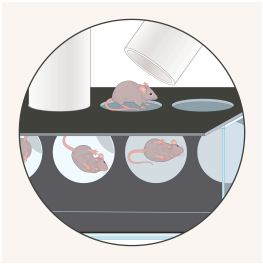
Aug 16, 2025

A behavioral assay for measuring acute itch in mice



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Seemay Chou[1], Behnom Farboud[1], Claire Kwon[1], MaryClare Rollins[1]

[1]Arcadia Science

Seemay Chou: Conceptualization, Supervision
Behnom Farboud: Methodology
Claire Kwon: Methodology
MaryClare Rollins: Conceptualization, Methodology, Supervision

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We use this protocol and it's working

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Abstract

Here we present a protocol for measuring mouse behavioral responses to acute itch stimuli. The experimenter injects mice intradermally with a pruritogen, then allows them to move freely within an enclosure. Behavior is recorded with HD video.

Image Attribution

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Materials

- Video cameras
  Sony [alpha]7sIII camera
  Sigma 24-70mm f/2.8 DG DN lens
  Tripod (example: Magnus TR-13 travel tripod with ball head)
- LED light (example: Godox M1 RGB Mini Creative On-Camera Video LED Light)
- Animal enclosure
- Insulin syringes (example: BD 8 mm 31G 0.5 mL)

Before start

Before conducting any experiments involving animals, obtain approval from IACUC or equivalent ethics committee(s).

Acclimatization procedure

1 **Day 1: Introduce mice to the enclosure**

Before beginning an experiment, mice must be acclimated to the enclosure and experimental procedures. Acclimatization ensures that animals are comfortable and familiar with their environment during an experiment, which facilitates behavioral responses to experimental stimuli, rather than to novel sounds, smells, or other distractions. This is particularly important when measuring itch-related behaviors because itch signals, transmitted by unmyelinated C-fibers, can be superseded by faster signals from myelinated pain or sensory neurons.

We acclimatize our mice in four sessions, each 24–48 hours apart.

1.1 Assemble your mouse enclosure wherever you plan to conduct experiments.

Note

We designed an experimental enclosure that allows simultaneous video recording of four mice.

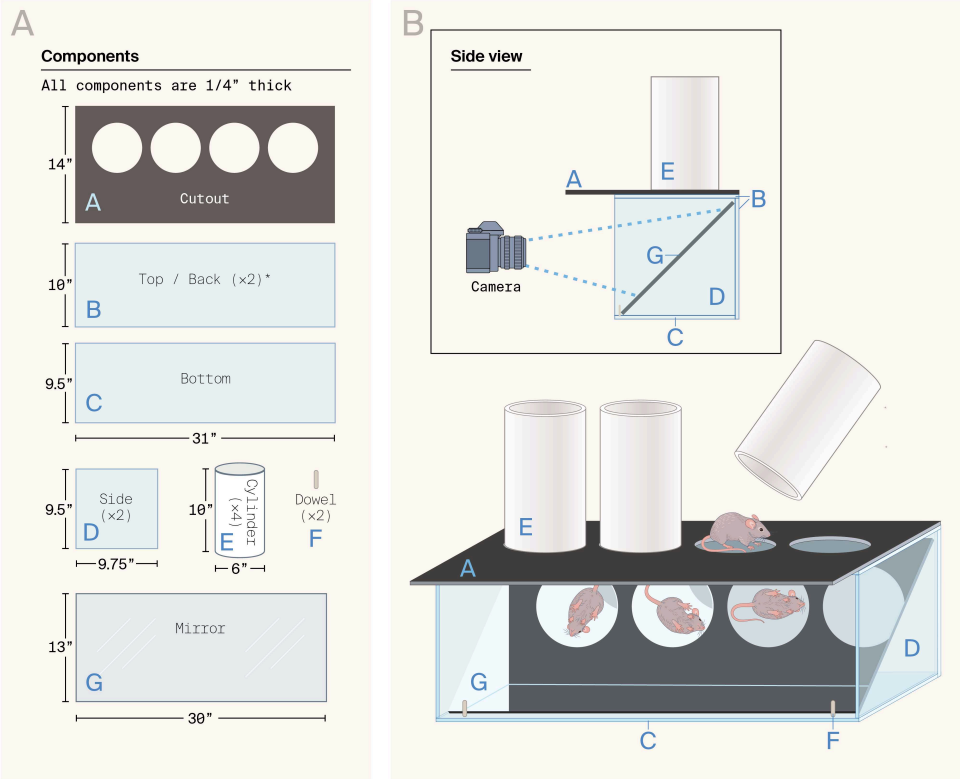

Figure 1. An experimental mouse enclosure for measuring itch-related behavior.
We designed and built an enclosure to enable video recording of mouse scratching and other itch-related behaviors.
(A) Components of the enclosure, made from acrylic. Note: We recommend using scratch-resistant acrylic for the top of the box (one of the panels designated 'B').
(B) Front and side views of the assembled enclosure. Acrylic panels form a box that's open on one side. An opaque panel with four circular cutouts is placed on top of the box. Cylinders fit exactly into the cutouts, and mice are placed in individual cylinders. A mirror is placed inside the box at a 45° angle, reflecting the view from beneath the mice. The mirror allows video recording of the mice without inverting the camera.

This protocol is written with our enclosure design in mind, but you can easily adapt it to suit other enclosures.

1.2 Establish the conditions you intend to use during your experiment (e.g., low light, quiet).

Note

We recommend using LEDs to provide low light in your experiment area. In our experience, mice are more active in low light and will scratch more when treated with pruritogens, compared to the same treatments in bright light.

If you're recording high-frame-rate video (which we recommend if you want to automate behavioral analysis with ML-based tools like DeepLabCut; see our **automated analysis resource**), we suggest using an LED light like the one we've listed in the equipment box below to provide consistent illumination without the flickering that occurs with fluorescent lights (which fluctuate at 120 Hz). For our experiments, we use one Godox M1 RGB to light a 156 sq ft room (settings: 91% brightness intensity, 5700 K color temperature). We point the LED toward the wall opposite the enclosure so the light bounces off the wall rather than reflecting off the enclosure mirror.

Equipment

M1 RGB Mini Creative Light	NAME
Godox	BRAND
https://www.amazon.com/Godox-M1-Color-Video-Light/dp/B0936S2N1M	LINK

1.3 Transfer mice to experiment area and gently lower each mouse into its assigned cylinder within the enclosure.

1.4 Allow mice to move freely within their cylinders for 10–15 minutes before transferring them back to their cages.

1.5 Clean and dry the enclosure between groups of mice. We recommend 70% ethanol or laboratory-specific sani-wipes.

2 **Day 2: Repeat enclosure training**

2.1 Set up the mouse enclosure as before.

2.2 Establish the conditions you intend to use during your experiment (e.g., low light, quiet).

2.3 Transfer mice to experiment area and gently lower each mouse into its assigned cylinder.

2.4 Allow mice to move freely within their cylinders for 10–15 minutes before transferring them back to their cages.

2.5 Clean and dry the enclosure between groups of mice. We recommend 70% ethanol or laboratory-specific sani-wipes.

2.6 At some point after completing enclosure training, anesthetize the animals and shave the fur from the nape of the neck. We recommend using an electric beard trimmer or pet hair trimmer.

Note

Shaving the mice on day 2 means the injection site will be clearly visible at the start of the experiment, but will allow enough time to resolve any local inflammation from micro-abrasions incurred during shaving.

3 **Day 3: Enclosure training, introduce injections**

3.1 Set up the mouse enclosure as before.

3.2 Establish the conditions you intend to use during your experiment (e.g., low light, quiet).

3.3 Transfer mice to experiment area and gently lower each mouse into its assigned cylinder.

3.4 Allow mice to move freely for 2–5 minutes. During this time, draw up the injection. We recommend warming the loaded syringe briefly in your closed hand.

3.5 Lift the first cylinder, remove the mouse from the top of the enclosure and replace the cylinder. Scruff the mouse using your thumb and forefinger to lift and lightly pinch the

skin at the nape of the neck. With an insulin syringe (31G needle), inject 30–50 µL of PBS or saline intradermally. The injected volume should bulge visibly beneath the skin. Lower the mouse back into the cylinder.

Note

Intradermal injections are shallow; hold the needle at 5–15° relative to the skin surface. Be careful not to inject too deeply, as subcutaneous delivery of some pruritogens can cause pain.

3.6 Repeat until all mice have been injected.

3.7 Allow mice to move freely within their cylinders for 10–15 minutes before transferring them back to their cages.

3.8 Clean and dry the enclosure between groups of mice. We recommend 70% ethanol or laboratory-specific sani-wipes.

4 Day 4: Enclosure training, introduce pruritogen injection

4.1 Set up the mouse enclosure as before.

4.2 Establish the conditions you intend to use during your experiment (e.g., low light, quiet).

4.3 Transfer mice to experiment room and gently lower each mouse into its assigned cylinder.

4.4 Allow mice to move freely for 2–5 minutes. During this time, draw up the injection. We recommend warming the loaded syringe briefly in your closed hand.

4.5 Lift the cylinder, remove the mouse and replace the cylinder. Scruff the mouse using your thumb and forefinger to lift and lightly pinch the skin at the nape of the neck. With an insulin syringe (31G needle), inject 30–50 µL of 200 µM serotonin in PBS (or a low concentration of your pruritogen of choice). The injected volume should bulge visibly beneath the skin. Lower the mouse back into the cylinder.

4.6 Repeat until all mice have been injected.

4.7 Allow mice to move freely within their cylinders for 10–15 minutes before transferring them back to their cages.

Note

The purpose of this step is to expose the animals to a mild itch stimulus while they're in the enclosure. The low concentration pruritogen injection should elicit a noticeable scratch response, but not so much that the skin becomes excoriated or inflamed.

4.8 Clean and dry the enclosure between groups of mice. We recommend 70% ethanol or laboratory-specific sani-wipes.

Experiment procedure

5 Set up the experiment enclosure and video cameras.

Note

We use two video cameras to record four mice. The cameras are pointed at the angled mirror, and the position and zoom are set such that each camera's field of view captures only two mice. Using two cameras means we can increase the number of pixels per cylinder, which improves the resolution of the recorded movement. If you're using QR codes to automate video labeling/processing (see our pub **here**), make sure the QR codes are also in frame.

Equipment

alpha7sIII camera	NAME
Camera	TYPE
Sony	BRAND
https://www.bhphotovideo.com/c/product/1577838-REG/sony_ilce7sm3_b_alpha_a7s_iii_mirrorless.html	LINK

Equipment

24-70mm f/2.8 DG DN lens	NAME
Lens	TYPE
Sigma	BRAND
https://www.bhphotovideo.com/c/product/1827260-REG/sigma_57a965_24_70mm_f2_8_dg_dn.html	LINK

Equipment

TR-13 travel tripod with ball head	NAME
Tripod	TYPE
Magnus	BRAND
https://www.bhphotovideo.com/c/product/1438201-REG/magnus_tr_13_travel_tripod_with.html	LINK

Equipment

NAME	TYPE	BRAND	LINK
TAB-4M Four-Mount Tripod Accessory Bar	Tripod cross beam	Oben	https://www.bhphotovideo.com/c/product/1448792-REG/oben_tab_4m_4_mount_tripod_accessory_bar.html

6 Establish experimental conditions.

Note

See our earlier note (go to step #1.2) for recommendations.

7 Transfer mice to experiment room and gently lower each mouse into its assigned cylinder.

8 Allow mice to move freely for 2–5 minutes. During this time, draw up the injection. We recommend warming the loaded syringe briefly in your closed hand.

9 Lift the first cylinder, remove the mouse and replace the cylinder. Scruff the mouse using your thumb and forefinger to lift and lightly pinch the skin at the nape of the neck. With an insulin syringe (31G needle), inject 30–50 µL of your pruritogen of choice, intradermally. The injected volume should bulge visibly beneath the skin. Lower the mouse back into the cylinder.

10 Press the record button on both cameras. Record for desired time (we recommend 15–30 minutes, depending on pruritogen). While recording, minimize noise and other distractions

11 Clean the enclosure between groups of mice. We recommend 70% ethanol or laboratory-specific sani-wipes.

Data processing and analysis

12 Scratching and other behaviors can be quantified manually by watching videos, but automating this process can significantly accelerate analysis.

For a description of our automated analysis pipeline, including video pre-processing, ML-guided body position tracking, and identification and quantification of scratching, see our resource **here**.

Acknowledgements

Additional contributors:

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- Kira E. Poskanzer — Conceptualization, Supervision
- Peter S. Thuy-Boun — Methodology