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

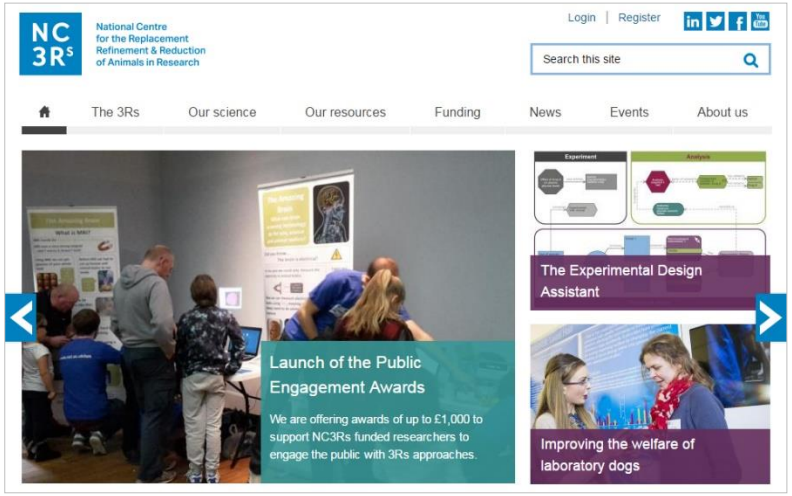
# Refined mouse handling: resources to support implementation

**Dr Mark Prescott**
**Director of Policy and Outreach**

Sixth UK-China Seminar on Research Animal Welfare and Ethics,
Nanjing, China, 23 May 2019

# About the NC3Rs

- Independent, scientific organisation.
- Established by UK Government in 2004 to lead the UK's 3Rs agenda.
- Work across the bioscience sector, with research funders, academia, industry and regulators.
- Use the 3Rs to support better science, innovation and animal welfare.
- 38 staff based in London and regionally.
- Budget ~ £10 million per year.
- Activities divided between funding research and innovation, and our own office-led science programmes.

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National Centre for the 3Rs

# Taming anxiety in laboratory mice

UNIVERSITY OF LIVERPOOL

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

BBSRC bioscience for the future

Jane L Hurst & Rebecca S West

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.

NATURE METHODS | VOL.7 NO.10 | OCTOBER 2010 | 825

NC 3Rs 3Rs Prize 2010

iat ANDREW BLAKE TRIBUTE AWARD 2017

AWARD 2017

## Tail

✗

## Tunnel

✓

## Cup

✓

NC 3Rs

# Refined handling: strength of the evidence base

- Well designed experiments, published in high ranking journals.
- Original paper from leading welfare scientist won 3Rs prize (independent panel).
- Range of behavioural measures of welfare (e.g. tests of anxiety; stress; avoidance of the handler; anhedonia), with good concordance between them.
- Large effect sizes and highly significant differences between tail-handled and tunnel-handled / cupped mice.
- Research findings generalise across mouse strains and sexes, and handlers.
- Liverpool findings replicated in other laboratories – e.g. Cincinnati, Newcastle, Tokyo, Hokkaido, Covance.
- Physiological as well as behavioural evidence for refinement (e.g. glucose homeostasis, corticosterone secretion).
- Animal welfare benefits alone would justify considering changing handling method.
- Scientific benefits, in addition (e.g. improved performance in behavioural tests, improved responsiveness to reward, reduced variation in behavioural tests data).
- Wide uptake would benefit millions of animals and scientific outcomes globally.
- Many UK institutions now using the refined methods.

# Resources to support implementation

www.nc3rs.org.uk/
how-to-pick-up-a-mouse

## Video tutorial


View our video tutorial on the refined mouse handling methods.

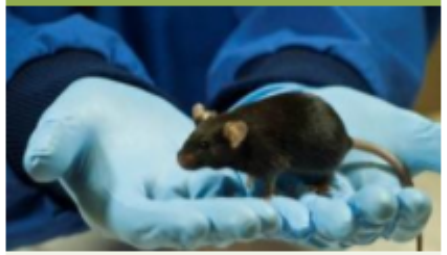
## Frequently asked questions

Read answers to frequently asked questions about tunnel and cup handling.

## Posters


Request copies of our mouse handling poster for display in your facility.

## Tips for implementation



Tips and strategies for rolling out the refined handling methods in your facility.

## Testimonials

Hear from those who have switched to the refined techniques.

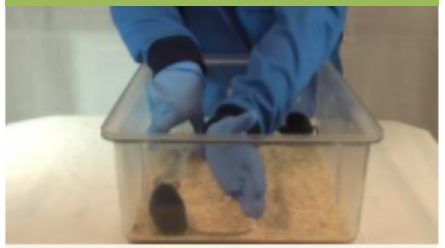
## Research papers


Read the underpinning research and related papers.

## Mouse handling webinar


Professor Jane Hurst describes the evidence supporting refined handling techniques and practical tips

## Video clips

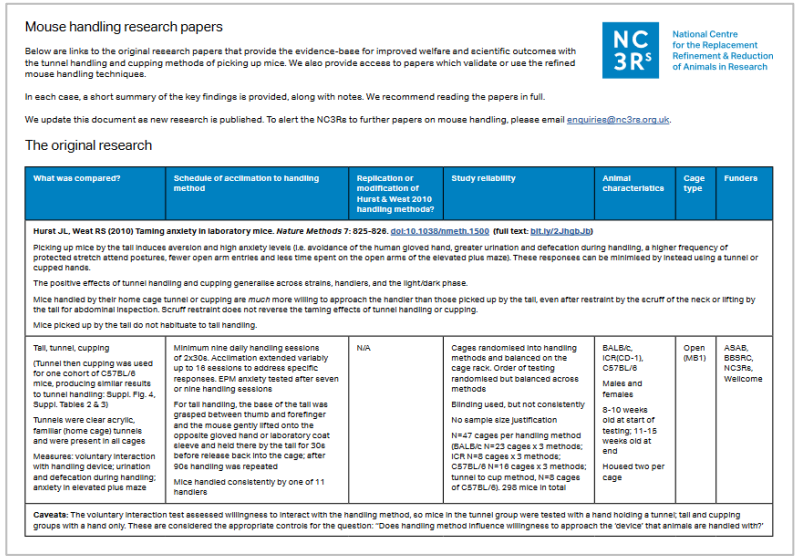


Download short video clips for use in in-house training.

# Summary of the research papers

**New!**

- Table summarising the research published to-date on the tunnel handling and cupping methods (11 papers). In each case, we note:
  - The key findings
  - What was compared (e.g. tail, tunnel, cupping) and the measures used
  - Animal characteristics (i.e. strain, sex and age of mice) as well as the tunnel and cage type
  - The schedule of acclimation to handling
  - An assessment of the study reliability and any caveats.
- We also provide quick links to published evidence addressing common questions about the refined handling techniques ("Where is the evidence for….?")



Mouse handling research papers

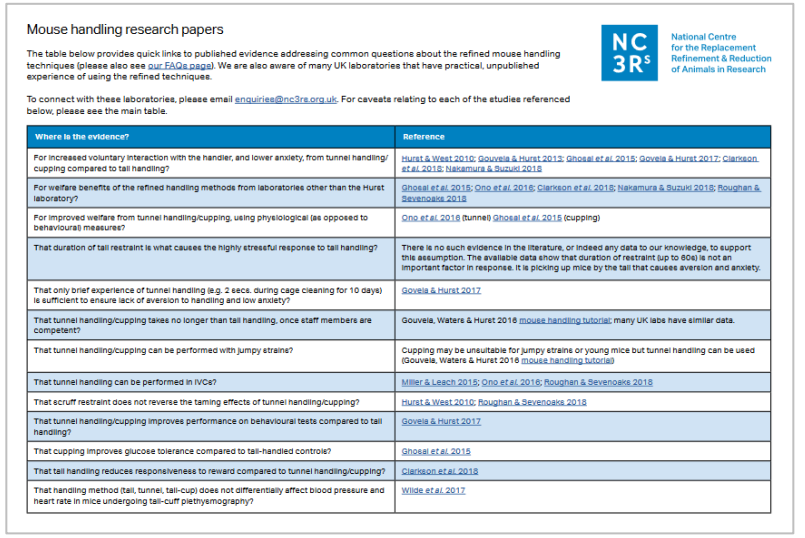
Below are links to the original research papers that provide the evidence-base for improved welfare and scientific outcomes with the tunnel handling and cupping methods of picking up mice. We also provide access to papers which validate or use the refined mouse handling techniques.

In each case, a short summary of the key findings is provided, along with notes. We recommend reading the papers in full.

We update this document as new research is published. To alert the NC3Rs to further papers on mouse handling, please email enquiries@nc3rs.org.uk.

The original research

| What was compared? | Schedule of acclimation to handling method | Replication or modification of Hurst & West 2010 handling methods? | Study reliability | Animal characteristics | Cage type | Funders |
|---|---|---|---|---|---|---|
| **Hurst JL, West RS (2010) Taming anxiety in laboratory mice.** ***Nature Methods*** **7: 825-826.** doi:10.1038/nmeth.1500 **(full text:** bit.ly/2JhqbJb**)**<br>Picking up mice by the tail induces aversion and high anxiety levels (i.e. avoidance of the human gloved hand, greater urination and defecation during handling, a higher frequency of protected stretch attend postures, fewer open arm entries and less time spent on the open arms of the elevated plus maze). These responses can be minimised by instead using a tunnel or cupped hands.<br>The positive effects of tunnel handling and cupping generalise across strains, handlers, and the light/dark phase.<br>Mice handled by their home cage tunnel or cupping are *much* more willing to approach the handler than those picked up by the tail, even after restraint by the scruff of the neck or lifting by the tail for abdominal inspection. Scruff restraint does not reverse the taming effects of tunnel handling or cupping.<br>Mice picked up by the tail do not habituate to tail handling. | | | | | | |
| Tail, tunnel, cupping<br>(Tunnel then cupping was used for one cohort of C57BL/6 mice, producing similar results to tunnel handling: Suppl. Fig. 4, Suppl. Tables 2 & 3)<br>Tunnels were clear acrylic, familiar (home cage) tunnels and were present in all cages<br>Measures: voluntary interaction with handling device; urination and defecation during handling; anxiety in elevated plus maze | Minimum nine daily handling sessions of 2x30s. Acclimation extended variably up to 16 sessions to address specific responses. EPM anxiety tested after seven or nine handling sessions<br>For tail handling, the base of the tail was grasped between thumb and forefinger and the mouse gently lifted onto the opposite gloved hand or laboratory coat sleeve and held there by the tail for 30s before release back into the cage; after 90s handling was repeated<br>Mice handled consistently by one of 11 handlers | N/A | Cages randomised into handling methods and balanced on the cage rack. Order of testing randomised but balanced across methods<br>Blinding used, but not consistently<br>No sample size justification<br>N=47 cages per handling method (BALB/c N=23 cages x 3 methods; ICR N=8 cages x 3 methods; C57BL/6 N=16 cages x 3 methods; tunnel to cup method, N=8 cages of C57BL/6). 298 mice in total | BALB/c, ICR(CD-1), C57BL/6<br>Males and females<br>8-10 weeks old at start of testing; 11-15 weeks old at end<br>Housed two per cage | Open (MB1) | ASAB, BBSRC, NC3Rs, Wellcome |
| **Caveats:** The voluntary interaction test assessed willingness to interact with the handling method, so mice in the tunnel group were tested with a hand holding a tunnel; tail and cupping groups with a hand only. These are considered the appropriate controls for the question: "Does handling method influence willingness to approach the 'device' that animals are handled with?" | | | | | | |



Mouse handling research papers

The table below provides quick links to published evidence addressing common questions about the refined mouse handling techniques (please also see our FAQs page). We are also aware of many UK laboratories that have practical, unpublished experience of using the refined techniques.

To connect with these laboratories, please email enquiries@nc3rs.org.uk. For caveats relating to each of the studies referenced below, please see the main table.

| Where is the evidence? | Reference |
|---|---|
| For increased voluntary interaction with the handler, and lower anxiety, from tunnel handling/cupping compared to tail handling? | Hurst & West 2010; Gouveia & Hurst 2013; Ghosal *et al.* 2015; Gouveia & Hurst 2017; Clarkson *et al.* 2018; Nakamura & Suzuki 2018 |
| For welfare benefits of the refined handling methods from laboratories other than the Hurst laboratory? | Ghosal *et al.* 2015; Ono *et al.* 2016; Clarkson *et al.* 2018; Nakamura & Suzuki 2018; Roughan & Sevenoaks 2018 |
| For improved welfare from tunnel handling/cupping, using physiological (as opposed to behavioural) measures? | Ono *et al.* 2016 (tunnel) Ghosal *et al.* 2015 (cupping) |
| That duration of tail restraint is what causes the highly stressful response to tail handling? | There is no such evidence in the literature, or indeed any data to our knowledge, to support this assumption. The available data show that duration of restraint (up to 60s) is not an important factor in response. It is picking up mice by the tail that causes aversion and anxiety. |
| That only brief experience of tunnel handling (e.g. 2 secs. during cage cleaning for 10 days) is sufficient to ensure lack of aversion to handling and low anxiety? | Gouveia & Hurst 2017 |
| That tunnel handling/cupping takes no longer than tail handling, once staff members are competent? | Gouveia, Waters & Hurst 2016 mouse handling tutorial; many UK labs have similar data. |
| That tunnel handling/cupping can be performed with jumpy strains? | Cupping may be unsuitable for jumpy strains or young mice but tunnel handling can be used (Gouveia, Waters & Hurst 2016 mouse handling tutorial) |
| That tunnel handling can be performed in IVCs? | Miller & Leach 2015; Ono *et al.* 2016; Roughan & Sevenoaks 2018 |
| That scruff restraint does not reverse the taming effects of tunnel handling/cupping? | Hurst & West 2010; Roughan & Sevenoaks 2018 |
| That tunnel handling/cupping improves performance on behavioural tests compared to tail handling? | Gouveia & Hurst 2017 |
| That cupping improves glucose tolerance compared to tail-handled controls? | Ghosal *et al.* 2015 |
| That tail handling reduces responsiveness to reward compared to tunnel handling/cupping? | Clarkson *et al.* 2018 |
| That handling method (tail, tunnel, tail-cup) does not differentially affect blood pressure and heart rate in mice undergoing tail-cuff plethysmography? | Wilde *et al.* 2017 |

# Answers to frequently asked questions

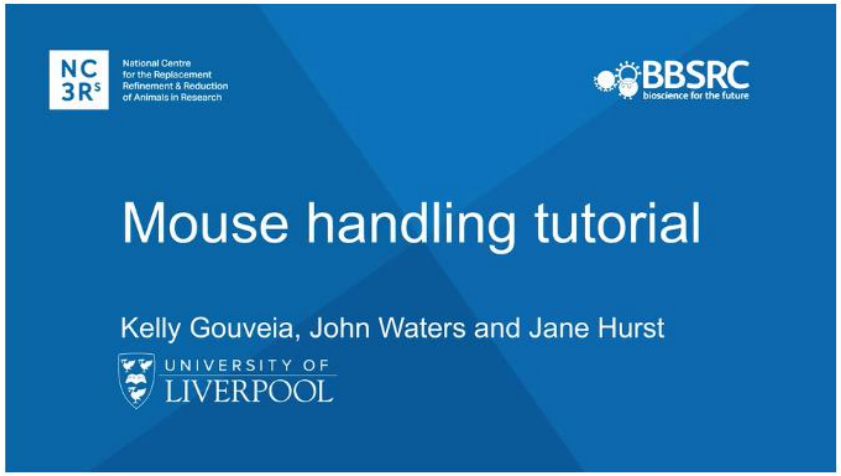
Professor Jane Hurst and Mr John Waters answer frequently asked questions about the techniques, e.g.

- Do tunnel handling and cupping take longer than tail handling?
- How long does it take to transition to the refined methods?
- Do the refined methods work for young mice, both sexes, all strains?
- Can the tunnel handling and cupping methods be used in IVCs?
- What if I need to health check or conduct a procedure?
- What size tunnel is appropriate for my cage?
- Do I have to take environmental enrichment out of the cage if using a tunnel?
- Can I share a tunnel between cages? How often should I clean the tunnel and how?
- Where can I find a supplier of clear tunnels, suitable for autoclaving?

# Video tutorial and webinar

18-minute tutorial covers:

- The impact of handling on anxiety and stress in mice.
- Handling techniques to improve welfare and reliability of mouse studies.
- Best practice in handling mice by different methods.
- How to incorporate best handling practices into routine husbandry and experimental procedures.





53-minute webinar “Mouse handling made easy” in which Professor Jane Hurst shares the evidence base supporting refined mouse handling techniques, as well as practical advice and tips for implementation.

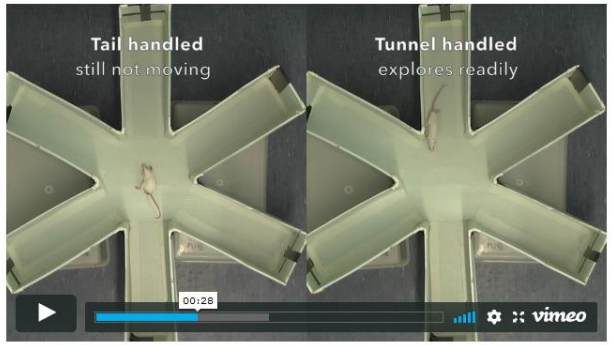
# Downloadable video clips

Short, instructional video clips available to download for use in staff training.

Cupping technique (2.52 mins.)


Tunnel handling at IVC cage cleaning (1.49 mins.)


Radial maze exploration (1.35 mins.)




Tunnel handling and scruffing

# Posters for display in laboratories

## New methods to handle mice – time for a change

*John Waters, Kelly Gouveia and Jane L. Hurst. Mammalian Behaviour & Evolution Group, University of Liverpool, Leahurst Campus, Neston CH64 7TE, UK*

The way that you pick up laboratory mice influences their stress, longer-term anxiety and reliability in scientific testing. Picking up mice by the tail induces negative responses [1-3]. In contrast, picking them up in a tunnel, or cupping them on the hand, considerably reduces stress and anxiety, and results in animals that are much more willing to interact with you voluntarily [3-5]. Below are simple tips for implementing these methods. Your choice should be compatible with your local biosecurity rules.

For further details and advice, see our free video tutorial at **www.nc3rs.org.uk/mouse-handling-tutorial**

### Tunnel handling

- Guide mouse into tunnel with free hand
- Lift mouse inside tunnel
- Remove mouse by tipping out backwards, with end of tunnel just above surface
- Mice habituate very quickly to tunnels
- Ideal for less experienced handlers
- Minimal risk of being bitten
- Abnormal behaviour easily spotted

### Cup handling

- Scoop mouse onto one or both palms
- Lift hand(s) clear of cage
- Mouse sits on the hand without restraint
- A single hand is sufficient once mice are familiar with this method
- Mice slower to habituate to cup handling
- Needs more skill to prevent mice jumping, but can improve animal-handler bond

### Combined tunnel to cup handling

- Pick up mouse in a tunnel
- Tip backwards onto open hand
- Mouse should stay willingly on the hand

### Tips for good handling

- **Do not** be hesitant
- Use cage side and free hand to guide mouse quickly into the tunnel – **do not** chase the mouse with the tunnel
- **Do not** wait for the mouse to enter voluntarily, actively guide it in
- With good technique, mouse will go straight in – practice makes perfect!
- Mice familiar with tunnels enter more readily
- Provide mice with a tunnel in their home cage if possible [2]
- Mice stay in the tunnel when lifted up, but cover tunnel ends to move animals safely over a distance
- Tip mice out backwards, **do not** shake them out
- Smooth clear plastic tunnels are ideal, 50 mm in diameter

- Inexperienced mice may try to jump off open hand
- To familiarise, scoop between hands held loosely around mouse for a few seconds
- See video tutorial for more detailed advice

### Restraint for procedures

- Capturing and picking up mice by the tail should be avoided where possible
- Once picked up, mice can be restrained by the tail, e.g. for sexing
- They can also be restrained by scruffing as needed for scientific procedures
- **Restraint by the tail or scruff does not reverse the positive effects of tunnel and cup handling** [1]

### Benefits

- Mice are much less anxious than those picked up by the traditional tail method [1-3]
- You can still restrain mice manually by the tail base or scruff when needed [1]
- Mice show more reliable behavioural [3] and physiological responses [4]
- They only need brief experience of tunnel handling to habituate [2,5]
- Once skilled, you can pick up mice by tunnel or cup just as quickly as by tail. You will need to practice to become efficient – but it's worth it!

**Acknowledgements**

We thank Anna Rogers and other members of the Mammalian Behaviour & Evolution Group for their invaluable help. The work was funded by the NC3Rs, BBSRC and the University of Liverpool.

**References**

1. Hurst JL & West RS (2010) *Nature Methods* 7:825-6.
2. Gouveia K & Hurst JL (2013) *PLOS ONE* 8: e68401.
3. Gouveia K & Hurst JL (2017) *Scientific Reports* 7:44999.
4. Ghosal S et al. (2015) *Physiology & Behavior* 150:31-7.

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## 抓取小鼠的新方法——是时候作出改变了

哺乳动物行为和进化小组，约翰·沃特斯（John Waters）、凯利·古韦亚（Kelly Gouveia）和简·L·赫斯特（Jane L Hurst）。英国利物浦大学Leahurst校区，Neston CH64 7TE。

实验小鼠的抓取方式会影响它们在科学实验中的应激、长期焦虑和可靠性。提起小鼠尾巴会引起负面反应[1-3]。相反，使用圆筒抓取，或者用手捧起小鼠会大大减少应激和焦虑，让小鼠更愿意主动配合[3-5]。下文为您简要说明这些方法。您应选择符合您当地生物安全法规的方法。

欲了解更多细节和建议，请观看我们的免费视频教程 **www.nc3rs.org.uk/how-to-pick-up-a-mouse。**

### 圆筒式抓取

- 徒手引导小鼠进入圆筒
- 提起小鼠所在的圆筒
- 倾斜圆筒后端，使出口贴近平面上方，使小鼠从圆筒中倒退出来
- 小鼠能够很快适应圆筒式抓取
- 非常适合经验不足的操作人员
- 被咬的风险降到最低
- 易发现异常行为

### 手捧式抓取

- 用单手或双手将小鼠置到手掌中
- 提起手掌，离开笼子
- 小鼠坐在手上可以不受束缚
- 一旦小鼠熟悉了这种方法，单手便可操作
- 小鼠需要较长时间适应手捧式抓取
- 需要具备一定技能的人员操作来防止小鼠跳跃，但是可以改善动物与操作人员的关系

### 圆筒和手捧结合

- 用圆筒提起小鼠
- 倾斜圆筒后端，使小鼠退到手掌中
- 小鼠应自愿停留在手上

### 良好抓取的技巧提示

- **不要**犹豫
- 使用笼子边和空着的手引导小鼠快速进入圆筒——**不要**用圆筒追逐小鼠
- **不要**等待小鼠自觉进入；要积极引导它
- 若操作技术良好，小鼠会径直进入圆筒——熟能生巧！
- 熟悉圆筒的小鼠更容易进入圆筒
- 如果有条件，可以在小鼠住的笼子里放置一个圆筒[2]
- 提起圆筒时，小鼠会在圆筒中停留，但移动时要善住圆筒两端来保证小鼠安全
- 倾斜圆筒后端，使小鼠倒退出圆筒，**不要**把小鼠晃出来
- 光滑透明、直径50毫米的塑料圆筒最为理想

- 没有经验的小鼠可能会试图跳出手心
- 双手轻轻包住小鼠几秒钟，可以使小鼠熟悉手掌式抓取
- 欲了解更多细节，请观看我们的视频教程

### 操作保定

- 尽可能避免抓取小鼠的尾巴
- 提起后，小鼠便可通过尾巴进行保定，例如交配时
- 根据科学流程需要，小鼠还可以从颈背处进行保定
- **从尾巴或者颈背进行保定不会影响圆筒式和手捧式抓取的正面作用**[4]

### 益处

- 这种方法比用尾巴提起的传统方法显著减少了小鼠的焦虑[1-3]
- 必要时，您仍然可以从小鼠尾巴或颈背进行手动保定[1]
- 小鼠表现出更可靠的行为反应[3]和生理反应[4]
- 小鼠只需要短暂的接触圆筒抓取，便可习惯这种操作方法[2,5]
- 若操作熟练，圆筒或手捧的方式可以像捉其尾巴的方式一样快速地抓取小鼠，勤加练习便可熟能生巧，而且这些方式大有裨益。

**鸣谢**

我[illegible]对实验小鼠的其他成员提供的宝贵帮助表示感谢。本项研究由NC3Rs（英国国家3R动物研究中心）、BBSRC（英国生物技术与生物科学研究理事会）和利物浦大学资助。

**参考文献**

1. Hurst JL & West RS (2010) *Nature Methods* 7:825-6.
2. Gouveia K & Hurst JL (2013) *PLOS ONE* 8: e68401.
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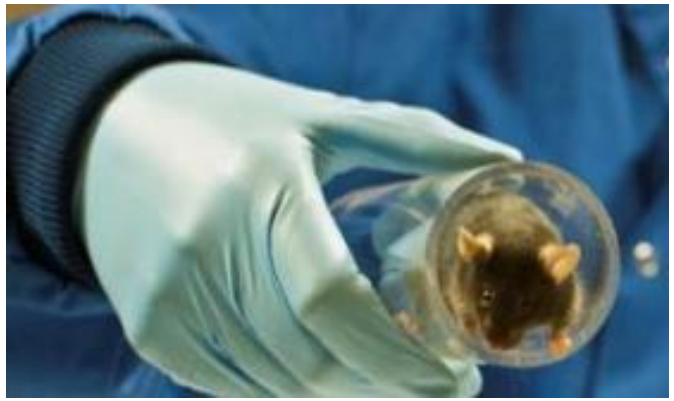
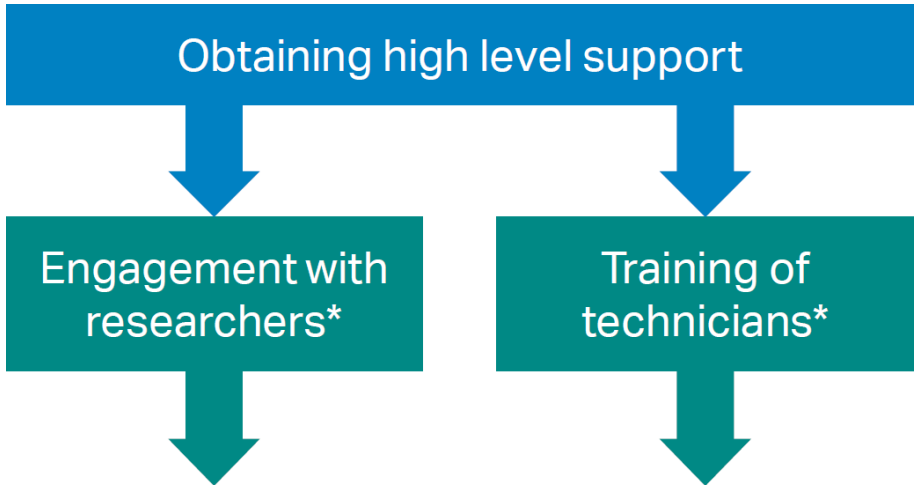
# Example implementation strategy

**Obtaining high level support**

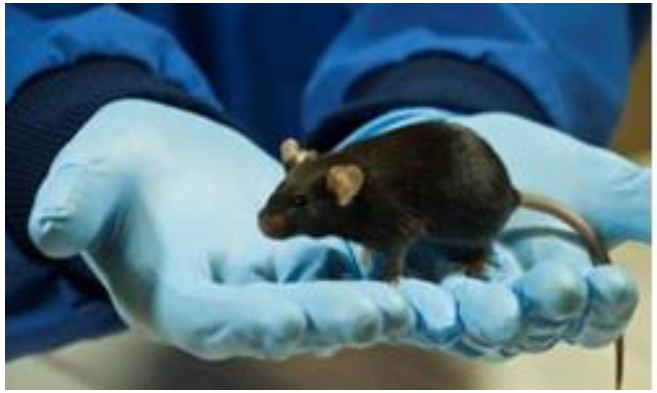
- Gain approval from Animal Facility Director, Head of College, Academic Lead, IACUC, etc.
- Introduce on a trial basis, with a timeline for review and to gather feedback.
- IACUC oversight can be helpful.



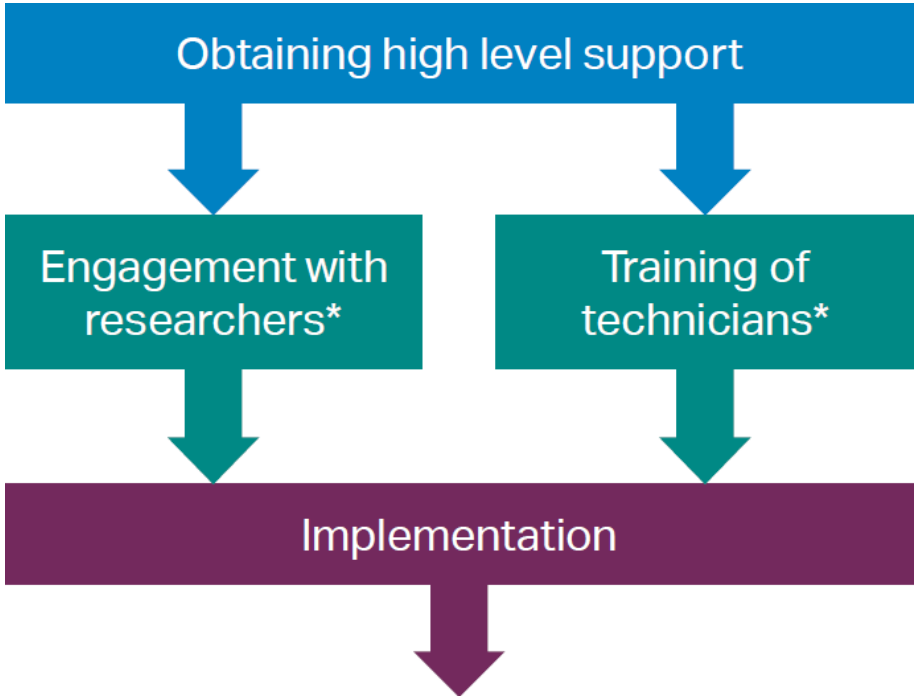
# Example implementation strategy




- Hold workshops and open discussion sessions for animal technicians – listen to concerns.
- Provide practical training, with Training Coordinators.
- Present the refined handling approaches and the local strategy to the animal facility users.
- Gain support from individual researchers – local champions.

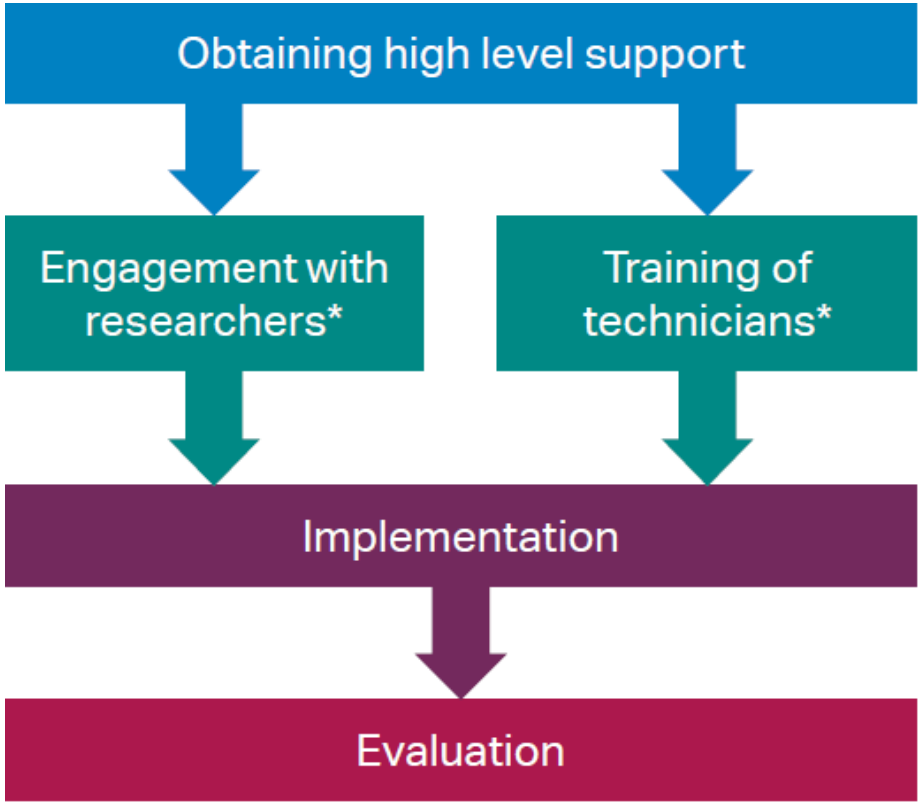


# Example implementation strategy




- Use a staggered approach – e.g. room by room
- Ensure the same handling method is being used consistently – communication among the project team, and with all staff, is important

# Example implementation strategy




- Monitor how the refined handling methods are working in practice
- Share experiences with peers (internally and externally)

# Testimonials

*"We wanted to make sure everyone understood why we were introducing the cupping technique and its benefits. The majority of our technicians are now trained and our standard operating procedures updated. Once technicians are used to the method, they confirm that the mice are easier to handle, appear less anxious, and that it does not require a large increase on their time."*
**Kiya Robinson, Associate Licensee, Covance, Harrogate**

***"**Changing to using tunnels for picking up mice has been straightforward. We've used the pre-existing tunnels provided for enrichment and the mice enter them easily. Another bonus: I can quickly health-check both ends of the mouse."* ***Sue Ecob, Animal Technician, University of Nottingham***

*"We moved a room at a time to the tunnel technique to manage the workload. The cost of purchasing polycarbonate tunnels has been offset by reduced need for disposable cardboard tunnels, now that each cage has a tunnel at all times. We've seen a huge improvement in interaction between mice and handler, and definitely wouldn't want to go back to tail capture."*
**Lisa Wright, NACWO/Facility Manager, University of Cambridge**

*"We have found no major issues with the time taken to handle mice using the new methods, once staff are trained. The NC3Rs video has helped us to illustrate the benefits to staff, especially those with reservations or with long-standing tail handling skills."*
**Andy Milner, Technical Manager, University of Portsmouth**

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# Thank you!

## For more information


- mark.prescott@nc3rs.org.uk
- www.nc3rs.org.uk
- www.facebook.com/NC3Rs
- @NC3Rs


## Keep in touch

Our monthly newsletter provides the latest updates from the NC3Rs:

www.nc3rs.org.uk/register


**www.nc3rs.org.uk/how-to-pick-up-a-mouse**

**Pioneering Better Science**