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Version 2

Mouse Habituation - Head Fixation on Disk V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the typical workflow for the habituation of a mouse for awake behavioral experimentation that utilizes a disk during head fixation.

Mouse habituation is a common technique utilized for mice undergoing behavioral experimentation and/or head fixation. Habituation allows the mouse to acclimate to new environments, handlers, and handling techniques, lowering the mouse's stress.

Habituation timeline summary: Mice are habituated for a total of 5 days, involving daily handling and increasing durations of head fixation in the behavior enclosure. Mice can be either water restricted or on Adlib water during habituation. In days 1 and 2 mice are habituated to handling, and on days 3-5 head fixing is introduced.

Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

Utilize proper PPE when handling mice. Prioritize handling the mouse over the cage to minimize risk of mouse jumping/falling. Keep mouse cage covered when not attending to mouse. Follow IACUC and veterinary requirements for handling mice.

Mice under water restriction should be closely monitored for health concerns due to the effects of dehydration. All mice should receive 1.0mL of water daily. Follow IACUC and veterinary requirements for water restricting mice.

Animal Behavior:

Utilize a 12-hour light cycle.

Mice should have access to ad-lib food and water until placed on diet/liquid restriction.

Single house mice on diet or water restriction.

Ensure cleanliness of experimental environment.

Materials

Materials:

	Tool / Supply	Manufacturer / Supplier	Part Number
	70% Ethanol (Diluted in house)	Sigma Aldrich	459836
	Kimwipes	Kimberly - Clark Professional	S47299A
	Liquid Reward	-	-
	Nalgene® 125 ml PET Square Media Bottles, Sterile	CP Lab Safety	342040-0125
	1mL Luer-Lok Syringe	Becton Dickinson and Company	309628
	25G 5/8 Needle *	Advantor	89134-134
	1000mL Nalgene™ Straight-Sided Wide-Mouth Polycarbonate Jars	Fisher Scientific	21161000
	Ohaus® Scout® Portable Balance	Sigma Aldrich	OH30253024
	Mouse Igloo® and Fast Tracs, Certified	Bio-Serv	K3327
	HEPA Replacement Filter for DC515	Dewalt	DC5151H
	18V Cordless 1/2 Gallon Wet/Dry Portable Vac (Tool Only)	Dewalt	DC515B
	Swiifer Sweeper	Uline	H-1960
	Swiffer Sweeper Pads - Wet Cloths	Uline	S-13901
	Water Bowl	Made in-house	0146-000-01
	Cart Cover	Life Science Products	CC-34X19X38Z
	Wheel Foam (MC® Solid Grip Black 18 in. x 4 ft.)	Kittrich	04F-187110-06
	Precision Screwdriver Hex 1/16th	WIHA	084705263137

Supplies can be substituted with their equivalent.

** = Optional*

Equipment:



	Material	Part Number
	Behavior Stage 1 (Behavior Platform, Behavior Training, Visual Behavior, Hotshoe Version 2) AND	0107-032-00
	Behavior Stage 2 (Passive Behavior Shroud Assembly)	0312-300-00

All equipment can be substituted with their equivalent.

Key:

AND = Including the tool/supply in row below.

Personal Protective Equipment (PPE):

	Suggested PPE
	Gloves
	Disposable lab coat
	Disposable face mask
	Scrubs
	Biohazard sharps disposal container
	Biohazard waste disposal container

Utilize PPE in accordance with IACUC and veterinary requirements.

Safety warnings

! Personal Protective Equipment (PPE) should be used at all times while operating this protocol.

Ethics statement

Research focused rodent behavior must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).

PHS Assurance : D16-00781

AAALAC : Unit 1854

Before start

Reference protocol below for specific start-up and take down procedures for behavioral training hardware and software:

Amaya, A. M., Swapp, J., Groblewski, P. A. (ND). Using behavioral training clusters. protocols.io. Unpublished.
<https://www.protocols.io/view/using-behavioral-training-clusters-c8vbw2n>

Reference water restriction protocol below for starting and maintaining a mouse on water restriction:

Protocol

NAME

Mouse Water Restriction

CREATED BY

Avalon Amaya

Preview

Day 1: Habituating a Mouse on a Disk

- 1 Handle mouse for 2-5 min.
 - 1.1 Scoop/cup mouse out of cage. Cup/cover mouse with hands for around 30 seconds to 1 minute.

Note

This act of cupping/covering the mouse acts to "calm" the mouse down and to prevent mouse from immediately jumping out of hands.



Hands cupping mouse over cage.

- 1.2 Uncup/cover hands, and let mouse explore the environment of your open hand for another 1-4 minutes.
 - 1.3 Weigh mouse and return back to cage.
 - 1.4 Maintain mouse on water restriction if applicable (See above section "Maintaining Water Restriction").
- 2 Clean and disinfect behavior room.
 - 2.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.



2.2 Wipe lickspouts with alcohol wipes.

2.3 Sweep floors.

Day 2: Habituating a Mouse on a Disk

3 Handle mouse for 2-5 min.

3.1 Repeat Day 1 handling steps  [go to step #1.1 - #1.2](#) .

3.2 Periodically touch the mouse's headframe to habituate mouse to future head fixation.

3.3 Maintain mouse on water restriction if applicable (see Before Start section).

4 Clean and disinfect behavior room.

4.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.

4.2 Wipe lickspouts with alcohol wipes.

4.3 Sweep floors.

Day 3: Habituating a Mouse on a Disk

5 Handle mouse for 1-2 min, secure mouse to head fixation stage, and place mouse within designated behavioral training box for 5 minutes.

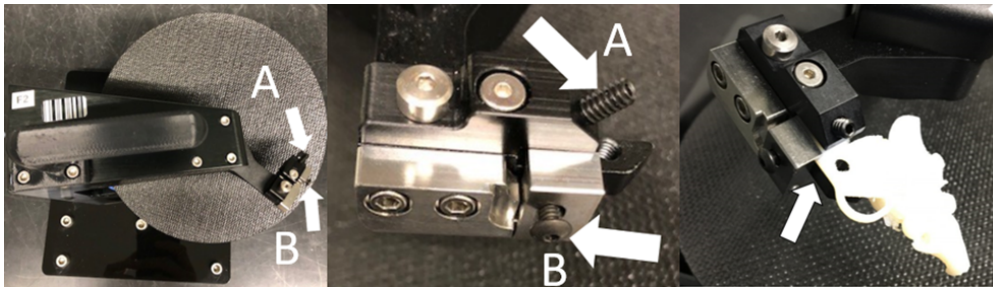
5.1 Cup or grab mouse out of cage by the tail.

- 5.2 Let mouse explore the environment of your hands for 1 minute. Periodically touch the mouse's headframe to habituate mouse to head fixation.



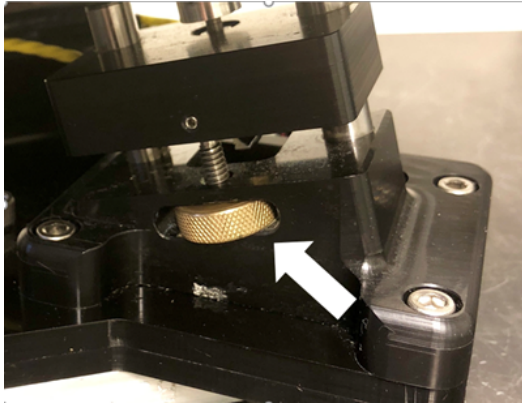
Hands holding mouse head frame and tail over cage.

- 5.3 While grabbing the mouse's headframe in one hand and the mouse's tail in the other. Secure mouse to disk head fixation stage.



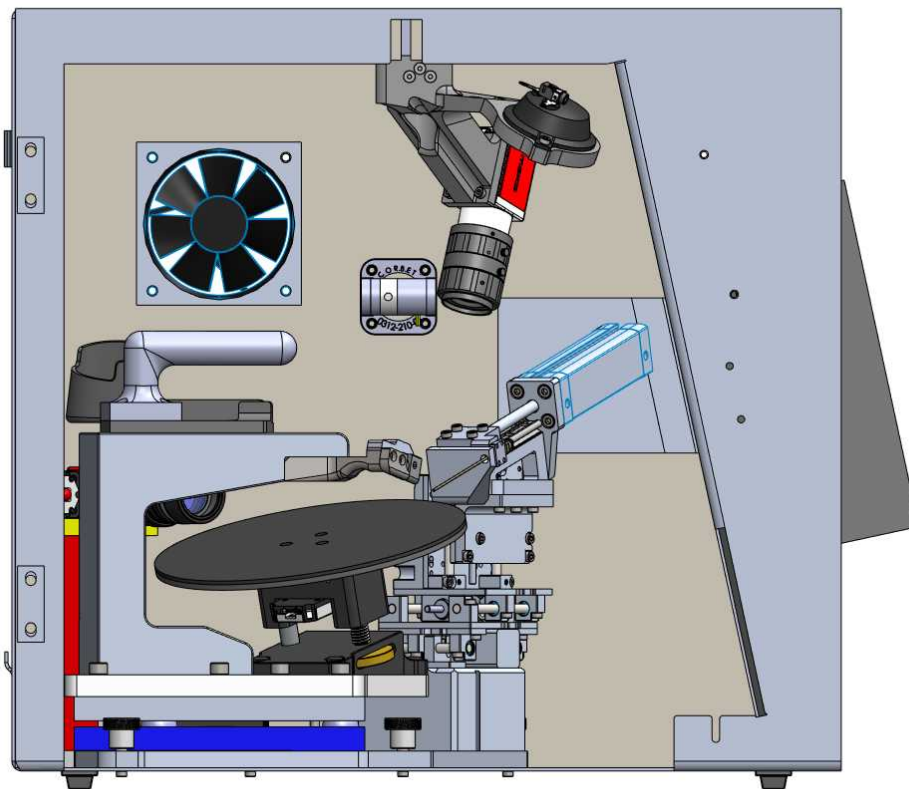
Stage, screws, and model of attached headframe.
A & B labels for securing points of contact in operational order.

- 5.4 Adjust disk height to allow mice to run/walk at a natural and comfortable gait.



Knob for disk height adjustment

- 5.5 Place mouse secured to head fixation stage within designated behavioral training box for 5 minutes.



Computer model design of inside of behavior box with behavior stage.



- 5.6 Remove mouse from box and head fixation stage.
- 5.7 Maintain mouse on water restriction if applicable (see Before Start section).
- 6 Clean and disinfect behavior room.
- 6.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.
- 6.2 Wipe lickspouts with alcohol wipes.
- 6.3 Sweep floors.

Day 4: Habituating a Mouse on a Disk

- 7 Handle mouse for 1-2 min, secure mouse to head fixation stage, and place mouse within designated behavioral training box for 10 minutes.
- 7.1 Repeat Day 3 handling and head fixing steps [↪ go to step #5.1 - #5.4](#) .
- 7.2 Place mouse secured to head fixation stage within designated behavioral training box for 10 minutes.
- 7.3 Remove mouse from box and head fixation stage.
- 7.4 Maintain mouse on water restriction if applicable (see Before Start section).
- 8 Clean and disinfect behavior room.
- 8.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.



8.2 Wipe lickspouts with alcohol wipes.

8.3 Sweep floors.

Day 5: Habituating a Mouse on a Disk

9 **(Passive Behavior):**

Handle mouse for 1-2 min, secure mouse to head fixation stage, and place mouse within designated behavioral training box for 10 minutes.

9.1 Repeat Day 4 steps  [go to step #7](#) - #8.3 .

10 **(Active Behavior):**

Handle mouse for 1-2 min, secure mouse to head fixation stage, place mouse within designated behavioral training box, and run 15 minute training session.

10.1 Open MouseDirector and load your username and the mouse ID onto its designated behavioral training box.

10.2 Repeat Day 3 steps  [go to step #5.1](#) - #5.5

10.3 Use lickspout controller to find Mouse Offset.
Mentioned in protocol *Using Behavioral Training Clusters*, section *Aligning Lickspout*

Amaya, A. M., Swapp, J., Groblewski, P. A. (ND). Using behavioral training clusters. protocols.io. Unpublished. <https://www.protocols.io/view/using-behavioral-training-clusters-c8vbw2n>

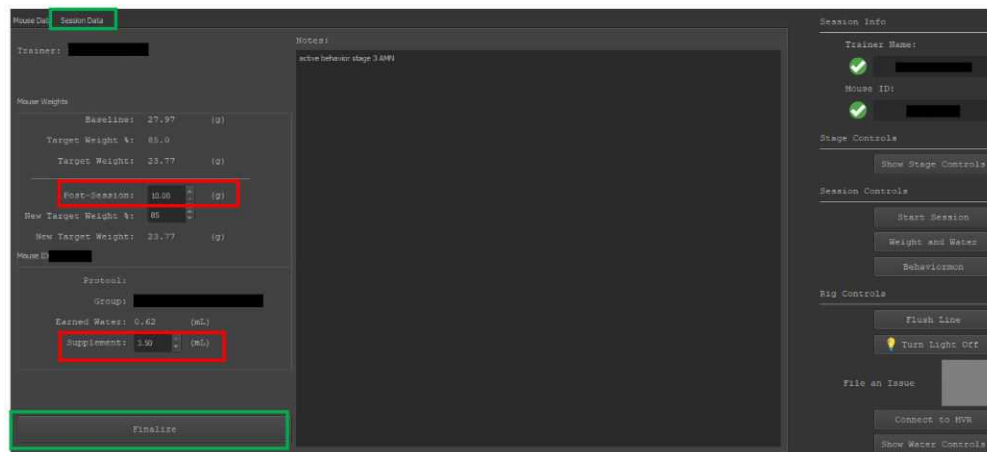
10.4 Confirm script regimen and the mouse ID.

10.5 Press "start session" to begin auto train session.

10.6 Confirm that script has begun via camera view (typically static grating images).



- 10.7 Monitor mouse behavior. If not licking or if tongue is not able to make contact with lickspout, move lickspout closer to mouth.
- 10.8 Once session is completed, press the "safe" button on Mouse Director to retract the lickspout.
- 10.9 Remove mouse from box and head fixation stage.
- 10.10 Weigh mouse.
- 10.11 Return mouse to cage.
- 10.12 Click "Session Data" tab on Mouse Director.
- 10.13 Enter mouse weight in "Post-Session: " text box.



The screenshot shows the Mouse Director software interface. The 'Session Data' tab is selected and highlighted with a green box. In the 'Mouse Data' section, the 'Post-Session' field is outlined in red. In the 'Mouse Info' section, the 'Supplemental' field is outlined in red. At the bottom of the interface, the 'Finalize' button is outlined in green. The right sidebar contains various control buttons and status indicators.

Session Data tab in Mouse Director.

Post-Session weight and Supplemental water calculation boxes outlined in red.

Session Data tab and Finalize button outlined in green.

**Note**

Mouse baseline weight and restriction percentage should already be associated with the mouse ID if water restricted through Waterlog. See water restriction protocol in above section.

- 10.14 Provide mouse with amount of supplemental water as written in "Supplemental:" box.

Note

If the supplemental water text box displays 0.00mL, ensure mouse has received at least 1.0mL of water for the session.

- 10.15 Press the "Finalize" to save session data.

- 11 Clean and disinfect behavior room.

- 11.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.

- 11.2 Wipe lickspouts with alcohol wipes.

- 11.3 Sweep floors.

Clean

- 12 Clean and disinfect behavior room.

- 12.1 Use 70% Ethanol spray and Kimwipes to clean weigh boats and surface areas.



Note

Make sure to remove all fecal matter from behavior boxes.

12.2 Wipe lickspouts with alcohol wipes.

12.3 Sweep floors.

12.4 Mop floors once a week at the end of the day once all mice have left the room.

Note

Make sure there are no animals present in room. Mop when room has sufficient time to air out.

12.5 Vacuum behavior boxes once a month at the end of the day once all mice have left the room.

Note

Use HVAC certified vacuum. Make sure no animals are present in room.

Habituating a Mouse Quick Guide

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Active Habituation

	M	T	W	Th	F
Week 1			Weigh	Weigh	Weigh Set baseline weight Water restrict
Week 2	Handle 2-5 minutes	Handle 2-5 minutes	Handle 1-2 minutes, headfix, place stage in box for 5 minutes	Handle 1-2 minutes, headfix, place stage in box for 10 minutes	Handle 1-2 minutes, headfix, 15 minute autoreward script
Week 3	Training day 1	Training day 2	Training day 3	Training day 4	Training day 5

Passive Habituation

	M	T	W	Th	F
Week 1	Handle 2-5 minutes	Handle 2-5 minutes	Handle 1-2 minutes, headfix, place stage in box for 5 minutes	Handle 1-2 minutes, headfix, place stage in box for 10 minutes	Handle 1-2 minutes, headfix, place stage in box for 10 minutes
Week 3	Training day 1	Training day 2	Training day 3	Training day 4	Training day 5

Quick Guide:

Typical first 2-3 week schedule of an experimental behavior mouse.

The intended use for quick guide is to reference once familiarized with habituating a mouse.