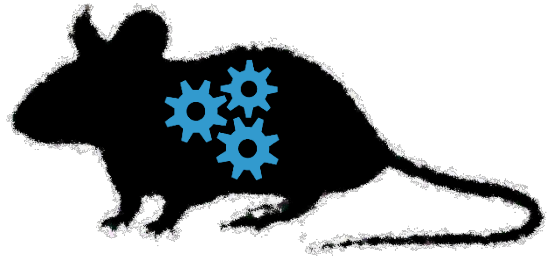
And in practice….

Practical considerations when including both sexes in experimentation

Sex-differences in phenotypes

Strategies for breeding and welfare

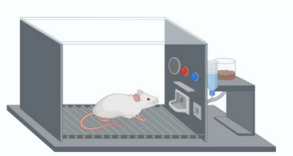


Tackling practical challenges

Phenotype variation

- Chromosomal sex
- Genetic background
- Gene alteration

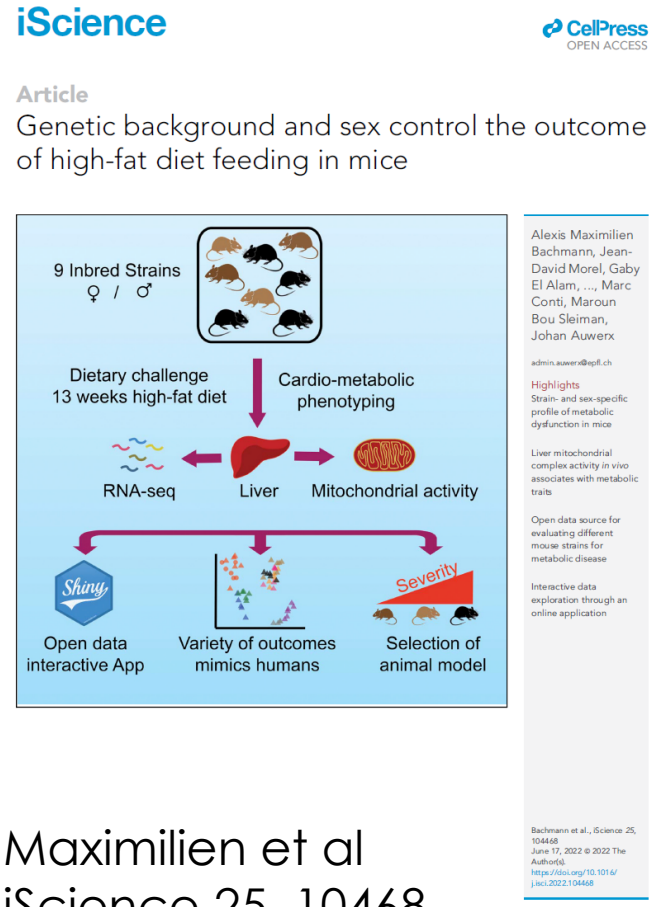
- Feed
- Caging
- Husbandry regimes
- Microbiome



- Equipment
- Protocol
- Metadata
- User

Data may vary according to combinations of all these factors.

Data differences- strains vs sex



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iScience 25, 10468

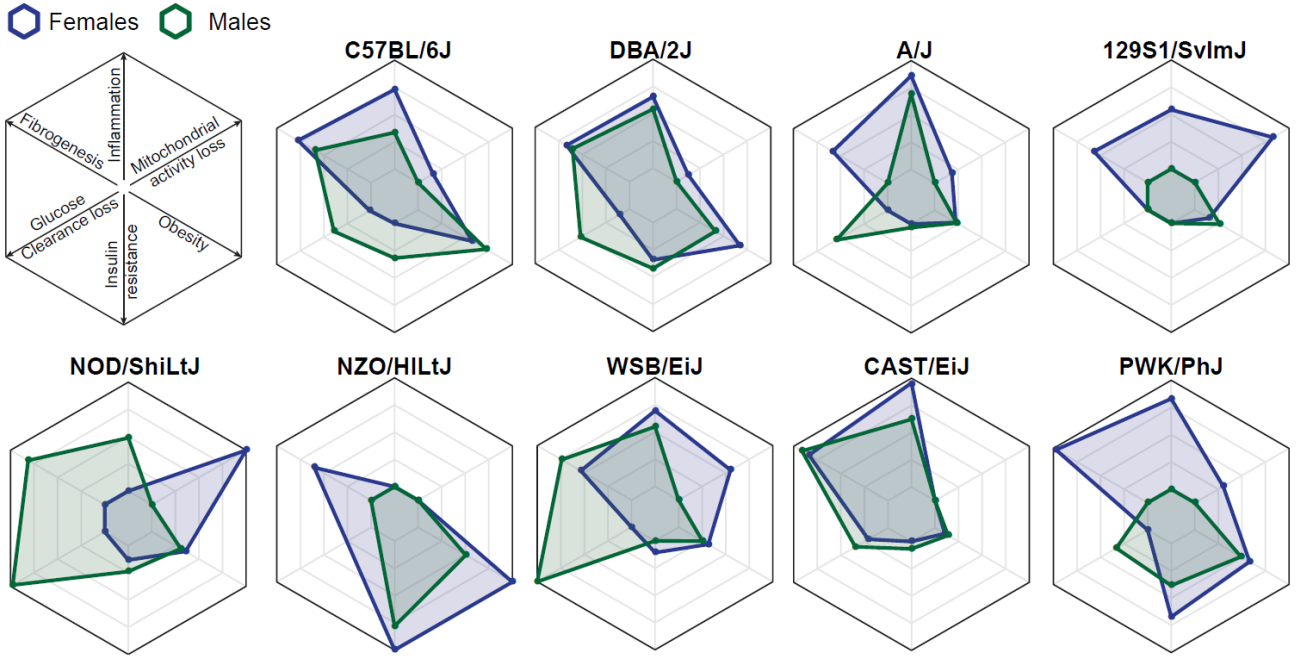
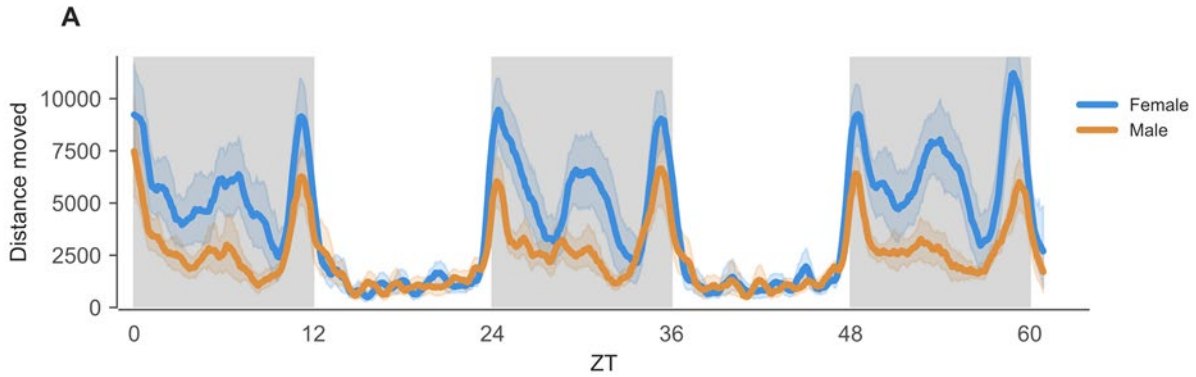


Figure 7. Strain-specific response signatures to obesogenic diet

The more we look…

Home cage monitoring-

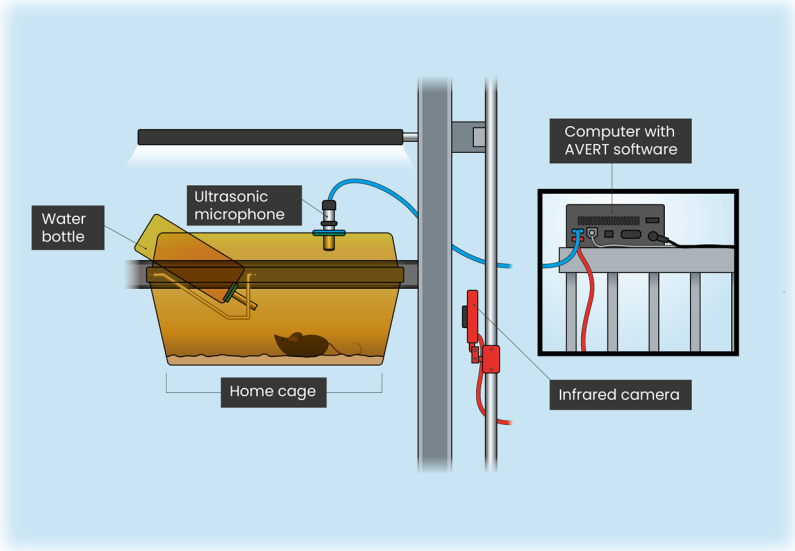
24/7 recording of activity via RFID chips

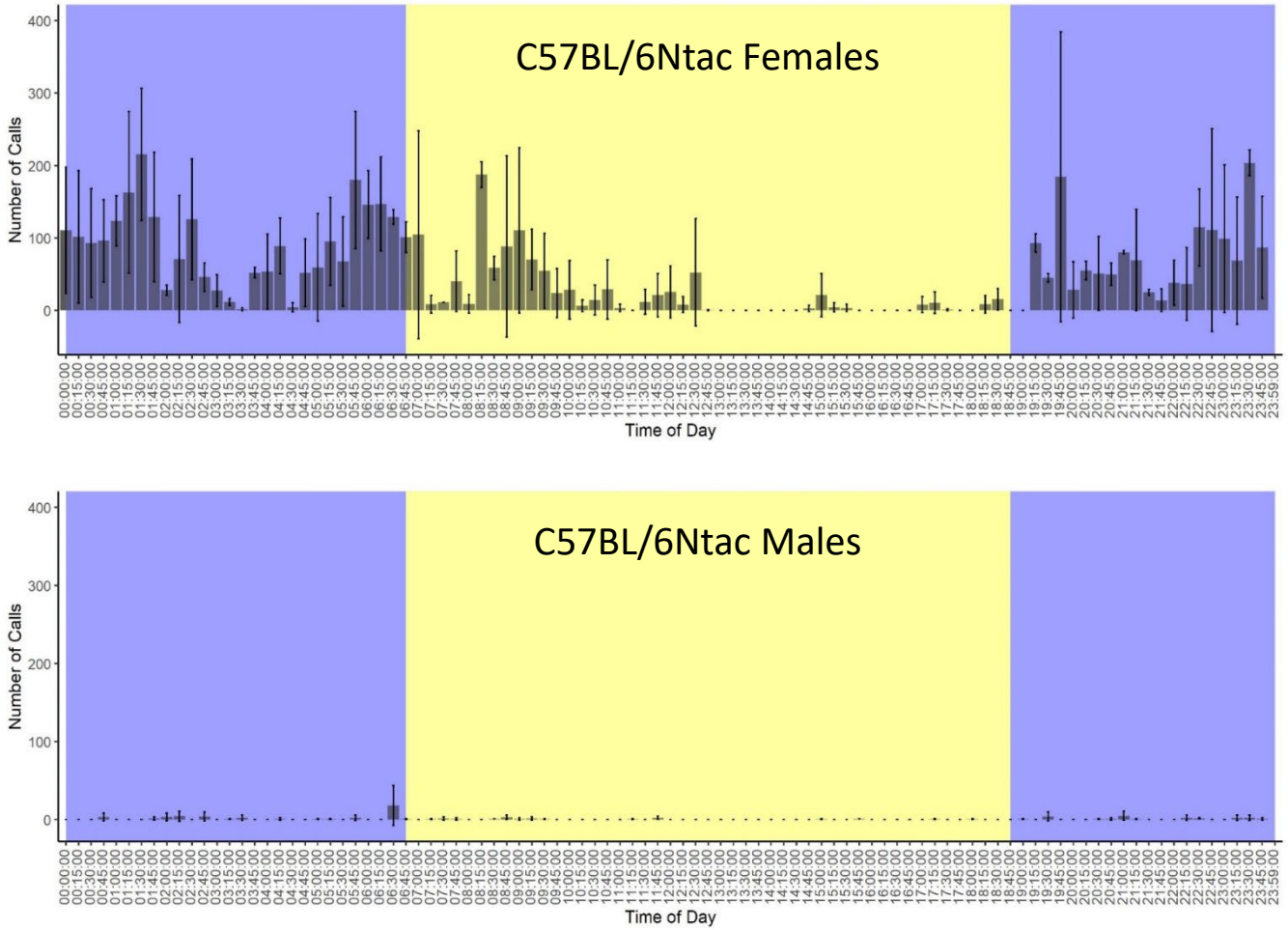


NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Ultrasonic Vocalisation

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



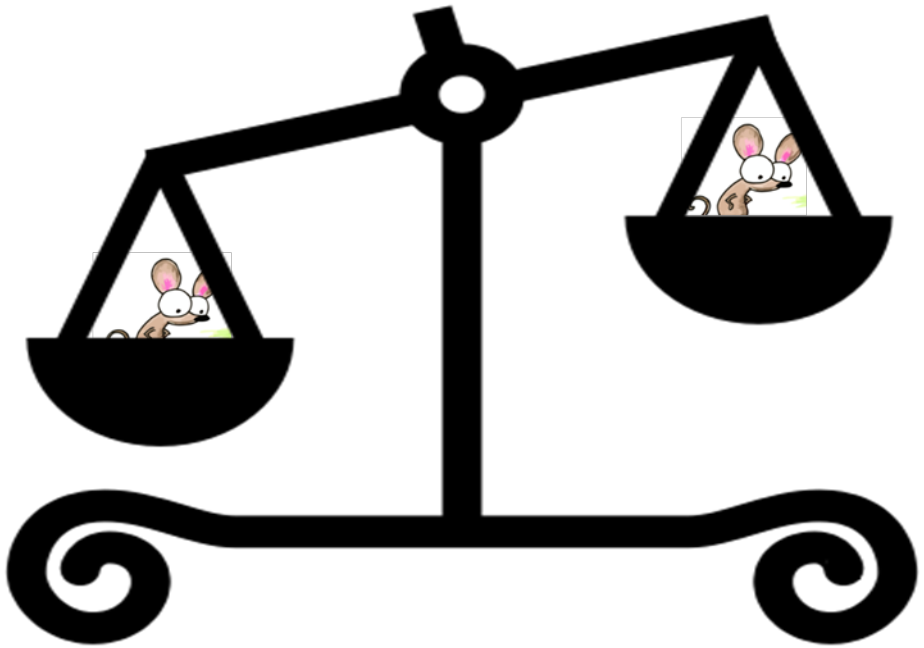


Monitoring groups of 3- 4 individuals

Husbandry challenges

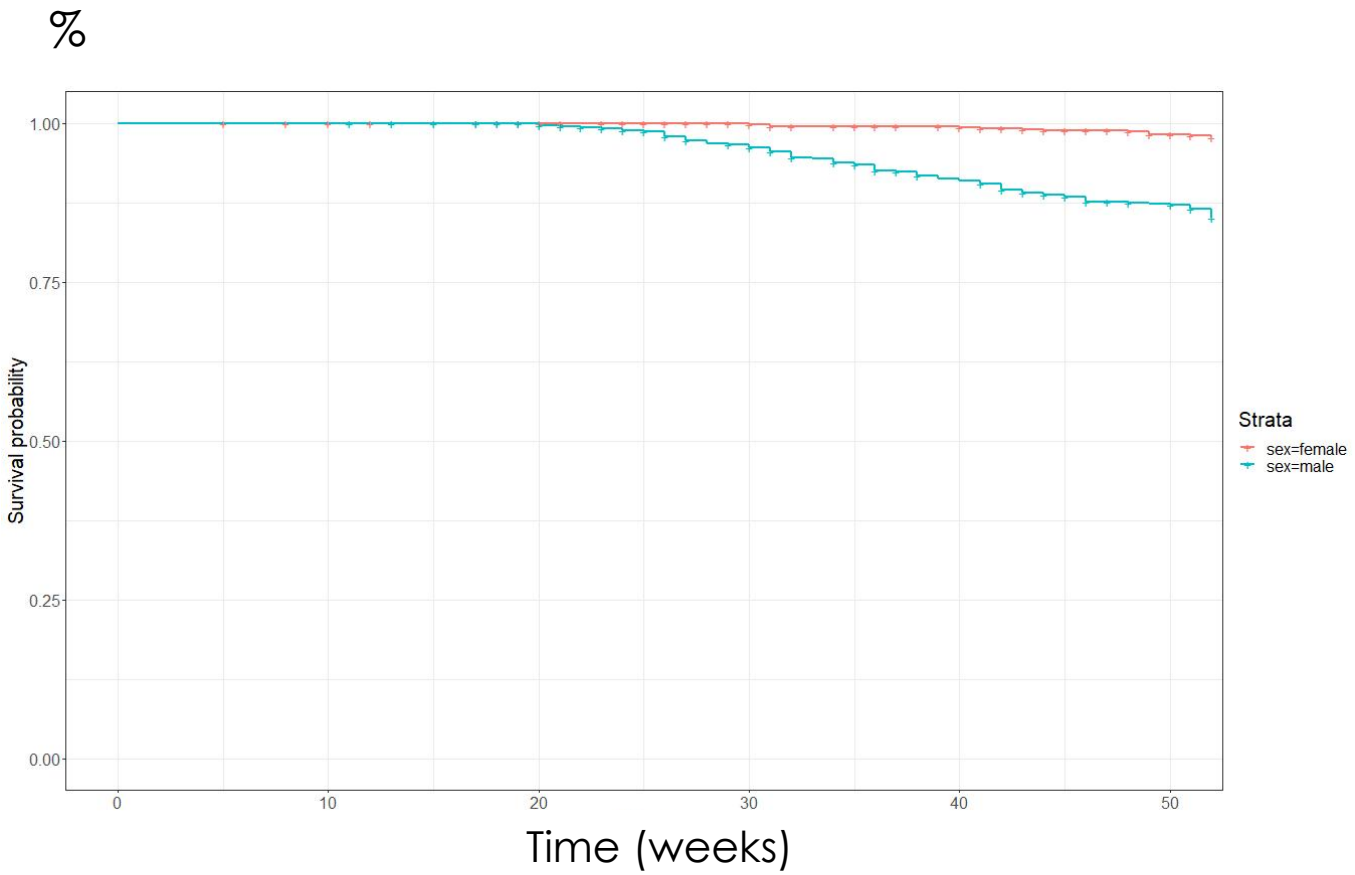
- Attrition, ageing and sex-specific welfare issues
- Single housing, aggression
- Randomisation

Risk of individual harm?



'Cost' of replacing that individual?

Attrition- a sex-specific effect



sex	animals (n)	Attrition (%)
female	961	2.2
male	910	14.9

C57BL/6Ntac – IMPC project

- Found Dead (2)
- Welfare concerns (weight loss, aggression and tarsal injury)- (152)
- Removed due to single housing- (5)

Tarsal Injury in ageing males

Harwell NACWO observed hind paw at an abnormal angle in a C57BL/6N ageing male

Tarsal injuries

normal

caudo-dorsal dislocation of the calcaneus

- Contacted individual centres
- Visited 2 centres and looked at their ageing stocks
- Discovered this was occurring in all international colonies examined
- Likely to be a common occurrence everywhere

Frequencies of injuries

	Substrain	Number of mice (male)	Age range (weeks)	Number affected	Earliest age affected (weeks)	Frequency (%)
The Centre for Phenogenomics, Canada	C57BL/6NCrl	235	5-59	21	20	8.9
The Jackson Laboratory, USA	C57BL/6NJ	1440	4-78	58	11	4.0
MRC Harwell Institute, UK	C57BL/6NTac	174	16-59	21	18	12.1
GMC Helmholtz Zentrum, Germany	C57BL/6NTac and C57BL/6NCrl	413	4-62	7	4	1.7
Baylor College of Medicine, USA	C57BL/6NJ	250	16-52	30	20	12

- Male only
- Stock cages
- C57BL/6N (likely other strains also)
- Affects phenotyping

- **Additional welfare checks**
- **Removed from pipeline**


PLOS ONE

RESEARCH ARTICLE

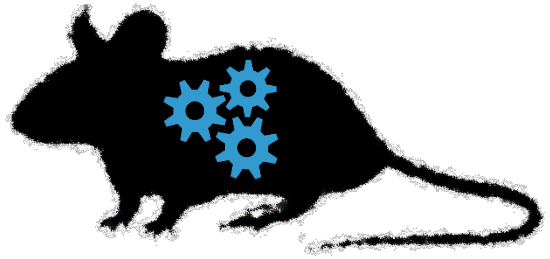
The occurrence of tarsal injuries in male mice of C57BL/6N substrains in multiple international mouse facilities

Eleanor Herbert[1], Michelle Stewart[2], Marie Hutchison[2], Ann M. Flenniken[3,4], Dawei Qu[3,4], Lauryl M. J. Nutter[3,5], Colin McKerlie[3,5], Liane Hobson[2], Brenda Kick[6], Bonnie Lyons[6], Jean-Paul Wiegand[6], Rosalinda Doty[6], Juan Antonio Aguilar-Pimentel[7], Martin Hrabe de Angelis[7,8,9], Mary Dickinson[10], John Seavitt[10], Jacqueline K. White[3], Cheryl L. Scudamore[2], Sara Wells[2]*

1 Department of Pathobiology and Population Sciences, Royal Veterinary College, Hertfordshire, United Kingdom, 2 Mary Lyon Centre, MRC Harwell Institute, Oxfordshire, United Kingdom, 3 The Centre for Phenogenomics, Toronto, Ontario, Canada, 4 Lunenfeld-Tanenbaum Research Institute, Sinai Health, Toronto, Ontario, Canada, 5 The Hospital for Sick Children, Toronto, Ontario, Canada, 6 The Jackson Laboratory, Bar Harbor, Maine, United States of America, 7 German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany, 8 School of Life Science Weihenstephan,

Check for updates

Aggression



Know your strain

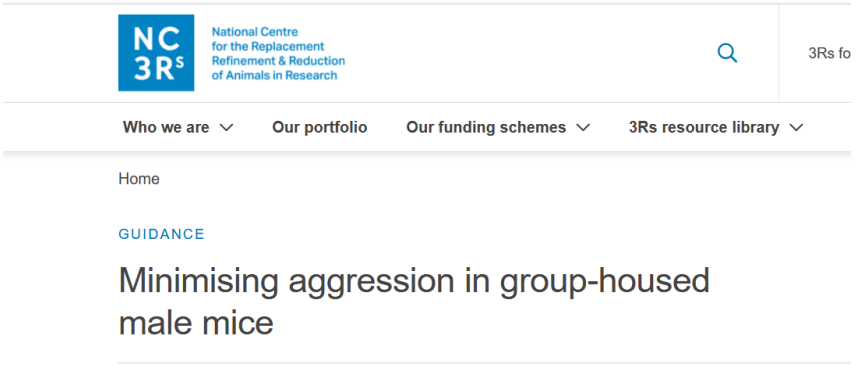
a) Is it suitable for your study

b) Are there strain-specific husbandry regimes which would reduce aggression?

Modify husbandry regimes

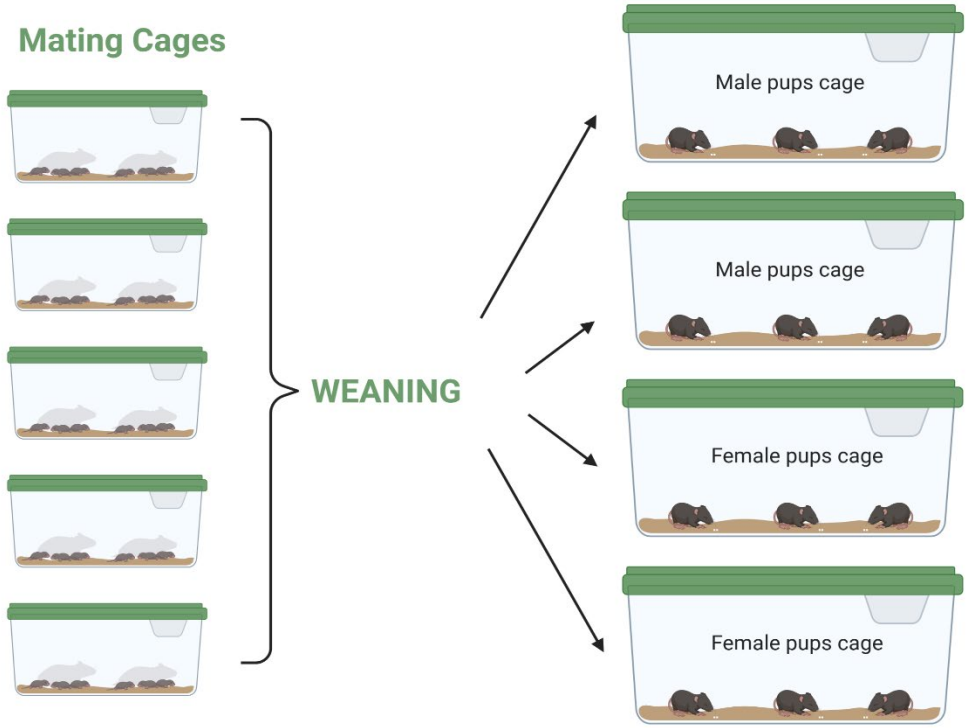
a) Avoid scent contamination between cages

a) Some types of enrichment strains may have reduced aggression



Grouping

Mixing animals at different life stages (especially males) may cause issues with aggression and behavioural changes



Randomisation at weaning if possible

- Mixing males of some strains later will lead to aggression
- Requires very controlled breeding

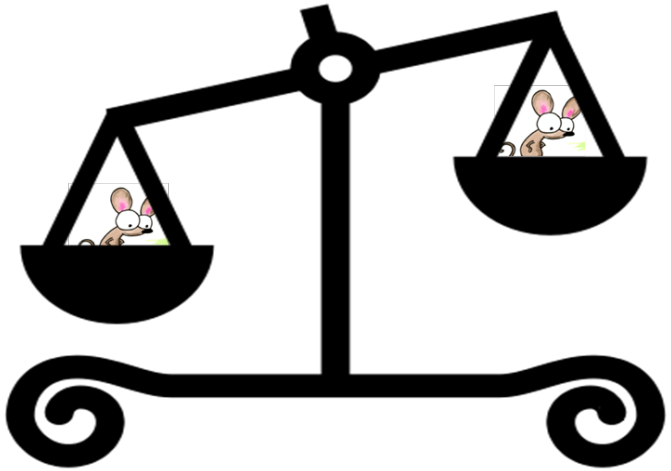
Housing- Welfare as well as £

100s of papers each year documenting behavioural, physiological and welfare changes between animals socially-housed and those kept singly.

Singly housing is costly in terms of cash, data and welfare.

Summary

- Sexually dimorphic data is complex and dependent upon the interplay of many factors.
- Different sexes may have different welfare and housing challenges and this needs to be factored into the experimental plan and explained in a grant application.
- Grouping needs to be planned carefully to avoid mixing cages of older animals.
- Housing has a profound affect on data and welfare



Both sexes of animals should be included as is appropriate for the particular experiment.

Reasons for conducting research in a single sex given by applicants will be considered as part of the peer review process. These may include logistical or ethical considerations and should have robust justification.