

Facts and Demonstrations: Exploring the effects of enrichment on data quality

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RSPCA strategy

- Challenge necessity and justification
- Where animals are used, implement the 3Rs:
 - Replace animals with humane alternatives
(Replacement – **the ultimate goal**)
 - Reduce suffering and improve welfare
(Refinement)
 - Reduce numbers of animals used
(Reduction)



Photo: RSPCA

Some ethical dilemmas for the RSPCA

- Veterinary vaccines and treatments
- Quarantine and rabies vaccination
- Badgers and TB - developing vaccines
- Developing alternative 'pest' 'control' strategies
- Wildlife rehabilitation



Photos: RSPCA

Broad work areas

- UK/EU law, international guidelines
- Training scientists and animal technologists
- Assessment and alleviation of pain, suffering and distress - especially 'severe' suffering
- 3Rs in biotechnology (genetic alteration)
- Developing processes of ethical review
- International work - e.g. eastern Europe and Asia
- Promoting high standards of husbandry and care



Photo: RSPCA

Definition of enrichment

- Enrichment is a **dynamic process for enhancing animal environments** within the context of the animals' behavioral biology and natural history. Environmental changes are made with the goal of **increasing the animal's behavioral choices and drawing out their species-appropriate behaviors**, thus enhancing animal welfare (Association of Zoos and Aquariums)
- Environmental enrichment is any modification to the environment of the captive animal that seeks to **enhance physical and psychological well-being** by **providing stimuli meeting the animals' species-specific needs** (Vera Baumans, 2000)

Some examples

- Group housing for social animals
- Three-dimensional environment
- Solid floor
- Nesting material
- Refuges
- 'Toys', manipulanda
- Foraging opportunities
- Positive interactions with humans
- Species-appropriate lighting
- Good quantity of space



Photo: Chris Sherwin

Some examples

- Group housing
- **Is this really 'enrichment', or should it be the baseline?**
- **'Environmental refinement' or 'environmental stimulation'?**
- Species-appropriate lighting
- Good quantity of space



Photo: Chris Sherwin

Why did I choose the topic of 'effects of enrichment on data quality'?

- Broad support for the concepts that 'better welfare = better science'
- ... and that environmental enrichment improves welfare
- **BUT ...**



Photo: RSPCA

Still some resistance to EE

- On economic grounds (as evidenced by some responses to new *US Guide*)
- Due to perception that there is insufficient proof that animals benefit (see above)
- Because of concerns that variability will increase, or a confound will be introduced, and data quality will be affected



Photo: LBS (Serving Biotechnology) Ltd

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Why might this be a problem?

1. Validity of the science within the project, as another variable will have been introduced
2. Comparability of data with previous studies conducted without enrichment
3. Potential for increases in animal numbers – refinement vs. reduction



Photo: RSPCA

John Ruskin (1819-1900)

The work of science is to substitute **facts** for appearances, and **demonstrations** for impressions



Photo: commons.wikimedia.org

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Photo: Novartis AG, Flickr Creative Commons

1: Scientific validity



- *US Guide*: Supports enrichment, presents as 'default' taking scientific goals into account
- Enrichment affects phenotype and may affect experimental outcome ... it should be considered an experimental variable and appropriately controlled
- Directive 2010/63/EU: Minimise restrictions on ability to satisfy physiological and ethological needs ... but exemptions allowed for scientific, health or welfare reasons

What is genuine scientific justification, and what is erring on the side of caution?



Photo: Novo Nordisk

Effects on variability

- Already studies and reviews in the literature
 - See handout for examples
- Effects that are significant
- Effects that are not significant
- No apparent effects at all
- Interpretation is important



Photos: AHVLA

Translatability/validity

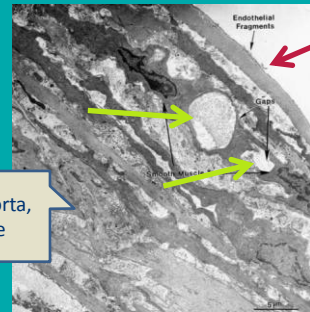
- Translatability of animal studies is coming under increasing scrutiny
- 'Standard' housing has been recognised as an issue in a number of fields
- CNS disorders:
 - 'One limitation of current approaches is that most studies are conducted on sedentary, unstimulated animals with unlimited access to food in the home cage, this leading to metabolic and physiological compromise'

Burrows EL & Hannan AJ (2013) CNS Neurol Disord Drug Targets 12: 587-592

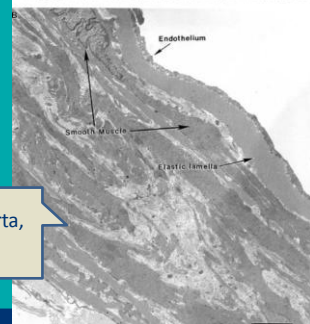
Validity: Fibulin-4^{+/-} mice

- Fibulin-4^{+/-} mice and wild types housed four/cage in 'standard' cages or 2/cage in larger cages, each with tunnel and wheel
- Arterial pathologies reduced by enrichment

Fibulin-4^{+/-} aorta,
standard cage



Fibulin-4^{+/-} aorta,
enriched cage



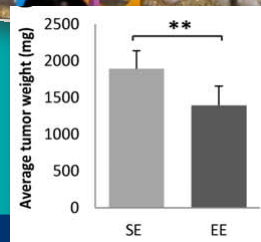
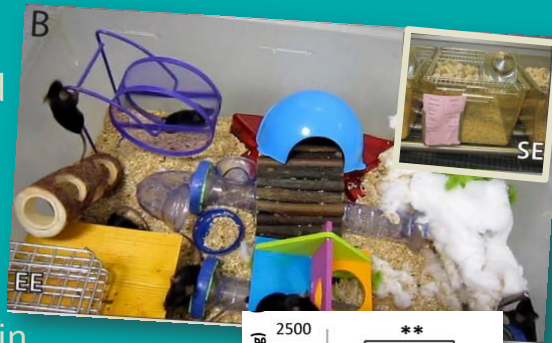
Cudilo E et al.(2007) PLoS ONE 2(2): e229. doi:10.1371/journal.pone.0000229

Authors' analysis

- Previous findings assumed to be due to genetic factors, but environmental factors may play a more important role than previously thought
- Different mechanisms suggested for environmental influence
- Suggestions for clinical benefit for human conditions, e.g. Marfan syndrome

Validity: tumour growth

- C57BL/6 mice housed in 'standard' or enriched cages
- Decrease in intratumoral COX-2 activity and increase in plasma ratio of adiponectin/leptin levels



Nachat-Kappes R et al. (2012) PLoS ONE 7(12): e51525. doi:10.1371/journal.pone.0051525

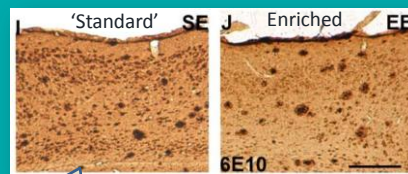
Authors' analysis

- Standard housing affects basic biological processes, such as mammary gland development and pathogenesis
- 'Many standard control rats and mice are sedentary, obese, glucose intolerant and on a trajectory to premature death' – could account for increased tumour growth in 'standard' mice
- Evidence for positive impacts of physical and social wellbeing

Martin et al. (2010) PNAS 107: 6127-6133

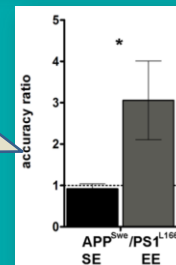
Validity: amyloid plaques

- 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. doi:10.1371/journal.pone.0069381

Authors' analysis

- 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

Mice are ...



Nocturnal and crepuscular	Housed in bright light
Highly dependent on small and scent markings	Markings are totally destroyed whenever cage cleaned
Sensitive to ultrasound	Many sources in lab
Feel secure when touching objects	Housed in barren cages

N Latham & G Mason (2004) *Appl. Anim. Behav. Sci.* 86: 261-289
 Castelhana-Carlos MJ & Baumans V (2009) *Laboratory Animals* 43: 311-327

Mice are ...



Photo: RSPCA

Master diggers	Have no opportunity to burrow
Highly social (sex and strain dependent)	Housed in inappropriate groups or singly
Capable of covering large distances	Housed in small cages
Omnivorous, trying new foods from different feeding sites	Fed boring, monotonous diets from hoppers

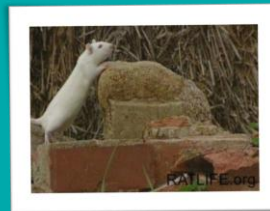
N Latham & G Mason (2004) *Appl. Anim. Behav. Sci.* 86: 261-289
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'Ratlife' DVD

- *The laboratory rat: a natural history*
- 'We may have taken the rat out of the wild, but have we taken the **wild** out of the **rat**?'

www.ratlife.org/



Photos: Manuel Berdoy/Ratlife



[Bar mousing video]

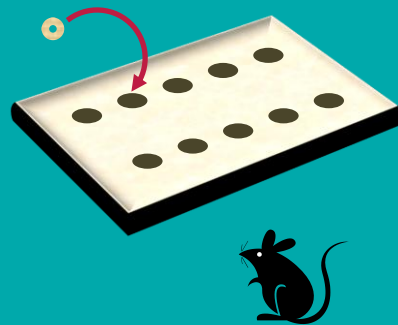
The bottom line

Inadequate housing and care, and unrefined procedures, can lead to stress and abnormal behaviours that are likely to be confounds in themselves

Photos: Naomi Latham; video: Hanno Würbel
<http://www.aps.uoguelph.ca/~gmason/StereotypicAnimalBehaviour/library.shtml>

Understanding the animal

- DBA mice were tested using a modified hole board
- Behavioural inhibition and cognitive disruption under white light, in comparison with dark/red light



Roedel A et al. (2005) *Laboratory Animals* 40: 371-381



Refining administration of substances

- Retinoic acid (RA) is used in developmental studies
- Traditionally administered by gavage
- When given in **chocolate treat**, bioavailability is increased & dose-dependent relationship observed



Photo: Mark Maconochie

Maconochie M et al. (2012) *Developmental Dynamics* 241: 741-758



Other benefits ...

Photo: Novartis AG, Flickr Creative Commons

Nest building behaviour

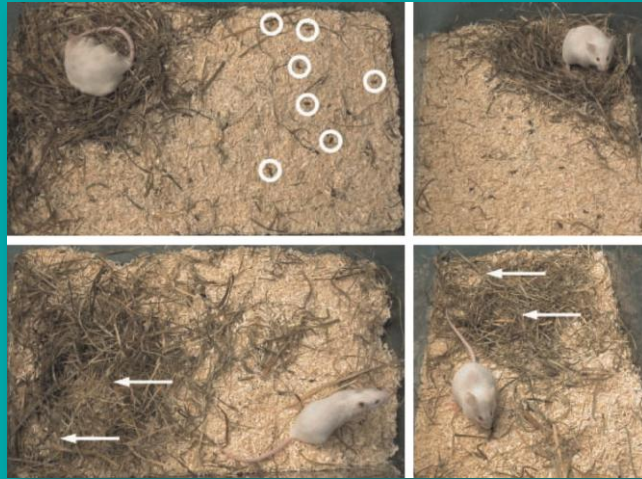


Photo: Arras M et al. 2007; <http://www.biomedcentral.com/1746-6148/3/16>

Other recent concepts ...

- Nest scoring in Alzheimer's research



- Burrowing behaviour
 - Increased latency to burrow with postoperative pain or when sick



Deacon R (2012) *J. Vis. Exp. (JOVE)* 59: e2607, doi:10.3791/2607

Jirkof P et al. *Front. Behav. Neurosci.* 4:165, doi: 10.3389/fnbeh.2010.00165

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Photo: RSPCA

2. Comparability

- May be perceived as obstacle to enrichment in regulatory toxicology
- Important to evaluate whether
 - data will be comparable
 - any differences will be systematic and can be taken into account
 - data quality will be improved
- The quality of the science should take precedence over comparability



Photo: Michael Brown

Regulatory toxicology



- OECD: 'proper conditions should be established and maintained for the storage, housing, handling and care of biological test systems'
- UK GLP: care, housing and containment should 'prevent stress and other problems which could affect the test system and hence the quality of data'
- ICH: recognises that data from unstressed animals will be of better quality
- In the experience of a major CRO, most clients will accept enrichment as long as there are no changes within studies

Analogy: Diving physiology

- Studies used to be conducted by restraining animals and forcibly immersing them
 - intense **bradycardia**
- Now telemetry is used to monitor animals diving freely
 - '**exercise-like**' response

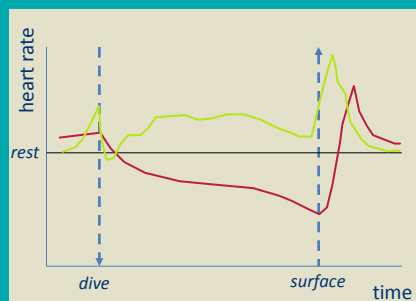


Photo: ChristopherWoo, Flickr Creative Commons

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Photos: MSD/RSPCA

3. An ethical issue



- Numbers may not increase, but if they do:
- Reduction isn't everything
- If suffering is reduced, or welfare improved, for each individual - then an increase in numbers may be justifiable
- Openness is key



UK public perceptions on animal experimentation



- 21 % are not bothered if animals are used in experiments
- 19 % agree that animal experiments should be banned
- 68% agree with animal use for all types of medical research, where there is no alternative
- 50% agree that 'animal experimentation for medical research purposes should only be for life-threatening diseases'
- 71% can accept as long as there is **no unnecessary suffering** to the animals

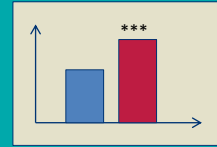
Polls by Ipsos-MORI, <http://www.ipsos-mori.com/>

Enrichment should be the norm – and the burden of proof should lie with those who propose to withhold it



Photos: Noldus Information Technology; RSPCA; LBS (Serving Biotechnology) Ltd

Conclusion: Facts ...



- Never assume that refinement will adversely affect results without evaluating whether this is the case, e.g. pilot studies
- Interpret findings of evaluation studies with an open mind
- **Different** does not mean **wrong!**
 - The data from animals in suboptimal housing may be the data that is 'flawed'
 - It may be possible to accommodate the differences
 - Consult literature, colleagues; use discussion groups
- Look critically at regulatory requirements and make sure they have not been 'over-interpreted'

*****Challenge the status quo!*****

... and demonstrations



- Include refinements, especially enrichment, in materials and methods sections of papers, posters and talks
 - See the ARRIVE guidelines
 - If it was not possible to refine for justifiable scientific reasons, **demonstrate why**
- Be aware that this is a constantly-evolving field; ensure that you maintain up to date knowledge
- Actively make sure that there are adequate information channels at your facility

www.NC3Rs/ARRIVE/
Photo: RSPCA



Thank you!

Photo: RSPCA