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

HOUSING HANDLING HABITUATION

The 3Hs Initiative

Improving the lives of laboratory rodents one lab at a time

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BBSRC bioscience for the future

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University of BRISTOL

Our background

- Work in our laboratory uses behavioural studies alongside neuropharmacological and neurochemical approaches to study the role of specific neural systems in the control of behaviours relevant to psychiatric disorders.

- To create meaningful models or detect sensitive behaviours, our controls must be happy and healthy.

- This led to us to think carefully about the lifetime experience of the lab rodent.

Contents

Factors which contribute to cumulative suffering

1. Laboratory caging systems and management methods

- Small cages restrict natural behaviours
- Limited opportunities for sensory enrichment
- Frequent social stressors e.g. cage cleaning

2. Impacts of repeated use of physical restraint

- Routine husbandry
- Common procedures e.g. substance administration

3. Negative associations with humans

- Natural fear
- Aversive experiences

Why does it matter?

Stress in lab rodents has been found to affect:

- Severity of disease model
- Response to pharmacological treatments
- Morbidity & mortality
- Vary between subjects e.g. active vs passive coping

Inaccurate conclusions (reproducibility crisis)
Greater variation
More n numbers required = greater animal wastage

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

Core aims of the 3Hs

Reduce use of physical restraint

Provide more naturalistic environments (within constrains of facility)

Develop habituation methods preceding procedures

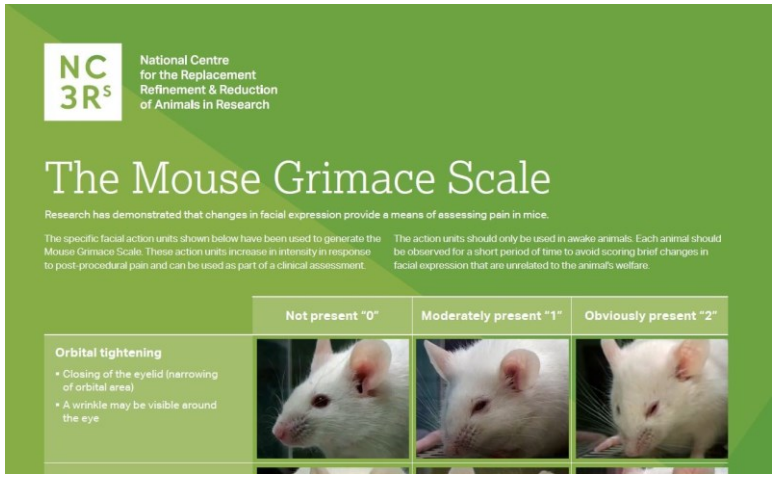
Produce quantitative evidence of welfare benefits

In this talk I will provide examples of some of our refinements across handling, housing and habituation

Our welfare assessment tools

Commonly used methods may lack sensitivity

- Plasma, salivary or faecal corticosterone
- Faecal pellets*
- Overt signs of distress
- Grimace scales
- Score sheets for specific procedures

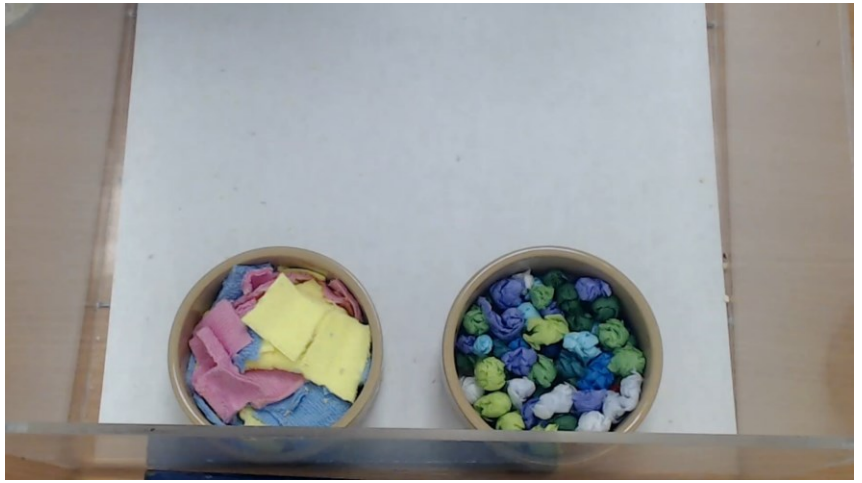


Objective methods based on neuropsychological model of affective biases in mood disorders

- Affective bias test – measures acute changes in affective state e.g. impacts of acute restraint
- Reward learning assay - measures changes in core affective state e.g. impacts of different housing conditions

Our welfare assessment tools

Affective bias test-Rats



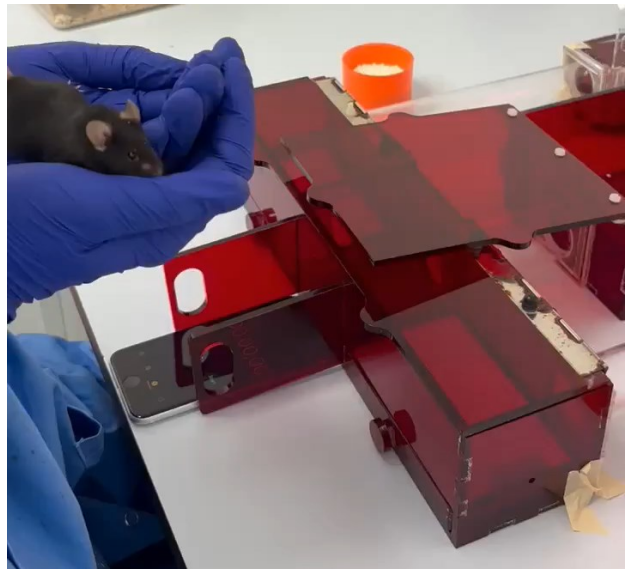
- Each rat learns two independent cue-reward associations (digging in bowl, finding a reward).
- Absolute value of each experience is the same
- Same rat experiences one under control conditions and one following a manipulation of their affective state
- Each rat is ‘asked’ to recall their memory of each experience and decide which they prefer

= % choice bias

Our welfare assessment tools

Reward learning task-Mice

- A T maze task where the mouse learns to associate a patterned floor with a high (2 pellets) versus low(1 pellet) reward.
- Bias for learning high over low reward calculated (% bias).
- A healthy mouse should learn the high reward rule more quickly than the low reward.



= % choice bias

Our welfare assessment tools

A positive bias indicates a positive affective state while a negative bias indicates a negative affective state.

We can use this output measure to understand whether a certain condition changes affective state.

Examples from Housing

Single housing mice

Playpens

Housing mice

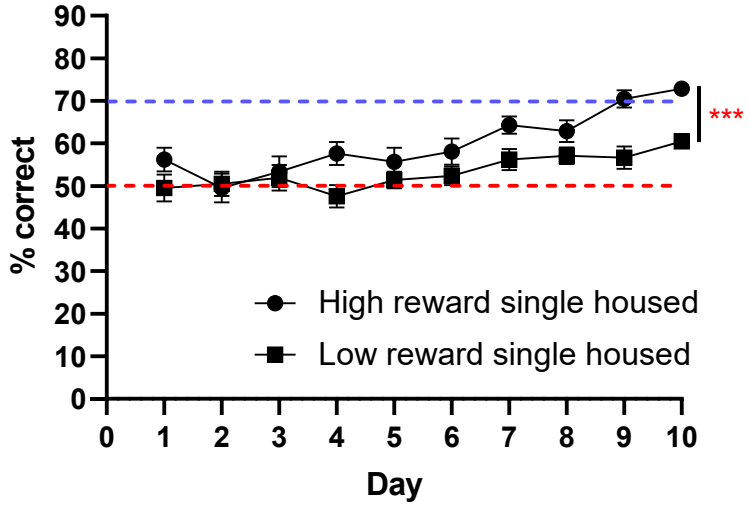
What does the ethology of the species tell us? Are all species social?

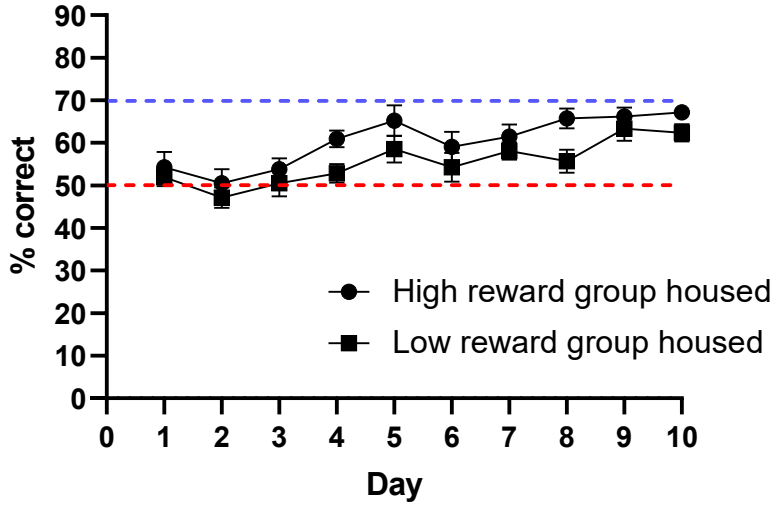
- Mice are thought of as a social animal and guidance suggests group housing is optimal for welfare.
- However, high levels of infighting are often observed, often leading to injury, separation or death.
- This can have profound effect on study quality and outcome.

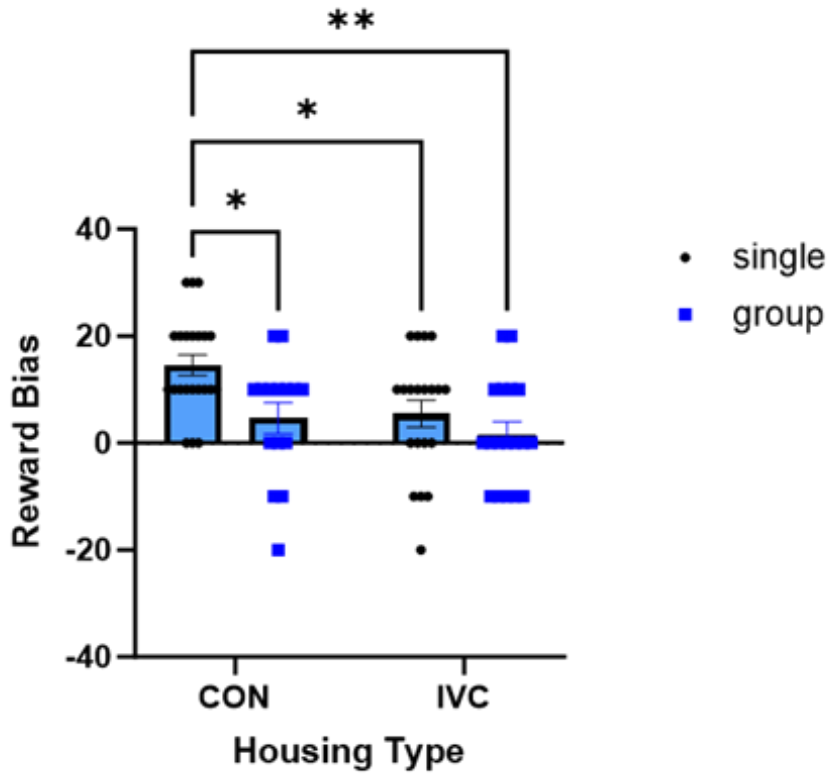
What is the impact of IVC caging?

Housing mice

Applying our welfare assessment tool







Singly housed mice in conventional caging learn the high reward the best...

Male mice are in a more positive affective state when housed alone

Playpens allow naturalistic behaviours

Rat Playpen

Mouse Playpen

- Particularly beneficial for social but singly housed animals.
- Can be used for enrichment, as a reward and to reduce the impact of aversive procedures.
- Examples in many other species e.g., pigs, rabbits, dogs

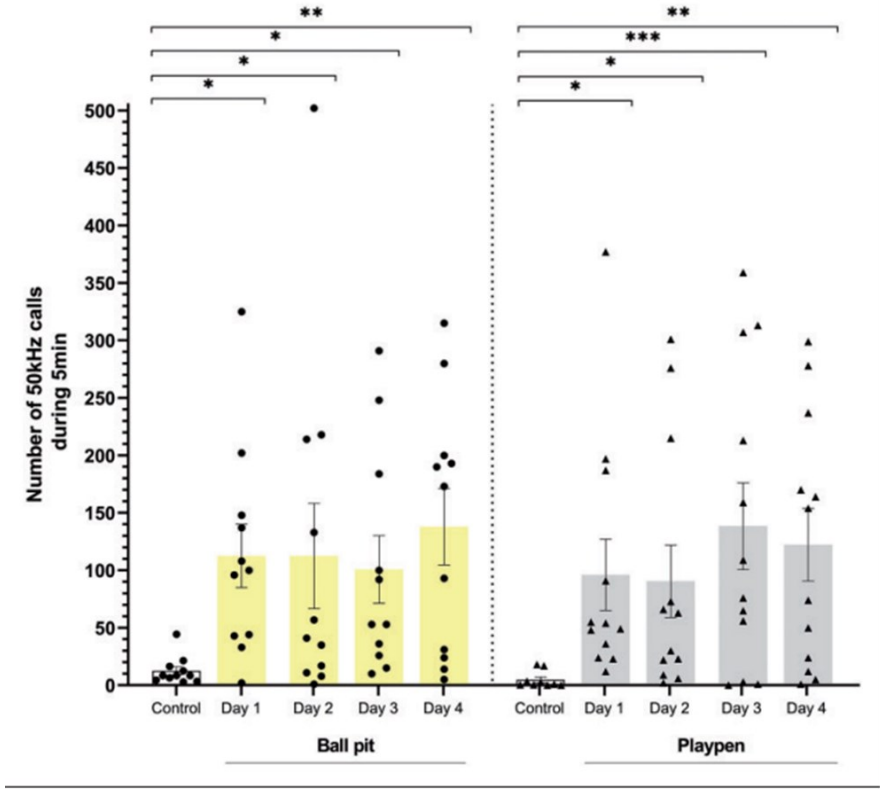
Playpens facilitate naturalistic behaviours

Pig Playpen

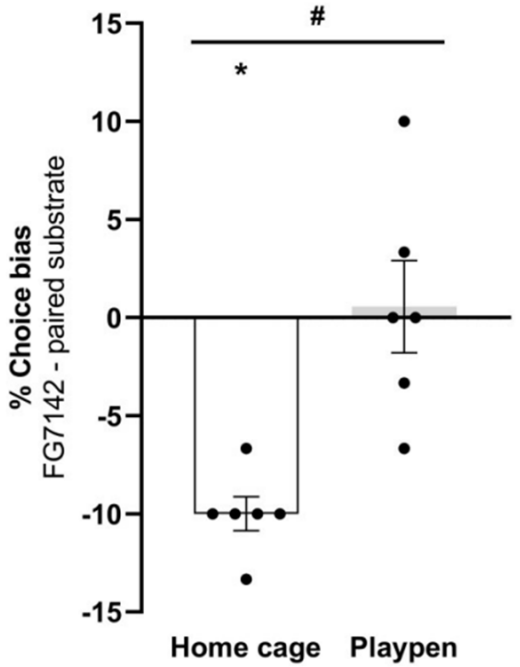
- Particularly beneficial for social but singly housed animals.
- Can be used for enrichment, as a reward and to reduce the impact of aversive procedures.
- Examples in many other species e.g., pigs, rabbits, dogs

Playpens induce a positive affective state

...and block the negative impact of aversive procedures



Lots of positive 50khz calls!



Playpen time blocked formation of a negative bias

Time in a playpen induces positive USVs and blocks a negatively induced bias

Examples from Habituation

Habituation to handling mice

Habituation to handling rats

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Types of reward

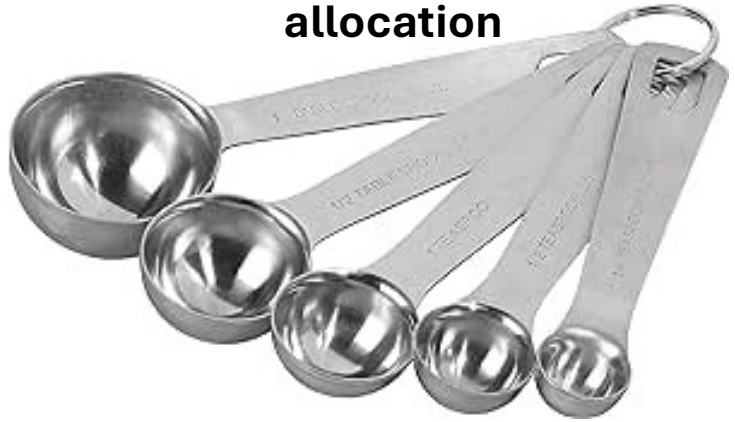
Liquid reward – can be diluted to reduce nutritional value

Mealworms can be crushed or liquidised to create a crumb

Lab diet suppliers offer a variety of treat options suitable for different species and management systems

Human foods

Measuring spoons can be used to enable correct portion allocation



Habituation: a step wise approach

A step wise approach minimises initial stress and formation of negative associations

Mice

Day 1: gently tube mouse onto palm and allow to move off.

Food reward at every stage!

Day 2-3: gently tube mouse onto palm, move between palms

Day 4-5: gently guide mouse onto palm from home cage

Always account for individual variability

Mice will sit on the open palm

Habituation: a step wise approach

Rats

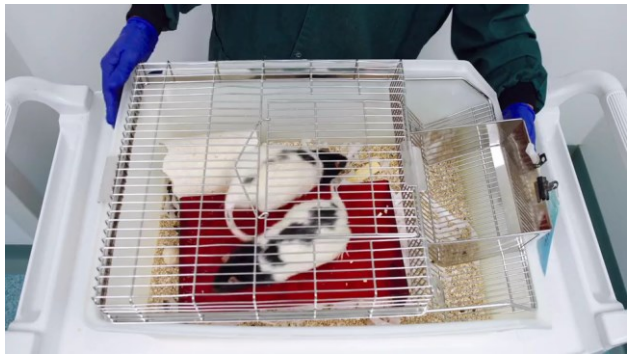
Day 1: gentle lifting from cage and back

Day 2: gentle lifting and transfer to travel box

Day 3: Wait for approach

Day 4: Introduce dosing positions

Food reward at every stage!



Examples from Handling

Positive context

Positive handler association

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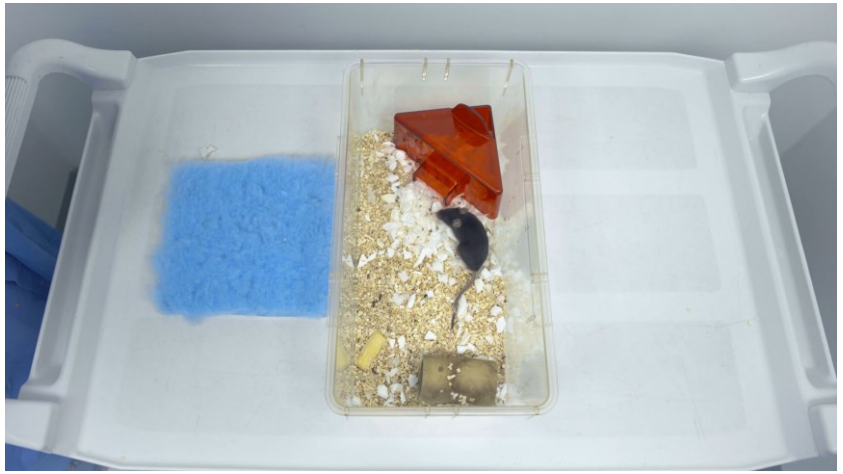
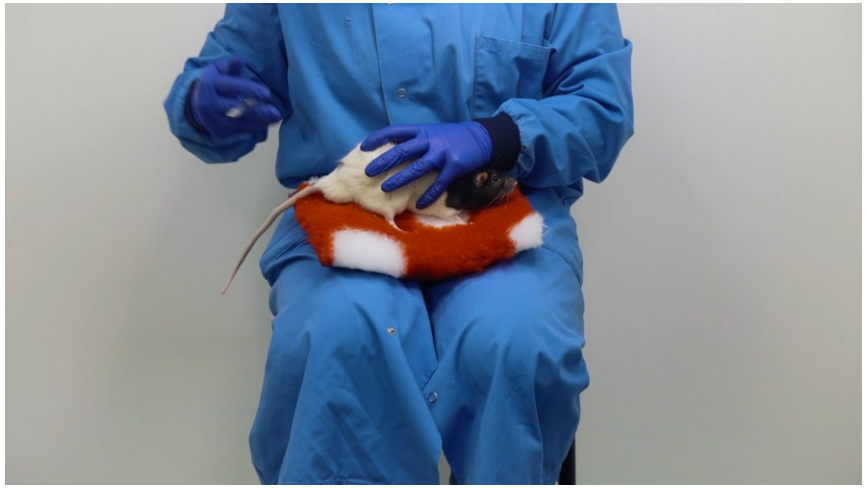
Handling-mitigating impact of restraint with positive context

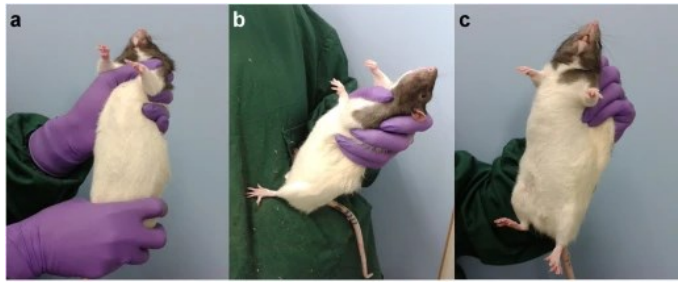
- Rodents need to be securely restrained for a range of different procedures (dosing, sampling etc).
- They can be habituated to a vetbed using food reward.
- A positively associated environment minimises the aversive impact of restraint.





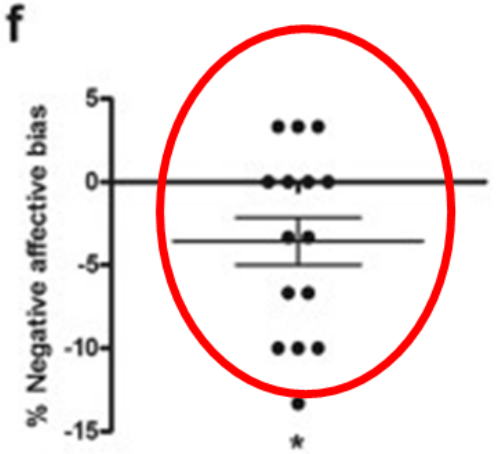
Handling-mitigating impact of restraint with positive handling

- IP injection in rats



Conventional methods:

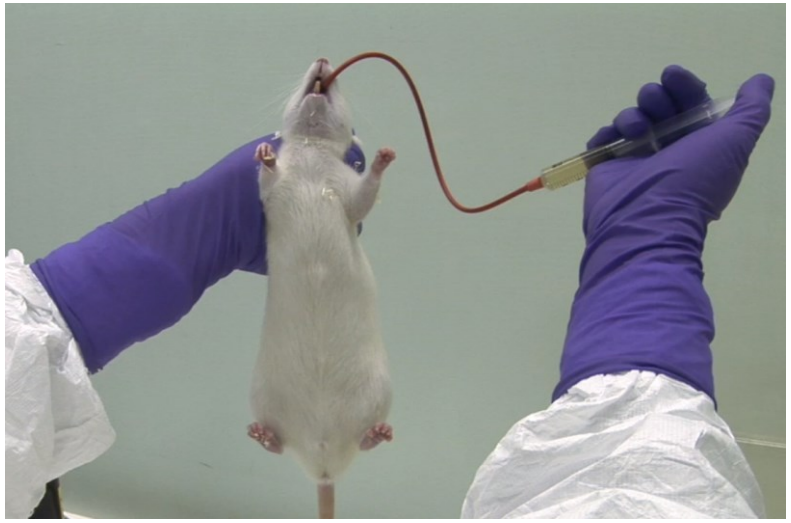
- Involve physical restraint
- Animals can sensitise
- Stressful for technical staff



Refined method:

- Reduced physical restraint with reduced overt signs of distress
- Animal's abdominal muscles are relaxed reducing pain
- No evidence of intestinal damage
- Less likely to bite handler as they are not the source of discomfort

Do they need to be restrained at all? Oral palatable dosing



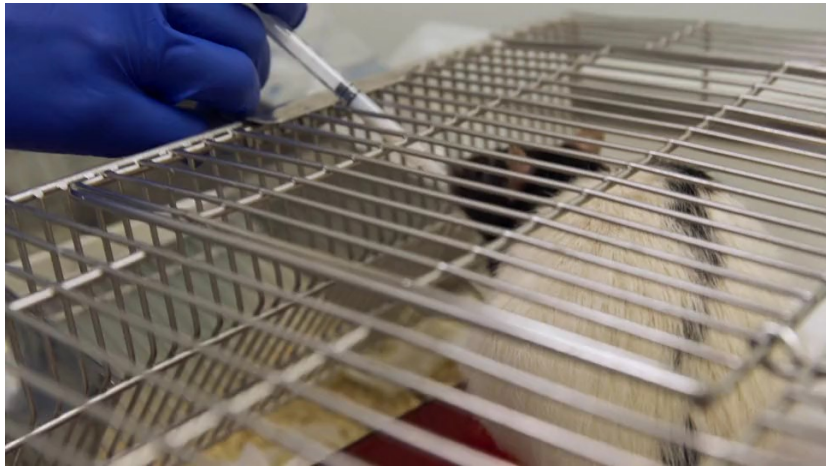
Conventional methods:

- Involve physical restraint
- Risk of adverse events
- Requires high level of technical skill

Refined method:

- Avoids physical restraint
- No risks associated with the administration procedure
- Very reliable
- Potential to delegate

Self-ingestion



Trans-species applications

Facilitating species-specific natural behaviours

Stepwise habituation to procedures

Formation of positive association with handler

Conclusion

- We should aim to refine the lifetime experience of the lab species, not just procedures.
- We provide objective evidence of welfare benefits using our refined protocols.
- There are opportunities to apply the framework to larger animals.