Implementation of refinement for laboratory species

Dr Chloe Stevens

What we will cover

- The 3Rs and refinement

- Why does refinement matter?

- The process of refinement
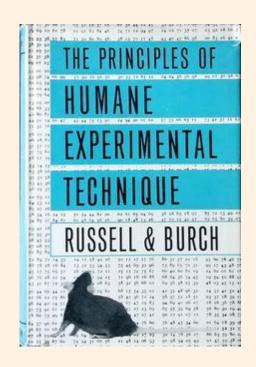
- Examples of good practice

- Useful resources

The 3Rs

Replacement: Methods which avoid or replace animal use

Reduction: Ensuring that the minimum number of animals is used to answer the scientific question

Refinement: Any measure that will reduce animal pain, suffering or distress, or improve welfare

The 'R' of refinement
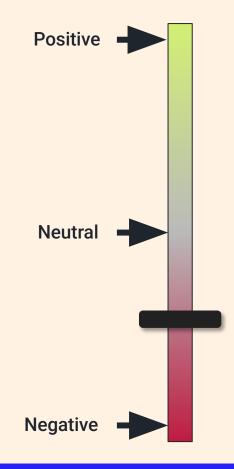
💉 Less invasive procedures

💊 Appropriate pain relief

🏠 Improved housing and care

🔍 Better welfare assessment

🛑 Appropriate humane endpoints

Positive

Neutral

Negative

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Why refine?

Why refine?

ETHICS

Ethics

"The question is not, 'Can they reason?' nor, 'Can they talk?' but, Can they suffer?"

Jeremy Bentham, 1789

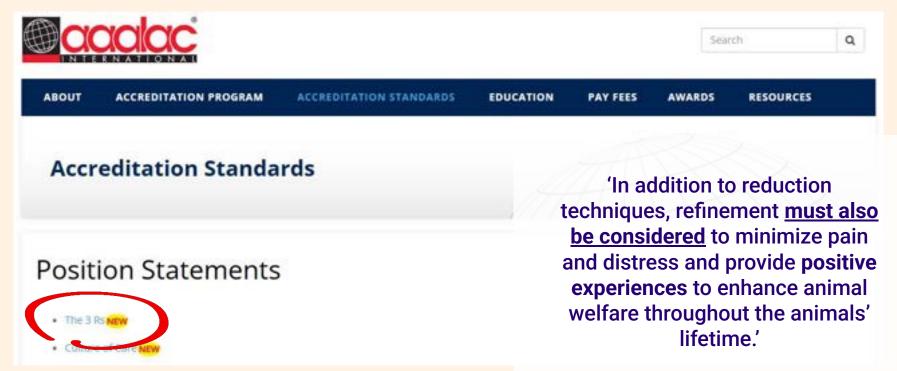*"Create all the happiness you are able to create; remove all the misery you are able to remove"*

Why refine?

Ethics

Legal/ regulatory

ANIMALS FOR SCIENTIFIC PURPOSES ACT,
B.E. 2558 (A.D. 2015).

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Standards and guidelines

Accreditation Standards

Position Statements
- The 3 Rs NEW
- Culture of Care NEW

'In addition to reduction techniques, refinement **must also be considered** to minimize pain and distress and provide **positive experiences** to enhance animal welfare throughout the animals' lifetime.'

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Why refine?

Ethics

Legal/ regulatory

Public opinion

Scientific outcomes

'Stressed animals do not make good research subjects'

American Medical Association

Happy animals make good science

Trevor Poole

Universities Federation for Animal Welfare, 8 Hamilton Close, Potters Bar, Hertfordshire EN6 3QD, UK
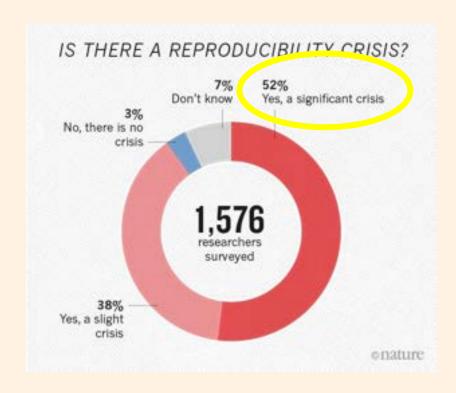
Better welfare = better science

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Stress in rodents affects…

Severity of disease model

Morbidity

Mortality

Thermoregulation

Response to treatments

Immune response

Pain response…

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Reproducibility

- The results of many scientific studies are difficult or impossible to replicate or reproduce on subsequent investigation - either by independent researchers or by the original researchers themselves.

- According to a 2016 poll of 1,500 scientists reported in the journal *Nature*:
  - **70%** had failed to reproduce at least one other scientist's experiment
  - **52%** agreed that there is a reproducibility crisis in science

IS THERE A REPRODUCIBILITY CRISIS?

7% Don't know

52% Yes, a significant crisis

3% No, there is no crisis

1,576 researchers surveyed

38% Yes, a slight crisis

nature
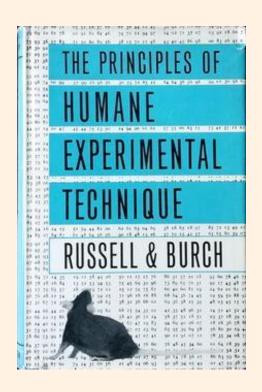
Risks of poor quality science

- More data variability

- More animals needed

- Resources wasted

- Poor translational value

- Safety tests could be flawed

- Treatments could be delayed for patient groups…

Why refine?

'it is widely recognised that the humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments'

Russell and Burch, **1959**

THE PRINCIPLES OF HUMANE EXPERIMENTAL TECHNIQUE

RUSSELL & BURCH

Science Museum, UK, c. 1961-1970

NC3Rs, UK

RISE, Sweden

How do we approach refinement?

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Important considerations about refinement

- Not just a 'one-off' at the project planning stage

- Not just a matter of following in-house refinement protocols

- Not just a matter of copying 'standard' welfare indicators, e.g. piloerection

Four key steps

1. What is 'normal'?

2. Identify potential sources of suffering

3. Identify ways to reduce or avoid suffering

4. Be able to assess welfare

Four key steps

1. **What is 'normal'?**

2. Identify potential sources of suffering

3. Identify ways to reduce or avoid suffering

4. Be able to assess welfare

What is 'normal'?

- Appearance/external anatomy
  - E.g. Coat, eyes, ears
  - E.g. Body condition
- Posture and gait
  - E.g. Ear position
- Behaviour
  - E.g. Activity, resting, feeding, drinking, social interactions
- Physiology
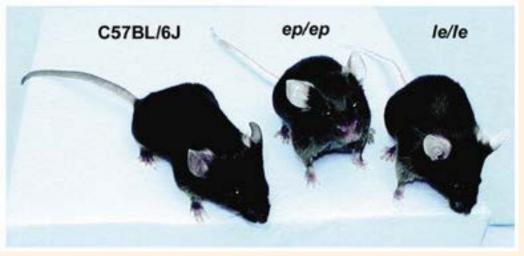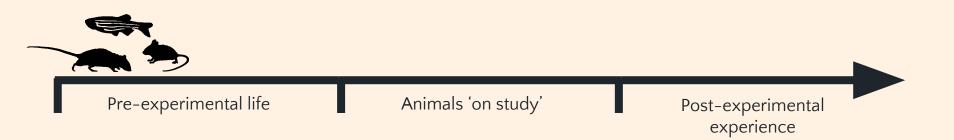 - E.g. Heart rate
  - E.g. Body temperature

What is 'normal'?

- Appearance/external anatomy
  - E.g. Coat, eyes, ears, fins
  - E.g. Body condition
- Posture and gait
  - E.g. Ear position, swimming patterns
- Behaviour
  - E.g. Activity, resting, feeding, drinking, social interactions
- Physiology
  - E.g. Heart rate
  - E.g. Body temperature
  - E.g. Opercular beat rate

What is normal?

- Can also vary depending on strain, age, sex…

Suzuki et al, 2002

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Challenging assumptions

- 'Animals are adapted to life in the laboratory, so it meets their needs'

- 'Plenty is known about their behaviour and welfare needs'

www.youtube.com/watch?v=giu5WjUt2GA
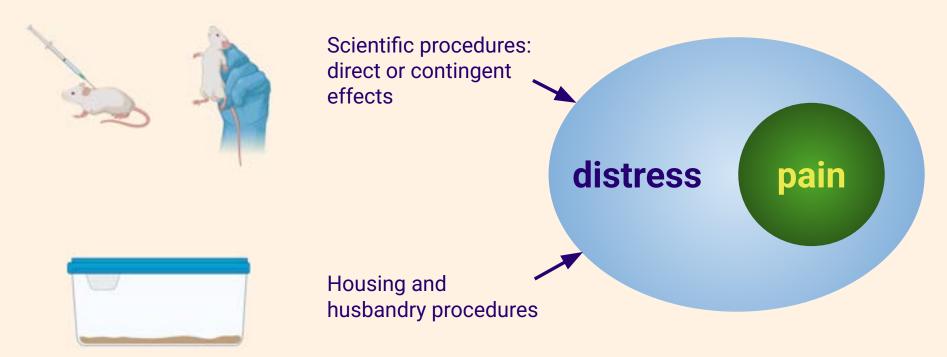
Four key steps

1. What is 'normal'?

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4. Be able to assess welfare

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Distress and pain

Scientific procedures: direct or contingent effects

Housing and husbandry procedures

distress pain

Lifetime experiences

Pre-experimental life | Animals 'on study' | Post-experimental experience

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Sources of suffering

Breeding

Capture from the wild

Transport

Marking for identification

Genotyping

Housing

Husbandry procedures

Handling and restraint

Scientific procedures

Effects of procedures

Humane killing

Rehoming or release

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focusonseveresuffering.co.uk/lifetime-experiences/

Thinking from the animal's perspective

Nocturnal and crepuscular

Omnivorous

Naturally fearful of humans

Highly dependent on smell

Sensitive to ultrasound

Motivated to dig

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Thinking from the animal's perspective

Cumulative effects

Severity

Procedure

Procedure

Procedure

The animal has become sensitised to the procedure and has not had enough time to recover between procedures 2 and 3

Time

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*"Even good laboratory conditions of
housing and husbandry will compromise the animal's well-being to some
degree..."*

UK Home Office 2015

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Life in the lab is inherently stressful

Some 'standard' cages *"provide mice and rats with a place to sleep but nothing really to do"*

Rodents in standard cages experience:

- higher severity for cancer, cardiovascular disease, stroke, anxiety…
- 50% higher chance of dying
- median lifespan reduced by 9%

Cait et al. BMC Biology   (2022) 20:15
https://doi.org/10.1186/s12915-021-01184-0

BMC Biology

RESEARCH ARTICLE   **Open Access**

Conventional laboratory housing increases morbidity and mortality in research rodents: results of a meta-analysis

Jessica Cait[1], Alissa Cait[2], R. Wilder Scott[3], Charlotte B. Winder[4] and Georgia J. Mason[1*]

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

ELSEVIER   journal homepage: www.elsevier.com/locate/applanim

How much 'enrichment' is enough for laboratory rodents? A systematic review and meta-analysis re-assessing the impact of well-resourced cages on morbidity and mortality

Jessica Cait[a,b], Charlotte B. Winder[b,c], Georgia J. Mason[a,b,*]

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Four key steps

1. What is 'normal'?

2. Identify potential sources of suffering

3. **Identify ways to reduce or avoid suffering**

4. Be able to assess welfare

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Aggregation of marginal gains

If you improve every area by even just 1%, those small gains can add up to remarkable improvement!

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Breeding

- Potential sources of stress:
  - Being paired with an unfamiliar animal
  - Separation of dams and litter
  - Disturbance during cage change

Breeding

- Consider appropriate social groupings
  - E.g. Breeding rats should be housed in stable pairs or trios, breeding female rabbits can be housed in groups with a male
- Review cage cleaning frequency
  - Rats – disturbance before pups are 2 days old can lead to cannibalism
  - Mice – pup mortality may be greater when cleaned out weekly compared with every 2 weeks
- Limit parental stress…

Applied Animal Behaviour Science
Volume 114, Issues 1–2, November 2008, Pages 235–247

Effects of cage-cleaning frequency on laboratory rat reproduction, cannibalism, and welfare

Charlotte C. Burn, Georgia J. Mason

The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages

C. K. Reeb-Whitaker, B. Paigen, W. G. Beamer, R. T. Bronson, G. A. Churchill, I. B. Schweitzer & D. D. Myers
The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA

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Transport

- Many institutions now send fresh or frozen embryos (e.g. mice), sperm (e.g. xenopus), eggs or early larvae (e.g. zebrafish) – avoids welfare problems associated with transport

- Consider acclimation periods
  - E.g. Rats transported by van for 5 hrs took 3 days for their heart rate, body temperature and activity patterns to return to normal
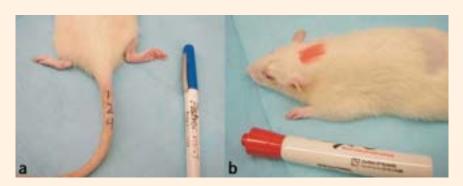
Marking for identification

- Potential source of stress:
  - Capture and restraint
  - Pain from invasive techniques

animals MDPI

Article

The Pen Is Milder Than the Blade: Identification Marking Mice Using Ink on the Tail Appears More Humane Than Ear-Punching Even with Local Anaesthetic

Charlotte C. Burn [1,*], Nur H. B. Mazlan [3,4], Natalie Chancellor [1] and Dominic J. Wells [2,*]

animals MDPI

Review

Refinement of Animal Experiments: Replacing Traumatic Methods of Laboratory Animal Marking with Non-Invasive Alternatives

Ilya Klabukov [1,2,3,4,*], Victoria Shestakova [1,2], Olga Krasilnikova [1], Anna Smirnova [1,2], Olga Abramova [1], Denis Baranovskii [1,3], Dmitri Atiakshin [5], Andrey A. Kostin [3], Peter Shegay [1] and Andrey D. Kaprin [3,3]

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Genotyping
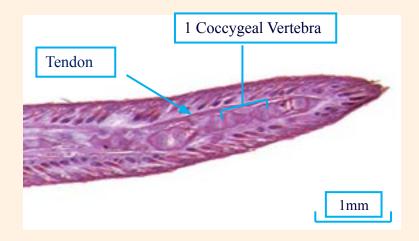
- Potential sources of stress
    - Capture and restraint
    - Pain from invasive techniques
    - Discomfort and stress from swabs

Genotyping

- Even at 2 weeks, there is some formation of bone within 1-2mm of tail tip of a mouse pup
  - Ear notching is considered more refined
- Take minimum amount of tissue
- Use local anaesthesia/topical analgesia
- Consider non-invasive approaches
  - E.g. swabbing, faecal pellets

1 Coccygeal Vertebra

Tendon

1mm

Genotyping
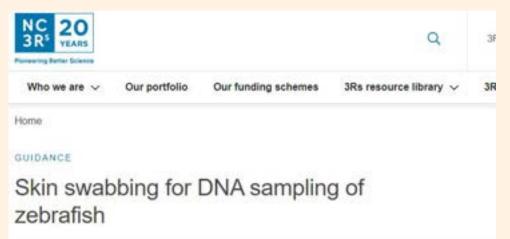
- Skin swabbing is a refined technique for genotyping zebrafish

Housing

- Potential sources of stress
    - Insufficient space
    - Lack of stimulation
    - Lack of control and choice
    - Environmental factors (noise, humidity, temperature, lighting, etc)
    - Inappropriate/unstable social groupings...

Housing - temperature

- Most facilities house mice at 20-24°C – up to 10°C *lower* than they prefer, and outside thermoneutral zone (29-34°C)

- Mice show increased aggression as temperatures increase from 20 to 25°C

- Provide all mice with 8-10g each of nesting material to allow them to control their microenvironment

Individually Ventilated Cages Impose Cold Stress on Laboratory Mice: A Source of Systemic Experimental Variability
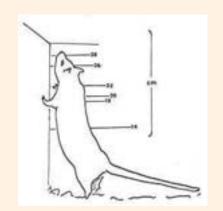
John M David,[1,*] Scott Knowles,[3] Donald M Lamkin,[2] and David B Stout[1]

Housing - space

- Rearing is an **important exploratory behaviour** for many rodents – adult rats can rear up to 30 cm

- Recommended minimum cage height for rats in 'the Guide for the Care and Use of Laboratory Animals (8th Ed) is 17.8 cm

ROYAL SOCIETY OPEN SCIENCE

rsos.royalsocietypublishing.org

Research

Cite this article: Makowska IJ, Weary DM. 2016 The importance of burrowing, climbing and standing upright for laboratory rats. R. Soc. open sci. 3: 160136.
http://dx.doi.org/10.1098/rsos.160136

The importance of burrowing, climbing and standing upright for laboratory rats

I. Joanna Makowska and Daniel M. Weary

Animal Welfare Program, University of British Columbia, 2357 Main Mall, Vancouver, British Columbia, Canada V6T 1Z4

IJM, 0000-0001-8873-1008

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Housing

This is how we house our rats, in modified former rabbit cages.

Housing - enrichment

Review
Environmental Enrichment for Rats and Mice Housed in Laboratories: A Metareview

Anna S. Ratuski and Daniel M. Weary

Housing - enrichment

How much 'enrichment' is enough for laboratory rodents? A systematic review and meta-analysis re-assessing the impact of well-resourced cages on morbidity and mortality

Jessica Cait [a,b], Charlotte B. Winder [b,c], Georgia J. Mason [a,b,c]

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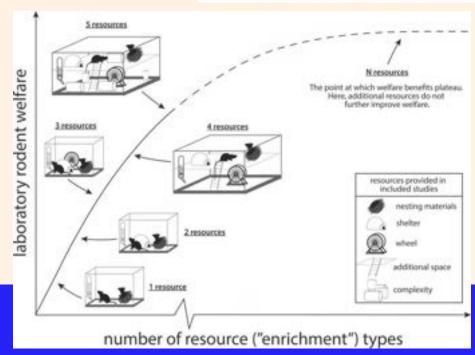
Home > 3Rs resource library

HUSBANDRY

Evaluating environmental enrichment

Supporting technicians in assessing the welfare impact of new enrichment.

nc3rs.org.uk/3rs-resources/evaluating-environmental-enrichment

The 3Hs Initiative

Housing, Handling and Habituation

Improving the lives of laboratory rodents one lab at a time

Framework Concept

To focus on the lifetime experience of laboratory mice and rats and methods which increase their positive affective experiences and reduce cumulative suffering.

Housing

Housing of laboratory mice and rats is constrained by many factors important to both managing their welfare and being able to carry out scientific procedures.

Table of Contents

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HOUSING
HANDLING
HABITUATION

Original Article

The use of ball pits and playpens in laboratory Lister Hooded male rats induces ultrasonic vocalisations indicating a more positive affective state and can reduce the welfare impacts of aversive procedures

Justyna K Hinchcliffe , Megan G Jackson , and Emma SJ Robinson

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Housing

UNIT FOR LABORATORY ANIMAL MEDICINE
UNIVERSITY OF MICHIGAN

Photo: Anna Ratuski

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Handling

Handling

Handling

Handling

An introduction to effective handling techniques for labratory rodents.

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Restraint

Habituation

Habituation

An introduction to animal habituation.

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Positive reinforcement training

- Training animals with rewards to encourage desired behaviours

- E.g. climbing on scales for weighing, moving into equipment to be transported, voluntary oral dosing, sample collection

- Can be used in a range of species

Scientific procedures

- Route and site of injections
- Properties of the substance (e.g. temperature, sterility, toxicity, irritancy, concentration)
- Dose volume, timing and frequency
- Aseptic surgical technique
- Appropriate use of anaesthesia/analgesia
- Effective welfare assessment
- Nutritional support (e.g. wet mash, jelly)
- Additional nesting material
- Early humane endpoints
- Humane killing

More ideas…

THINKING ABOUT THE LIFETIME EXPERIENCES OF EACH ANIMAL

There is huge potential for reducing suffering and improving welfare by thinking carefully about how every event might be experienced by the animal and how each one can be optimally refined.

Breeding ›

Capture from the wild ›

Transport ›

Marking for identification ›

Genotyping ›

Animals may find it disorientating to be taken from their home cage and placed, maybe with unfamiliar individuals, into a confined container. They will likely be distressed if they experience vibrations, a wider range of temperatures, noise and new smells, e.g. whilst in a vehicle or airplane. They will be met by unfamiliar animal care staff.

Examples of actions: Where possible, send eggs, sperm or embryos; minimise journey duration, noise and vibration; review reception, quarantine and health screening protocols. Plan and liaise with colleagues to minimise moving animals within the animal house unless this is for essential scientific or animal welfare reasons, e.g. to an exercise area.

Husbandry procedures ›

Handling and restraint ›

Effects of procedures ›

Humane killing ›

Housing ›

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focusonseveresuffering.co.uk/lifetime-experiences/

Four key steps

1. What is 'normal'?

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3. Identify ways to reduce or avoid suffering

4. Be able to assess welfare

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Welfare assessment

- Monitoring animals for signs of pain, suffering and distress associated with procedures and their effects

- Day-to-day assessment of all animals to see how they're coping with their environment and to detect health and welfare issues

Detecting distress

- This can be really difficult!

- Some species may conceal signs of pain or distress

- Many animals will alter their behaviour when being observed

- We might be focussing on the wrong things

Misunderstood: research shows link between dog attacks and 'misunderstanding of dog behaviour'

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Detecting distress

- This can be really difficult!

- Some species may conceal signs of pain or distress

- Many animals will alter their behaviour when being observed

- We might be focussing on the wrong things

Do "Prey Species" Hide Their Pain? Implications for Ethical Care and Use of Laboratory Animals

Larry Carbone

Independent scholar; 351 Buena Vista Ave #703E, San Francisco, CA 94117, USA

larrycarbonedvm@gmail.com

In-tank underwater cameras can refine monitoring of laboratory fish

T Ellis*[1], GSE Rimmer[1], S-J Parker[1], C Joiner[1], M Sebire[1], DW Verner-Jeffreys[1] and J Lines[2]

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Detecting distress

- This can be really difficult!

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- We might be focussing on the wrong things

PLoS one
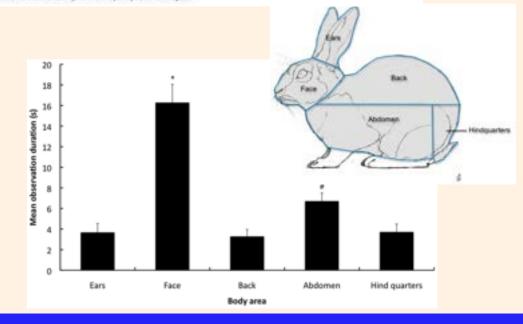
Are We Looking in the Wrong Place? Implications for Behavioural-Based Pain Assessment in Rabbits (*Oryctolagus cuniculi*) and Beyond?

Matthew C. Leach*, Claire A. Coulter, Claire A. Richardson, Paul A. Flecknell
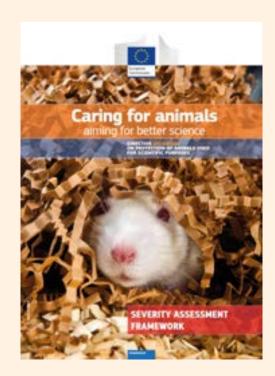
Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, United Kingdom
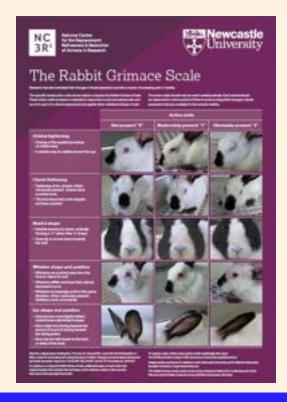
Welfare assessment
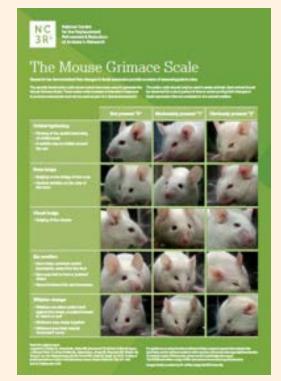
- **Appearance**, including body, coat and skin condition; for example unkempt coat, porphyrin staining

- **Body functions**, such as reduced food intake, changes in body temperature

- **Environment** within the enclosure; for example, nest quality, consistency of faeces

- **Behaviours**, including social interaction, posture, gait, and undesirable behaviours such as stereotypies

- **Procedure-specific indicators**, for example, tumour size in cancer studies

- **Free observations**, for observers to enter their own text should they see an indicator of suffering that was not predicted

Caring for animals
aiming for better science

SEVERITY ASSESSMENT FRAMEWORK

Grimace scales

Body condition scoring

RSPCA

Nest building

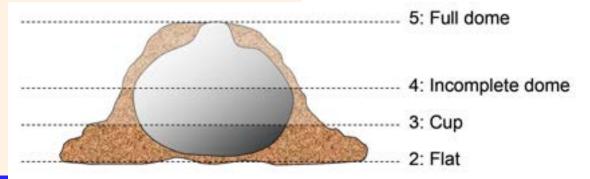## Nest Building as an Indicator of Health and Welfare in Laboratory Mice

Brianna N. Gaskill,[1] Alicia Z. Karas,[2] Joseph P. Garner,[3,4] and Kathleen R. Pritchett-Corning[1]

• Author information • Copyright and License information   PMC Disclaimer

Abstract

Go to: ▸

The minimization and alleviation of suffering has moral and scientific implications. In order to mitigate this negative experience one must be able to identify when an animal is actually in distress. Pain, illness, or distress cannot be managed if unrecognized. Evaluation of pain or illness typically involves the measurement of physiologic and behavioral indicators which are either invasive or not suitable for large scale assessment. The observation of nesting behavior shows promise as the basis of a species appropriate cage-side assessment tool for recognizing distress in mice. Here we demonstrate the utility of nest building behavior in laboratory mice as an ethologically relevant indicator of welfare. The methods presented can be successfully used to identify thermal stressors, aggressive cages, sickness, and pain. Observation of nest building behavior in mouse colonies provides a refinement to health and well-being assessment on a day to day basis.

5: Full dome

4: Incomplete dome

3: Cup

2: Flat

Score sheets

- Standardise and formalise welfare assessment

- Aid in record-keeping

- Should be tailored to research protocol and to animals to be scored

- Clear criteria for taking action

	Score 0	Score 1
Observation of the animal		
Spontaneous behavior	Sleeping, resting, digging, running, walking, rearing, climbing, eating, drinking, grooming, sniffing	Sudden movements, backwards movements, transient involuntary muscular contraction of any body part, kicking with hind paws, licking/biting the wound
Posture	Lying, sitting, moving.	Hunched, arched back, crouched
Breathing	Undisturbed, regular	Exerted, irregular
Coat condition	Clean, smooth, well-groomed	Ruffled, dirty, unkempt, piloerection, hair loss (alopecia)
Eyes	Clear, bright	Discharge
Body condition	Good, unchanged as judged from external appearance	Sunken flanks, swollen areas, ascites
Wound	Clean, dry, smooth	Dirty, bloody, uncleaned, signs of self-injury, signs of inflammation or necrosis, i.e., unusual color (e.g., red, pale) or swollen
Behavior after provocation/weighing	Alert, ready to take flight	Apathetic, sedated, highly aggressive, increased vocalization
Movement after provocation/weighing	No aberration in moving pattern	Decelerated/slowed, crawling, immobile, lameness, tiptoe gait
Appearance of cage		
Condition of nest	A nest clearly identifiable	Either no nest identifiable or multiple fragmentary nest-like resting places at different locations
Condition of territory	Cage area clearly structured, i.e., obvious areas for defecation and sleeping	Areas of defecation and sleeping indistinguishable, feces either adhering to nesting materials or not visible
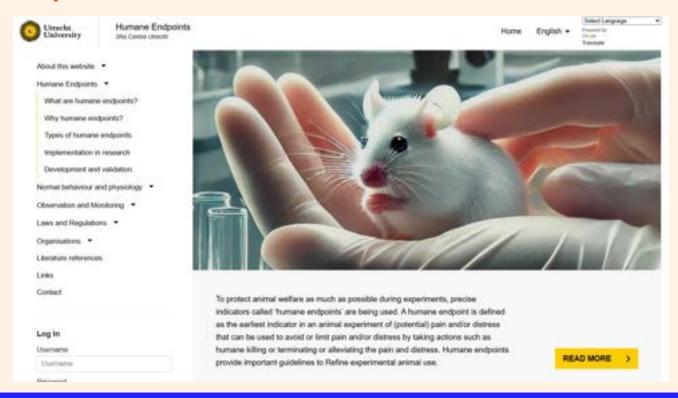
Humane Endpoints

General tips for welfare assessment

- Tailored to species and procedure
- Ensure understanding of normal physiology and behaviour
- Add scientific parameters if appropriate
- Think about how indicators will be used in practice (don't cut and paste!)
- Involve scientist, vets and technicians
- Ask IACUC for guidance
- Consider a 'welfare pilot study'
- Include final welfare assessment protocol in publications

Resources

THE ROADMAP TO REDUCING SEVERE SUFFERING

CULTURE → ANALYSIS → EVALUATION → IDENTIFY ISSUES → OVERCOME OBSTACLES

Practical aspect

APPROACHES TO REFINEMENT
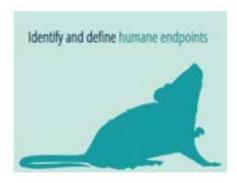
Refinement begins with reviewing and considering the lifetime experiences of each animal at the project planning stage, with input from people with different expertise including the scientist, the veterinarian, and animal technologists. The AWERB, AWB, IACUC or AEC should be able to provide useful input.

MAJOR AIMS ARE TO:

Identify as many sources of pain, suffering or distress (harms) as possible, so that refinements can be researched and included in experimental protocols and within housing, husbandry and care

Identify opportunities to improve welfare

Determine indicators of suffering that are tailored to the species, strain (if appropriate) and procedure for day-to-day welfare assessment

Identify and define humane endpoints

focusonseveresuffering.co.uk/refinement/

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Predicted lifetime experiences (not including procedures, which are addressed in sheet 2)

Project licence number	
Protocol number	

Factor	Experience of the animal	Welfare issues	Ways of mitigating these
Sourcing			
Transport			
Identification			
Genotyping			
Housing and environment			

FOCUS ON SEVERE SUFFERING

Focus on procedures

| Project licence number | |
| Protocol number | |

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects and indicators of these	Methodology and interventions	Humane endpoints

focusonseveresuffering.co.uk/roadmap/

norecopa

Search:

About Norecopa   Alternatives   Databases & Guidelines   Education & training   Legislation   Meetings   More resources   News   PREPARE   Species   Wiki

PREPARE

PREPARE checklist

Comparison with ARRIVE

Endorsements

Film

1-Literature searches

2-Legal issues

PREPARE

The PREPARE Guidelines, and this section of the Norecopa website, have been developed with the involvement and support of the RSPCA.

As part of ongoing efforts to reduce waste, promote animal alternatives (all the three Rs), and increase the reproducibility of research and testing, a group of experts from the UK and Norway, led by Norecopa, has produced a set of guidelines for **planning** experiments:

PREPARE *(Planning Research and Experimental Procedures on Animals: Recommendations for Excellence)*

PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

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Science Museum, UK, c. 1961-1970

NC3Rs, UK

RISE, Sweden

RSPCA

Summary

- Think about how all the different **events impact on the animals you are interacting with**

- **Never assume** that current or established methods are the best, either for the science or the animal

- There is great potential to make an **immediate difference** as improvements can often be made quickly, easily and cheaply

- Refinement is a **team effort** between technicians, vets and scientists

- **Animal welfare** and **science** will both benefit

- **There is always more we can do**

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Thank you

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