The R of Refinement: still a “Cinderella”?

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Why Cinderella?

Cinderella is somebody or something that is suddenly raised from obscurity to honour or importance.

The New Penguin English Dictionary (2000)

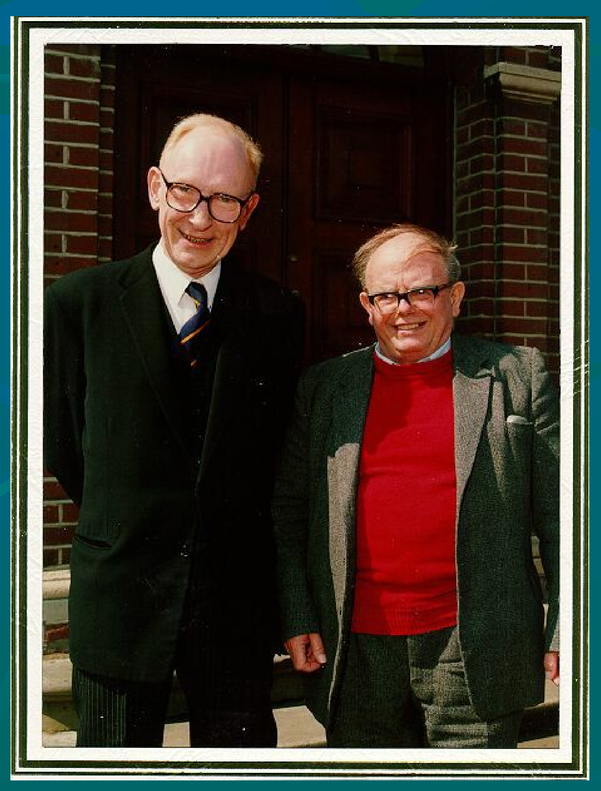
Welfare of Laboratory Animals - Role of Refinement?

Hypothetical ‘ideal’ level of welfare:

The state of being in animals when their nutritional, environmental, health, behavioural and mental needs are met

Hawkins P et al. A guide to defining and implementing protocols for the welfare assessment of laboratory animals. Laboratory Animals 45, 1-13, 2011

Russell and Burch
The Principles of Humane Experimental Technique, 1959



- Replacement
- Reduction
- Refinement

Refinement:

” Simply to reduce to an absolute minimum the amount of distress imposed on those animals that are still used”.

Russell and Burch, 1959

Aim of Refinement

- Enhance animal well-being
- Improve scientific quality of animal experiments

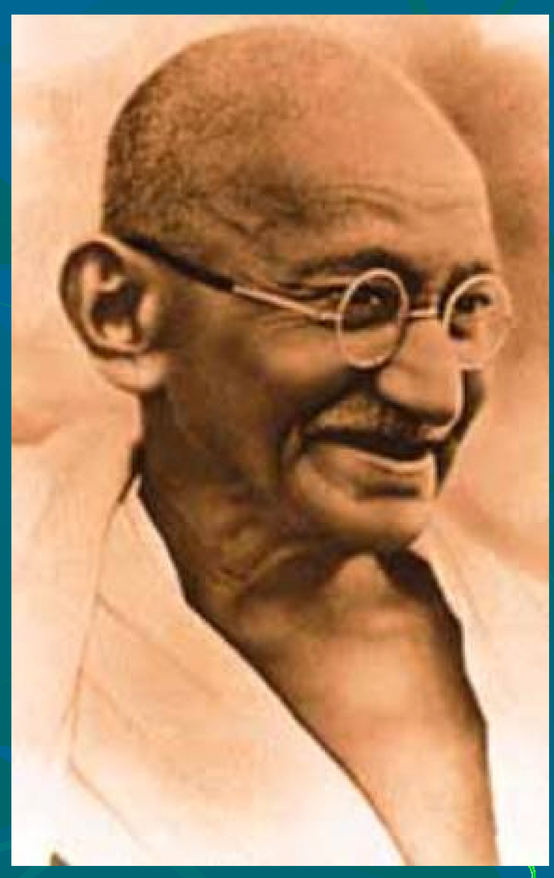
Why is Refinement important?

- Animal welfare
- Scientific results
- Legal obligation
- Judgement ethical admissibility

Legislation

The greatness of a nation and its moral progress can be judged by the way its animals are treated.

Gandhi



Directive 2010/63/EU: Procedures
Article 13: Choice of methods

- Par 1:
  A procedure shall not be carried out if another method not entailing the use of a live animal is recognised
- Par 2:
  In choosing between procedures, those shall be selected which:
  - Use the minimum number of animals
  - Involve animals with the lowest capacity to experience discomfort
  - Cause the least discomfort
- Par 3:
  Death as an endpoint shall be avoided as far as possible and replaced by early and humane endpoints

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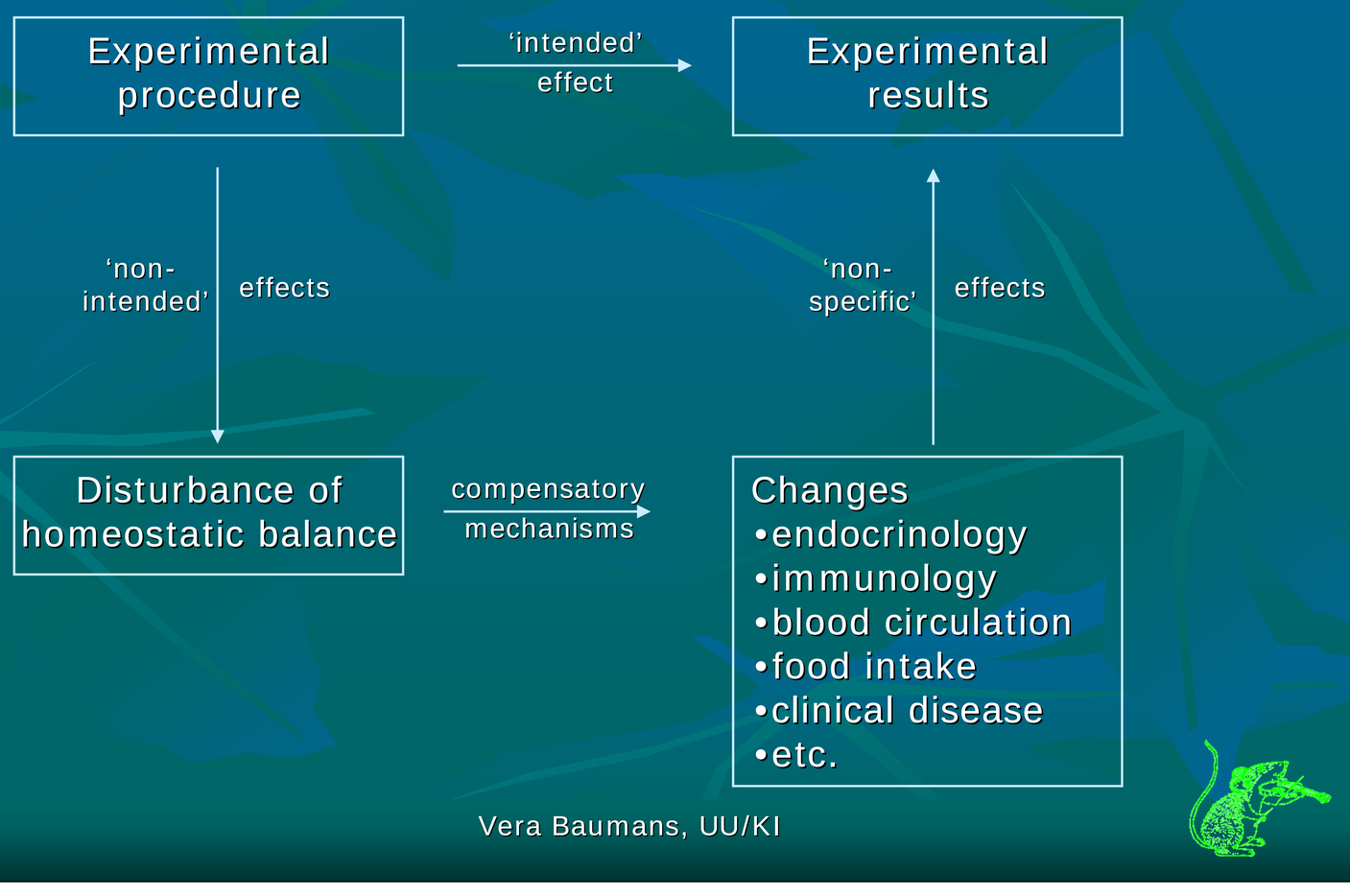
Intended and non- intended effects of procedures on experimental results



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Non- intended effects

- Environmental factors
- Pain, discomfort, distress
- ……..

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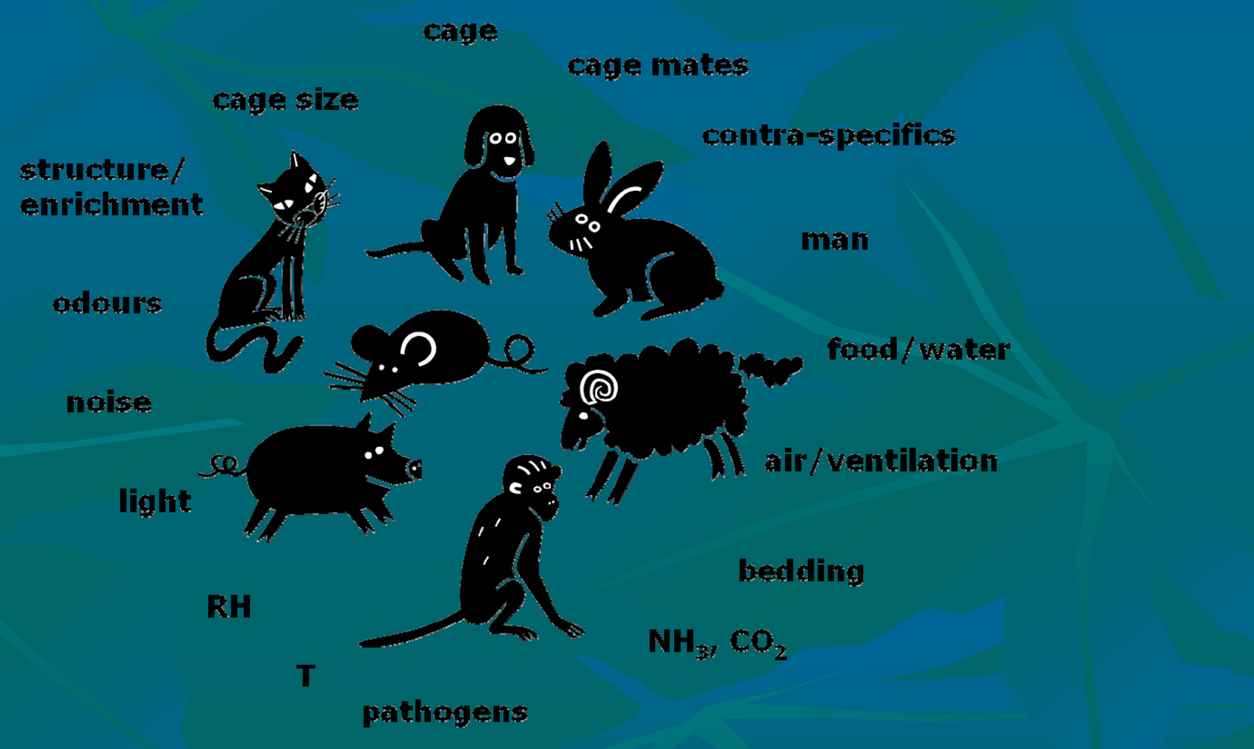
Environmental factors affecting laboratory animals



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Objectives of Refinement

- Reducing negative states
- Promoting positive mental and physical states
  - Knowledge of biological characteristics of animals and translation into housing, husbandry, nutrition etc
  - Improving performance of experimental procedures

Buchanan-Smith HM et al (2005). Anim.Welfare 14, 379-384
Verwer C (2011). Thesis Utrecht University

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Refinement = decrease in discomfort

- Translation biological characteristics/ needs of animals into husbandry e.g. environmental refinement/enrichment
- Adequate assessment of pain/discomfort
- Humane endpoint
- Anaesthesia/analgesia
- Improvement procedures e.g. handling, training, injections, surgery
- Skills researcher
- Adequate care

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Individual housing

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Pair housing

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Group housing

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Environmental Refinement / Enrichment

Any modification in the environment of the captive animal that seeks to enhance its psychological and physiological well being by providing stimuli, meeting the animal's species specific needs.

Newberry 1995,
Baumans 2000

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Environmental Refinement / Enrichment

- Complexity
- Opportunity to perform highly motivated behaviours e.g. social contacts, nest building, exploration, foraging
- Predictability
- Controllability

} Stress ↓

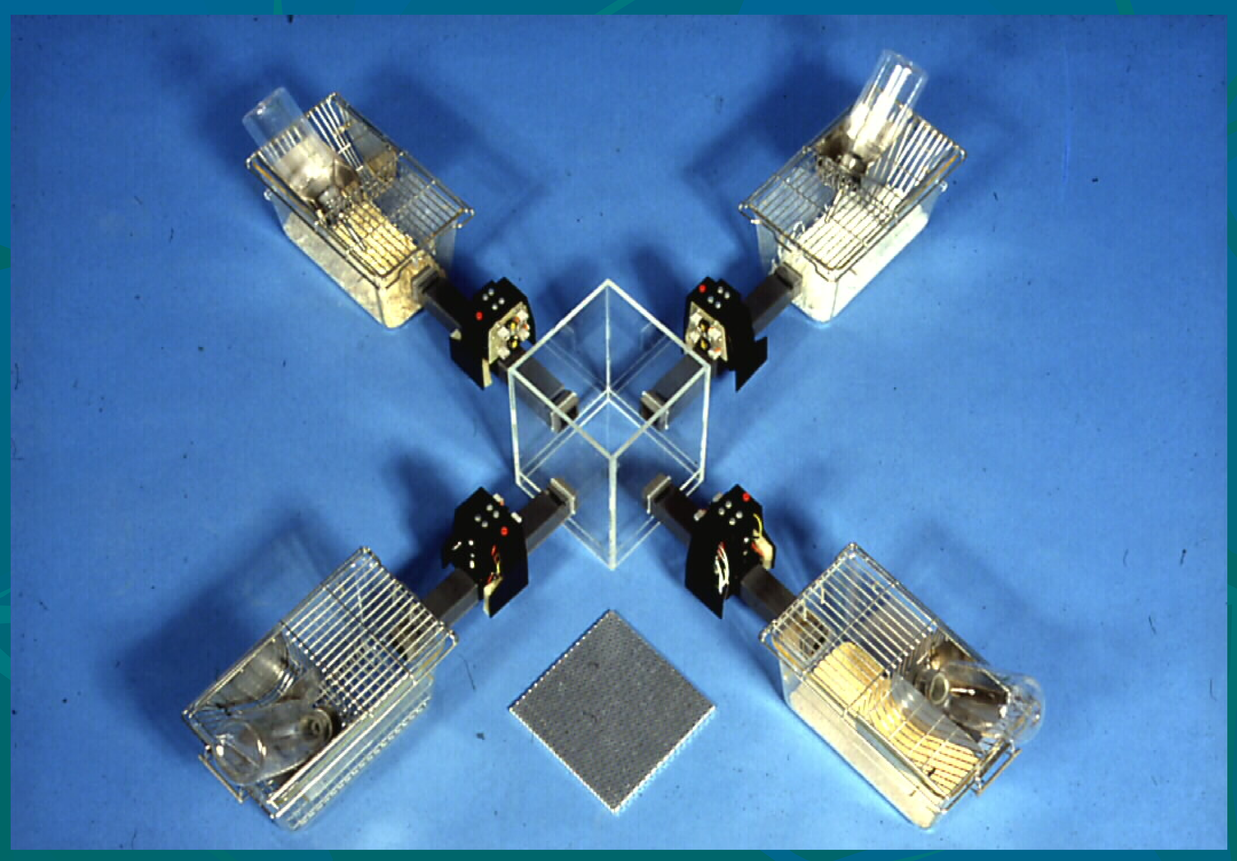
- Security (social partners, nest)
- Reduces the need for pain relief in mice (self administration of analgesics). Pham et al (2010).Physiol & Behav 99, 663-668
- No harm to animal, caretaker, experiment

How to find out needs of animals?

Preference tests

- ”Ask” animals what they prefer
- Strength of preference
- Combine with behavioural observation

Preference test



Important:

- Knowledge on normal animal behaviour
- Evaluation of the refinement/enrichment used
  - Animal behaviour
  - Experimental results
  - Practicality
- Previous refinement/enrichment
  - Breeder
  - Other animal unit

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Pain

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage

National Research Council, 1992

Distress

A subjective state that results from the inability to adapt to a chronic stressor or to a repeatedly occurring stressor resulting in negative effects on welfare

Balcombe et al (2004). Contemp.Top.Lab.Anim. 43,42-51

Possibilities to reduce pain / discomfort / distress

- Ethical admissibility
- In vitro tests
- Mathematical models
- Computer simulation
- Other animal model
- Methodology, e.g. other induction method
- Diagnostics, e.g. imaging (MRI, PETscan)
- Healthy animals
- Stimulating environment
- Anaesthesia/analgesics
- Skills
- Adequate care
- Humane endpoints

Humane end point

To define a set intervention point which allows the collection of quality scientific data but limits the amount of suffering for the animal.

Russell & Burch 1959

Hendriksen & Morton 1998

Humane end points

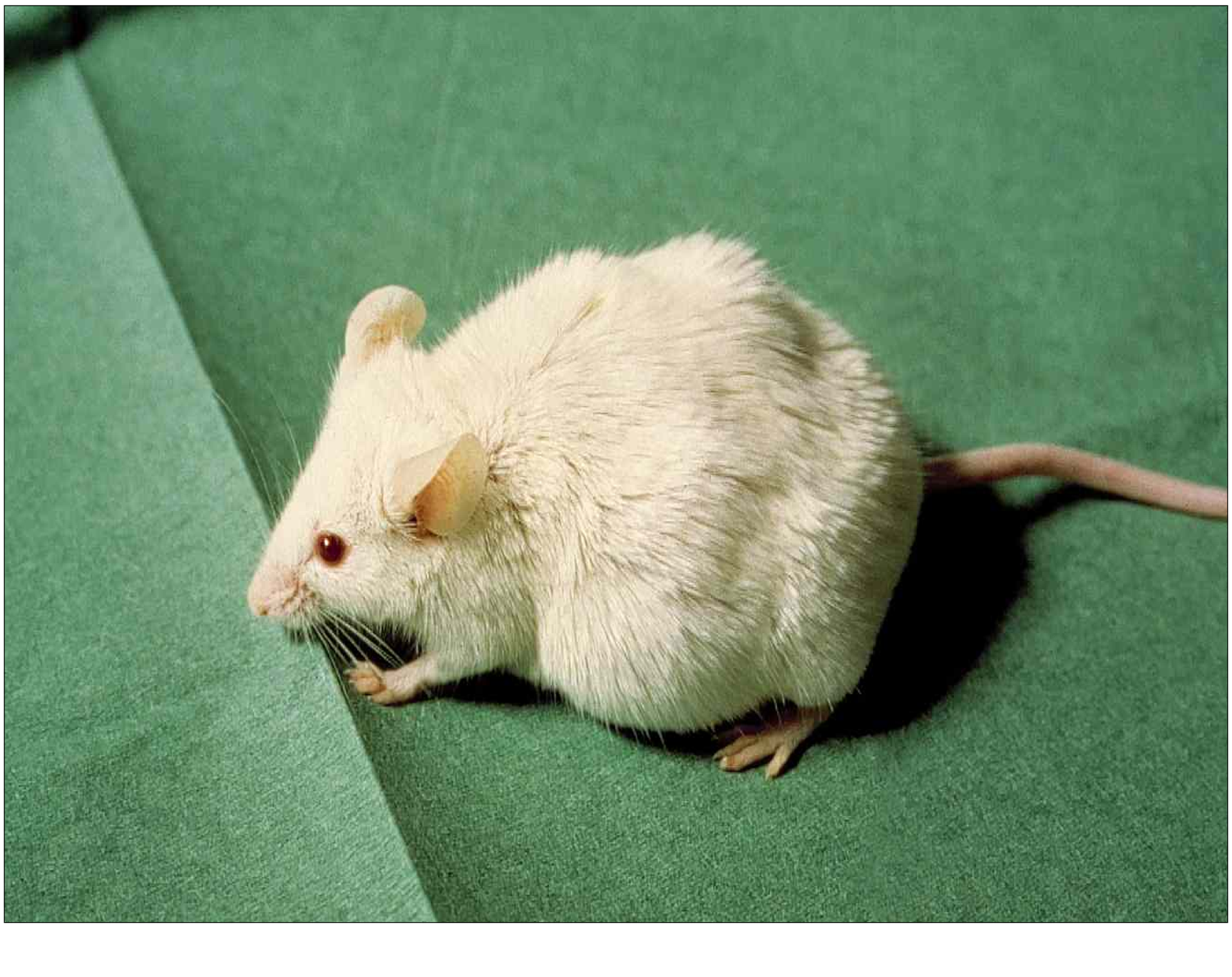
- 20% weight loss of normal weight
- 15% weight loss within 2 days
- Body condition score
- Drop in body temperature > 4 °C
- Tumour volume mouse max. 1.2 x 1.2 cm
- Tumour volume rat max. 2.5 x 2.5 cm
  (Workman et al. Brit.J.of Cancer,2010,102,1555)
- Tumour ulceration
- Ascites > 10% body weight
- Diarrhoea / incontinence > 48 hrs
- Abnormal behaviour/posture etc

How to establish humane endpoints?

- Pilot experiment
- Monitoring
- Authorisation to euthanize. Friday afternoon!

Assessment of discomfort

- Principle of analogy
- Sensitivity of tissues
- Parameters of pain/discomfort/distress



Parameters to assess pain and discomfort

- Behavioural
- Physiological
- Post-mortem

Behavioural parameters

- Abnormal behaviour (e.g. stereotypies, posture, gait). Knowledge on natural behaviour essential!
- Sudden fear/aggression
- Vocalising
- Auto-mutilation
- Grooming ⇩
- Activity ⇩ or ⇧
- Isolation
- Nest building
- Pain behaviour (back-arching, stagger, belly-press, twitch, writhe, flinch, rear leg lift, stretch
- Mouse / Rat Grimace Scale

Chromodacryorrhea

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Parameters to assess discomfort

- Behavioural
- Physiological
- Post-mortem

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Physiological Parameters

- Weight loss
- Food intake ⇩
- Body condition score
- Reproduction ⇩
- Pilo-erection
- Respiratory signs
- Cardiovascular signs
- Blood pressure (telemetry)
- Body temperature ⇩ or ⇧ (telemetry)
- Stress hormone levels (eg corticosterone, catecholamines)
- Immunosuppression
- Heart rate ⇩ or ⇧ (telemetry)

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Body condition score mouse

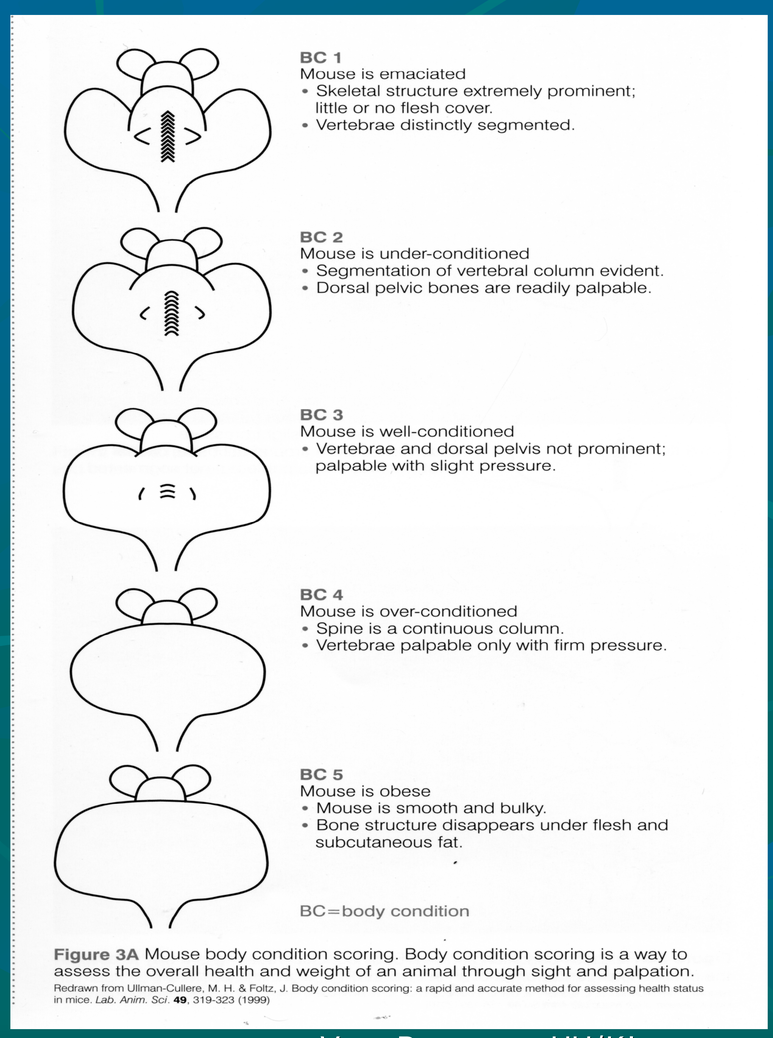

Figure 3A Mouse body condition scoring. Body condition scoring is a way to assess the overall health and weight of an animal through sight and palpation.
Redrawn from Ullman-Cullere, M. H. & Foltz, J. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab. Anim. Sci.* **49**, 319-323 (1999)

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Parameters to assess discomfort

- Behavioural
- Physiological
- Post-mortem

Post-mortem Parameters

- Fatty depots
- Muscle volume
- Fluid balance
- Lymphoid organ size
- Adrenal cortex size
- Infections
- Stomach ulcers

Animal welfare indicator record systems

- Numerical: quantify severity of adverse effects, score 0,1,2,3,4
  Data can be statistically analysed, but also subjective. Time consuming.
- Binary: adverse effects yes or no
  Less data, more objective, less time consuming.

Why is Refinement important?

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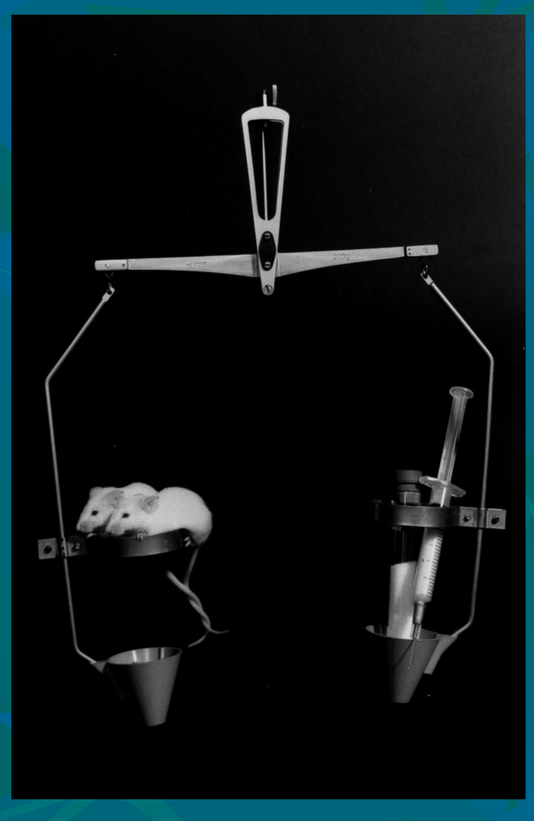
Animal Ethics Committee

Ethical Evaluation

Does the benefit of the proposed experiment outweigh the estimated suffering of the animals



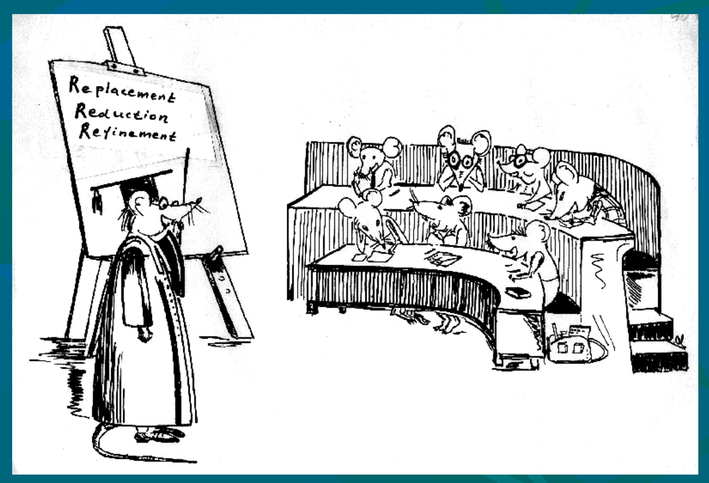
Animal Ethics Committees Tasks

Critical evaluation of:

- Benefit of research versus animal suffering
- Implementation of the Three Rs
- Competence of persons

Education and training

The welfare of animals must depend on an understanding of animals, and one does not come by this understanding intuitively; it must be learned.



P. Medawar,
The Hope of Progress,
1972

Education and training in Laboratory Animal Science

required for:

- Persons taking care of animals (animal caretakers)
- Persons carrying out experiments (animal technicians)
- Persons responsible for the design of the experiment (scientists)
- Persons who have specialised in LAS at post-graduate level (master students, animal welfare officers)

Directive 2010/63/EU: Authorisation
Article 23: Competence of personnel

Par 2:

- Member States shall ensure that staff is adequately educated and trained for:
  - Carrying out procedures on animals*
  - Designing procedures and projects**
  - Taking care of animals*
  - Killing animals*

* Staff should be supervised until they have demonstrated the requisite competence

** Staff should have a relevant scientific background and species-specific knowledge

Russell and Burch
The Principles of Humane Experimental Technique, 1959

- Replacement
- Reduction
- Refinement
- Responsibility

Our responsibility as researcher

- How important is it to use animals for this purpose?
- Can an alternative, non-animal method be used?
- Can the number of animals be reduced?
- Can pain/discomfort be reduced?
- What can be done to improve the living conditions of our animals?

Refinement is not a Cinderella anymore!