

Refined Mouse Handling: Practical Applications



The North American 3Rs Collaborative

A person wearing a grey sweater with brown patches on the elbows is sitting at a wooden table. They have their hands clasped in a thinking pose. In front of them is a chessboard with several wooden chess pieces. A wicker basket is visible on the left side of the table. The background is a blurred indoor setting.

**Some may not be convinced that this is the
RIGHT thing to do.**

Evidence?

Impact?

Practicality?

A wooden maze with a white text box at the top and three questions at the bottom.

Others may be running into roadblocks.

Time?

People?

Training?

**The 3RC was created by people who
have been in these situations firsthand**



The North American 3Rs Collaborative

Refine. Reduce. Replace.



**Collaborating to advance better science –
for both people & animals**

www.na3rsc.org | contactus@na3rsc.org





The 3RC partners with you across the field.



The 3RC's strategy is to identify initiatives with

Strong Evidence

Big Impact

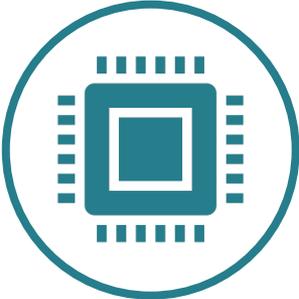
Real-World Practicality



The 3RC currently has six key 3Rs initiatives.



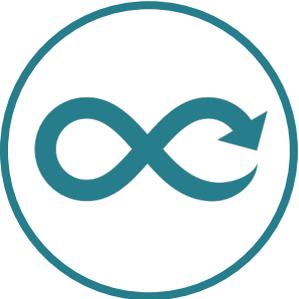
**Rodent Health
Monitoring**



**Microphysiological
Systems**



**Translational
Digital Biomarkers**



Refinement



**3Rs Certification
Course**



**Compassion
Fatigue Resiliency**

For each initiative we generally provide

Thought Leadership: expert consensus, real world experiences, & research

Benchmarking Use & Beliefs (e.g., Barriers): surveys & feedback

Education & Practical Resources: presentations, resource hubs, slide decks, SOPs, training courses, FAQs, etc.

Today's agenda covers the evidence, impact, & practicality of refined handling.



Megan LaFollette
NA3RsC

**Evidence &
Impact (15 min)**



**Donna Goldsteen &
Erin Straley, AstraZeneca**
**Practicality in
Pharma (10 min)**



**Liz Nunamaker & Shaina
Wallach, U. Of Florida**
**Practicality in
Academia (10 min)**



**Q&A
(55 min)**

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https://bit.ly/3RC_Newsletter



Refined handling was developed by Professor Jane Hurst, to study scent communication in wild mice.

When she began working with laboratory mice, she needed to use the same handling methods to get good results.

She conducted research comparing **tail handling, tunnel handling, & cupping** to pick up mice prior to conducting normal procedures (e.g., restraint, injection).



Hurst & West 2010, *Nature Methods*



Strong evidence indicates that refined handling is advantageous.

(>15 publications across 10 years from independent labs)

Refined handling = Non-aversive Handling

Refined handling is supported by many measures

- Voluntary interaction with the handler
- Attempt to avoid capture/ease of handling
- Stress during handling
- Anxiety following handling:
 - Elevated plus maze test
 - Open field test
 - Light-dark box test
 - Novel stimulus approach
- Physiological stress response:
 - Corticosterone
 - Blood glucose
- Response to reward / anhedonia / depression like behaviour
- Reliability of behavioural and drug testing
- Response to procedures:
 - Anaesthesia
 - Injection (SC, IP)
 - Gavage
 - Tattoo
 - Ear punch
 - Blood sampling

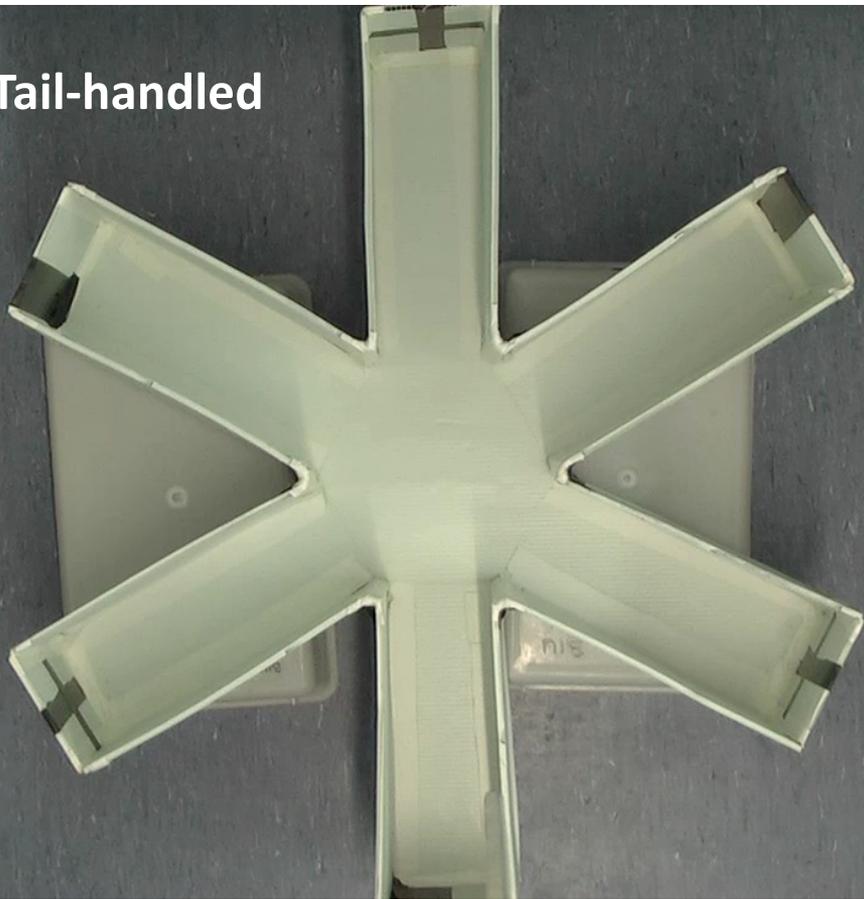
The consistent conclusion is that picking up mice by the tail has negative effects compared with refined methods

Let's dive into the evidence

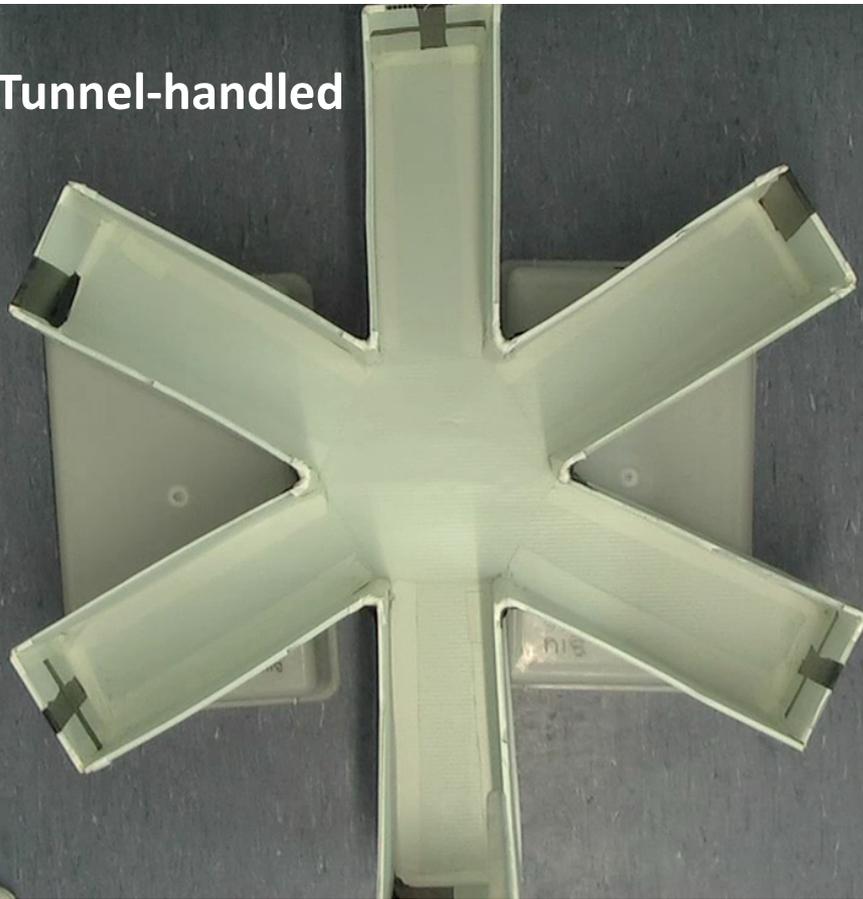


Refined handling **reduces anxiety.**

Tail-handled



Tunnel-handled



Refined handling reduces anxiety – across labs, strains, and tests

Elevated plus maze (n = 9)

- Hurst & West 2010, *Nature Methods*
- Gouveia & Hurst 2013, *PLoS ONE*
- Gouveia & Hurst 2019, *Sci Reports*
- Ghosal et al. 2015, *Physiol Behav*
- Clarkson et al. 2018, *Sci Reports*
- Nakamura & Suzuki 2018, *J Vet Med Sci*
- Roughan & Sevenoaks 2019, *JAALAS*
- Clarkson et al. 2020, *Sci Reports*
- Henderson et al. 2020, *Sci Reports*

Social novelty test

- Gouveia & Hurst 2017, *Sci Reports*
- Gouveia & Hurst 2019, *Sci Reports*
- Mertens et al. 2019, *PLoS ONE*

Open field test (n = 9)

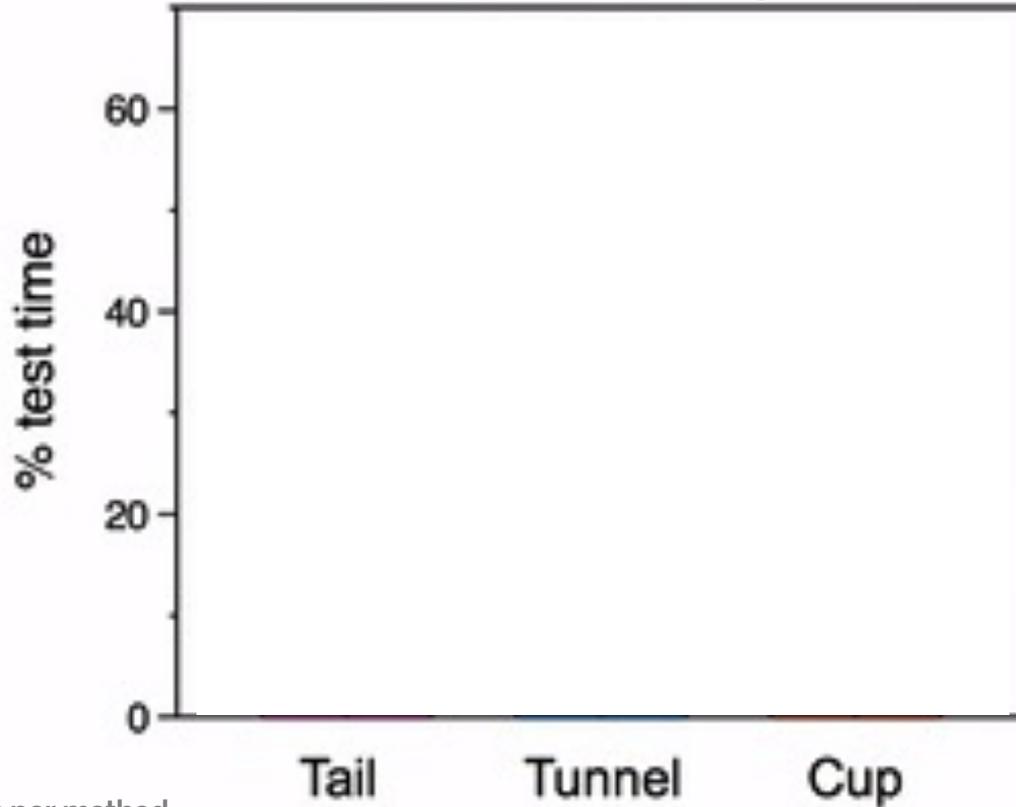
- Hurst & West 2010, *Nature Methods*
- Gouveia & Hurst 2017, *Sci Reports*
- Gouveia & Hurst 2019, *Sci Reports*
- Ghosal et al. 2015, *Physiol Behav*
- Clarkson et al. 2018, *Sci Reports*
- Nakamura & Suzuki 2018, *J Vet Med Sci*
- Roughan & Sevenoaks 2019, *JAALAS*
- Clarkson et al. 2020, *Sci Reports*
- Henderson et al. 2020, *Sci Reports*

Light-dark box test

- Mertens et al. 2019, *PLoS ONE*

Generally, no difference between tunnel vs. cupping

Refined handling reduces fear & increases voluntarily interaction



Mean \pm SEM, n = 55 cages per method.

(C57BL/6, BALBc, IRC(CD-1), 9 handlers, 9th handling session)

Hurst & West 2010, *Nature Methods*

Voluntary interaction results are **widely generalisable and reproducible**

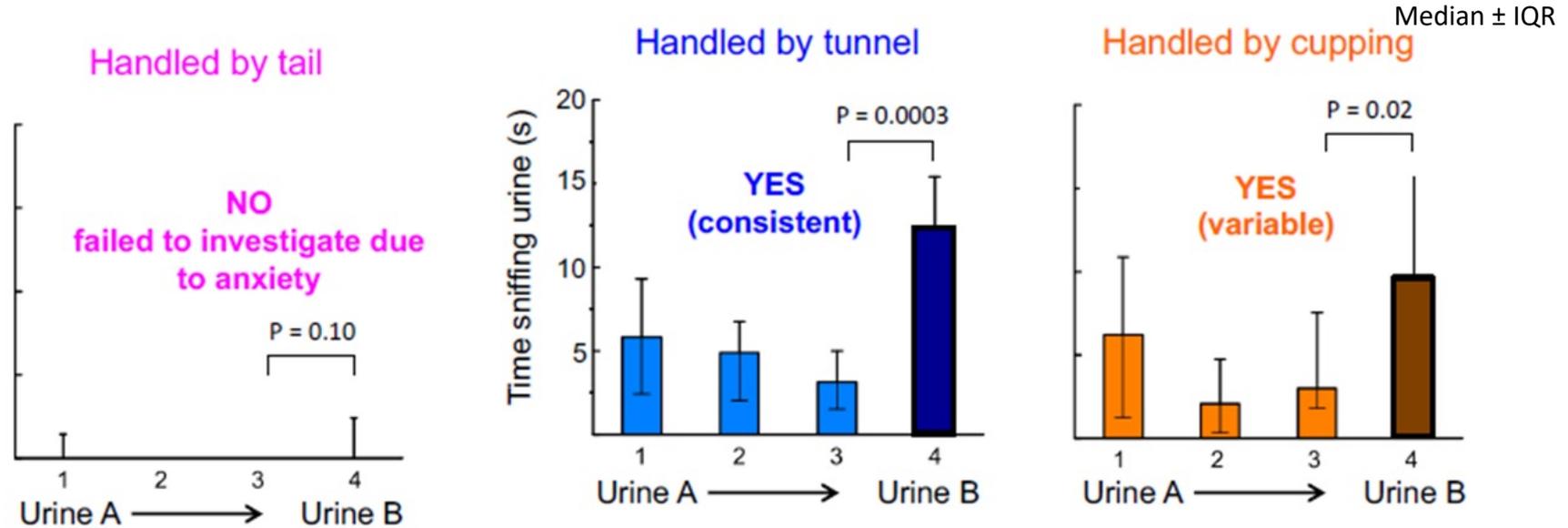
Generalises across:

- Strains
- Sexes
- Handlers
- Cage types
- Laboratories
- Light or dark

Strong reproducibility (n = 9):

- Hurst & West 2010, *Nature Methods*
- Gouveia & Hurst 2013, *PLoS ONE*
- Clarkson et al. 2018, *Sci Reports*
- Nakamura & Suzuki 2018, *J Vet Med Sci*
- Gouveia & Hurst 2019, *Sci Reports*
- Roughan & Sevenoaks 2019, *JAALAS*
- Clarkson et al. 2020, *Sci Reports*
- Henderson et al. 2020, *Sci Reports*
- Sensini et al. 2020, *Sci Reports*

Refined handling increases test reliability

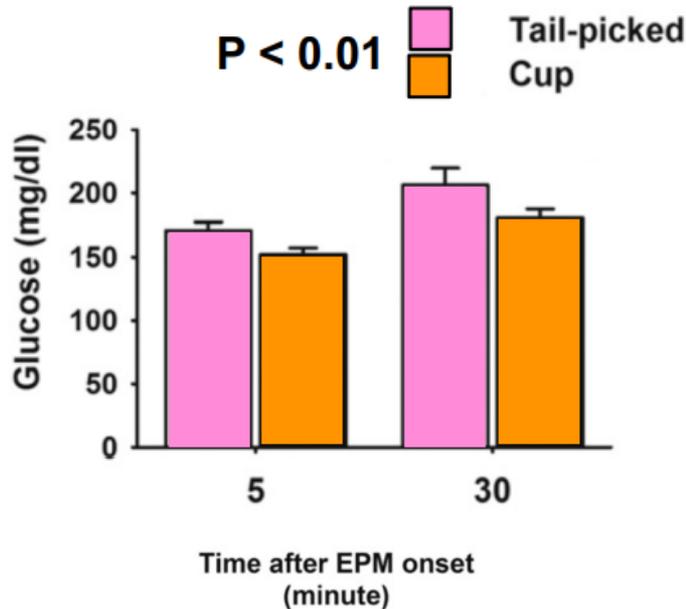


(n=16, brief handling at cage clean only)

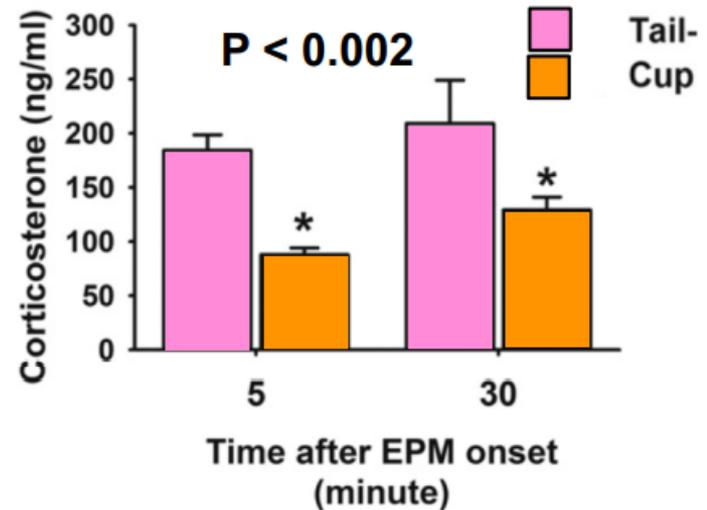
Decreasing anxiety from handling improves scientific quality.

Refined handling mitigates physiological confounds to metabolic endpoints

Cupping reduces glucose response



Cupping reduces corticosterone
(evidence of acute stress)



****Critical for diabetes research as it improves glucose tolerance****

(Ghosal et al. 2015, *Phys Behav*)

Refined handling **reduces depressive-like behavior & chronic stress**

- As measured by
 - Sucrose reward
 - Resilience to negative events
 - Forced swim test
 - Burrowing test
 - Adrenal gland size

(Clarkson et al. 2018, 2020; Sensini et al. 2020)

Refined handling improves breeding performance.

One extra pup weaned per breeding pair
during their reproductive lifespan

7% increase in proportion of litters successful weaned per pair

Fewer litter loses prior to weaning

20% lower risk of recurrent litter loss

(Hull et al., in 2022)

**So that's the
evidence & impact,
but is it practical?**

Once staff are trained, refined handling is
efficient & beneficial
even if only done at cage change.



(Gouveia & Hurst, 2019)

**Will common
procedures
counteract the
benefits of refined
handling?**

Refined handling is beneficial even in combination with **standard procedures**.

Scruff, Tail or Tube Restraint

(Hurst & West 2010; Roughan & Sevenoaks 2018; Gouveia & Hurst 2019; Henderson et al. 2020; Redaelli et al. 2021)

IP or SubQ Injections

(Gouveia & Hurst 2019; Henderson 2020)

Anesthesia

(5 mins. isoflurane; Henderson et al. 2020)

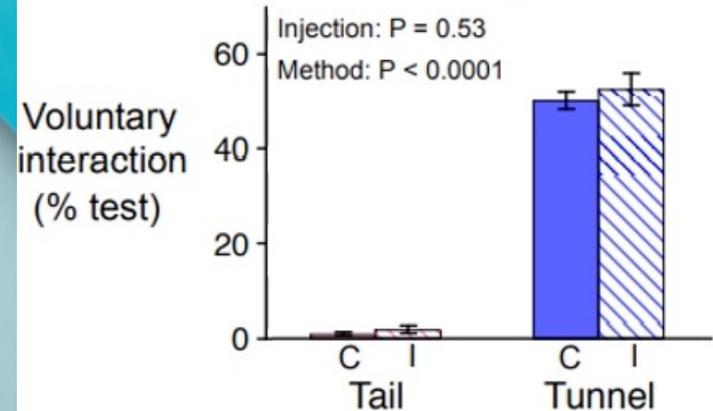
Oral Gavage

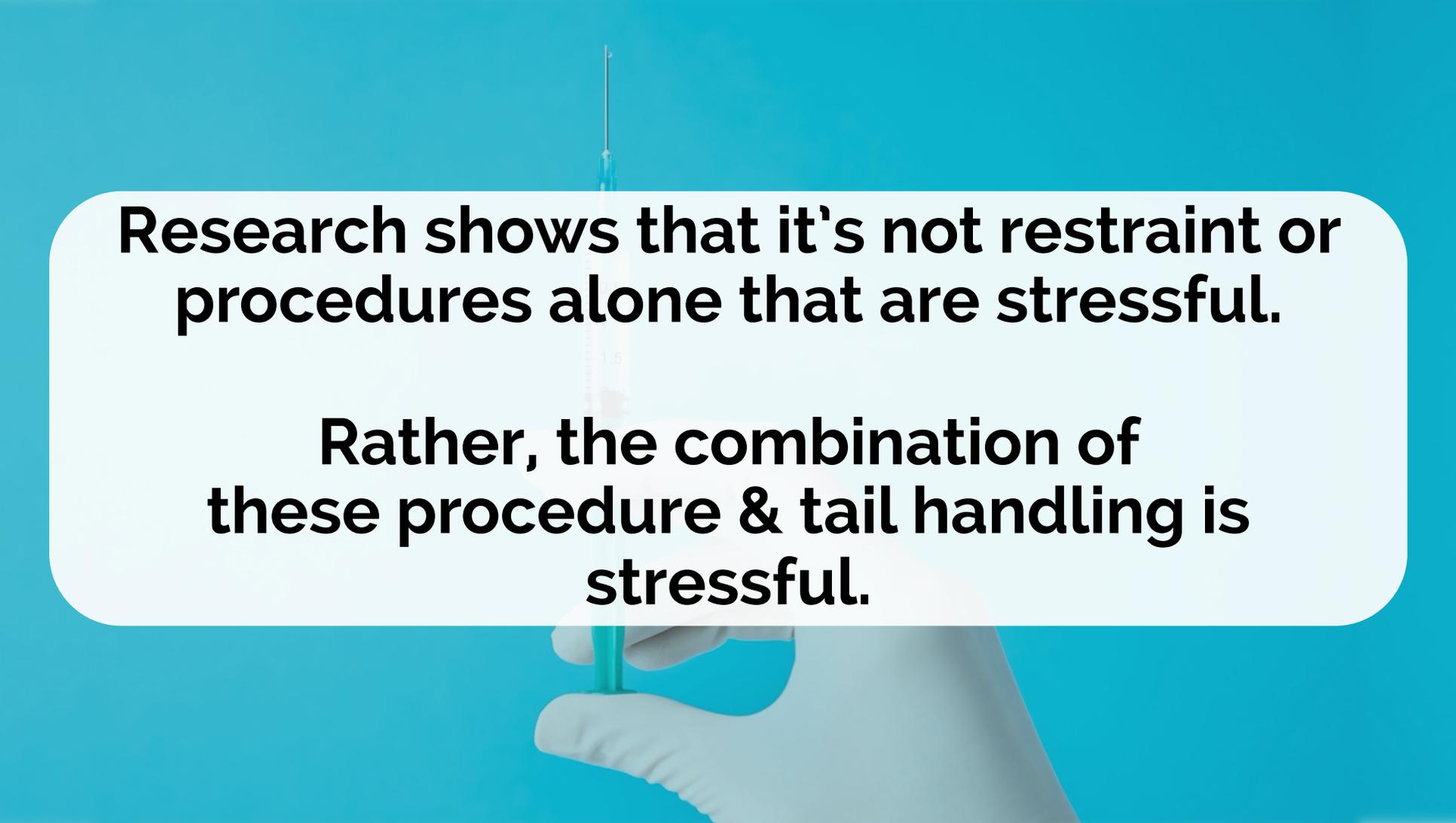
(Nakamura & Suzuki 2018)



(Gouveia & Hurst 2019, Sci Reports)

After 5th injection



A hand wearing a white latex glove is holding a clear syringe with a blue plunger and needle. The syringe is positioned vertically in the center of the frame. The background is a solid, vibrant blue. The text is overlaid on a white rounded rectangle in the center.

Research shows that it's not restraint or procedures alone that are stressful.

Rather, the combination of these procedure & tail handling is stressful.

Handlers report less aggression, fewer bites, & less cage escapes.

"I teach Mouse 101. Often, we would have mouse bites to brand new mouse handlers. **This was stressful to everyone involved.**

Since moving to tunnel trained mice... **we have not had any mouse bites** to brand new mouse handlers. ...This is great **welfare for the mice. And great for the handlers for confidence and learning.**"

For more details & references visit
na3rsc.org/picking-up-mice &
[nc3rs.org.uk/how-to-pick-up-a-
mouse](https://nc3rs.org.uk/how-to-pick-up-a-mouse)

NA3RsC's resource page gives a high-level overview.

Picking up mice

Learn why & how to use non-aversive handling to pick up mice.



Animal Welfare



Scientific Quality



Ease of Handling



Job Satisfaction

Strong evidence indicates that it is beneficial to animal welfare & scientific to picking up mice with non-aversive handling (i.e., tunnel handling or cupping) rather than by the tail. Picking mice up by the tail – even for only 2s at cage change – causes negative effects.

What is the evidence for this?

There have been 19 publications supporting the benefits of on-aversive handling improving welfare & scientific quality including:

- **Reducing anxiety** as measured by elevated plus maze, social novelty test, open field test, and light-dark box test (shown in 10 papers including Hurst & West 2010)
- **Reducing depressive-like behavior** as measured by sucrose reward, resilience to negative events (Clarkson et al. 2018, 2020), forced swim test and burrowing test (Sensini et al. 2020)
- **Reducing chronic stress** as measured by adrenal gland size (Clarkson et al. 2020)
- **Increasing test reliability** (Hurst & West, 2017)
- **Improving physiological parameters** such as improving glucose tolerance & reducing blood glucose & corticosterone (Ghosal et al. 2015; Ono et al. 2016)
- **Increasing voluntary interaction** with the handler (shown in 10 papers including Hurst & West 2010)
- **Improving breeding** as measured by larger pups (0.75g), more pups born (1), weaned (1.5), and longer breeding productive lifespan (20%; Hull et al., In Preparation).

For extensive details on the evidence base for non-aversive handling including a printable table of each study's key findings & methods see [NC3Rs mouse handling research summary](#).

Common concerns

Will this take longer for husbandry & procedures?

- Even **brief** tunnel handling during cage change is sufficient to deliver the positive benefits (Gouveia & Hurst 2019).
- Once staff are adequately trained husbandry and procedures **do NOT take any longer**.

What if I have to then restrain mice by the tail or perform aversive procedures?

The benefits of non-aversive persist even after standard procedures such as:

- Scruff or tail restraint (Hurst & West 2010; Roughan & Sevenoaks 2018; Gouveia & Hurst 2019; Henderson et al., 2020)
- Subcutaneous or intraperitoneal injection (Gouveia & Hurst, 2019; Henderson et al. 2020)
- Anesthesia (Henderson et al. 2020)
- Oral gavage (Nakamura & Suzuki, 2018)

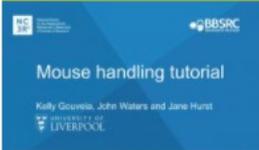
Tips for switching an entire institution to non-aversive handling

- Curate & identify champions for non-aversive handling from husbandry & scientific staff
- Get high-level support from management/regulatory bodies (IACUC, etc.)
- Consider rolling out tunnel handling in stages to address feedback/concerns by personnel type (husbandry versus clinical), project, research group, animal room, etc.
 - Similarly, it may work first to get veterinarian/administration support operations/husbandry support, and then scientific staff.
- Order adequate supplies
- Provide training on evidence base & methods
 - Consider bringing in an external expert
- Track & address concerns, issues, & acceptance

For more details & tips see [NC3Rs tips for implementation](#).

Nc3Rs' resource page has in depth information.

Video tutorial



Mouse handling tutorial

Kelly Gouveia, John Waters and Jane Hurst

UNIVERSITY OF LIVERPOOL

View our video tutorial on the refined mouse handling methods.

Frequently asked questions



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

Non-aversive mouse handling in practice



Hear from champions based at facilities around the UK who have successfully implemented non-aversive mouse handling methods.

Video clips



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

Posters



New methods to handle mice – time for a change

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

Research papers



Read the underpinning research and related papers.

Tips for implementation



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

Mouse handling webinar



Mouse handling made easy - Reducing anxiety in mice and their handlers

Professor Jane Hurst
27 April 2018

UNIVERSITY OF LIVERPOOL

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

Takeaway: refined handling is advantageous to animals, science, & people.



Improves animal
welfare



Increases
scientific
quality



Improves ease
of handling

Acknowledgments

- **Mark Prescott, NC3Rs**
- **Professor Jane Hurst**
- **NA3RsC Supporters**
- **NA3RsC Refinement Team**

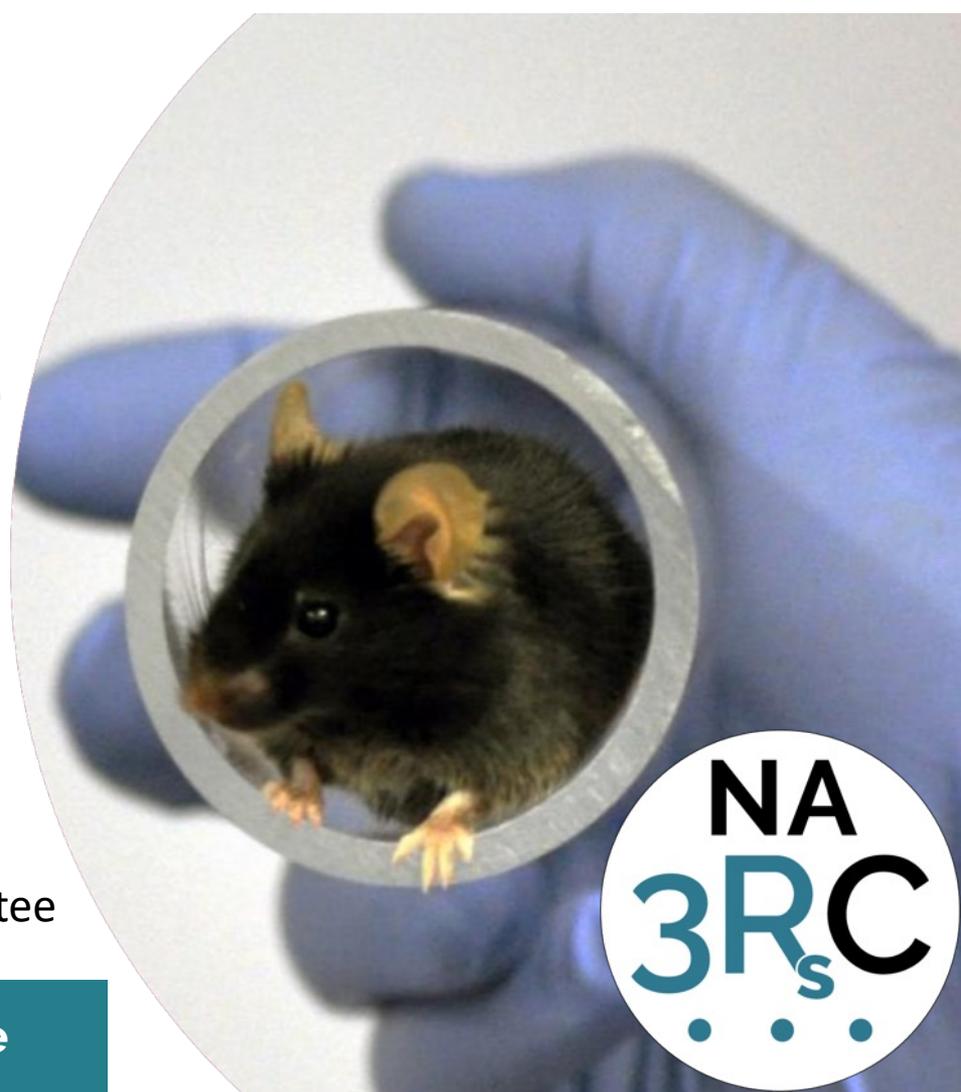
Acknowledgements: NA3RsC's sponsors



Changing the Culture of Mouse Handling

Donna Goldsteen, NA3RsC Refinement Committee

The North American 3Rs Collaborative



Animal Care Program at Large Pharmaceutical Company

- **20,000 sq. ft. animal facility, rodents only**
- **2021 animal census**
 - **38,220 mice**
 - **616 rats**
- **95% IVC caging with automatic watering**
- **Cages changed at least every 2 weeks**
- **Limited special housing – automated feed measurement cages (individually housed mice)**
- **Positive pressure isolator caging for germ-free mice**

Animal Care Program Components

- **Husbandry Staff:** cage changes, animal receipt, cage wash, facility maintenance, limited technical support (shaving, body weight collection)
- **Technical Staff:** technical support for therapy area scientists to include:
 - Dosing via various routes
 - Blood collection
 - Surgery support
 - Imaging support
 - Study data collection
 - Training animal care staff and scientists

Initial Implementation Path (2019)

1. Global support
2. Ordered clear tunnels
3. Bring in an external expert
4. Train husbandry & technical support staff

Later Implementation Path (2020)



1. Select PI champions
2. Tail handling no longer acceptable to PIs in July 2020
3. Track concerns, issues, & acceptance

Training



- **Humane Science refresher training November 2020**
 - **Discussed the implementation of alternative handling methods and emphasized no mice are to be picked up by the tail.**
 - **Provided training videos and literature on why this was necessary**
- **All new employee Humane Science training and Animal Facility training includes discussion on mouse handling methods.**
- **All new scientists hired must demonstrate their technical skills and proper handling methods will be assessed.**

Survey after One Year of Implementation

- **Sent survey to all animal facility users and AST staff about Mouse Handling**
 - **75% said they used non-aversive methods more than 50% of the time**
 - **25% use it less than 50% of time**
- **Only 2 people thought it was not at all useful**

Several misconceptions remain

- “Mice will need to be held by tail for IV injection anyway”
- “New mice are too fast and it takes too long to get them in the tunnel”
- “Takes too much time for specific procedures like randomization”
- “Tunnel handling hasn’t been validated for my models”

Refined Mouse Handling at AstraZeneca: Update

Erin Straley

Director, Animal Sciences and
Technologies, Gaithersburg Site Head

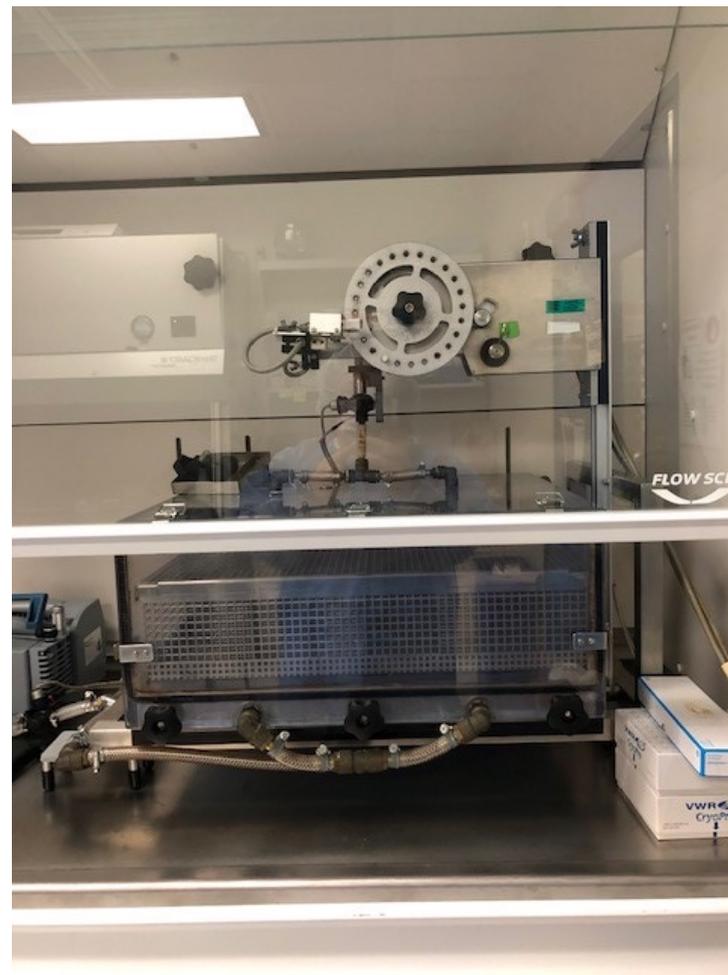


Specific Challenges Since Initiation

- Diabetic Mice – too big for tunnels! **CUPPING**
- Germ Free Mice for Microbiome Studies – concerns about contamination **CUPPING**
- Breeders **CUPPING-ISH**
- COPD Smoke Exposure – another alternative
- “Opportunistic” Handling
- Water Valve Leaks



Smoking Mouse Chamber



“Opportunistic” Handling Options



Sneak Peak: Survey Data

I still think the tunnel method is more stressful and takes way longer. I like the hand scoop method because the mice get used to being handled quicker and are calmer.

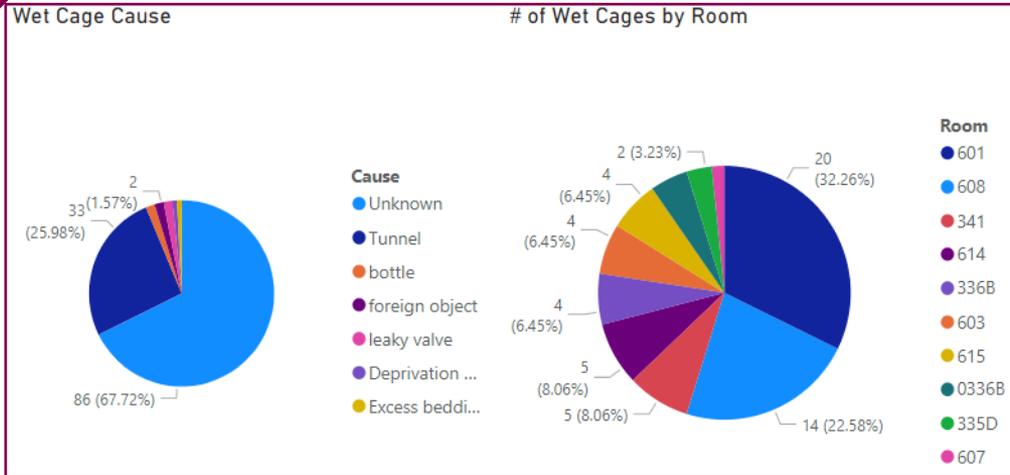
thicker mice do not fit in tunnel well, cupping works.

I still dont like or use the plastic tunnels as I use the hand scoop or cupping method.

There are no advantages to the tunnel in my opinion. I do like the hand scoop method.



Water Valve Challenges



Survey Data

- 84 respondents, 18 AST and 54 research scientists
- Never worked with lab mice to 34 years of experience (63 respondents with >10 years experience and only 5 with < 1 year)
- 96% of respondents say they are familiar with AST policies for handling and 87% say they are moderately or very familiar.
- 45% say they use non-aversive handling >90% of the time while 17% say they use it <5% of the time.
- 8% say they only use tail handling



Survey Data: Repeaters

- 22 respondents remember taking the survey last time
- Of those 22, 19 say it took them less than 1 month to get used to non-aversive handling and 13 of those said it took less than 2 weeks.
- In free text questions, we asked what their thoughts were before using non-aversive handling and after using it.
 - Before, only 5 had “positive” comments
 - After, 18 did
- Belief that the process takes too long is still the number one complaint (5 before, 7 after)

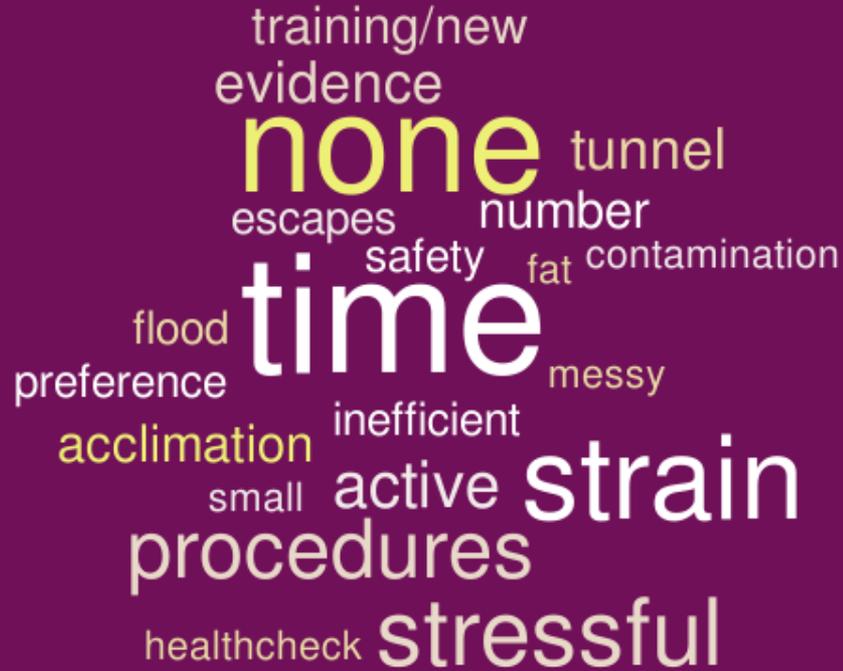


Survey Data: Comparison

	2021	2022
# Respondents	41	84
I am familiar w/ AST policy on NA handling	100%	96%
I am moderately/very familiar with the policy	85%	87%
I use NA handling >90% of the time	41%	45%
I use NA handling <5% of the time	17%	17%
Use tail restraint only	5%	8%
I'm confident I can use NA handling	93%	75%
I expect to use NA handling in the next year	90%	77%
I do not expect to use NA handling in the next year	2.5%	7%
It is expected that I will use NA handling	83%	75%
It is good for my mice to use NA handling	93%	75%
Using NA handling is the right thing to do	93%	74%



What factors or circumstances make it difficult for you to use non-aversive handling for mice?



Thank you!

- Gaithersburg AST Team
 - Shonda Hawkins
 - Carlos Gonzalez
- Gaithersburg TA Scientists
- Donna Goldsteen for her post-retirement support of our efforts
- Isaiah Straley – (my 13 year old) for help with PowerPoint Animations 😊



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Transitioning to Refined Handling in an Academic Institution

Elizabeth Nunamaker

Shaina Wallach



University of Florida

- Public institution
- 12 facilities
- 140 staff
- 25,000 mouse cages
- 598 active mouse protocols
- 288 mouse PIs



Need to garner support and generate buy-in.

Administration



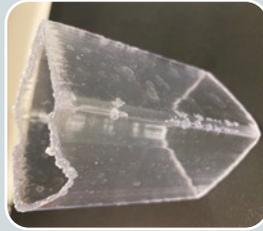
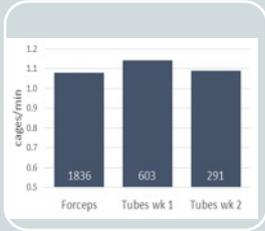
Operations



Investigator Staff



Pilot studies identified advantages & disadvantages of refined handling



Time to perform cage changes

Tunnel length



Tunnel longevity



Sanitation frequency

• monthly

Effects on breeding

• 20% ↑
• ↓ attrition

Effects on cage flooding

• 0.45% → 0.98%
• 70 cages

tunnel placement

Tunnels placed
in back of cage
may trigger ligit



tunnel placement t

Staff are
trained to
place tunnels
at the front of
the cage



Other Husbandry Considerations

- Initial learning curve
- More difficult to verify gender/check underside during cage change
- Cloudy/soiled tunnels may impede visualization of health concerns
- Excessively soiled tunnels require spot changing

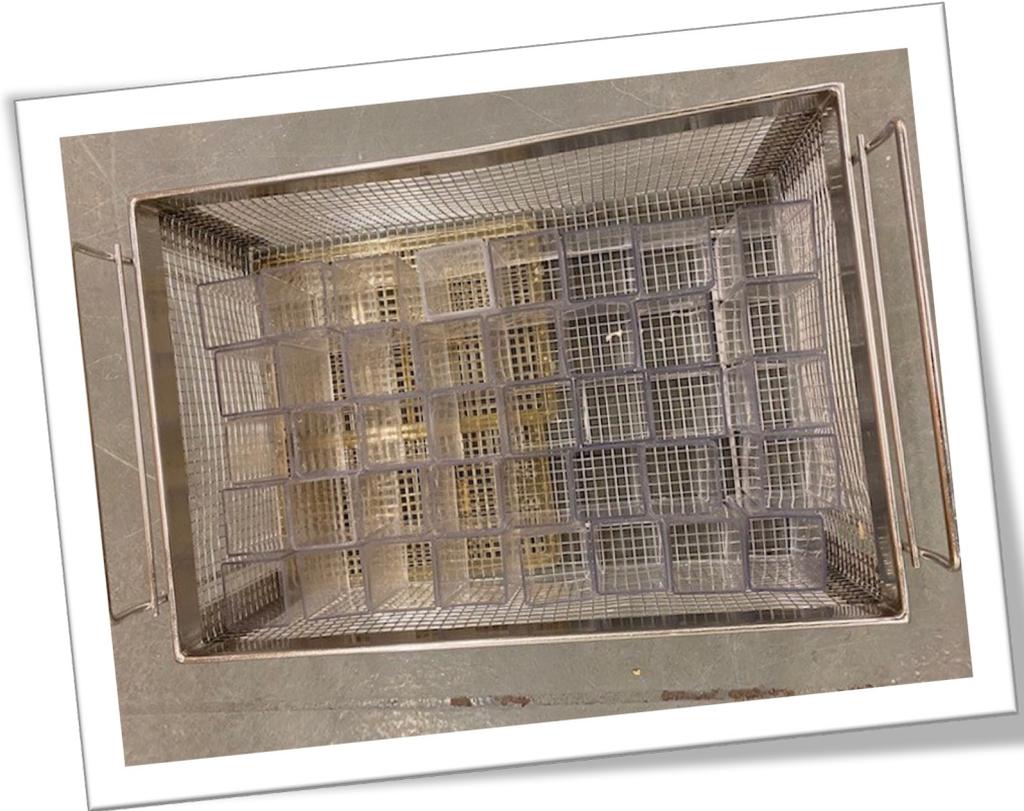
Cage Wash Considerations

- Excessively soiled tunnels may require soaking



Cage Wash Considerations

- **Increased active processing time:**
 - Remove tunnels from soiled cages
 - May need to hose/scrub if excessively soiled
 - Arrange in single layer for optimal automated washing
 - Inspect for cleanliness/cloudiness
 - Add to clean completes or package for autoclaving



A laboratory setting with a gloved hand holding a small mouse in a clear plastic container. The background is blurred, showing laboratory equipment and a person in a white lab coat.

Questions?

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shaina11@ufl.edu

<https://na3rsc.org/rodent-handling/>

