

# The science behind non-aversive mouse handling



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Mammalian Behaviour & Evolution  
[www.liv.ac.uk/mbe](http://www.liv.ac.uk/mbe)



# Working with wild rodents

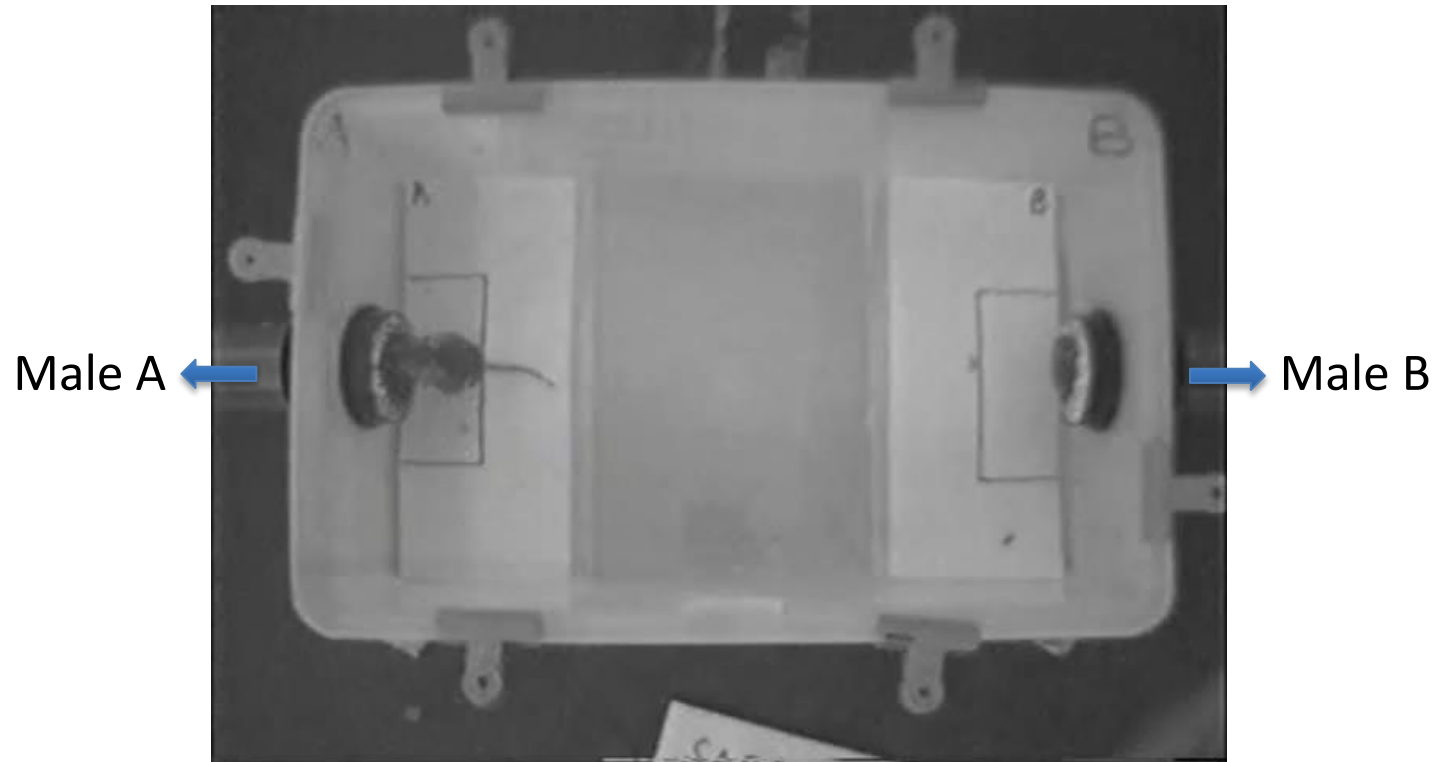


- Understanding behaviour in naturalistic environments
- But detailed understanding requires controlled tests

Henry Wellcome Laboratory of  
Mammalian Behaviour & Evolution

# Bioassays under controlled conditions

- ▶ Wild mouse co-operation requires much care in approach and handling
- ▶ Lab mice used as model for wild mice
  - Difficult to replicate normal mouse behaviour
  - Anxiety in lab mice is a major problem



Female wild mouse choosing between males in side tunnels



# Reproducibility & animal stress

## Lack of Reproducibility Plagues the Scientific Community

researchers at pharmaceutical giant Amgen were not able to reproduce data from 47 out of 53 groundbreaking publications on drug discoveries in cancer research.



**Mouse tales.** Researchers are increasingly worried about their inability to reproduce the results of studies involving laboratory animals, such as mice.

V. Altounian/Science

NIH Takes Steps to Improve Reproducibility

- Why do lab mice show such high anxiety?
- Laboratory has major confounding influence on behavioural phenotypes influenced by stress  
*Crabbe et al. (1999) Science*
- Experimenter outweighs genotype in mouse pain studies  
*Chesler et al. (2002) Nature Neuroscience*

# Taming anxiety in laboratory mice

nature|methods

Brief Communication

## Taming anxiety in laboratory mice

Jane L Hurst  & Rebecca S West

*Nature Methods* **7**, 825–826 (2010)

doi:10.1038/nmeth.1500

[Download Citation](#)

[Behavioural methods](#) [Mouse](#)

Received: 30 June 2010

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### Abstract

Routine laboratory animal handling has profound effects on their anxiety and stress responses, but little is known about the impact of handling method. We found that picking up mice by the tail induced aversion and high anxiety, whereas use of tunnels or open hand led to voluntary approach, low anxiety and acceptance of physical restraint. Using the latter methods, one can minimize a widespread source of anxiety in laboratory mice.

### NC3Rs 2010 Prize



ASAB

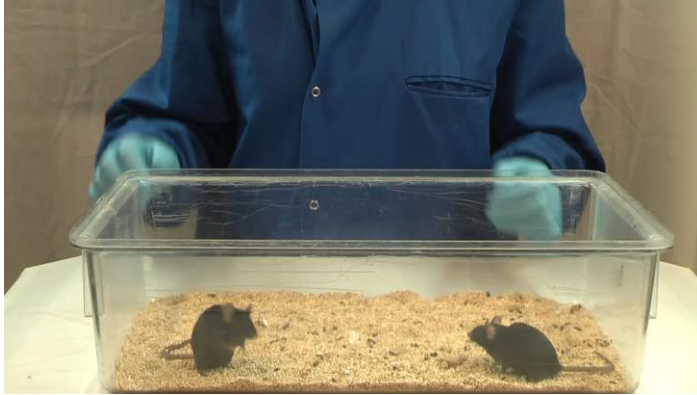


National Centre  
for the Replacement  
Refinement & Reduction  
of Animals in Research

wellcome trust



# Handling methods compared



**Traditional tail handling**



**Picked up in tunnel**



**Cupped on open hand**



Hurst & West (2010) *Nature Methods*

# Measuring the difference

Assessed from many different angles:

- ▶ Voluntary interaction with the handler
- ▶ Attempt to avoid capture
- ▶ Stress during capture
- ▶ Anxiety following handling
  - Elevated plus maze tests
  - Open field tests
- ▶ Reliability in behavioural testing
- ▶ Response to injection
- ▶ Effects on physiological responses (other labs)

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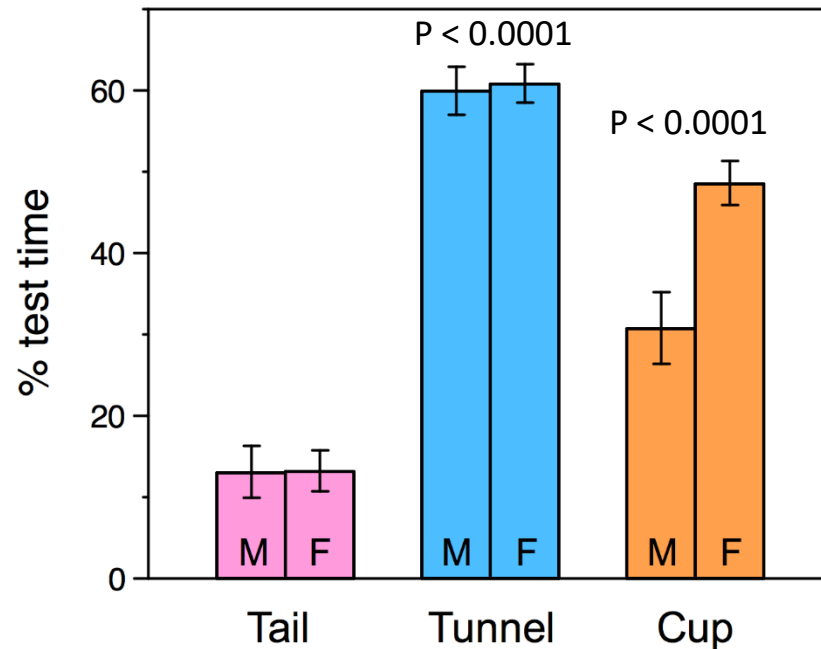
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Consistent conclusion:  
Picking up mice by tail has  
negative effects compared to  
alternative methods



# Voluntary interaction with handler

- ▶ Tunnel or cup handled mice are much more willing to interact voluntarily with their handler



- ▶ No major differences between
  - Strains
  - Handlers – regardless of experience
  - Light or dark period

Tail handled

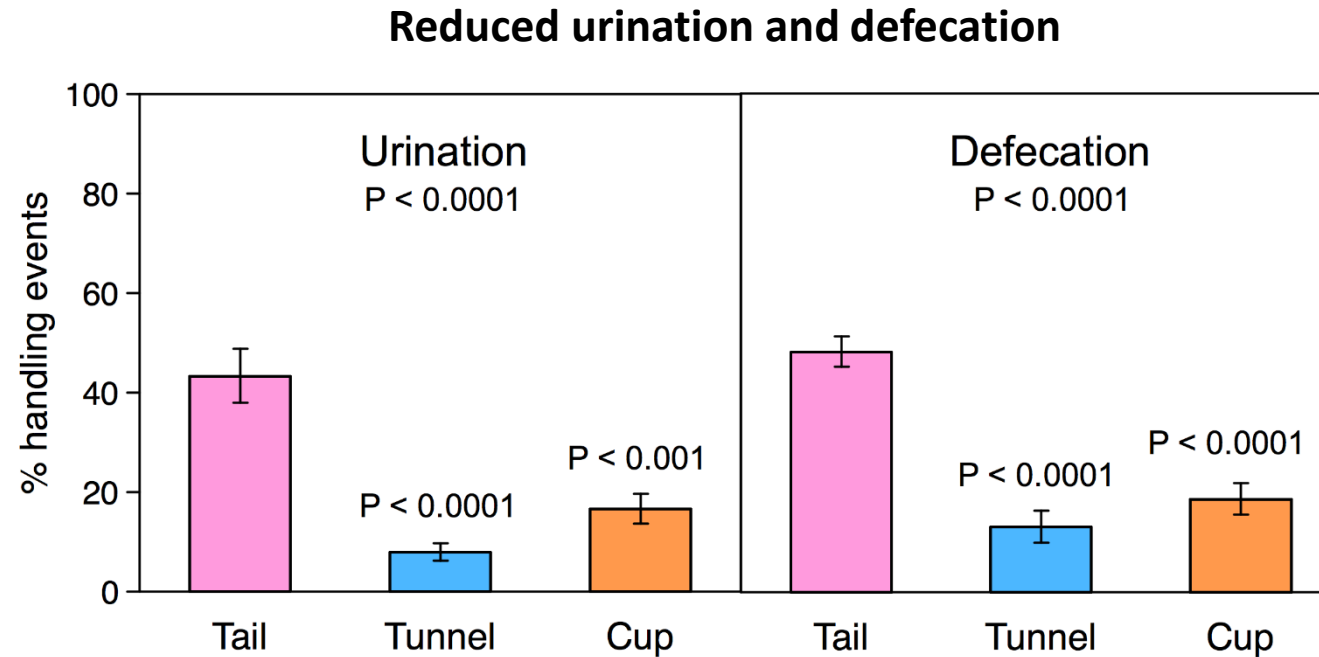


Tunnel handled

(cage enrichment removed for filming)

# Stress during handling

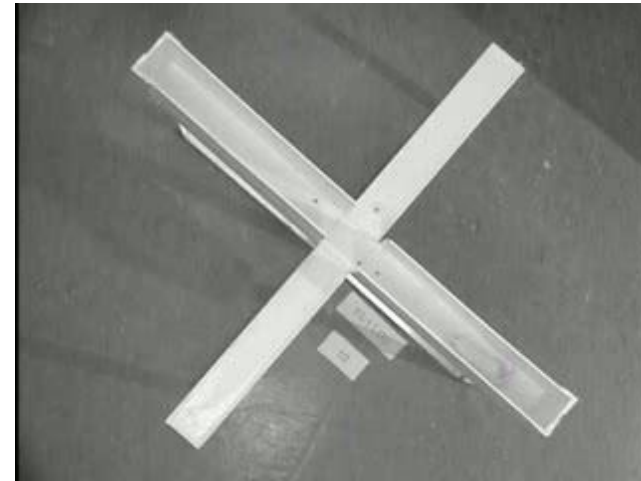
- ▶ Tunnel and cup methods reduce handling stress



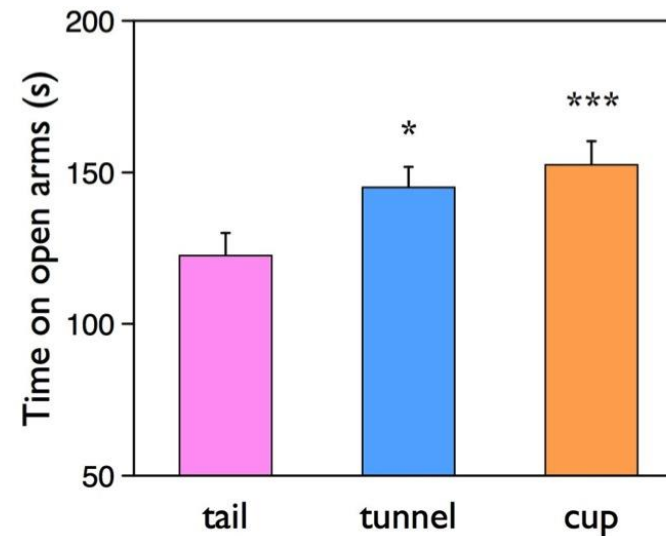
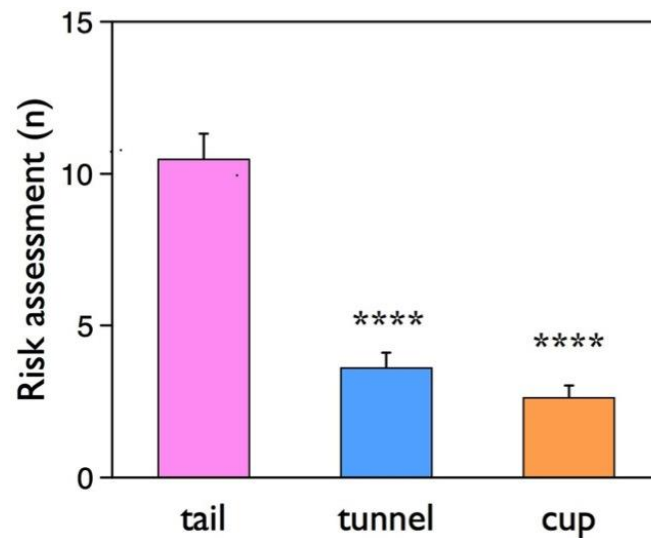
Scored each time a mouse was picked up  
BALB/c mice, n = 864 handling events

# Tests of anxiety

- Anxious animals
  - avoid open areas
  - show more risk assessment behaviours
- **Elevated plus maze test**
  - Tail handled mice consistently show greater anxiety

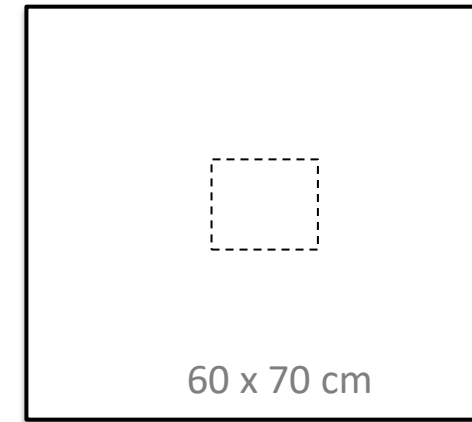


300s test



# Tests of anxiety

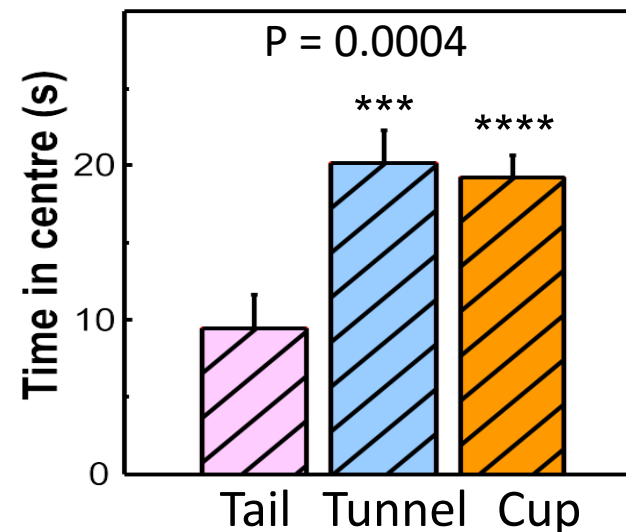
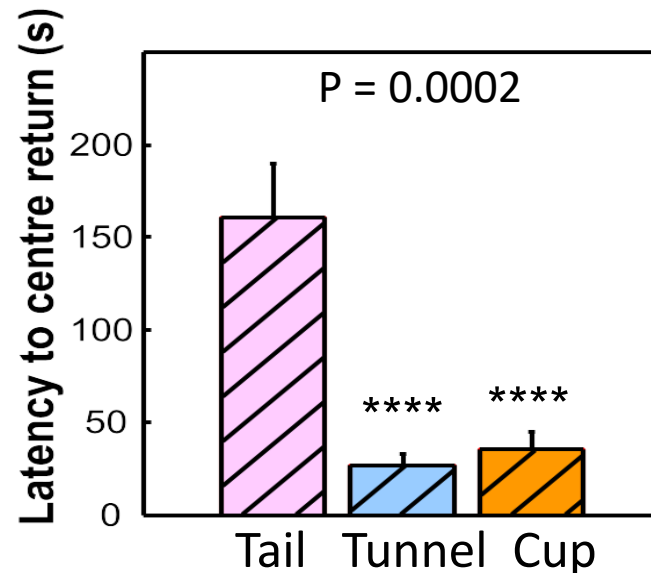
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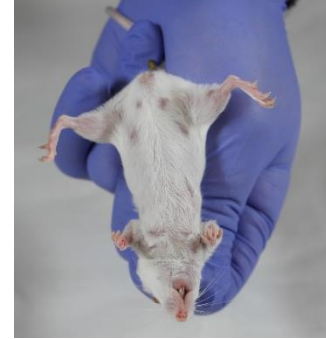
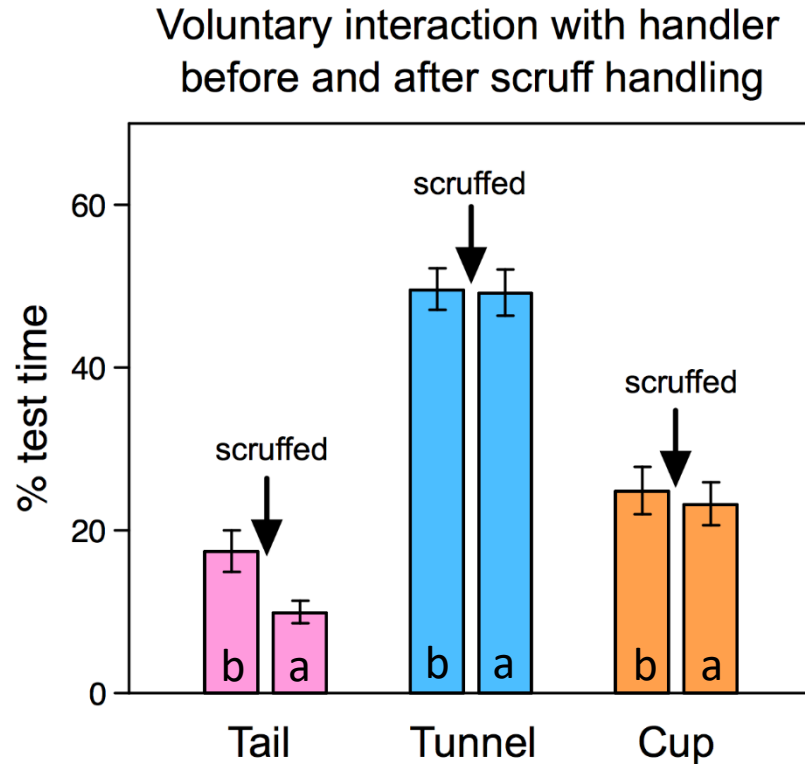
300s test

- **Open Field Test**

- Tail handled mice consistently show greater anxiety in open field



# Response after scruff restraint



- ▶ Scruff restraint does not reverse taming by tunnel or cup handling
- ▶ Only mice picked up by tail avoid handler after scruff restraint

Methods differ both before (b) and after (a) scruffing,  $P < 0.001$

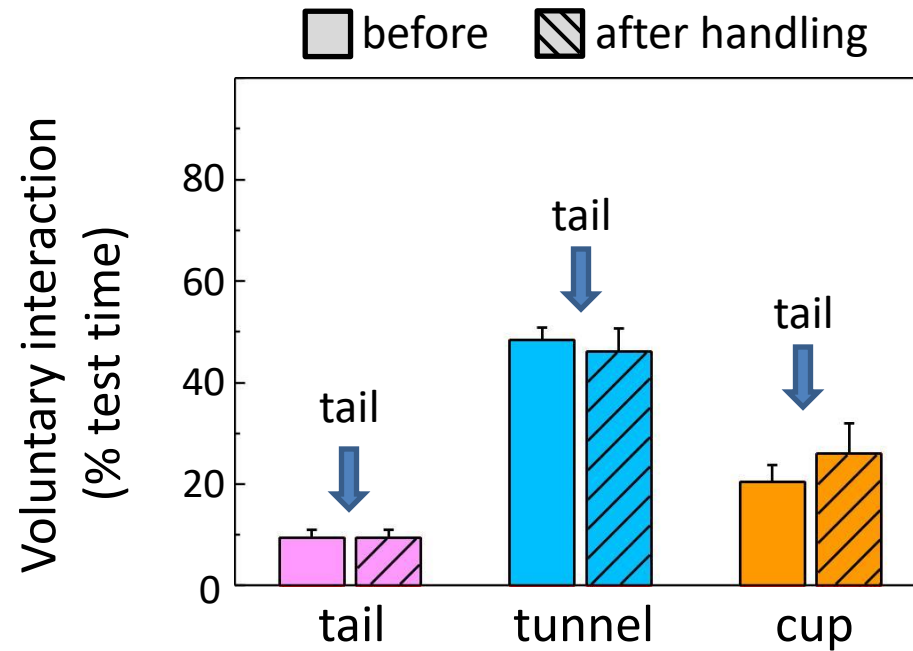
Mean  $\pm$  sem,  $n = 24$  cages per method,  
three strains combined



# Tail manipulation

Aversion is response to capture & pick up by tail

- ▶ Lifting by tail after pick up for abdominal inspection is not aversive



- ▶ Mice picked up by normal method
- ▶ Placed on hand  
(tunnel mice tipped out onto hand)
- ▶ Restrained and lifted by tail to inspect abdomen

# Implementation of non-aversive handling

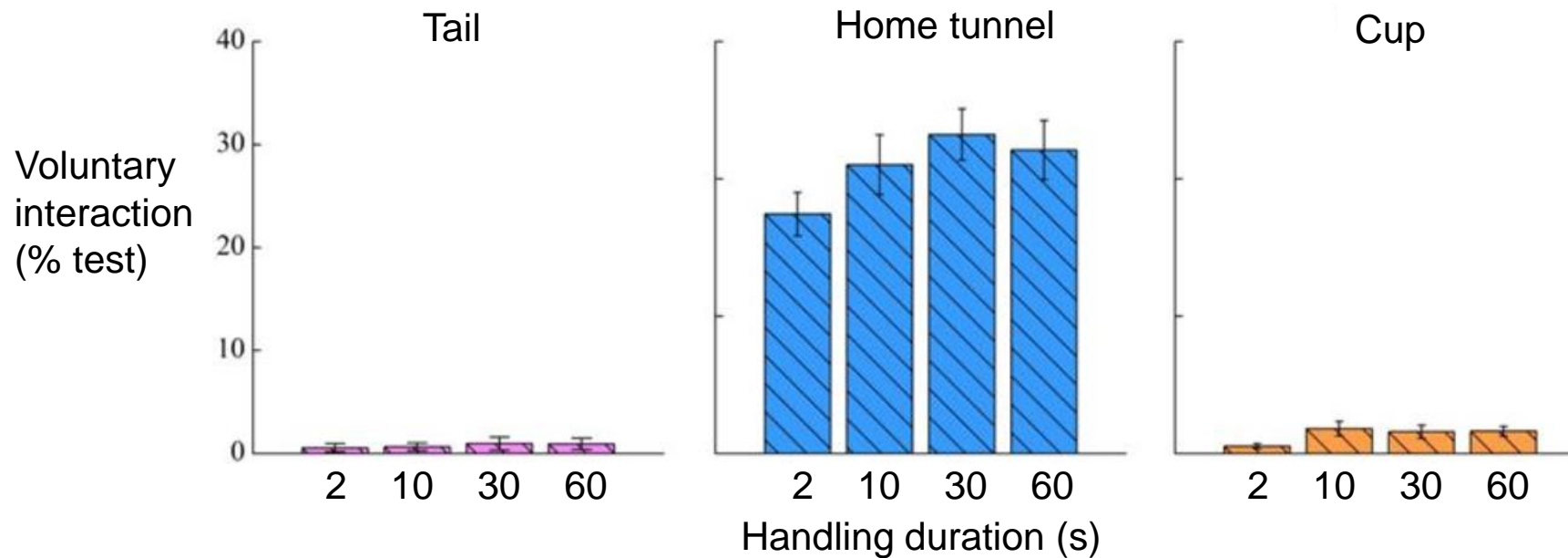
- ▶ How practical is it to use tunnel and cup for routine mouse handling?
  - ▶ Duration & frequency of handling required
  - ▶ Do tunnels need to be present in home cages?
  - ▶ Response to restraint & injection
- ▶ What is the impact of handling method on scientific data?
- ▶ How consistent are responses between laboratories?
  - ▶ Cancer Research UK, AstraZeneca, Liverpool
  - ▶ Evidence from other laboratories
- ▶ What are the barriers to adoption?
  - ▶ Animal care & researcher perspectives
  - ▶ Online tutorial and other materials



Kelly Gouveia  
NC3Rs PhD student

# Brief handling - duration

- ▶ No difference in response whether held for 2s, 10s, 30s or 60s
- ▶ 5 daily handling sessions insufficient to accustom mice to cupping

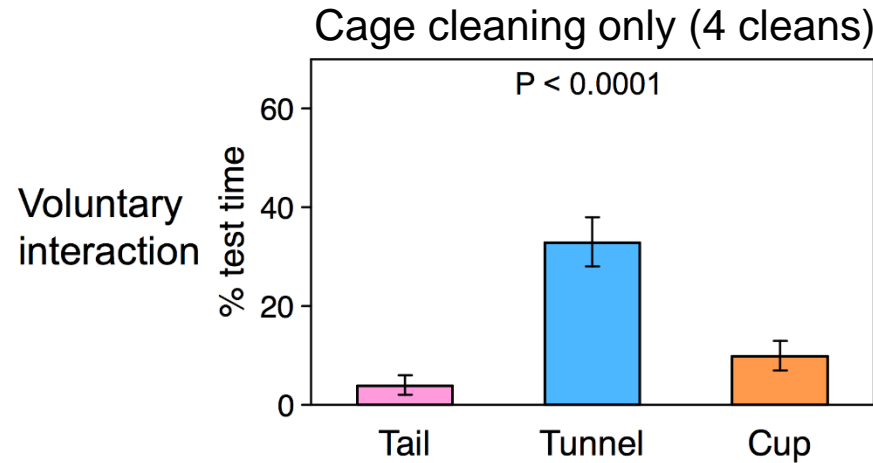


Data for 160 C57BL/6 mice  
(both sexes) after 5 daily handling sessions

Gouveia & Hurst (*data in prep*)

# Brief handling - frequency

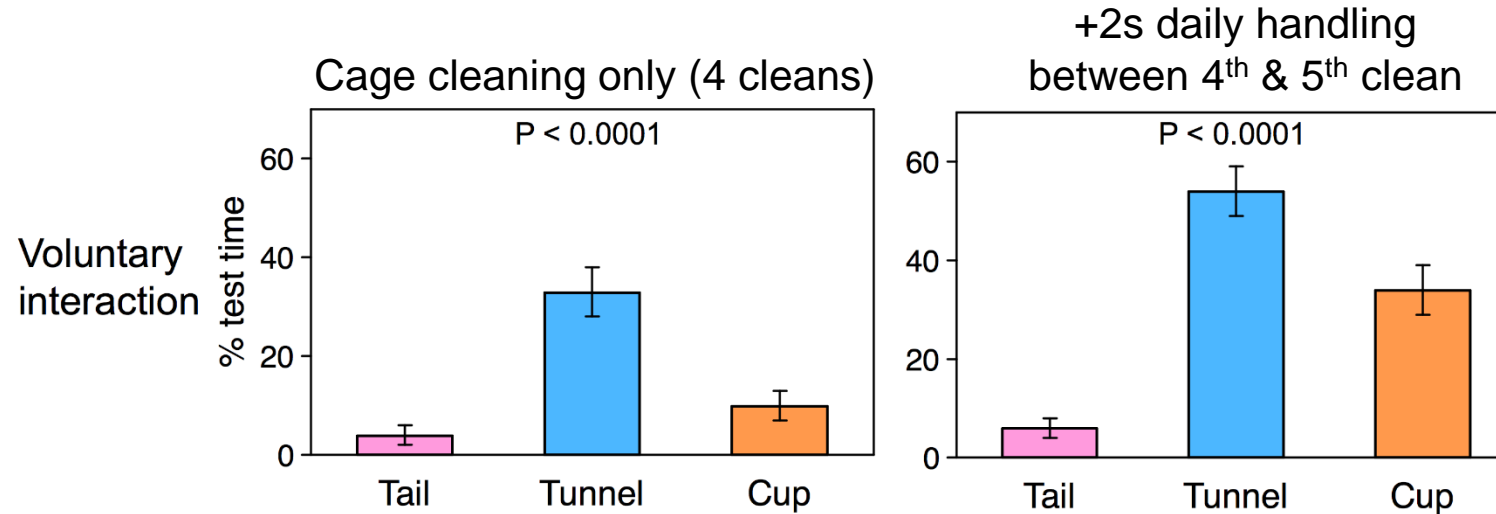
- ▶ Brief handling at cage cleaning (c. 2s) is sufficient to accustom to tunnels



Data for 96 BALB/c mice

# Brief handling - frequency

- ▶ Brief handling at cage cleaning (c. 2s) is sufficient to accustom to tunnels
- ▶ Cupping requires more frequent handling (but brief handling sufficient)

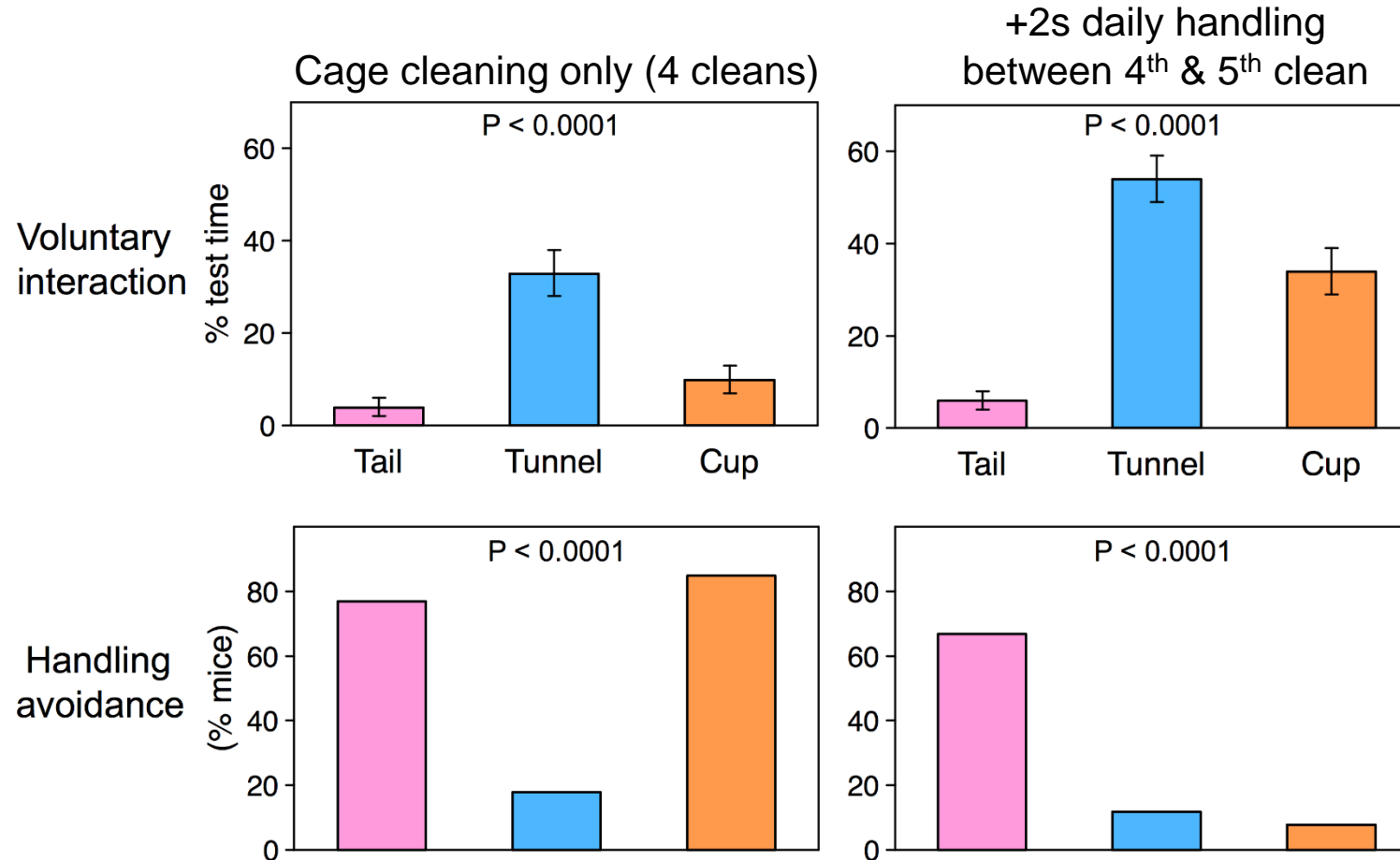


Data for 96 BALB/c mice



# Brief handling - frequency

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# Mouse tunnel experience

OPEN ACCESS Freely available online

PLOS ONE

## Reducing Mouse Anxiety during Handling: Effect of Experience with Handling Tunnels

Kelly Gouveia, Jane L. Hurst\*

Institute of Integrative Biology, University of Liverpool, Neston, Cheshire, United Kingdom

- ▶ Home cage tunnels provide enrichment & familiarity
  - entered more readily than unfamiliar tunnel
  - particularly useful for more nervous strains
- ▶ Still positive response to unfamiliar tunnels
  - much better than tail handling ( $P < 0.0001$ )



Gouveia & Hurst (2013) *PLOS ONE* : e66401

# Testing reliability

[www.nature.com/scientificreports](http://www.nature.com/scientificreports)

## SCIENTIFIC REPORTS

OPEN

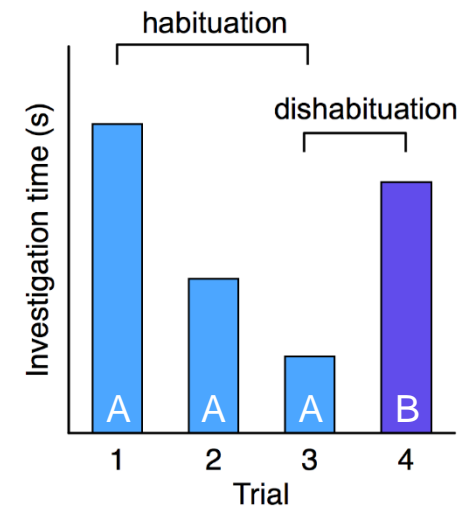
### Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling

Received: 01 September 2016

Accepted: 17 February 2017

#### Habituation – dishabituation test

- ▶ Tests ability to recognise a difference between stimuli
- ▶ Same stimulus presented repeatedly
- ▶ Novel stimulus → investigation increases



# Testing reliability

[www.nature.com/scientificreports](http://www.nature.com/scientificreports)

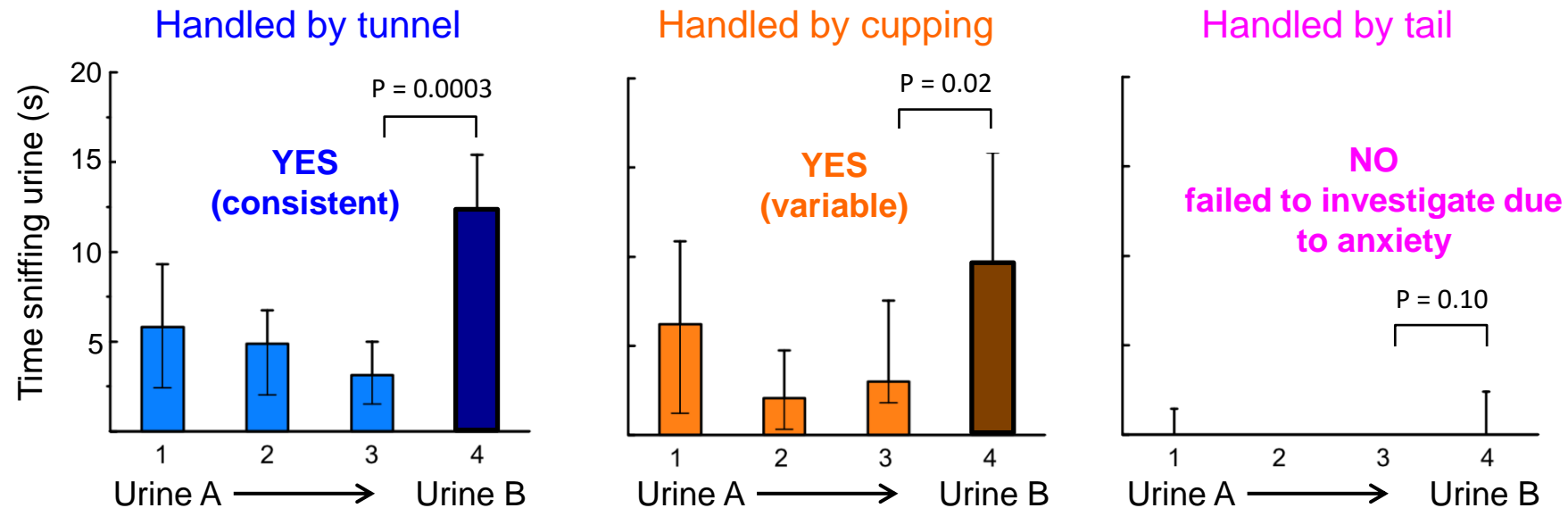
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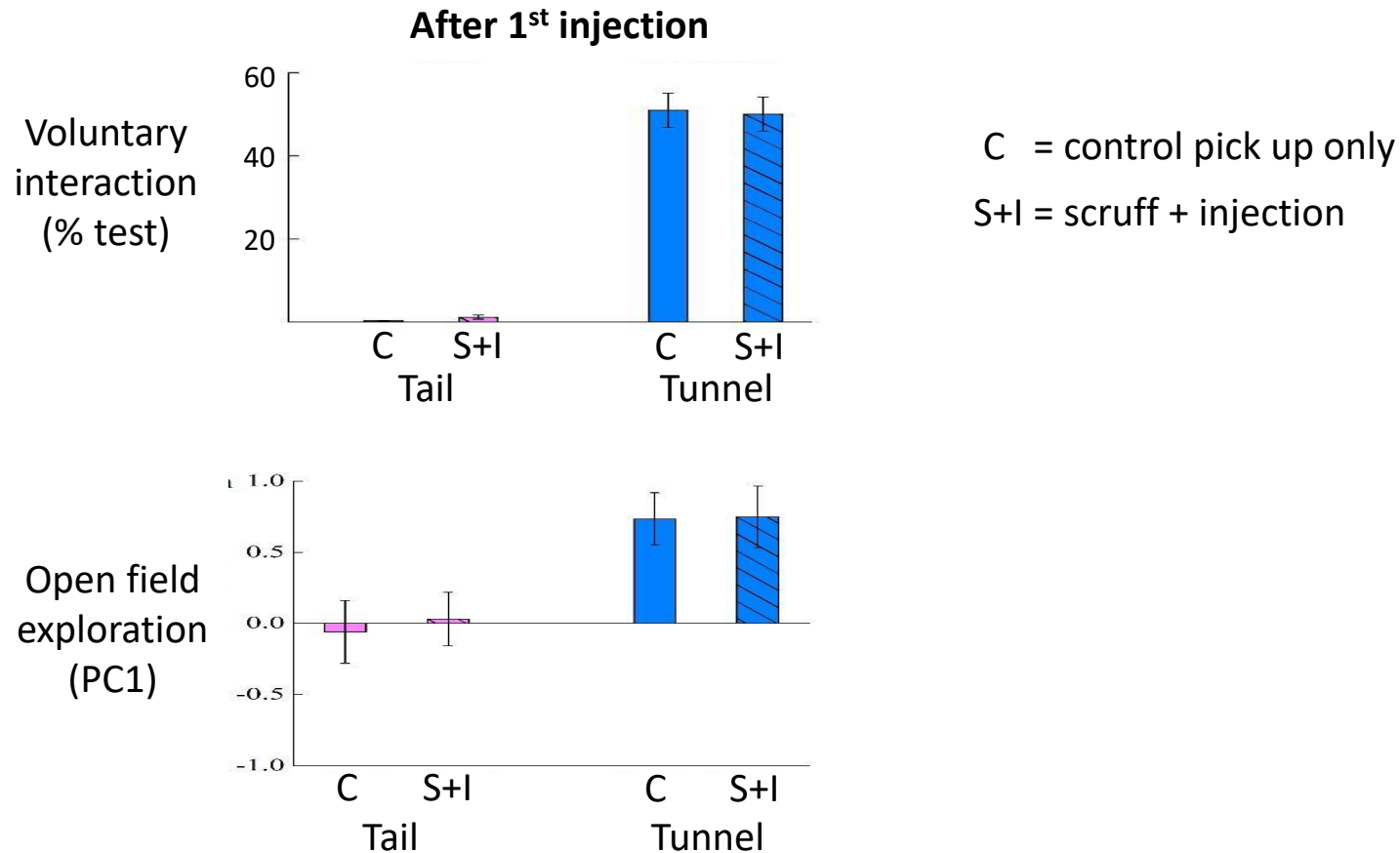


(brief handling at cage cleaning only)

Gouveia & Hurst (2017) *Scientific Reports*

# Response to injection

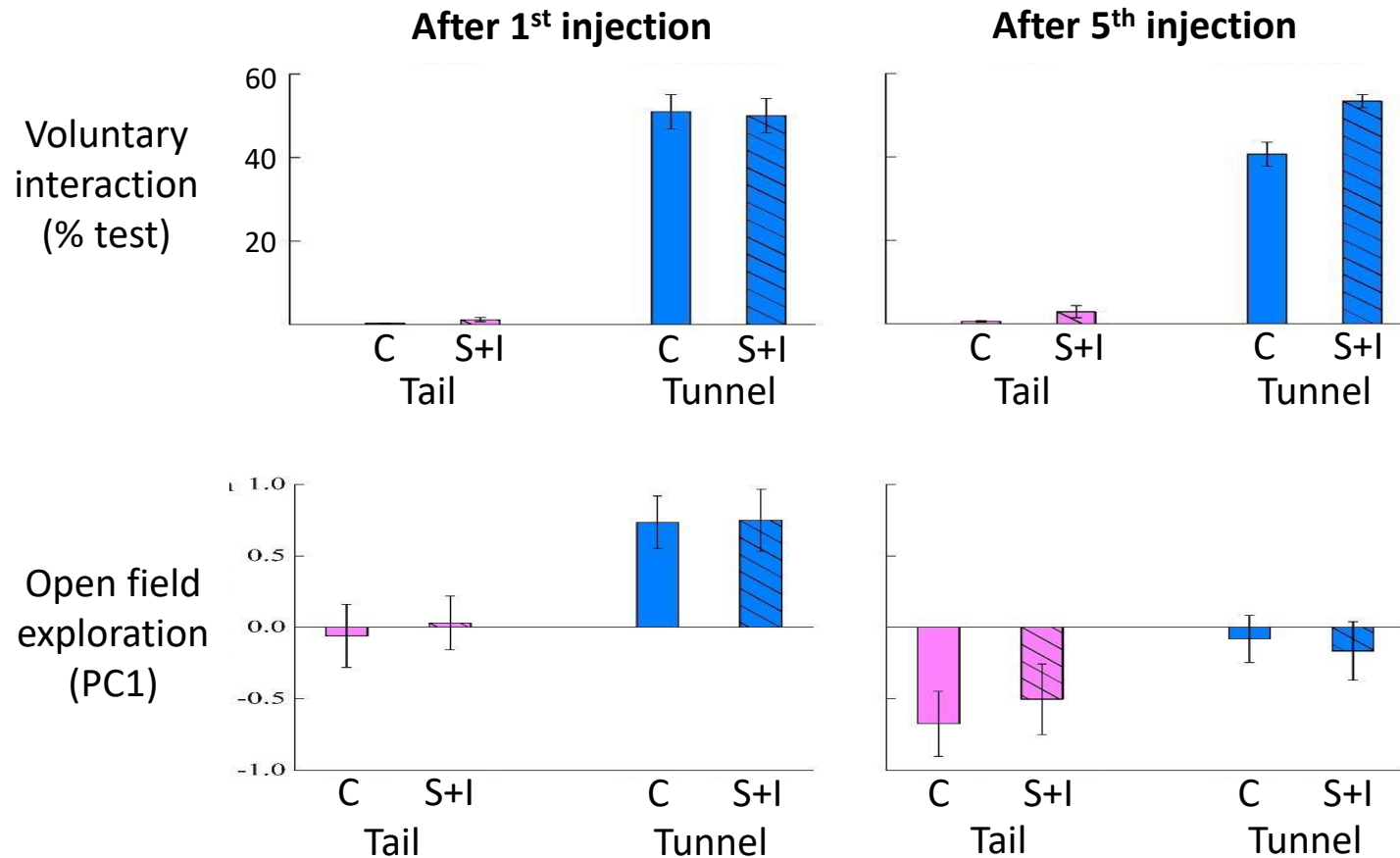
- ▶ Handling method strongly influences anxiety ( $P < 0.001$ , all measures)
  - Experience of subcutaneous injection with scruff restraint does not





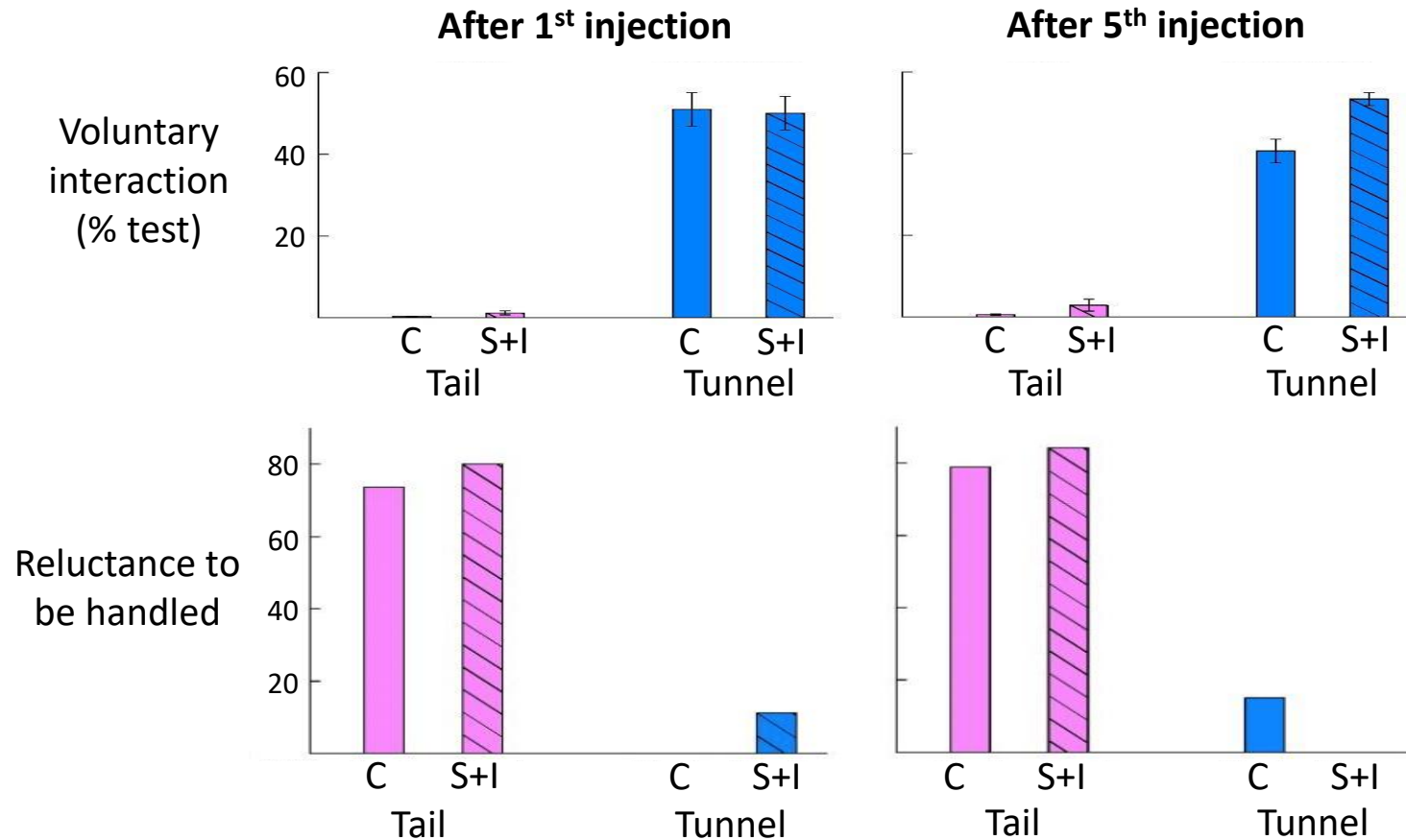
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# Measuring the difference

Assessed from many different angles in our lab:

▶ Voluntary interaction with the handler	 TAIL	 TUNNEL	 CUP
▶ Attempt to avoid capture	 TAIL	 TUNNEL	 CUP
▶ Stress during capture	 TAIL	 TUNNEL	 CUP
▶ Anxiety following handling			
• Elevated plus maze tests	 TAIL	 TUNNEL	 CUP
• Open field tests	 TAIL	 TUNNEL	 CUP
▶ Reliability in behavioural testing	 TAIL	 TUNNEL	 CUP
▶ Response to injection	 TAIL	 TUNNEL	

Responses generalises across mouse strains and handlers

# Evidence from other labs

- ▶ > 300 citations of our handling papers - No negative issues yet reported
- ▶ Studies in other laboratories replicate our findings
  - ▶ NC3Rs summary of mouse handling research papers:  
<https://www.nc3rs.org.uk/mouse-handling-research-papers>
- ▶ Comments & testimonials from those that have changed
  - ▶ Not as difficult as anticipated
  - ▶ Does not interfere with health checks
  - ▶ Animals more relaxed, easier to handle, fewer bites
  - ▶ New trainees less scared
  - ▶ Increased personal satisfaction
  - ▶ “Would not go back to tail handling”
  - ▶ No time difference once fully competent

**Andrew Blake  
Tribute Award 2017**



**John Waters**  
From sceptic to champion

# Practicalities: tunnel handling

## ▶ Common mistakes





# Tunnel features

- ▶ **Plastic transparent tunnels** : best for handling and health inspection

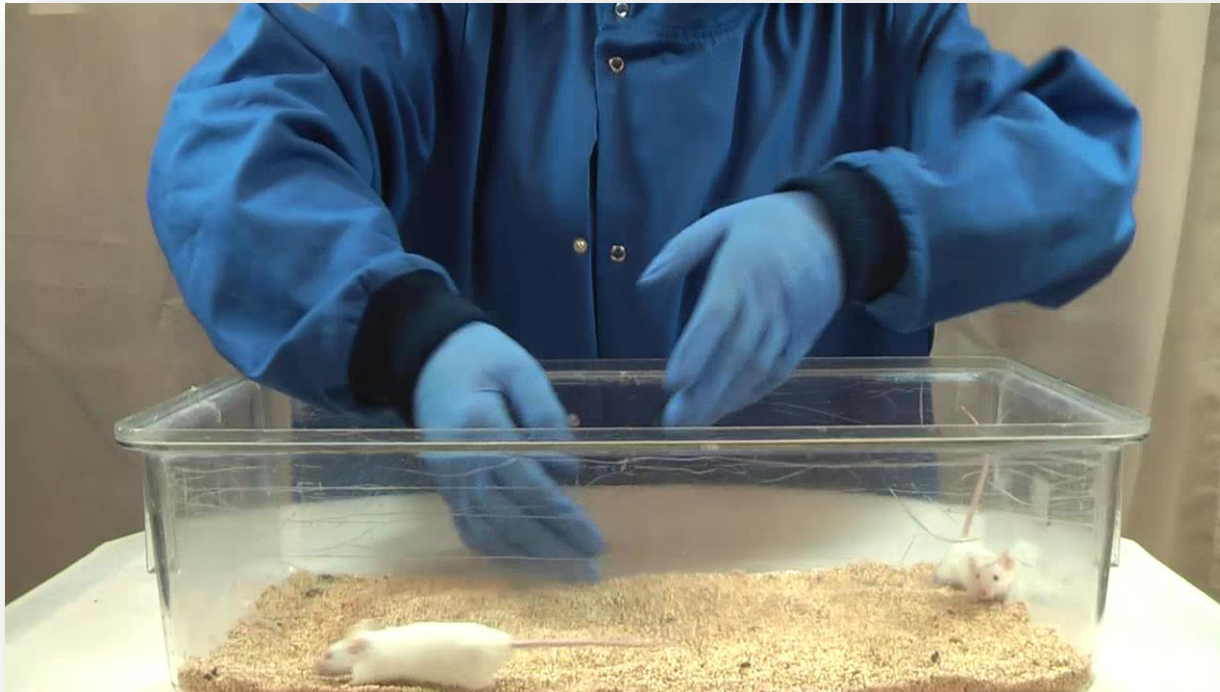
- ▶ **Ideal measurements:**

- 5 cm width
- 12-18 cm length



# Practicalities: habituation to cup

- ▶ Habituation to stay on the hand
  - On first handling, close hands loosely around mouse for up to 20s (5-10s often sufficient)
  - Experience required to assess steadiness



Can also habituate by

- ▶ holding between closed hands when transferring to clean cages

# Practicalities: tunnel to cup

- ▶ Habituate mice to being picked up in a tunnel
- ▶ Then tip mice onto hand



# Summary

- ▶ **Anxiety induced by handling can be a major confound in scientific research**
  - Strongly induced by capturing & picking up mice by the tail
  - Avoided by non-aversive handling methods – repeatable across labs
    - Use a tunnel to pick up mice
    - Or cup on open hand once habituated to being picked up
  - Extensive handling not needed to see major effects
    - Only brief experience of tunnel handling needed
    - Cupping on open hand needs more experience (strain/age dependent)
  - Can still restrain mice by tail base or scruff when needed
- ▶ **Improves reliability of behavioural & physiological responses**
- ▶ **Easily incorporated into normal husbandry**
  - Takes little or no longer than tail handling once competent

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Rob Beynon

## NC3Rs

Vicky Robinson

Mark Prescott

Kathryn Owen

Katie Lidster

NC3Rs team

## CRUK

AstraZeneca



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for the Replacement  
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