

# And in practice....

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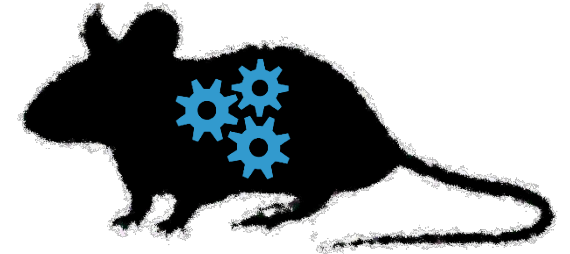
## Practical considerations when including both sexes in experimentation



Sex-differences  
in phenotypes



Strategies for  
breeding and  
welfare



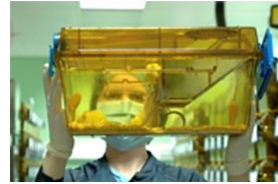
Tackling practical  
challenges

# Phenotype variation

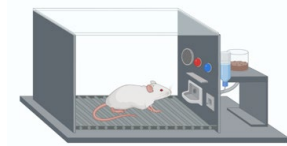
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- Chromosomal sex
- Genetic background
- Gene alteration



- Feed
- Caging
- Husbandry regimes
- Microbiome



- Equipment
- Protocol
- Metadata
- User

**Data may vary according to combinations of all these factors.**



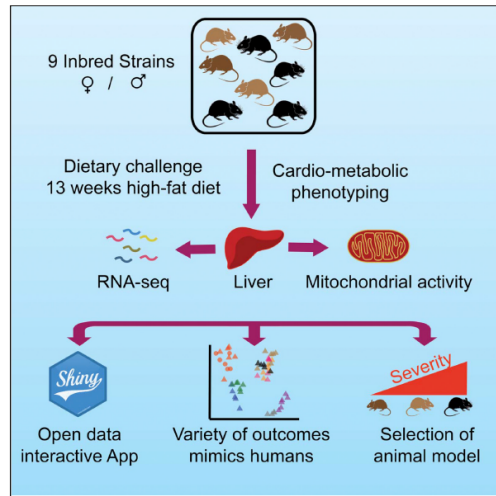
# Data differences- strains vs sex

iScience



Article

Genetic background and sex control the outcome of high-fat diet feeding in mice



Alexis Maximilien Bachmann, Jean-David Morel, Gaby El Alam, ..., Marc Conti, Maroun Bou Sleiman, Johan Auwerx  
 admin.auwerx@epfl.ch

**Highlights**  
 Strain- and sex-specific profile of metabolic dysfunction in mice

Liver mitochondrial complex activity in vivo associates with metabolic traits

Open data source for evaluating different mouse strains for metabolic disease

Interactive data exploration through an online application

Bachmann et al., iScience 25, 104468  
 June 17, 2022 © 2022 The Author(s)  
<https://doi.org/10.1016/j.isci.2022.104468>

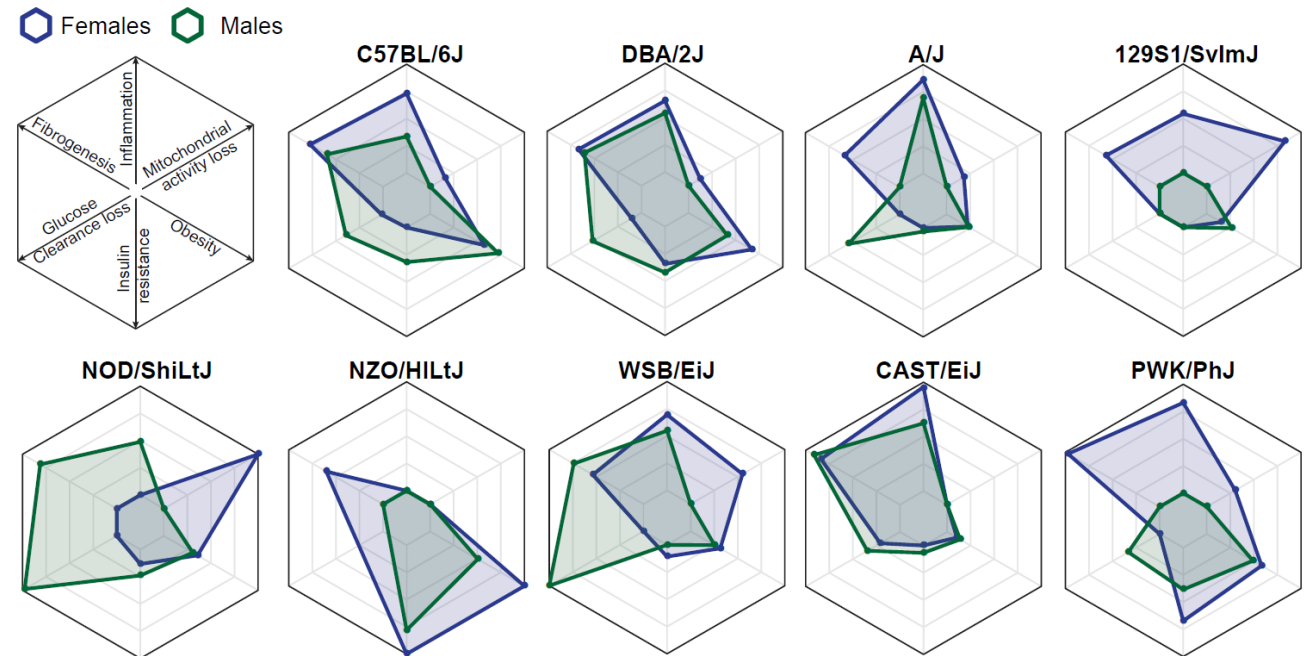


Figure 7. Strain-specific response signatures to obesogenic diet

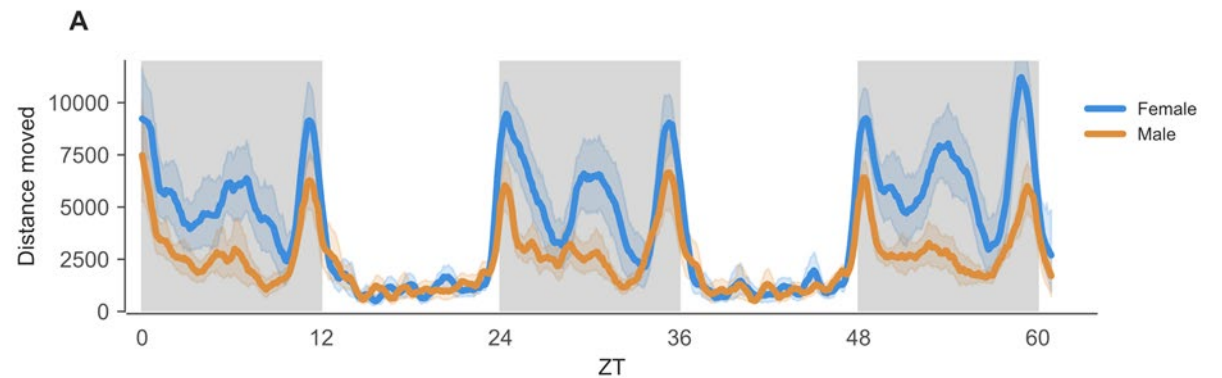
Maximilien et al  
 iScience 25, 10468

# The more we look...

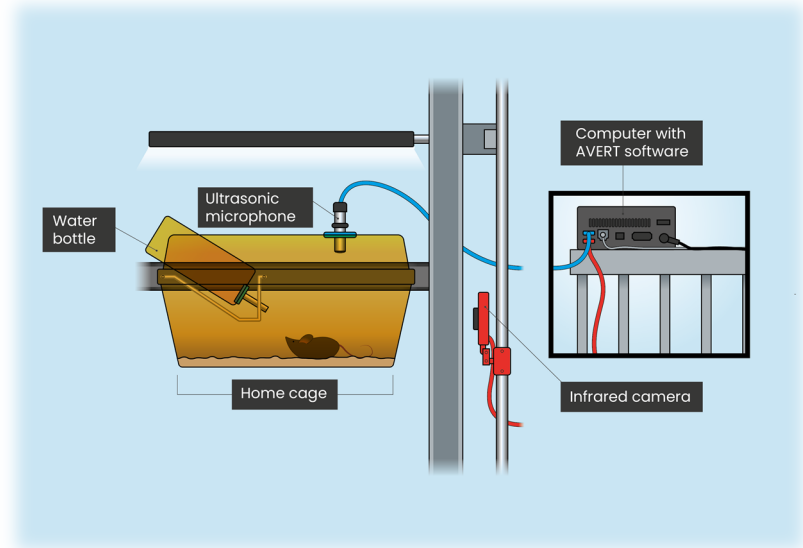


Home cage monitoring-

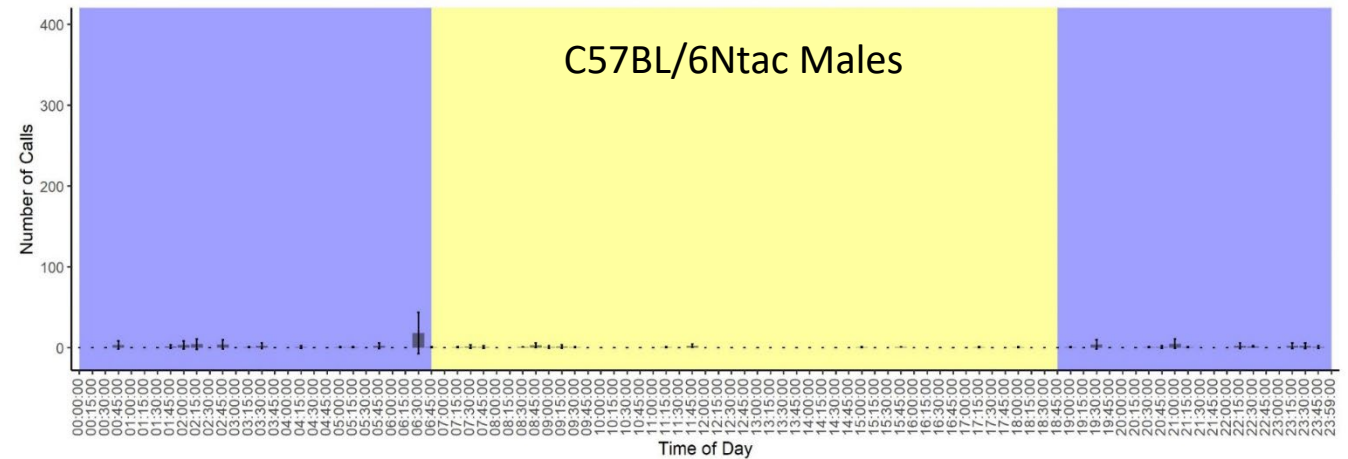
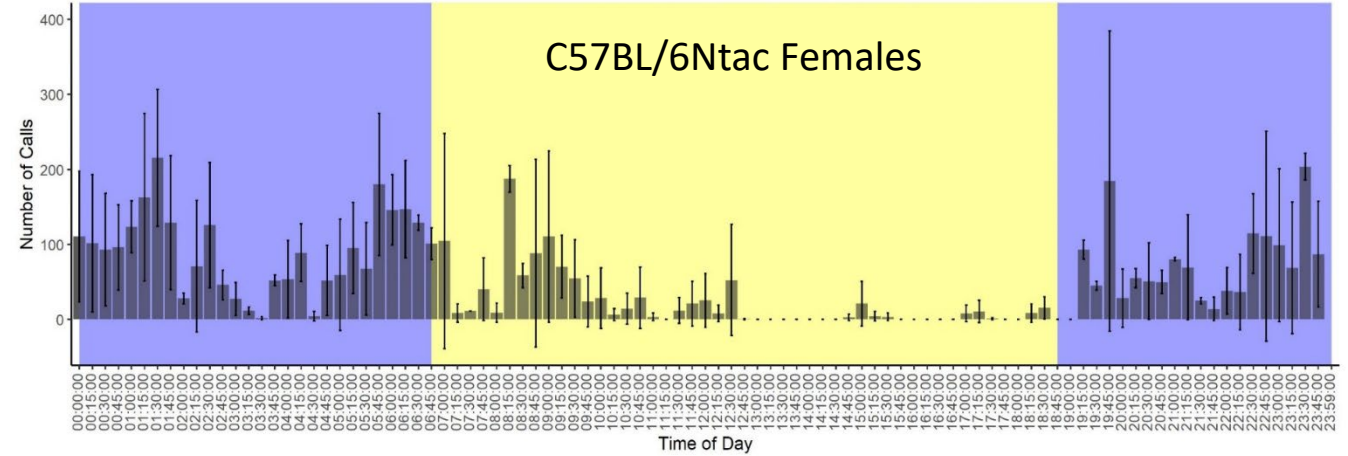
24/7 recording of activity via RFID chips



# Ultrasonic Vocalisation



Monitoring groups of 3- 4 individuals

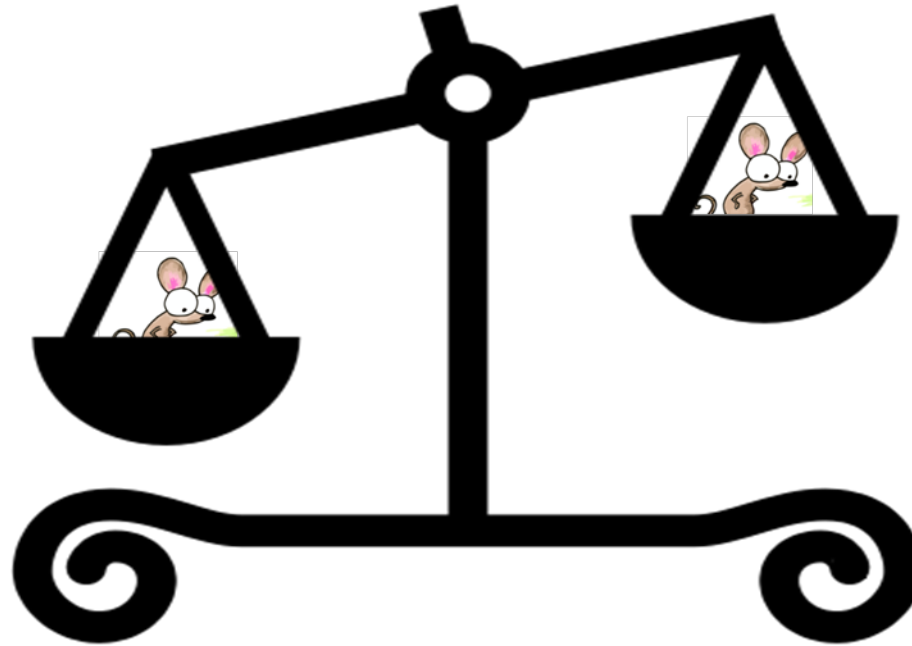


# Husbandry challenges

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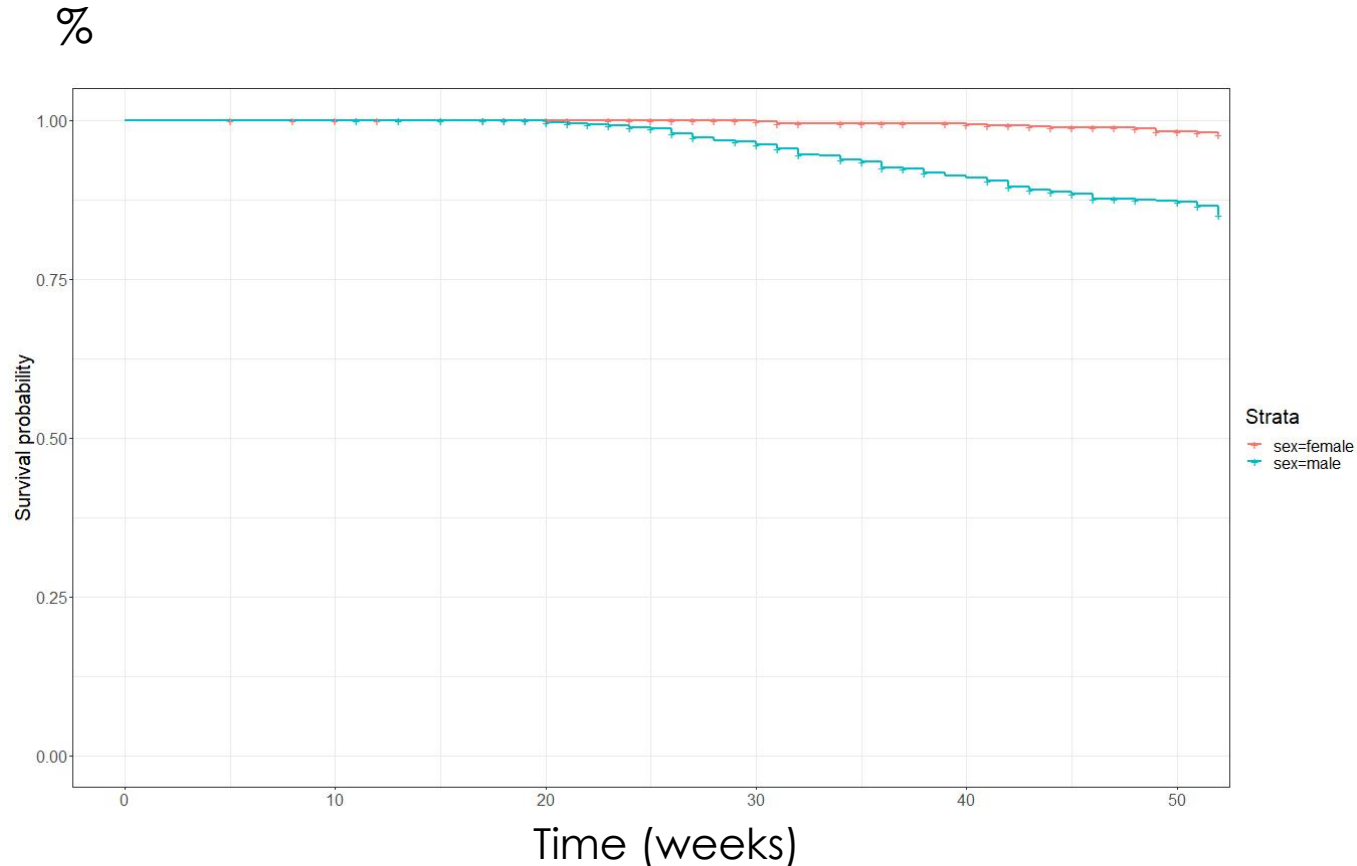
- Attrition, ageing and sex-specific welfare issues
- Single housing, aggression
- Randomisation

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

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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<sup>1</sup>, Michelle Stewart<sup>2</sup>, Marie Hutchison<sup>2</sup>, Ann M. Flenniken<sup>3,4</sup>, Dawei Qu<sup>3,4</sup>, Lauryl M. J. Nutter<sup>3,5</sup>, Colin McKerlie<sup>3,5</sup>, Liane Hobson<sup>2</sup>, Brenda Kick<sup>6</sup>, Bonnie Lyons<sup>6</sup>, Jean-Paul Wiegand<sup>6</sup>, Rosalinda Doty<sup>6</sup>, Juan Antonio Aguilar-Pimentel<sup>7</sup>, Martin Hrabe de Angelis<sup>7,8,9</sup>, Mary Dickinson<sup>10</sup>, John Seavitt<sup>10</sup>, Jacqueline K. White<sup>1</sup>, Cheryl L. Scudamore<sup>2</sup>, Sara Wells<sup>11</sup>

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# Aggression



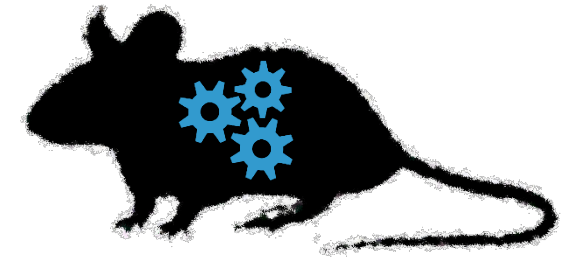
## Know your strain

- 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



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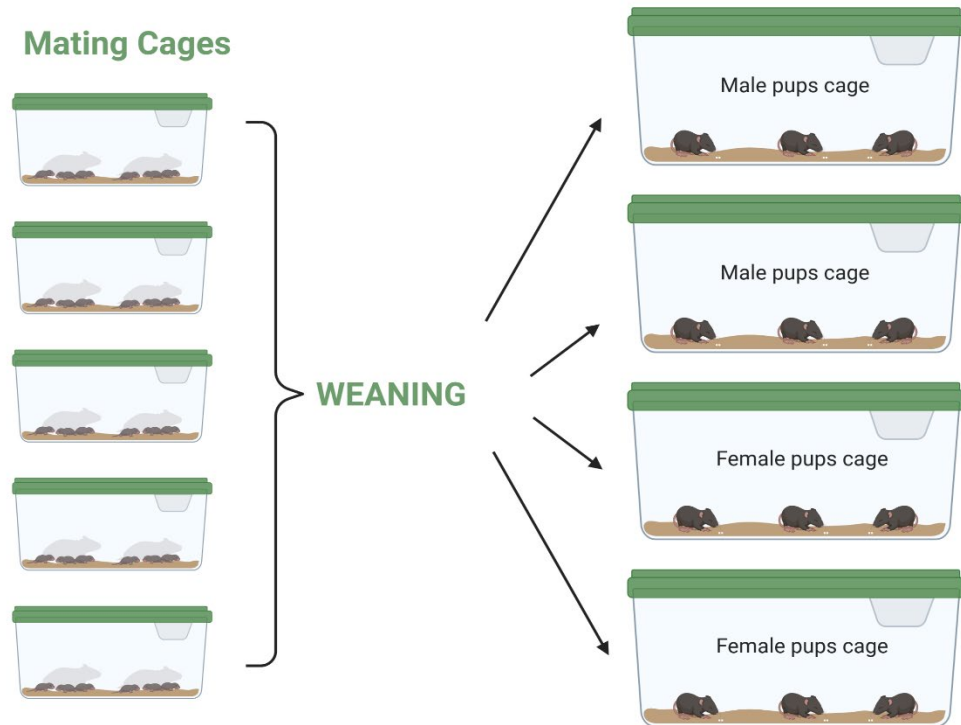
GUIDANCE

Minimising aggression in group-housed male mice



# 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.

scientific reports

OPEN **Effects of separated pair housing of female C57BL/6JRj mice on well-being**

A. Rex<sup>1</sup>, R. Palme<sup>1</sup>, C. Thöne-Reineke<sup>2</sup> & K. Ullmann<sup>1,2\*</sup>  
Common code of practice to house female mice in groups require to house them individually, even though social welfare, we introduced a separated pair housing system

ORIGINAL INVESTIGATION

**The interaction between Environmental Enrichment and fluoxetine in inhibiting sucrose-seeking renewal in mice depend on social living condition**

N. Pintori<sup>1,2</sup> · A. Piva<sup>3</sup> · V. Guardiani<sup>1</sup> · C. M. Marzo<sup>3</sup> · I. Decimo<sup>1</sup> · C. Chiamulera<sup>1</sup>

Received: 7 February 2022 / Accepted: 17 March 2022 / Published online: 30 March 2022  
© The Author(s) 2022

**Short-term Housing in Metabolic Caging on Measures of Energy and Fluid Balance in Male C57BL/6j Mice (*Mus musculus*)**

Ziegler, Alisha A<sup>1</sup>; Groba, Connie C<sup>2</sup>; Reha, John J<sup>1</sup>; Jensen, Eric S<sup>2</sup>; Thulin, Joseph D<sup>1</sup>; Segar, Jeffrey L<sup>1</sup>; Gi  
Journal of the American Association for Laboratory Animal Science, Volume 61, Number 2, March 2022, pp. 13  
er: American Association for Laboratory Animal Science  
ps://doi.org/10.30802/JAALAS-JAALAS-21-000087  
ous article · view table of contents · next article >

Current Biology

**Threat history controls flexible escape behavior in mice**

Highlights

- Individually housed, but not group-housed, mice show robust escape to looming stimuli

Authors

Stephen C. Lenzi, Lee Cossell, Benjamin Grainger, Sarah F. Oleson,

EJN European Journal of Neuroscience FENS Federation of European Neuroscience Societies

RESEARCH REPORT | Full Access

**Single housing-induced effects on cognitive impairment and depression-like behavior in male and female mice involve neuroplasticity-related signaling**

Na Liu, Yulu Wang, Aerin Y. An, Christopher Banker, Yi-Hua Qian, James M. O'Donnell

First published: 31 August 2019 | <https://doi.org/10.1111/ejn.14565> | Citations: 19

Edited by Christina Dalla

Na Liu, Yulu Wang and Aerin Y. An contributed equally to this work.

The peer review history for this article is available at <https://publons.com/publon/10.1111/EJN.14565>

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**ich alters anxiety-like and social behaviors in male mice**

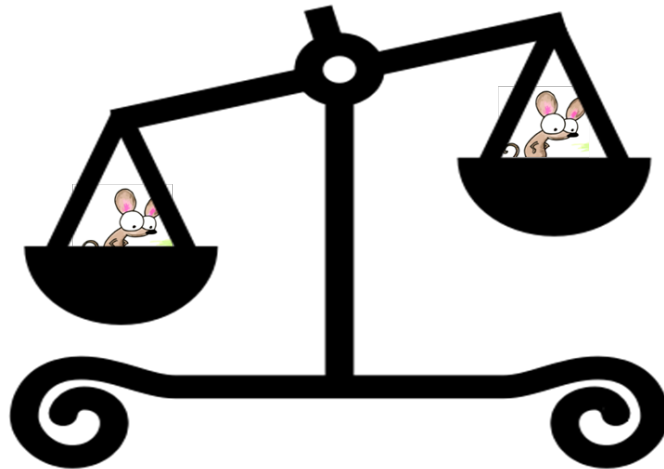
Zeng<sup>1</sup>, Yu-Hsin Chu<sup>2</sup>, Chih-Lin Lee<sup>3</sup>, Ching-Chuan Cheng<sup>4</sup>, Chen-  
γ Su<sup>5</sup>, Kai-Ti Lin<sup>3,4</sup>, and Tsung-Han Kuo<sup>1,2,4</sup>

National Tsing Hua University, Hsinchu, Republic of China<sup>1</sup> Department of Medical Science,  
Hsinchu, Republic of China<sup>2</sup> Institute of Systems Neuroscience, National Tsing Hua University,  
Institute of Biotechnology, National Tsing Hua University, Hsinchu, Republic of China<sup>3</sup> Brain  
ing Hua University, Hsinchu, Republic of China



# 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.