethics committees** and **Animal Welfare Bodies (AWBs)**

Why the AWB is a good thing



BENEFITS FOR ANIMALS, SCIENCE AND STAFF, AS SET OUT IN EC WORKING DOCUMENT (HERE)

- Provides motivation and support for animal welfare and the Three Rs
- Provides advice on the project application process
- Improves communication between scientists and animal technologists
- Fosters a good culture of care
- Improves public confidence in the quality of scientific work and care
- Improves the quality of science …

Pooling of Animal Experimental Data Reveals Influence of Study Design and Publication Bias

Malcolm R. Macleod, PhD; Tori O'Collins, BSci; David W. Howells, PhD; Geoffrey A. Donnan, MD

OPEN ACCESS Freely available online PLOS | MEDICINE

Threats to Validity in the Design and Conduct of Preclinical Efficacy Studies: A Systematic Review of Guidelines for In Vivo Animal Experiments

Valerie C. Henderson[1], Jonathan Kimmelman[1]*, Dean Fergusson[2,3], Jeremy M. Grimshaw[2,3], Dan G. Hackam[4]

PERSPECTIVE

doi:10.1038/nature11556

A call for transparent reporting to optimize the predictive value of preclinical research

Story C. Landis[1], Susan G. Amara[2], Khusru Asadullah[3], Chris P. Austin[4], Robi Blumenstein[5], Eileen W. Bradley[6], Ronald G. Crystal[7], Robert B. Darnell[8], Robert J. Ferrante[9], Howard Fillit[10], Robert Finkelstein[1], Marc Fisher[11], Howard E. Gendelman[12], Robert M. Golub[13], John L. Goudreau[14], Robert A. Gross[15], Amelie K. Gubitz[1], Sharon E. Hesterlee[16], David W. Howells[17], John Huguenard[18], Katrina Kelner[19], Walter Koroshetz[1], Dimitri Krainc[20], Stanley E. Lazic[21], Michael S. Levine[22], Malcolm R. Macleod[23], John M. McCall[24], Richard T. Moxley III[25], Kalyani Narasimhan[26], Linda J. Noble[27], Steve Perrin[28], John D. Porter[1], Oswald Steward[29], Ellis Unger[30], Ursula Utz[1] & Shai D. Silberberg[1]

OPEN ACCESS Freely available online PLOS | BIOLOGY

Evaluation of Excess Significance Bias in Animal Studies of Neurological Diseases

Konstantinos K. Tsilidis[1☯], Orestis A. Panagiotou[1☯], Emily S. Sena[2,3], Eleni Aretouli[4,5], Evangelos Evangelou[1], David W. Howells[3], Rustam Al-Shahi Salman[2], Malcolm R. Macleod[2], John P. A. Ioannidis[6]*

Lost in Translation

Bumps in the Road Between Bench and Bedside

Leonard A. Levin, MD, PhD
Helen V. Danesh-Meyer, FRANZCO

TRANSLATIONAL MEDICINE—TURNING BASIC SCIENCE discoveries into innovative therapies—has led to re-

obtained opposite results.[4,5] The difference in methods for increasing the intraocular pressure was apparently minor—one group cauterized the episcleral veins of the eye,[4] the other used hypertonic saline to sclerose them.[5] Yet this difference was enough to lead one group to report that the drug was neuroprotective, the other to report it was not. The dem-

312 Practical Neurology

EDITORIAL

Pract Neurol 2010; 10: 312–314

Animal models of neurological disease: are there any babies in the bathwater?

Malcolm Macleod,[1] H Bart van der Worp[2]

OPEN ACCESS Freely available online PLoS MEDICINE

Research in Translation

Can Animal Models of Disease Reliably Inform Human Studies?

H. Bart van der Worp[1]*, David W. Howells[2], Emily S. Sena[2,3], Michelle J. Porritt[2], Sarah Rewell[2], Victoria O'Collins[2], Malcolm R. Macleod[3]

1 Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht, The Netherlands, 2 National Stroke Research Institute & University of Melbourne Department of Medicine, Austin Health, Melbourne, Australia, 3 Department of Clinical Neurosciences, University of Edinburgh, Edinburgh, United Kingdom

RSPCA

… which is critically important given current issues with reproducibility

Many issues with reproducibility could be resolved with the application of empathy and resources

- **This would minimise confounds associated with unrelieved pain and avoidable distress; prevent data being lost due to mortality**
- The AWB's task list effectively covers all this (and experimental design) – *if properly implemented*
- Vets can do a lot to help support the AWB and can also use the AWB to channel their concerns

Empathy – understanding animal biology and behaviour helps to improve welfare

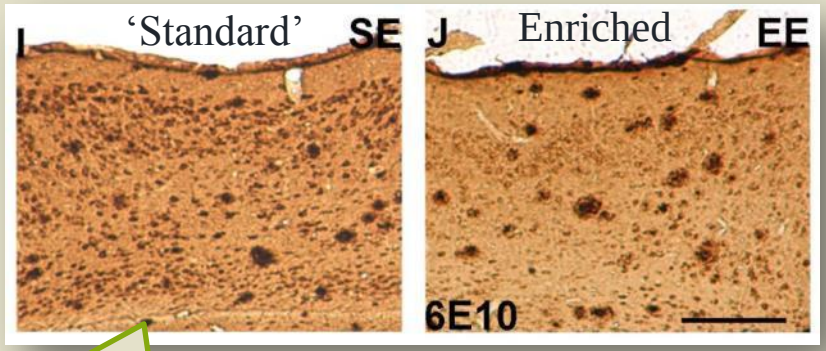
Mice and rats are …	… but
Nocturnal and crepuscular	they are housed in bright light and used during the day when they would normally be sleeping
Highly dependent on smell and scent markings	their markings are completely destroyed whenever the cage is cleaned
Made extremely anxious when captured by the tail (mice)	most people catch them by the base of the tail
Most comfortable at a temperature of 26 to 34 °C (mice)	many facilities house them at colder temperatures, and/or do not provide sufficient nesting material

Photos: Jane Hurst, Liverpool University

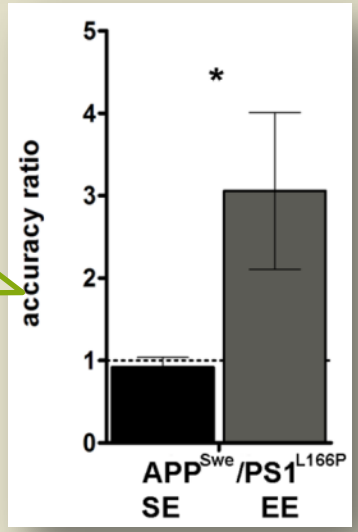
And better welfare = better science

Example: amyloid plaques

- $APP^{Swe}/PS1^{L166P}$ mice group housed in 'standard' conditions or 80x50x80 cm cage with several floors, wheels, refuges
- Enrichment transiently accelerated amyloid deposition but had protective effect on cognitive deterioration



Entorhinal cortex at 4 months



Average accuracy ratio at 6 months

Montarolo F et al. (2013) PLoS ONE 8(7): e69381

Author's analysis of these data

- Environmental factors can modulate symptoms and pathological progression in $APP^{Swe}/PS1^{L166P}$ mice
- Confirmed dissociation between amyloid burden and cognitive deterioration, as in human patients
- Reviewed and discussed other studies with different EE protocols – using enrichment as a tool to help understand effects of environment on pathology

Tasks of the AWB - 1

DIRECTIVE ARTICLE 27

(a) advise the staff dealing with animals on matters related to the welfare of animals, in relation to their acquisition, accommodation, care and use;

(b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep it informed of technical and scientific developments concerning the application of that requirement;

(c) establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment;

Tasks of the AWB - 2

DIRECTIVE ARTICLE 27

(d) follow the development and outcome of projects, taking into account the effect on the animals used, and identify and advise as regards elements that further contribute to replacement, reduction and refinement; and

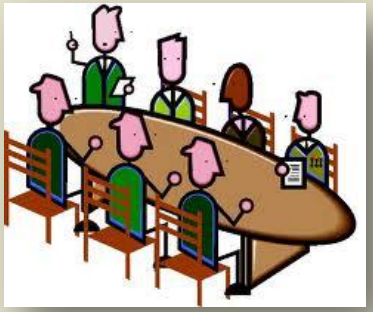
(e) advise on rehoming schemes, including the appropriate socialisation of the animals to be rehomed.

A point to note

- The AWB does not perform project evaluation - that is done by the competent authority
- … but the AWB's advice can help to ensure that proposed projects are justifiable, reproducible and conducted according to good practice

How do the AWB's tasks facilitate reproducibility, and how can the veterinarian contribute to the AWB?

The Animal Welfare Body



ARTICLE 26

The animal-welfare body shall include at least the person or persons responsible for the welfare and care of the animals and, in the case of a user, a scientific member. The animal- welfare body shall also **receive input from the designated veterinarian** (or the expert referred to in Article 25)

EC working document on AWBs and NCs

INVOLVING A WIDER MEMBERSHIP

Although the membership of the AWB does not require a veterinarian, their input is required under the Directive as it is considered to be very valuable. Therefore a number of Member States have mandated their formal inclusion in their AWBs.

UK ASPA: The NVS should be an active member of, and play a central role in, the AWERB at your establishment. (Note: At least one NVS at the establishment must be a full member of the AWERB.)

Tasks of the AWB - 1

ARTICLE 27

(a) advise the staff dealing with animals on matters related to the welfare of animals, in relation to their acquisition, accommodation, care and use;

(b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep it informed of technical and scientific developments concerning the application of that requirement;

(c) establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment;

EC guidance on task (a)

- AWB **should be the forum** at which new or revised practices relating to animal care and use can be considered and endorsed
- Developing local policies and standards including SOPs
  - refined admin and sampling; severity assessment; environmental enrichment
  - dealing with exceptions to SOPs (e.g. single housing)
- Disseminating information on standards and policies
  - standing agenda items, e.g. health reports from veterinarian

Tasks of the AWB - 1

ARTICLE 27

(a) advise the staff dealing with animals on matters related to the welfare of animals, in relation to their acquisition, accommodation, care and use;

(b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep it informed of technical and scientific developments concerning the application of that requirement;

(c) establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment;

EC guidance on task (b)

- Development of and input into guidance on application of the Three Rs for inclusion in the Education and Training Framework, including CPD
- Identification and dissemination on good practice on the Three Rs e.g. improvement of experimental design and optimisation of group sizes
- Ensuring Reduction and Replacement are specifically addressed … members with expertise in experimental design and developments in alternatives …

Tasks of the AWB - 1

ARTICLE 27

(a) advise the staff dealing with animals on matters related to the welfare of animals, in relation to their acquisition, accommodation, care and use;

(b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep it informed of technical and scientific developments concerning the application of that requirement;

(c) establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment;

EC guidance on task (c)

- Reviewing the effectiveness of systems in place to ensure that animals are adequately monitored
  - day to day monitoring cageside
  - observing and recording behaviour and clinical signs
  - using EC Working Document on Severity Assessment, including a 'team approach'

Tasks of the AWB - 2

ARTICLE 27

(d) follow the development and outcome of projects, taking into account the effect on the animals used, and identify and advise as regards elements that further contribute to replacement, reduction and refinement; and

(e) advise on rehoming schemes, including the appropriate socialisation of the animals to be rehomed.

EC guidance on task (d)

- Input at project planning and application stage can improve the quality of applications, ensure Three Rs implemented and check whether there are suitable facilities and expertise on site
- Mid-term project reviews can ensure projects are on track and identify Three Rs opportunities
- AWBs can work with project holders to tailor severity assessment

Three ways that vets can enhance the AWB

Photo: RSPCA

1. Understanding biology and behaviour

- Scientists are experts in their field, but many could learn more about their study species
- Veterinary expertise can improve the AWB's advice on 'matters relating to the welfare of animals', 'refinement', and 'technical and scientific developments'
- **This will reduce confounds due to animals being unable to express physiological and ethological needs**

2. Understanding harms to animals, and how to assess and alleviate these

- Vets can help to shift the AWB's thinking away from 'what will be done to the animal' to 'what will the animal experience?'
- Especially important for species where behaviour is difficult to interpret, or for cumulative severity
- Veterinary input into recognising, assessing and alleviating suffering is invaluable
- **This aids reproducibility by reducing confounds associated with unrelieved pain, or psychological distress**

3. Being an advocate for animals, including supporting animal technologists and lay members

- A specific perspective, to help ensure that the full spectrum of knowledge and opinion is included
- Vets have the authority to help other AWB members join discussions with confidence
- There may be an expectation that the vet will be a 'champion' for animals and 'make people think' (see tinyurl.com/y9mcha27)
- **Leadership skills, ability to influence and understanding of collective responsibility are all important for the AWB**

National Committees

ARTICLE 49

- Advise competent authorities and AWBs on matters dealing with the acquisition, breeding, accommodation, care and use of animals in procedures and ensure sharing of best practice
- Exchange information on the operation of AWBs and project evaluation and share best practice throughout the Union

Vets can help by supporting:

- Local AWBs
- Their National Committees (if they exist)
- National AWB networks, e.g. Netherlands, Belgium, UK
- Culture of Care network (see https://norecopa.no/alternatives/culture-of-care)

Take home messages …

- Thoughtful and robust implementation of the AWB's tasks would significantly help to solve the reproducibility crisis
- Take another look at the EC Working Document on AWBs and NCs
- You may like to reflect on your level of input into the AWB as a veterinarian, and how much support you receive
- If you want to become more involved, could you get further support from the 'person responsible for ensuring compliance', or ESLAV and ECLAM?