

**Website:** [www.3hs-initiative.co.uk](http://www.3hs-initiative.co.uk)

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



Improving the lives of laboratory  
rodents one lab at a time



**Professor Emma Robinson, University of Bristol, UK**

# Factors which contribute to cumulative suffering in laboratory rodents

## 1. **HOUSING** - laboratory caging systems and management methods

Small cages restrict natural behaviours

Limited opportunities for sensory enrichment

Frequent social stressors e.g. cage cleaning

## 2. **HANDLING** - Impacts of repeated use of physical restraint

Routine husbandry

Common procedures e.g. substance administration

## 3. **HABITUATION** - Negative associations with humans

Natural fear

Aversive experiences

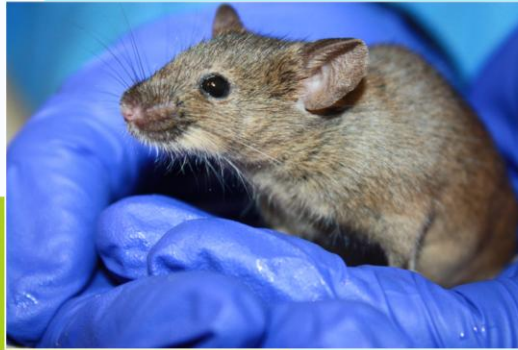


# Two simple strategies which benefit welfare without compromising scientific needs

1. Adopt methods which reduce the use of physical restraint and aversion associated with human handling
2. Provide positive affective experiences – anticipation of reward



**Housing**



**Handling**



**Habituation**

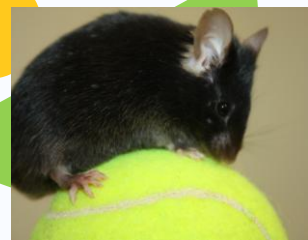
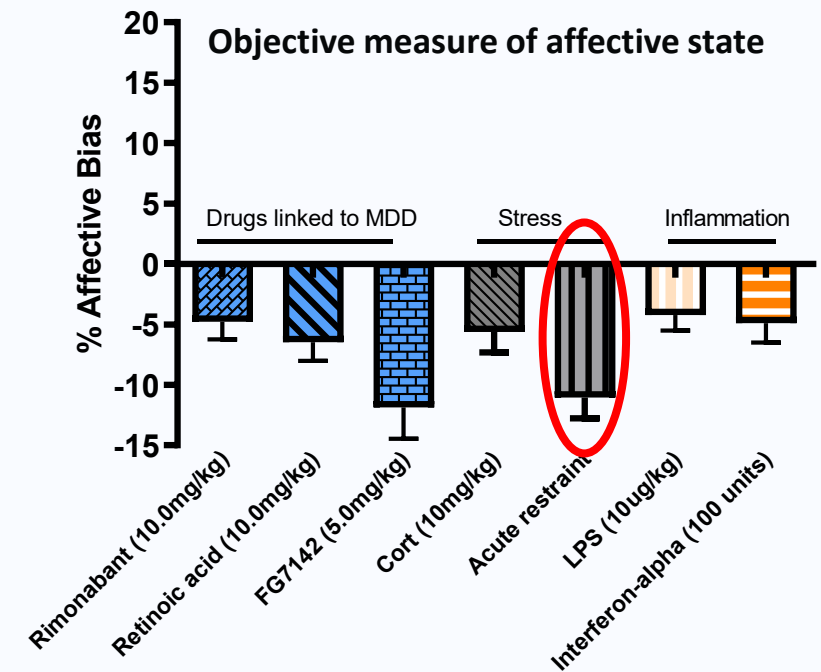
# Restraint induces a negative affective state

- We underestimate the impact of physical restraint within our protocols
- Acute or repeated physical restraint has effects on control data, data variability and reproducibility

**Stress matters for science AND animal welfare**

# Positive reinforcement induces a positive affective state

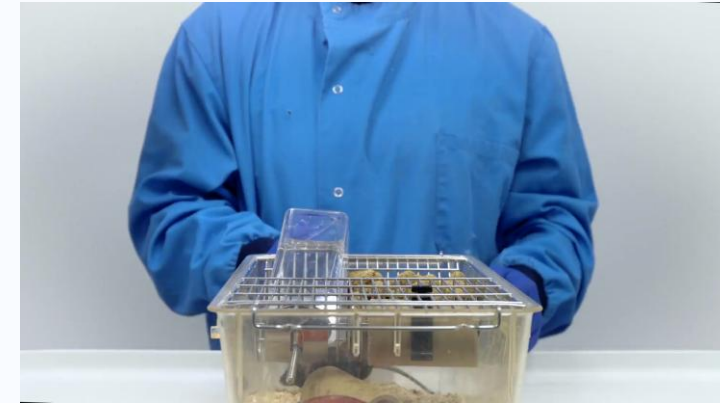
- Pairing experiences with reward leads to anticipation of positive events and a positive affective state
- Generates to a positive association with human interactions
- Pairing aversive procedures with rewards can reduce long term harms and cumulative suffering
- Can be used to counteract effects of restricted housing



# What are the aims of the 3Hs initiative?

- To reduce stress associated with handling
- To minimize the use of physical restraint
- To enhance the housing environment with species relevant enrichment compatible with scientific requirements
- To provide objective assessment of the welfare benefits to support policy change

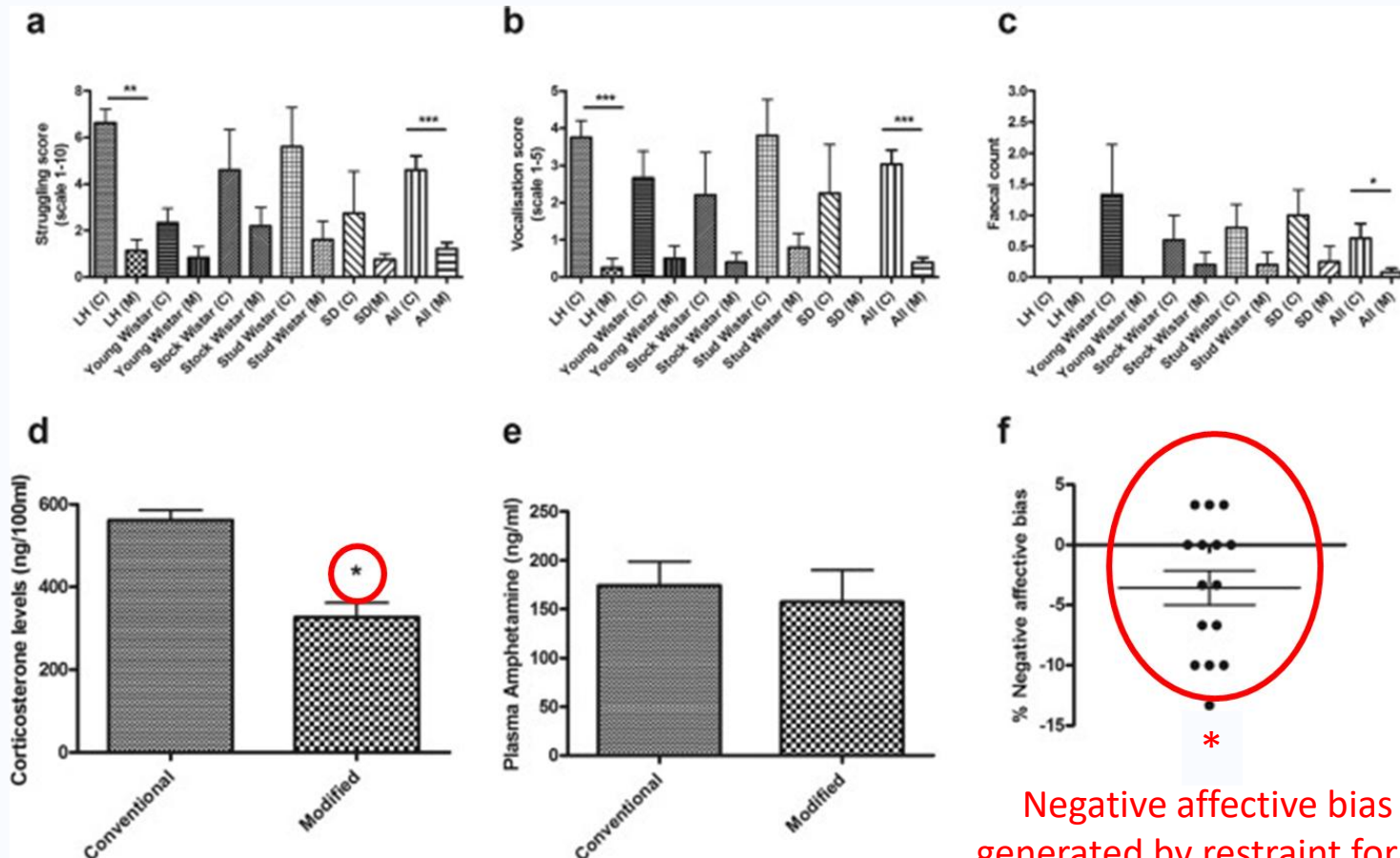
# Examples of the 3Hs in practice



Visit our website for more videos illustrating the practical applications of the 3Hs as well as guidance documents

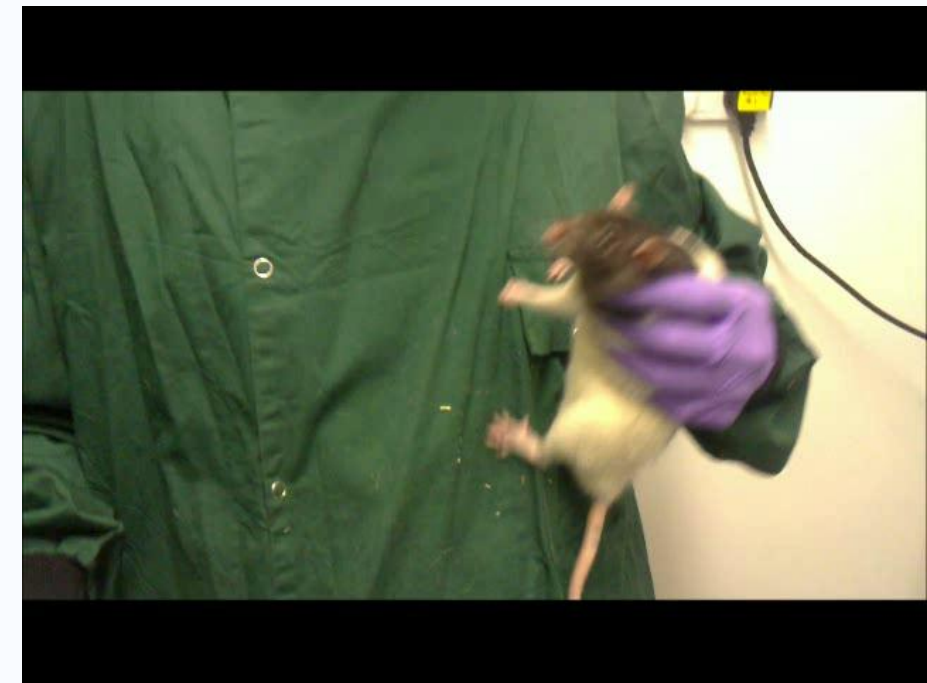
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# Objective evidence of welfare benefits of refinement handling for IP injections in rats



~40% reduction in corticosterone with modified method

Negative affective bias generated by restraint for IP injection vs modified method



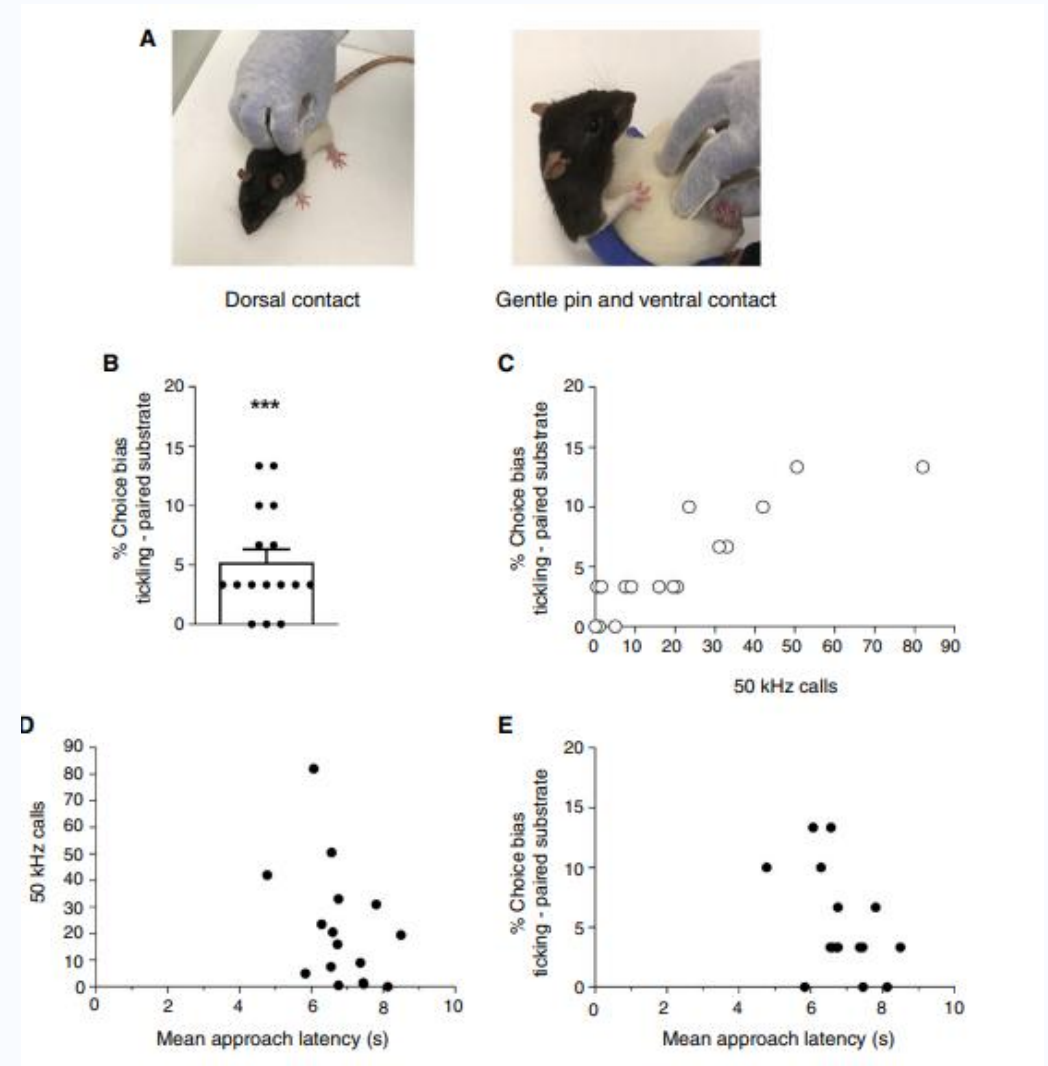
# Tickling in rats associated with positive affective state (for some but not all rats)

## Correspondence

## Rat 50 kHz calls reflect graded tickling-induced positive emotion

Justyna K. Hinchcliffe<sup>1</sup>,  
Michael Mendl<sup>2,\*</sup>,  
and Emma S.J. Robinson<sup>1</sup>

Current Biology 2021



# Positive welfare impacts of rat play pens

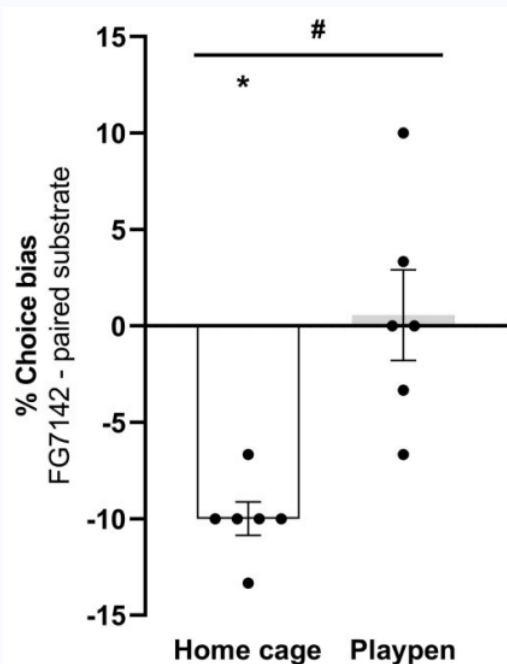
Original Article

**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

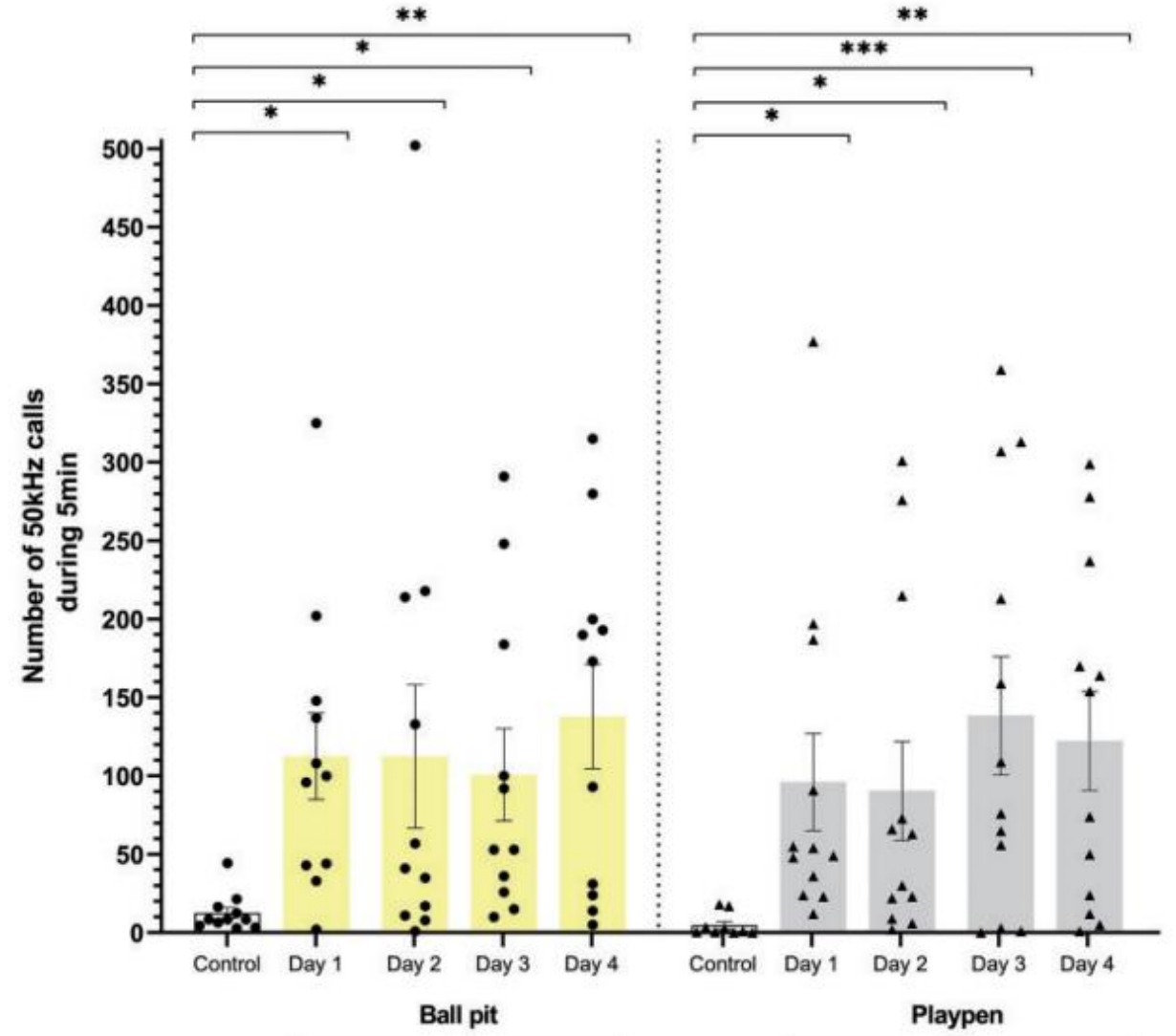


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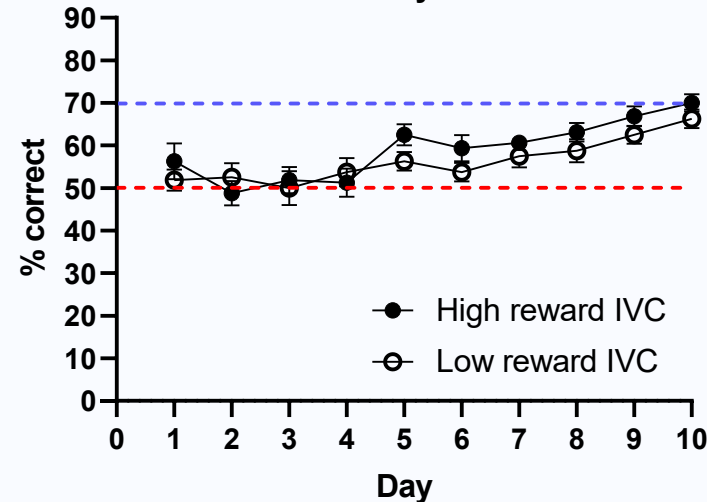
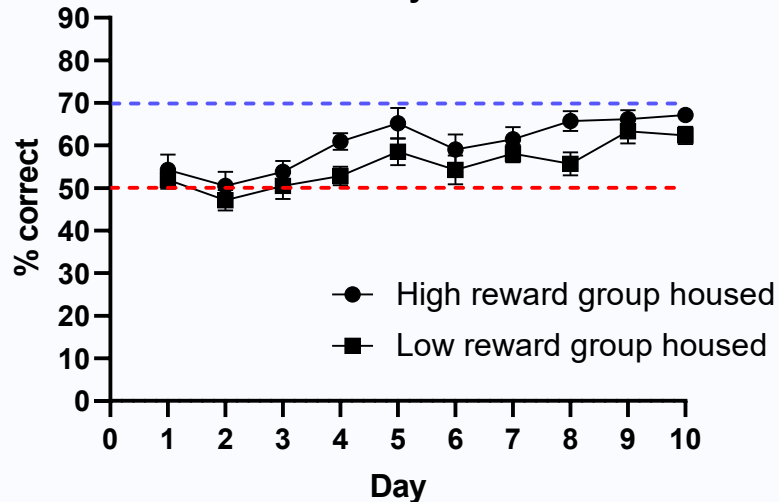
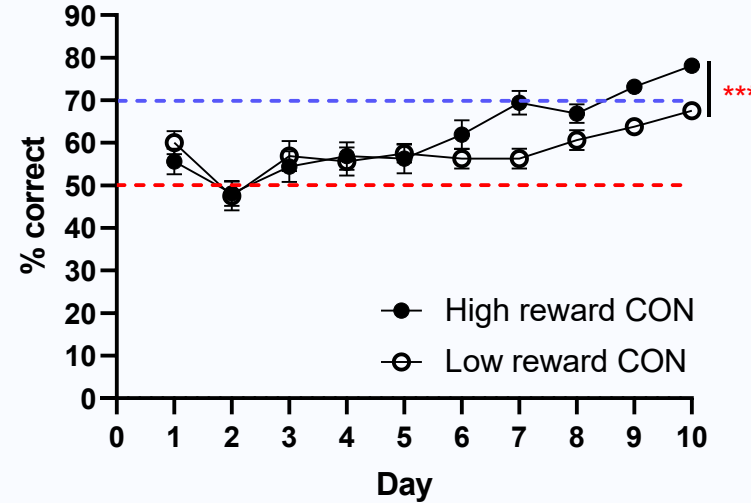
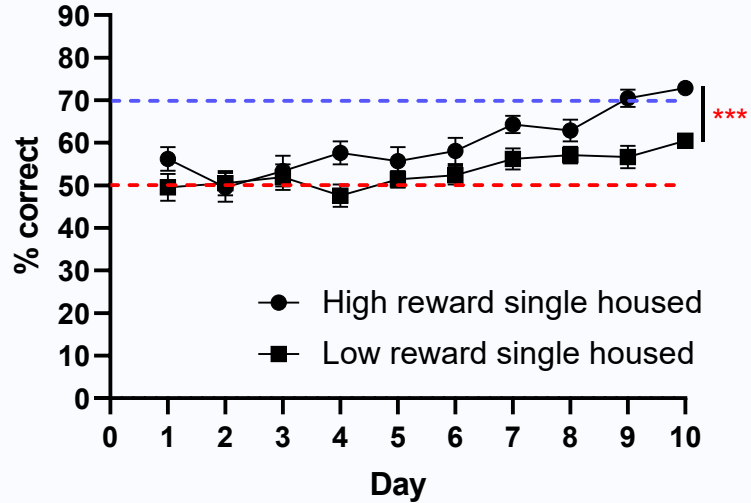



**Increases 50kHz USVs indicating positive affective state**

**Attenuates negative effects of acute aversive intervention**

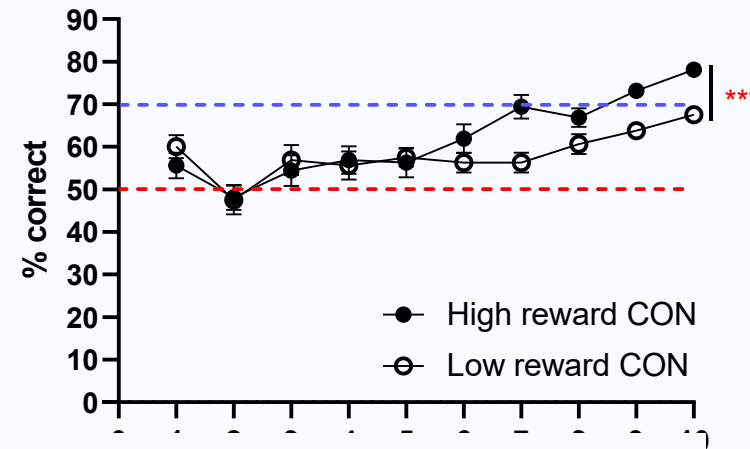
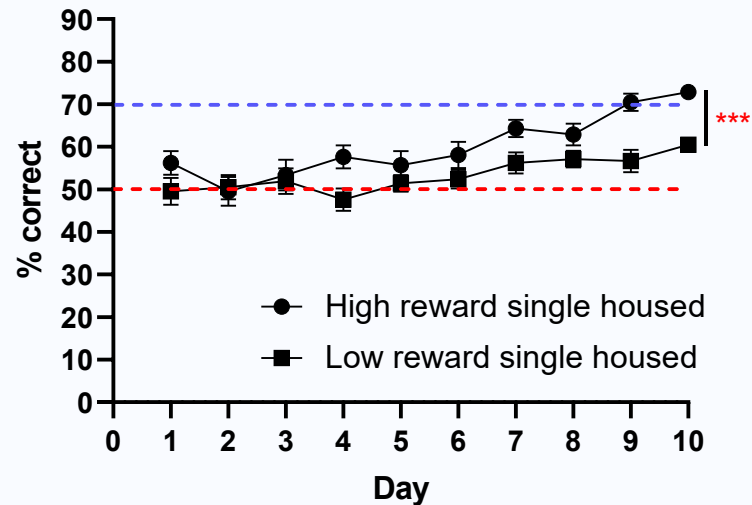


# Male mice prefer to live on their own (but they don't like IVCs)

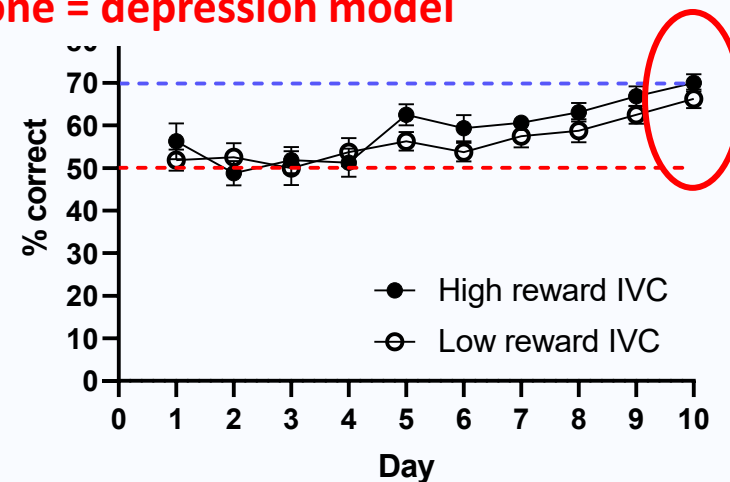
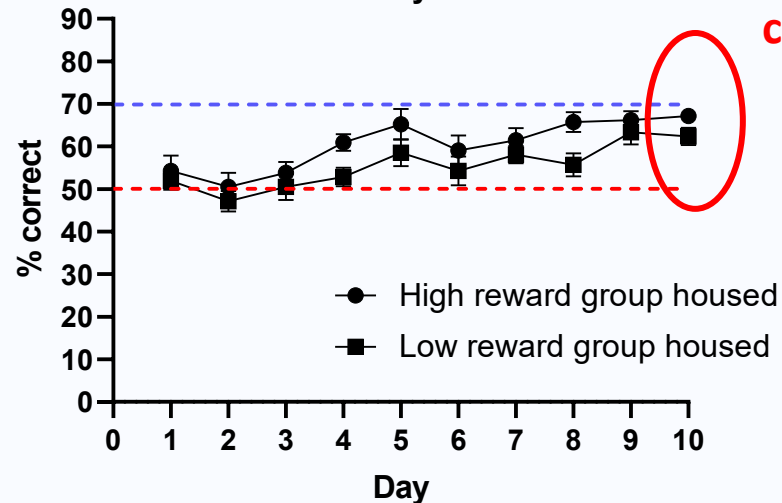


Development of a **reward-induced bias** in the provides an objective measure of core affective state in mice

# Male mice prefer to live on their own (but they don't like IVCs)



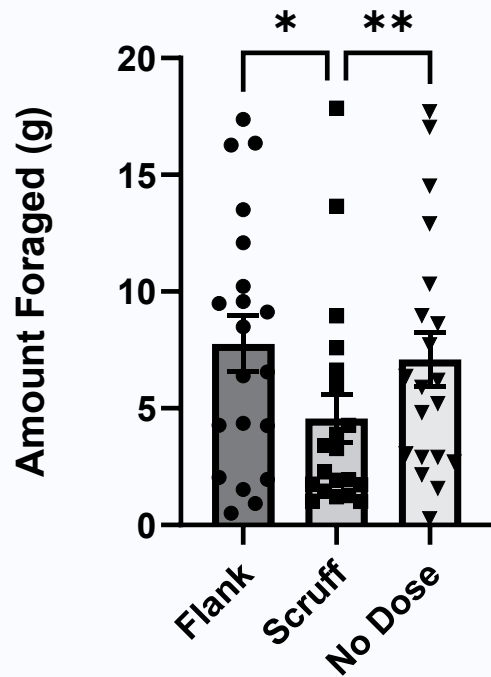
Equivalent to chronic exposure to corticosterone = depression model



Development of a **reward-induced bias** in the provides an objective measure of core affective state in mice

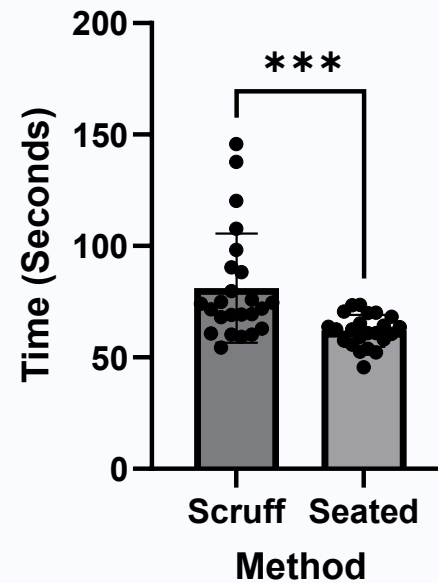
# Objective assessments of the impacts of restraint during common procedures

CD1 & C57BL/6 120 min Total foraged



Scruff handling for SC dosing leads to a sustained reduction in motivation to forage indicative of a negative affective state

Time to loss of righting reflex 2 methods



Time to loss of righting reflex following IP anaesthetic overdose is higher in scruff versus seated methods indicative of higher stress



# Positive reinforcement

- Pairing human interactions with a high value food reward to generate a positive affective experience
- Very small quantities of reward are needed – **‘rodent-sized’** portions
- Use after cage cleaning, welfare checks and regulated procedures
- Use to reinforce positive associations with handling
- Low calorie treat options e.g. diced apple



1x piece of popcorn = 2 slices of cake (mouse)



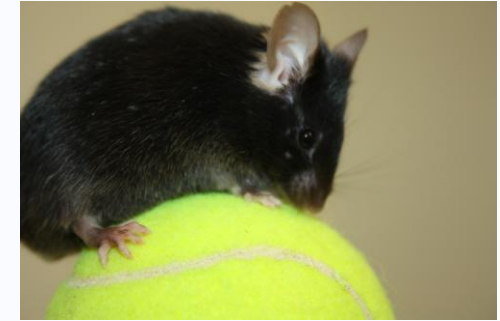
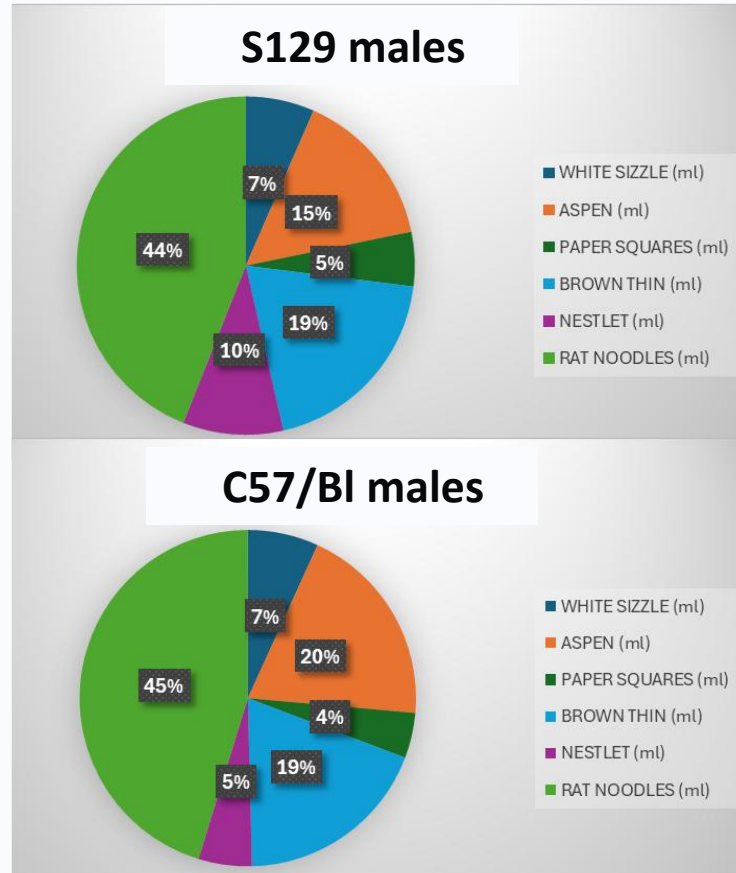
1x fat mealworm = 12-ounce steak (mouse)



# Providing more sensory diversity through home cage enrichment



When given the choice, mice make nests using multiple media – structure, insulation and liner



Olfaction and touch are rodent's dominant senses with vision and hearing prioritised to predator avoidance

# Replacing an aversive dosing method with a rewarding one



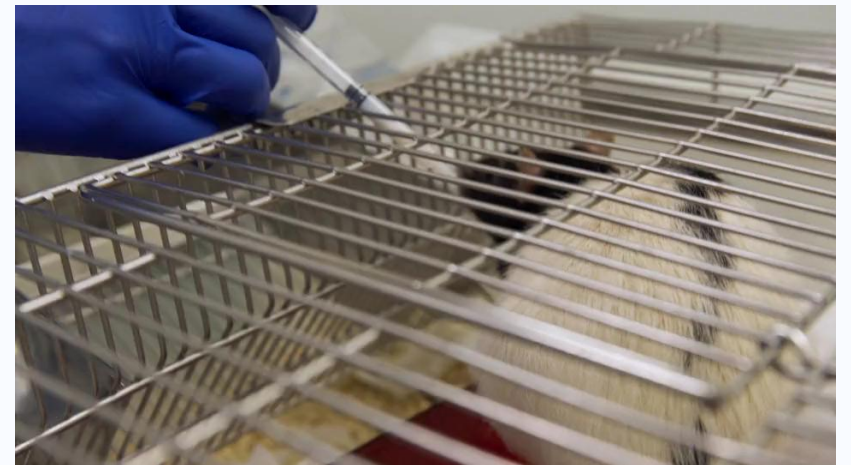
## Conventional oral gavage:

- Involves physical restraint and distress
- Risk of adverse events
- Requires high level of technical skill

## Refined method:

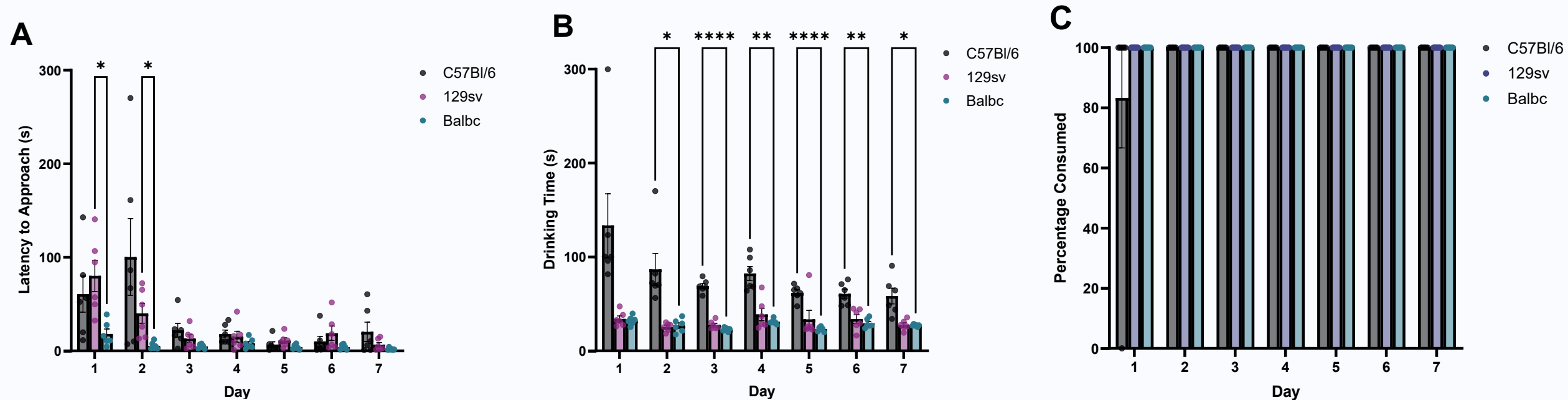
- Avoids physical restraint and provides a rewarding experience
- No risks associated with the administration procedure
- Optimised protocols
- Potential to delegate

## Self-ingestion



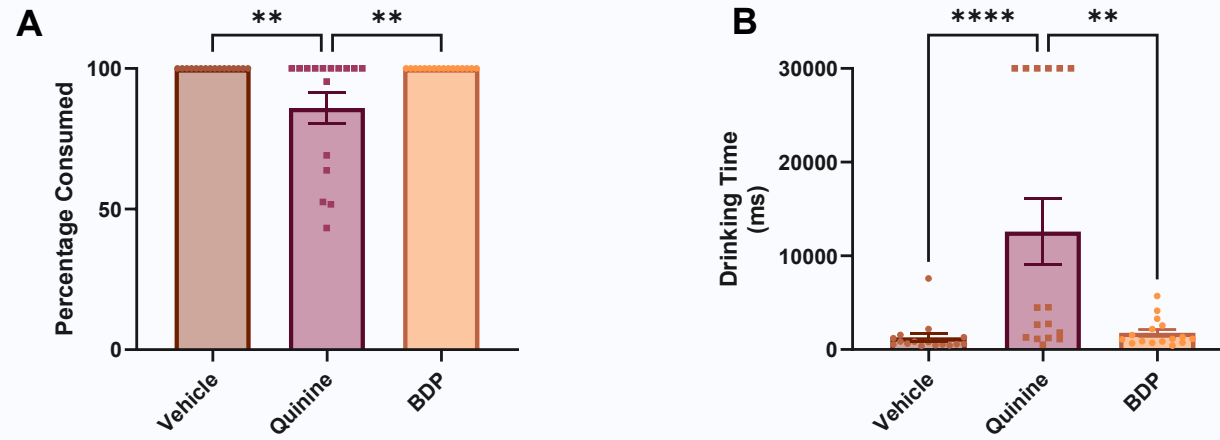


# Mice trained to self administer condensed milk from a syringe in less than 3 sessions

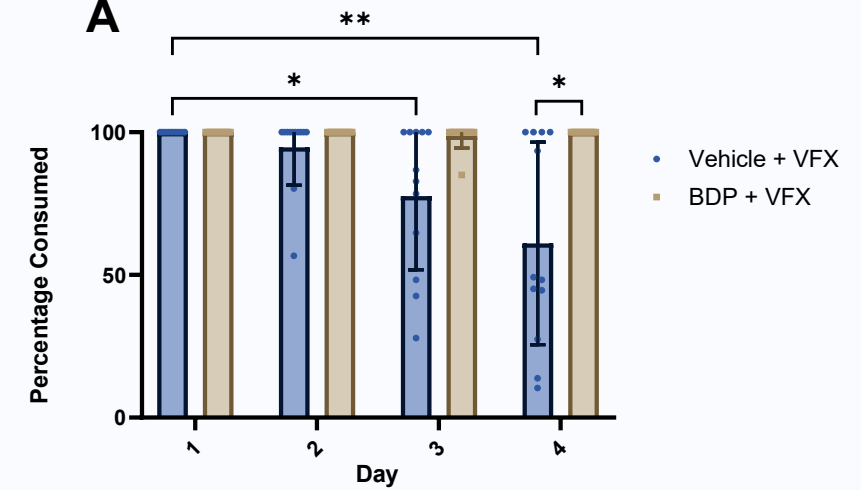


# Use of masking agents to improve intake of aversive flavoured substances

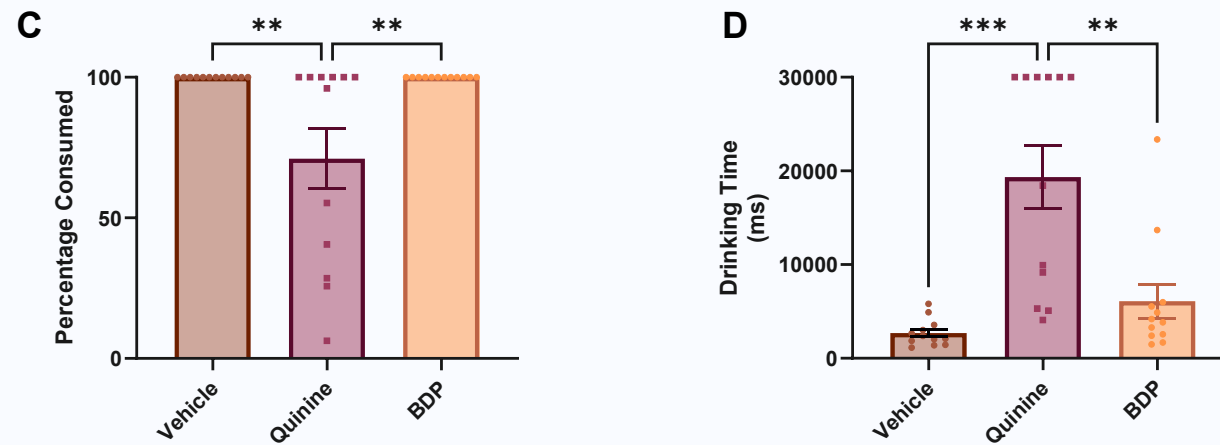
## Rat



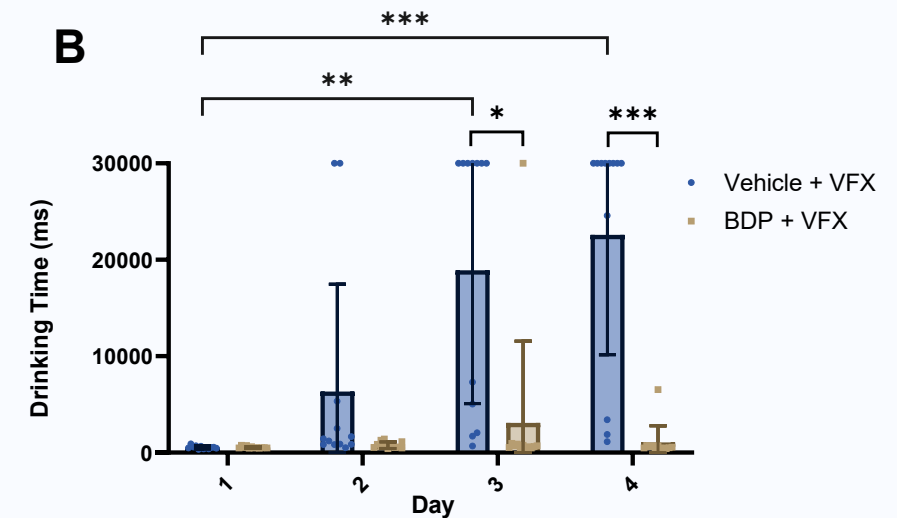
## A



## Mouse

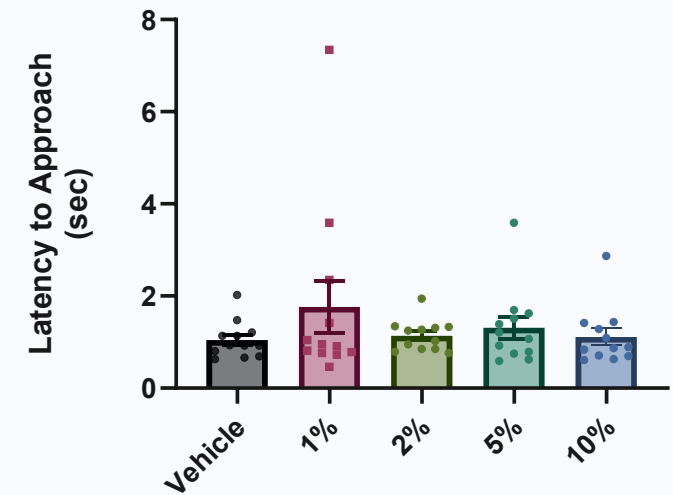
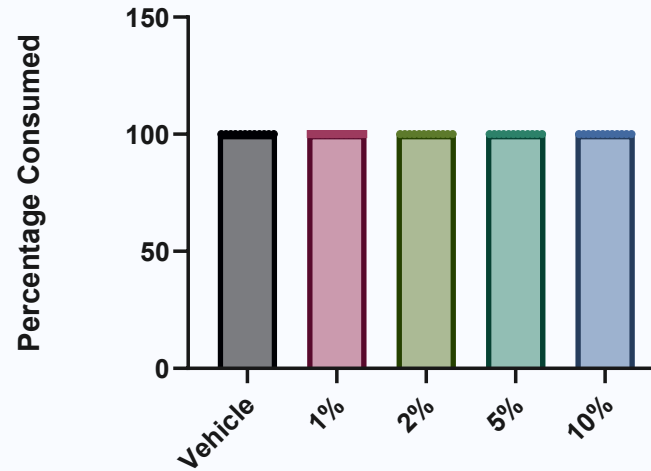
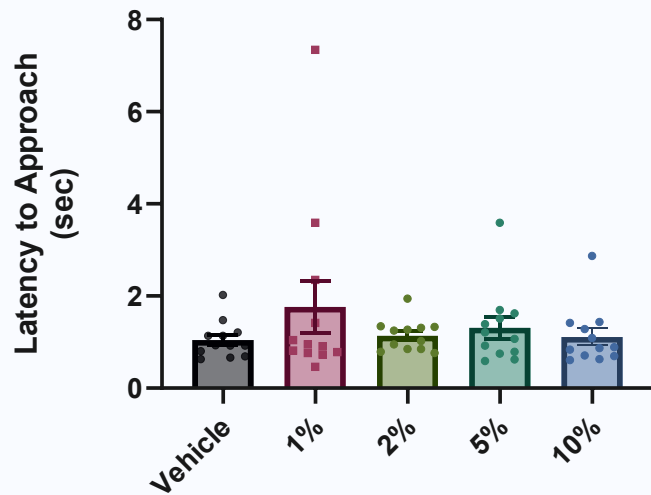


## B

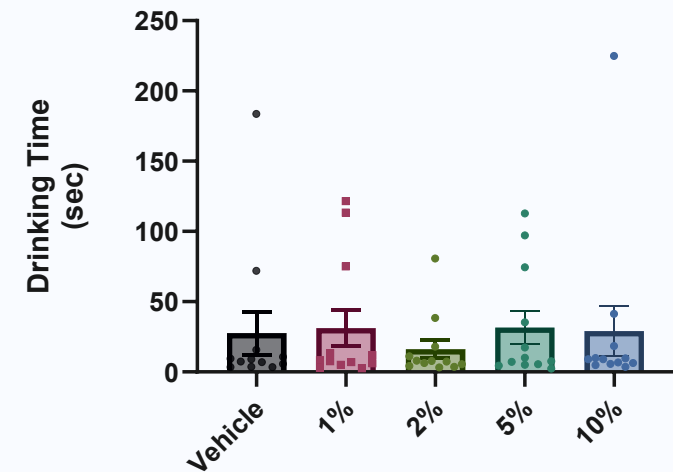
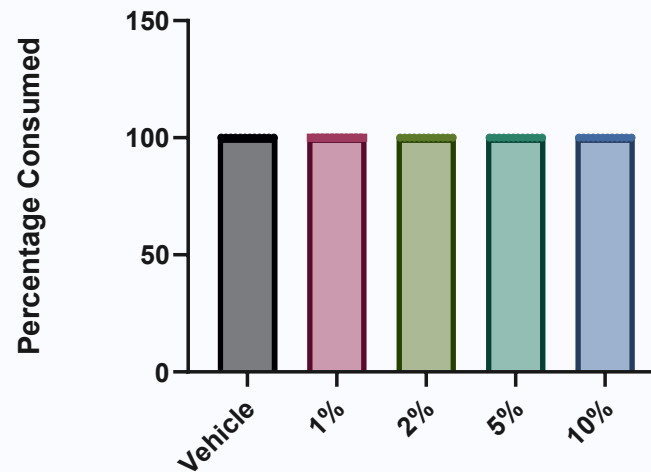
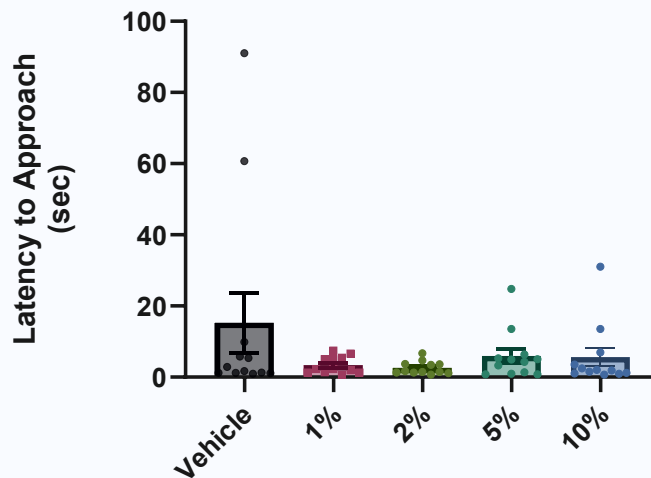


# DMSO up to 10% has no effects on voluntary ingestion of masking mixture

## RAT

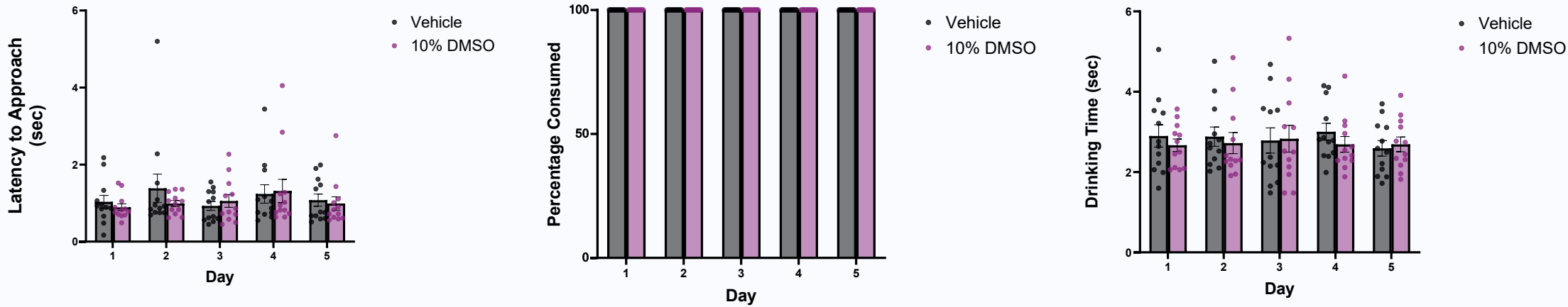


## MOUSE

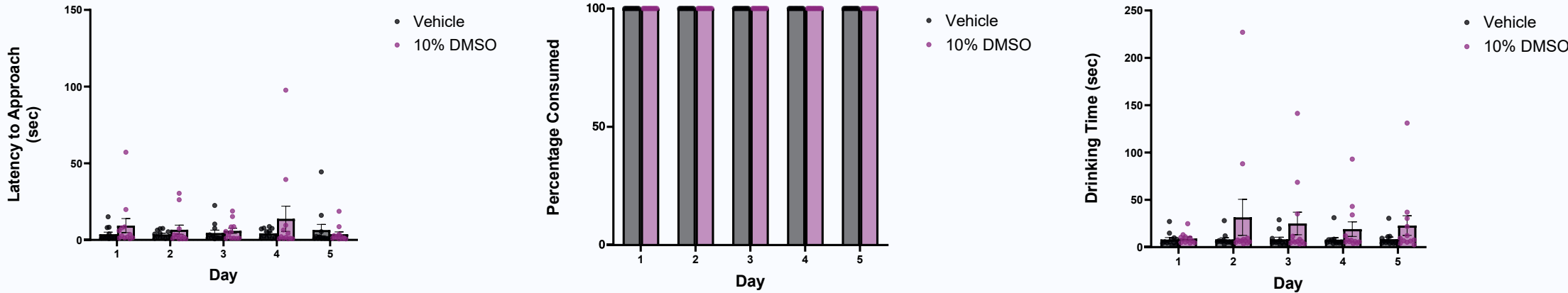


# Stable intake during repeated daily administration of 10% DMSO in masking mixture

## RAT



## MOUSE



# Oral administration of substances using palatable solutions

## Neophobia

- Introduce the palatable substance a week before you start training and dosing

## Development of aversion

- Use a masking agent to reduce the bitter taste of compounds in solution e.g. Bitter Drug Powder™
- To reduce any association of the drug's effect and the palatable substance (conditioned aversion):
  - use a daily dose of the vehicle before and during the study
  - Train animals to self-administer a variety of different flavoured vehicles e.g. strawberry, chocolate and banana milkshake and alternate throughout the study
  - Avoid regular routines to reduce associative learning



# Training rats and mice when restraint cannot be avoided e.g. tethering

Animals trained using positive reinforcement to accept brief periods of restraint



The rat 'burrito' – a method commonly used to physically restrain animals for tethering with head mounted devices



# Refined handling summary

- Habituation to handling with positive reinforcement fundamentally changes the affective state of laboratory mice and rats
- Providing agency is critical to success
- Rats can be managed for most scientific studies with no physical restraint
- Most restrained procedures in mice can be replaced with refined handling
- Positive handling generates more translational scientific outcomes



# Adapting the 3Hs framework for different management systems

## Principles of the 3Hs are relevant to all management systems



- Positive reinforcement during routine handling and procedures can be used in all management systems
  - Food rewards can be chosen based on scientific needs e.g. grain or protein-based treats
  - Providing treats in 'rodent size portions' unlikely to impact on overall nutritional intakes
- Habituation to handling
  - Can be integrated into all management systems
  - May take more time in IVC systems
- Reducing physical restraint
  - Handling methods can be adapted for IVCs e.g. using vet bed
- Play pens and some types of handling may not be feasible
  - Biosecurity concerns
  - Need to use laminar flow hoods and changing stations

**Ethical assessment should consider any scientific or management restrictions e.g. IVC caging, on positive welfare interventions in overall assessment of harm-benefits**

# Summary

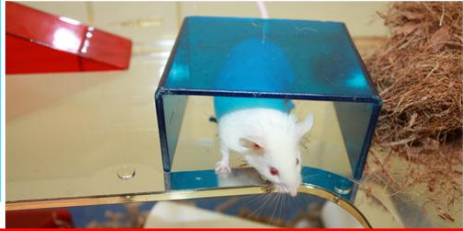
**Implementing the 3Hs may increase time spent with the animals  
BUT has major benefits for animal welfare and human wellbeing**



- Reduces cumulative suffering and refines lifetime experience
- Refinement can be achieved by:
  - Enhancing the housing environment
  - Reducing stress associated with handling and restraint
  - Habituation to procedures
  - Use of positive reinforcement
- We use objective methods to quantify the welfare benefits to support implementing best practise

# Summary

Implementing the 3Hs may increase time spent with the animals  
BUT has major benefits for animal welfare and human wellbeing



- Reduces cumulative suffering and refines lifetime experiences

**Habituated animals which associate human interactions with positive events are easier and quicker to manage**



- Enhance the housing environment
  - Reducing stress associated with handling and restraint
  - Habituation to procedures
  - Use of positive reinforcement
- 
- We use objective methods to quantify the welfare benefits to support implementing best practise

# Summary

Implementing the 3Hs may increase time spent with the animals  
BUT has major benefits for animal welfare and human wellbeing



Habit  
with

**Higher welfare supports better  
science and the 3Hs can help  
achieve this**

lifetime

eractions  
manage

and restraint



- Habituation to procedures
- Use of positive reinforcement
- We use objective methods to quantify the welfare benefits to support implementing best practise

# Acknowledgements

## Funding

NC3Rs and BBSRC

## Key researchers and co-developers

Megan Jackson, Julia Bartlett, Justyna Hinchcliffe,  
Gabriella Portlock, Jennifer Davies

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