

Nov 07, 2025

Running a Dynamic Foraging Behavior Task in Mice

DOI

<https://dx.doi.org/10.17504/protocols.io.5jyl8p4m6g2w/v1>



Ella Hilton-VanOsdall¹

¹Allen Institute for Neural Dynamics

Allen Institute for Neural...



Ella Hilton-VanOsdall

Allen Institute for Neural Dynamics

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Protocol Citation: Ella Hilton-VanOsdall 2025. Running a Dynamic Foraging Behavior Task in Mice. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5jyl8p4m6g2w/v1>



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Protocol status: Working

We use this protocol and it's working

Created: February 23, 2024

Last Modified: November 07, 2025

Protocol Integer ID: 95676

Keywords: Foraging, Dynamic Foraging, Behavior, Behavior Training, Foraging Behavior, Decision Making, Mouse behavior, foraging behavior task, behavior task in mice, dynamic foraging, behavioral task, thirsty mouse, water reward, behavioral training, mice, decisions on reward, procedure for behavioral training, course of the experiment, probabilistic reward, experiment, experimenter, reward, task, water, experimental setup, choice history

Abstract

This protocol describes the experimental setup and procedure for behavioral training of mice in a “dynamic foraging” task (as described in Bari et al., 2019; Grossman, et al., 2022). The behavioral task outlined in this protocol involves training a thirsty mouse to make decisions in a dynamic environment. Each trial begins with an auditory “go cue,” followed by a choice to lick toward either a left or right lickspout. Choices are followed by probabilistic rewards (drops of water). Reward probabilities change over the course of the experiment, so mice must base their decisions on reward and choice history to maximize their water reward. The task can be modified to reflect the experimenter’s specific question through alterations in the training parameters. Mice are trained 5-7 days per week, with a typical session lasting 75-90 minutes. This protocol outlines the materials and steps for preparing and running the experiment.

Guidelines

Mice should be single-housed on a 12-hour light cycle (8:30 AM - 8:30 PM dark). Active behavior must occur during the dark period. Mice must be provided ad-libitum food throughout the experiment - refer to protocol in *Before Starting* for details on water restriction. Experimenters must ensure cleanliness of the experimental environment at all times.

It is best practice to let the mouse's weight stabilize at their target weight (post water restriction) before starting training, and run experiments at approximately the same time each day to ensure the mouse is motivated by the water reward.

Materials

Materials (Software):

[Dynamic Foraging Task \(GitHub Repository\)](#)

[Auto-training for the Dynamic Foraging Task \(GitHub Repository\)](#)

[Code Ocean capsule for evaluation of mouse performance and training stage advancement](#)

Materials (Hardware):

Supplies can be substituted with their equivalent

	Material	Manufacturer	Part Number	Purpose
	Behavior boxes	Built in-house	-	Secure animal, run behavior. See Materials - Figure 1
	1mL Luer-Lok Syringe	Becton Dickinson and Company	309628	Supplemental water delivery to mouse
	Nalgene® 125 ml PET Square Media Bottles, Sterile	CP Lab Safety	342040-0125	Storage of supplemental liquid reward
	1000mL Nalgene Straight-Sided Wide Mouth Polycarbonate Jars	Fisher Scientific	21161000	Mouse weight collection
	KimWipes	Kimberly-Clark Professional	S47299A	Cleaning
	70% Ethanol	Sigma Aldrich	459836	Disinfecting hardware, diluted in house
	Ohaus® Scout® Portable Balance	Ohaus	OH30253024	Weight collection
	Water Reward (Reverse Osmosis)	-	-	Liquid reward, replaced weekly
	Lens Cleaning Wipes	Zeiss	B0030E4UIQ	Cleaning debris from optic fiber implants
	Water Bowl	Built in-house	-	Holds supplemental water for the mouse. See Materials - Figure 2



	Material	Manufacturer	Part Number	Purpose
	Cloth Cart Cover	Life Science Products	CC-34X19X38Z	Coverage of mouse cages to block potential light exposure
	Swiffer Sweeper	Uline	H-1960	Weekly mopping of experimental room
	Swiffer Sweeper Pads	Uline	S-13901	Weekly mopping of experimental room
	HEPA Filter Vacuum	Makita	XCV11Z	Weekly vacuuming of behavior boxes and room
	1/16" Hexagon Driver	DigiKey	431-1114-ND	Headfixing
	5MM Hexagon Driver	Bondhus	13164	Engaging magnetic base

Personal Protective Equipment (PPE)

	Materials
	Biohazard Sharps Disposal Container
	Disposable Face Mask
	Disposable Lab Coat
	Gloves

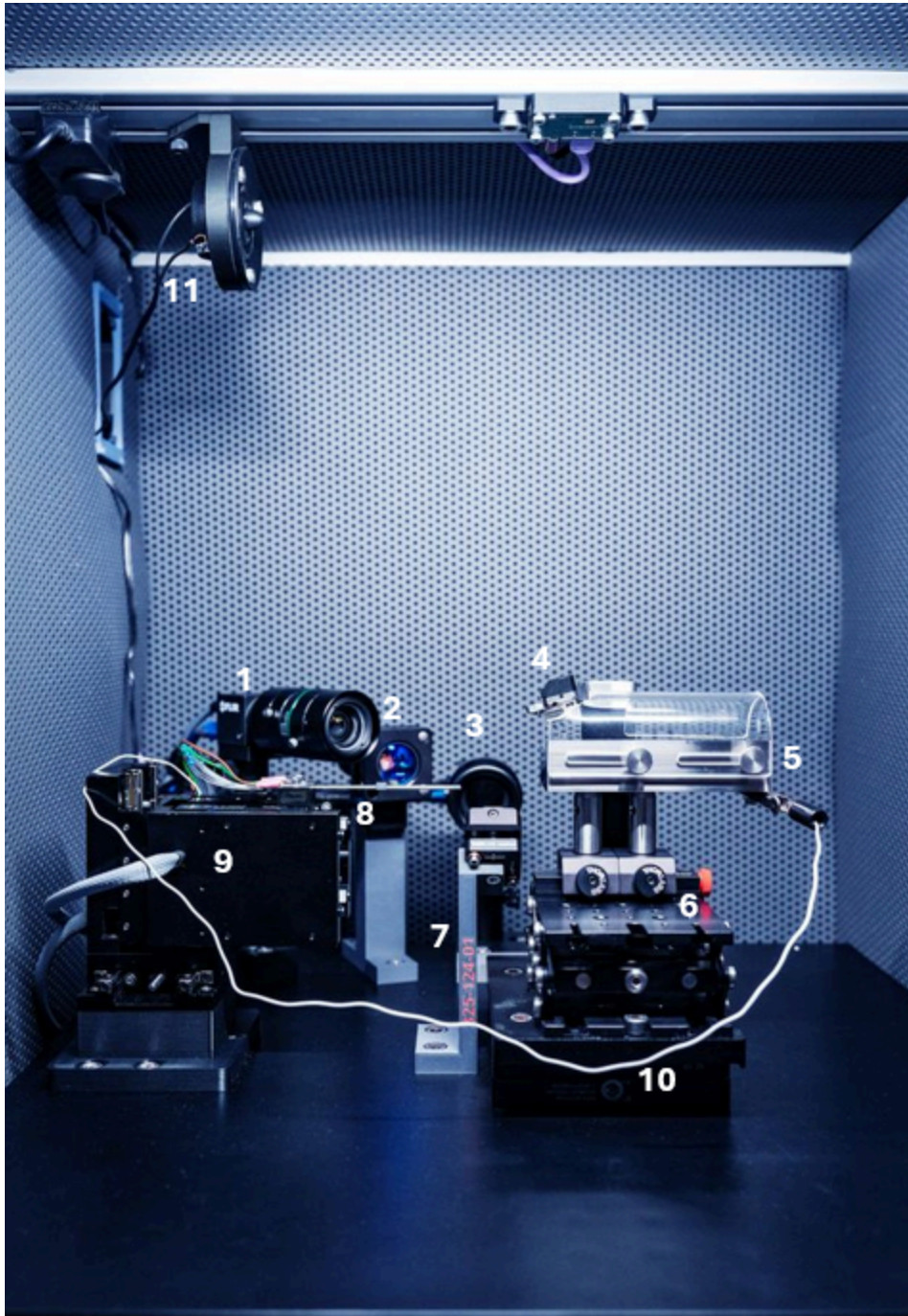


Figure 1.1: Behavior box (1 in Materials - Hardware)

- 1 = High speed video camera (side view)
- 2 = Infrared LED
- 3 = High speed video camera (bottom view)
- 4 = Head frame clamp*
- 5 = Metal mouse tube, alligator clip*
- 6 = Mouse stage apparatus*
- 7 = Mirror
- 8 = Lickspouts
- 9 = Motor stage
- 10 = Magnetic base
- 11 = Speaker

*= all components of the mouse stage apparatus



Figure 1.2: Behavior box tower



Figure 2 (10 in Materials - Hardware): water bowl attaches to cage food hopper



Safety warnings

- ❗ Ensure the following PPE is worn by experimenter:
 - Gloves
 - Disposable Face Mask
 - Disposable Lab Coat

Ethics statement

Research focused behavior experiments 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

Ensure that all animals meet IACUC and veterinary requirements. Whenever handling a water-restricted mouse, be sure to monitor the animal for signs of dehydration, referring to institutional veterinary staff. Mice must be habituated and headfixed using an approved head-fixing technique (see protocol below). Mice must be run in the dark period of their light cycle, as described in the guidelines section.

Running this experiment is dependent on a specific HARP/Bonsai configuration and software setup - see GitHub repository [here](#) for the software to run the task.

Protocol

NAME

Mouse Water Restriction

CREATED BY

Avalon Amaya

Preview

Protocol



NAME

Mouse Habituation - Head Fixation into Tube

CREATED BY

Avalon Amaya

Preview

Software Setup

- 1 Set up the software and GUI to enable running the task by ensuring connections between hardware and software are established (see Materials - Software). Launch Bonsai and the GUI by selecting the appropriate software on the computer.

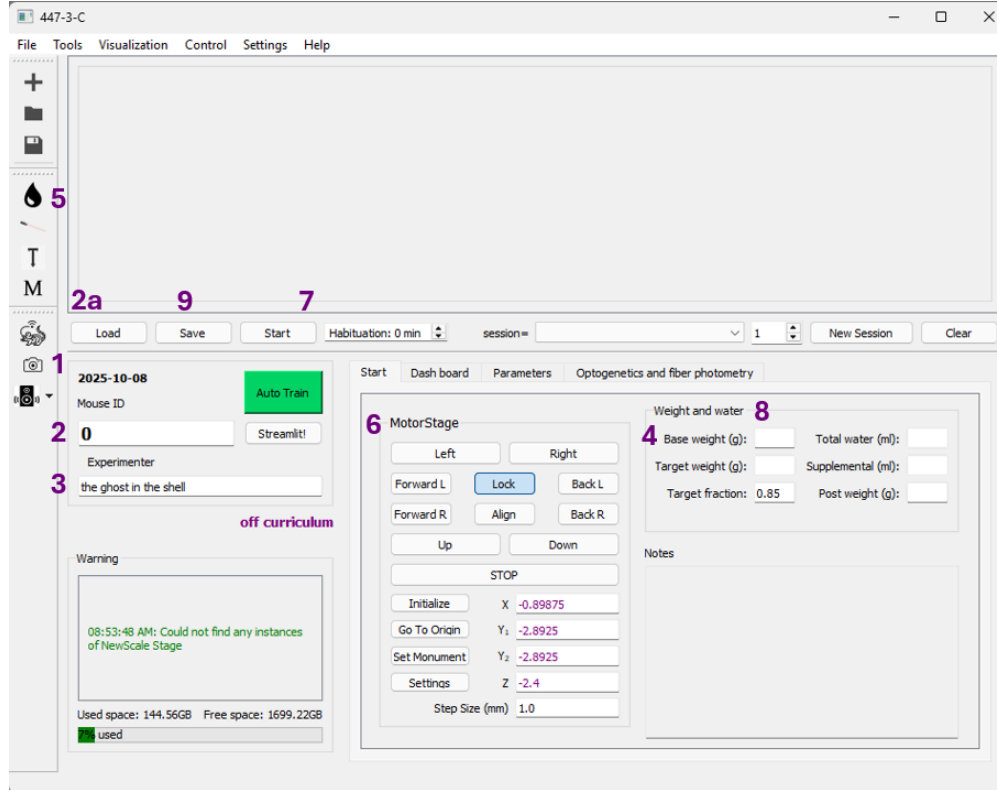


Figure 1: Dynamic Foraging GUI

- 1 = Camera control button
- 2 = Mouse ID box
- 2a = Load button
- 3 = Experimenter name box
- 4 = Baseline weight box
- 5 = Water calibration window button
- 6 = Motor stage control panel
- 7 = Start button
- 8 = Weight and water panel
- 9 = Save button

- 2 Launch cameras through the GUI so that the mouse can be visualized during training (see **Figure 1:** part 1).

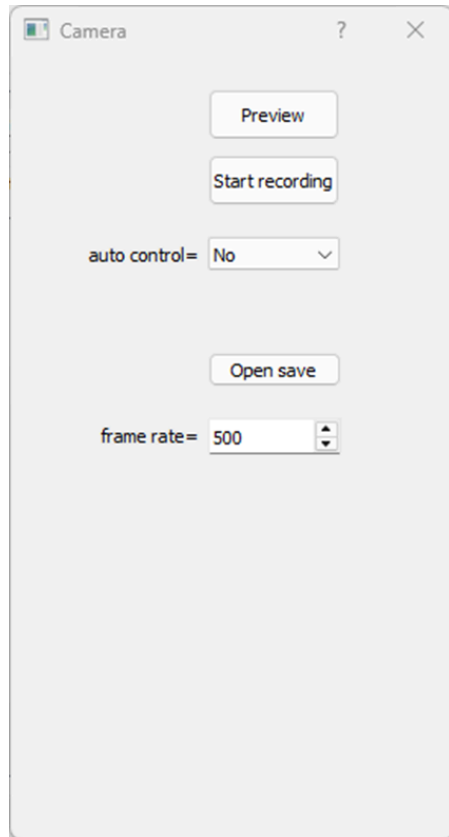


Figure 2.1: Dynamic Foraging GUI camera control window

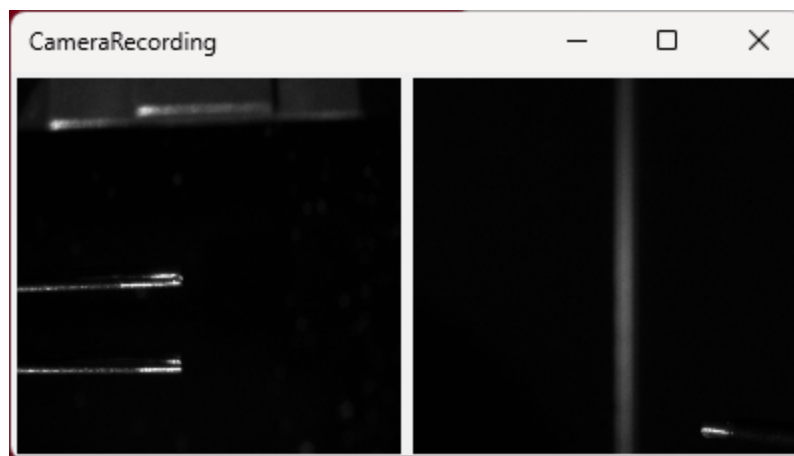


Figure 2.2: Dynamic Foraging GUI camera preview window

- 3 In the main window of the GUI, enter the mouse ID, experimenter name, and baseline weight (see **Figure 1:** parts 2, 3, and 4). Cross reference all mouse ID information (ear notch or tattoo with information in the GUI).

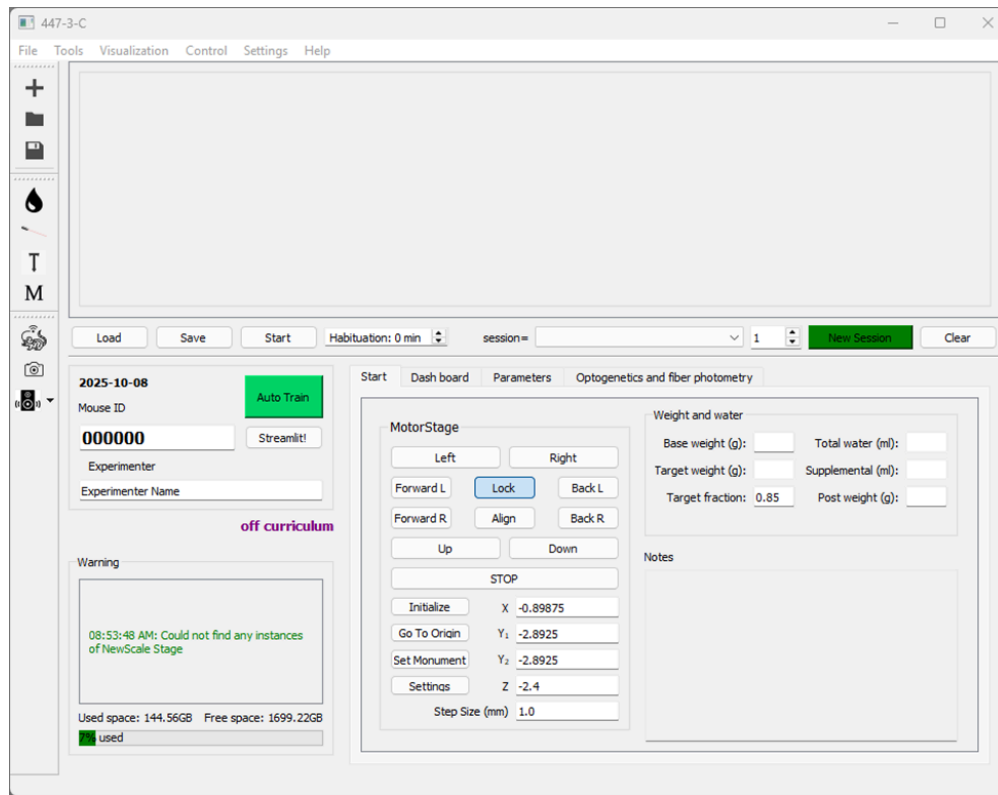


Figure 3: Dynamic Foraging GUI after entering mouse ID and experimenter name

Note

If loading a previously trained mouse, this can be done using the Load function (see **Figure 1: part 2a**).

Prepare Hardware

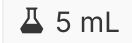
- 4 Open the water control panel (see **Figure 1: part 5**). Using the software control of the solenoids in the water calibration panel (see **Figure 4**), drain  5 mL of water from each lickspout. This ensures that all air bubbles are cleared from the tubing and that water delivery to mice during the task is reliable.



Figure 4: Dynamic Foraging GUI water calibration window



Note

Once a week, empty the water reservoir completely, and refill with reverse osmosis water. See the protocol below for water system maintenance.

Protocol



NAME

Solenoid Valve Calibration for Behavior Rigs Utilizing Water Reward

CREATED BY

Ella Hilton-VanOsdall

[Preview](#)

- 5 Refill water reservoirs to  50 mL .

Note

It is important that each water reservoir is filled to a consistent volume each session, as differences in water volume can affect the pressure in the water lines and subsequent water delivery.

- 6 Use a KimWipe moistened with 70% ethanol (EtOH) to sanitize each lickspout.
- 7 Ensure that the infrared LEDs are turned on to allow the experimenter to visualize the mouse through the highspeed video camera while minimizing the mouse's white light exposure.

Secure the Mouse to Behavior Apparatus

- 8 Remove the mouse tube from the behavior box and place it on the head-fixing station. Move tube apparatus into its lowest and most posterior position.



Protocol



NAME

Mouse Habituation - Head Fixation into Tube

CREATED BY

Avalon Amaya

[Preview](#)

9 Remove the mouse from its home cage and evaluate health conditions.

9.1 If the mouse does not appear BAR do not proceed with the experiment. Alert your animal care team and monitor the mouse's condition.

Note

Always follow all IACUC and veterinary requirements when handling water restricted mice.

10 Secure the mouse to the tube using a 1/16" hex driver and approved head-fixing technique (Step 7 in Mouse Habituation - Head Fixation into Tube).

11 Adjust the tube apparatus such that the mouse is in a comfortable position, as described in Mouse Habituation - Head Fixation into Tube [➡ go to step #8](#) . If the mouse shows signs of stress, such as vocalization, this is an indication that they are experiencing discomfort and action to mitigate this must be taken, such as adjusting the tube height and backward/forward positioning.

Position the Mouse in the Rig

12 Place the mouse tube apparatus in the rig and engage the magnetic mechanism (see **Figure 5:** part 10) with a 5mm hex screwdriver. This ensures that the mouse tube apparatus remains firmly in place during training.

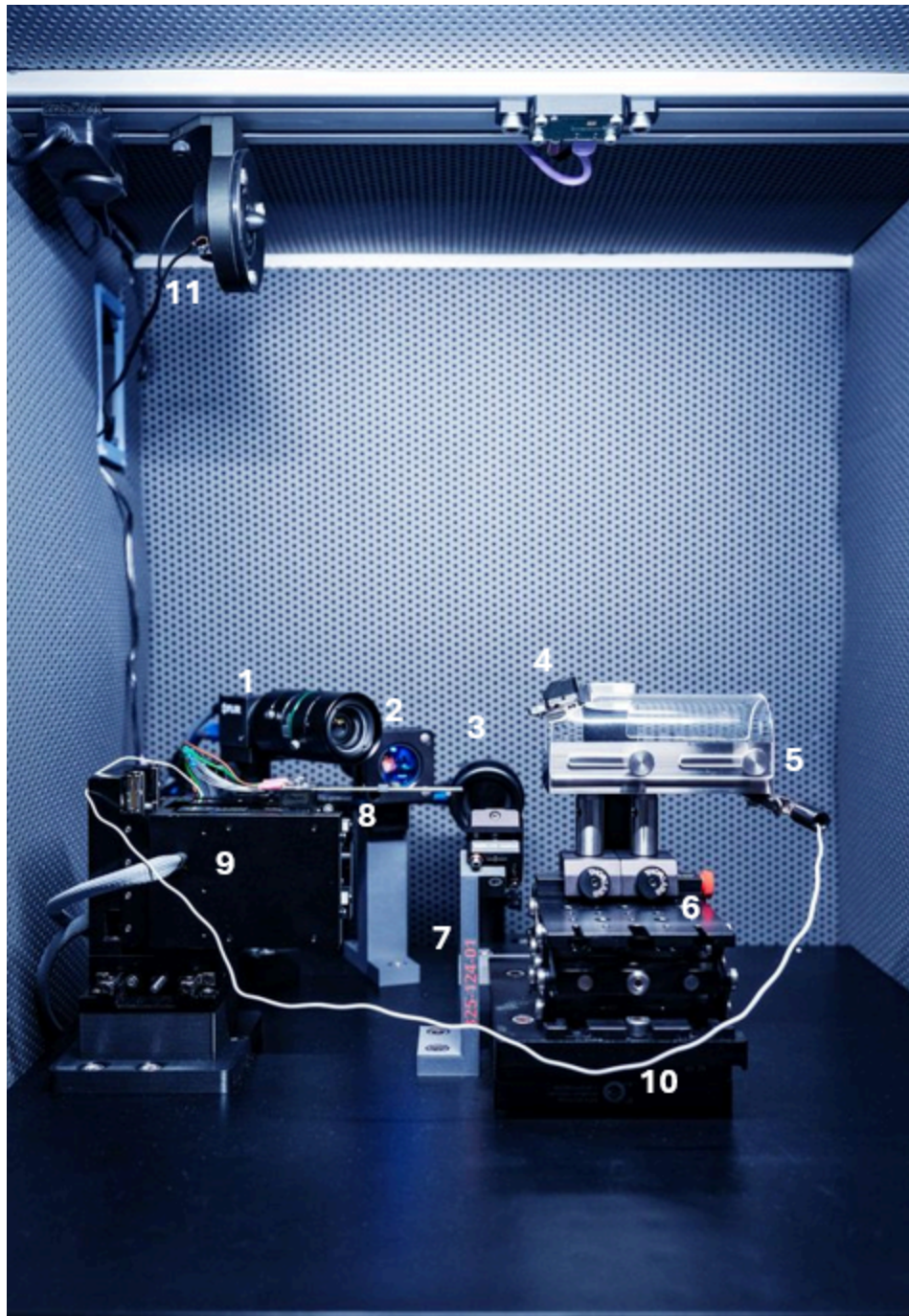


Figure 5: Behavior box

- 1 = High speed video camera (side view)
- 2 = Infrared LED
- 3 = High speed video camera (bottom view)
- 4 = Head frame clamp*
- 5 = Metal mouse tube, alligator clip*
- 6 = Mouse stage apparatus*
- 7 = Mirror
- 8 = Lickspouts
- 9 = Motor stage
- 10 = Magnetic base
- 11 = Speaker

*= all components of the mouse stage apparatus

- 13 Using an alligator clip, ground the mouse by connecting the alligator clip from the AIND motor stage (**Figure 5**: part 9) to the tube apparatus (**Figure 5**: part 5).

Note

Connecting the alligator clip to the conductive tube ensures that each time the mouse's tongue touches the lickspout during the session, the electrical circuit is completed and the mouse's licks are detected.

- 14 Close the door to the experimental box to ensure minimal sound contamination during the session.

- 15 While viewing the mouse through the side and bottom cameras (see **Figure 2.2**), adjust the lickspouts using the motor control in the GUI (see **Figure 1**: part 6) so that each spout is within reach of the mouse's tongue.

Note

Always move lickspouts slowly and in small increments (0.2 mm) to ensure mouse safety.

- 15.1 Lickspouts should be positioned such that:

1. The philtrum line between the nose and and mouth is positioned in the middle of the two lickspouts.
2. The tips of the lickspouts are aligned with the upper front teeth.
3. The Z position of the lickspouts is approximately 2mm below the mouth.

The mouse's tongue should be able to comfortably reach the end of the lickspout and make full contact with each lick. This placement can take a few days of training to refine, as new mice will likely need the lickspout close to encourage engagement. However, as the mouse learns the task, the lickspout should be lowered to 2 mm below the mouse to discourage excessive licking.

To minimize side bias the lickspout location may need to be adjusted to the left or right during the experiment. For example, a mouse that is left biased may need the lickspout offset, so that the right lickspout is closer to the center.

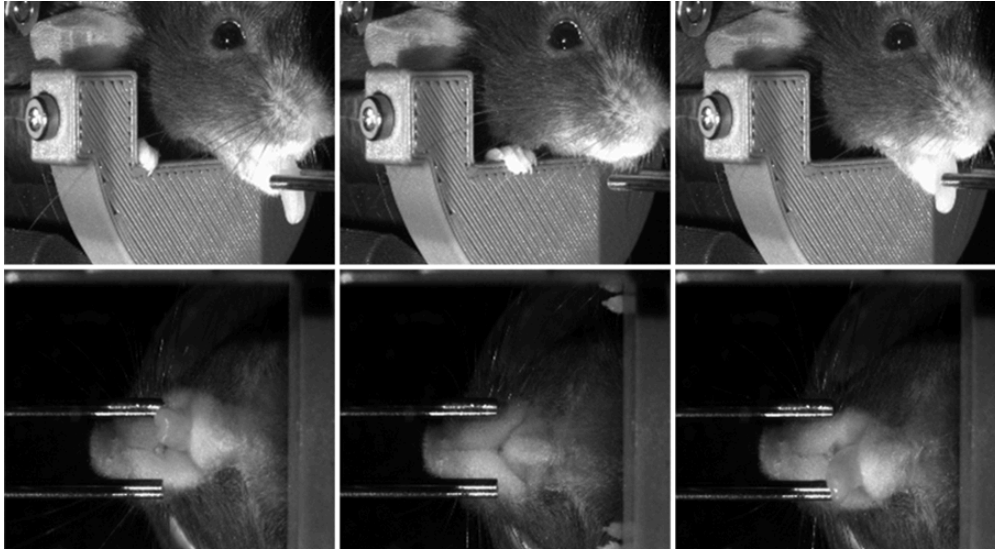


Figure 6: Example lickspout placement for an experienced mouse

Left: left licks

Middle: resting position

Right: right licks

Start the Behavior Session

- 16 Ensure that all training parameters are correct for the particular training stage that the mouse is on (see **Figure 8**). Training parameters should follow the below examples for each stage (Uncoupled Baiting Task, version 2.3).

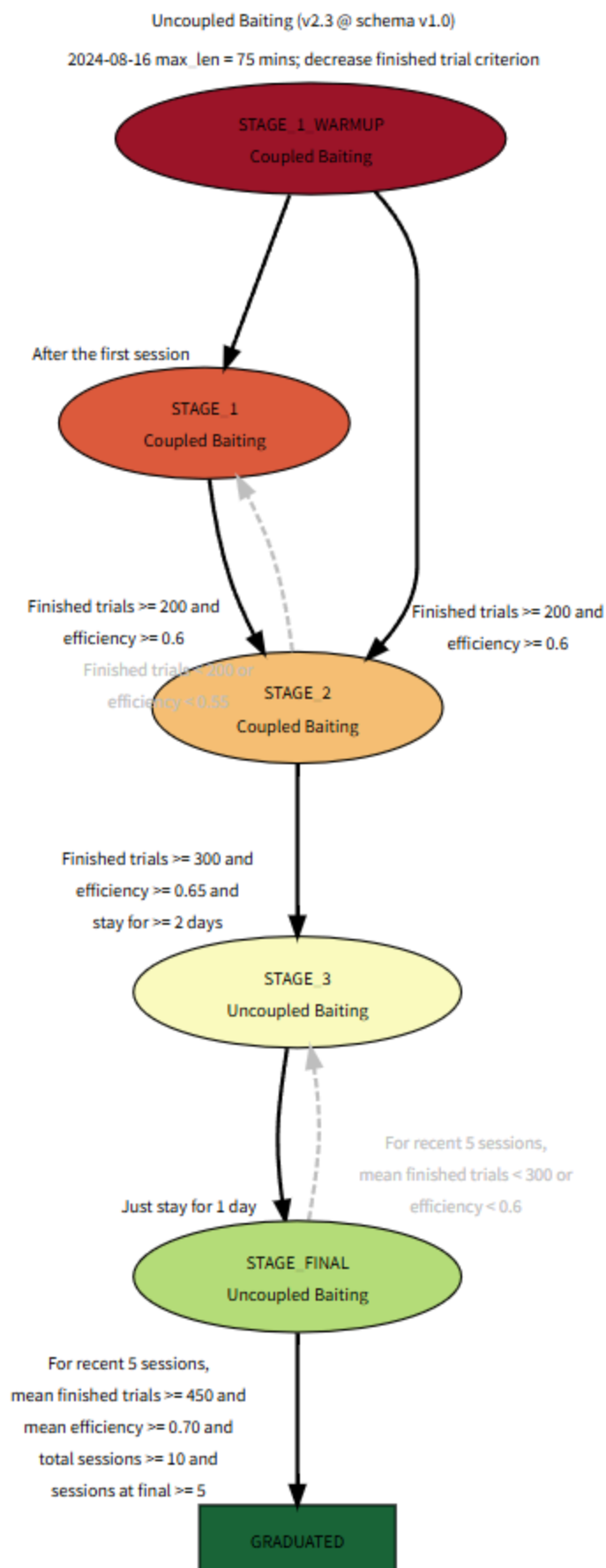




Figure 7: Curriculum stage advancement criteria for the Uncoupled Baiting Task version 2.3



	STAGE_1_WARMUP	STAGE_1	STAGE_2	STAGE_3	STAGE_FINAL	GRADUATED
BaseRewardSum	0.8	0.8	0.8	0.8	0.8	0.8
RewardFamily	3	3	1	1	1	1
RewardPairsN	1	1	1	1	1	1
UncoupledReward				0.1, 0.4, 0.7	0.1, 0.4, 0.7	0.1, 0.4, 0.7
Randomness	Exponential	Exponential	Exponential	Exponential	Exponential	Exponential
BlockMin	10	10	20	20	20	20
BlockMax	30	30	35	35	35	35
BlockBeta	10	10	10	10	10	10
BlockMinReward	0	0	0	0	0	0
DelayMin	0.1	0.1	0.3	0.5	1.0	1.0
DelayMax	0.1	0.1	0.3	0.5	1.0	1.0
DelayBeta	0.0	0.0	0.0	0.0	0.0	0.0
RewardDelay	0.0	0.0	0.0	0.0	0.0	0.0
AutoReward	True	True	True	True	False	False
AutoWaterType	Natural	Natural	Natural	Natural	Natural	Natural
Multiplier	0.5	0.5	0.5	0.5	0.5	0.5
Unrewarded	3	5	7	10	10	10
Ignored	3	5	7	10	10	10
ITIMin	1.0	1.0	1.0	1.0	1.0	1.0
ITIMax	7.0	7.0	10.0	15.0	30.0	30.0
ITIBeta	3.0	3.0	3.0	3.0	3.0	3.0
ITIIncrease	0.0	0.0	0.0	0.0	0.0	0.0
ResponseTime	5.0	5.0	3.0	2.0	1.0	1.0
RewardConsumeTime	1.0	1.0	1.0	1.0	3.0	3.0
StopIgnores	20000	20000	25	25	25	25
AdvancedBlockAuto	now	now	now	off	off	off
SwitchThr	0.5	0.5	0.5	0.5	0.5	0.5
PointsInARow	5	5	5	5	5	5
MaxTrial	1000	1000	1000	1000	1000	1000
MaxTime	75	75	75	75	75	75
RightValue_volume	4.0	2.0	2.0	2.0	2.0	2.0
LeftValue_volume	4.0	2.0	2.0	2.0	2.0	2.0
warmup	on	off	off	off	off	off
warm_min_trial	50	50	50	50	50	50
warm_max_choice_ratio_bias	0.1	0.1	0.1	0.1	0.1	0.1
warm_min_finish_ratio	0.8	0.8	0.8	0.8	0.8	0.8
warm_windowsize	20	20	20	20	20	20

Figure 8: Parameter changes across stages for the Uncoupled Baiting Task version 2.3

Note

As training continues, parameters become increasingly difficult as the mouse learns the task. If using the Dynamic Foraging GUI setup, mouse performance is evaluated at the end of each day, and the results saved in the Autotrain database (df_manager) for the rigs to query on the next day. If the criteria for advancement in **Figure 7** are met, parameters will automatically change the following training session, as outlined in **Figure 8**.

Refer to the [Training Parameters](#) section of the GitHub repository for parameter definitions. For technical details of defining and deploying the curriculum system, see this [GitHub repo](#). In particular, the script outlining advancement criteria [here](#), and the code driving advancement of mice through training stages [here](#) (evaluated in this [Code Ocean capsule](#)).

- 17 Ensure end-point criteria for the session are set such that the session will stop when training time reaches 75min or if the mouse ignores > 80% of the past 30 trials, whichever comes first (maximum time = 75min, auto stop ignore window = 30, auto stop ignore ratio threshold = 0.80).

Note

Experimenters can modify these criteria for their own purposes if needed.

- 18 Using the behavioral apparatus coupled with software control, start the session by pressing the Start button (see **Figure 1**: part 7).

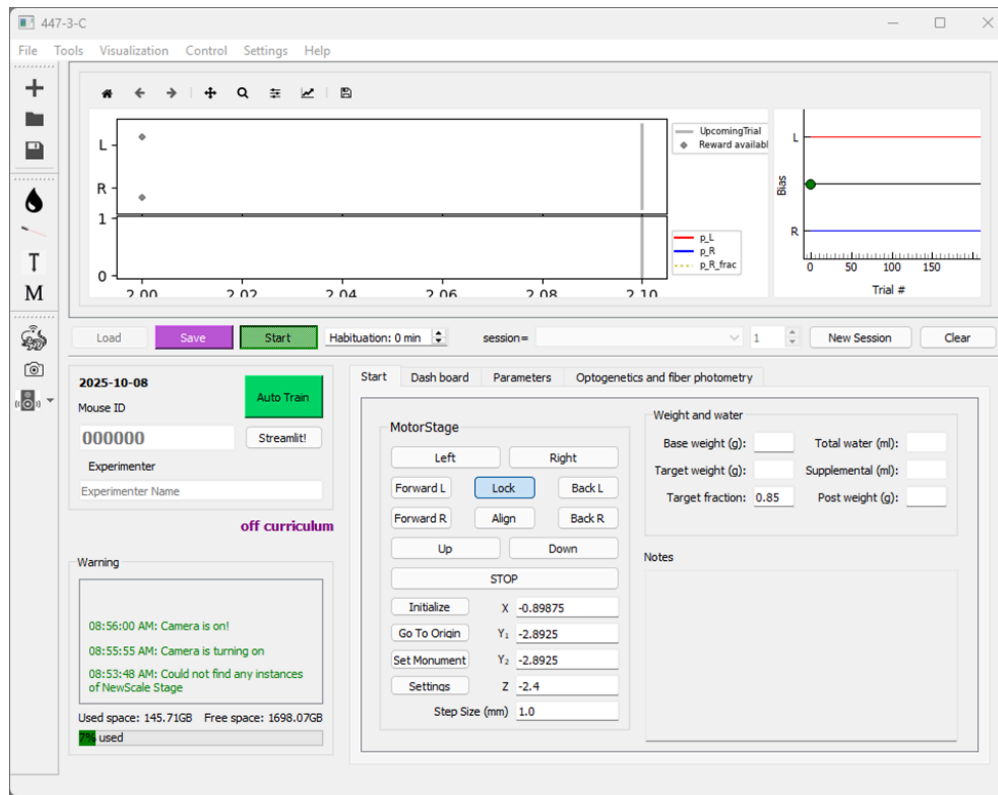


Figure 9: Dynamic Foraging GUI after starting the behavior session

Monitor Behavior and Hardware Performance

- 19 Aurally verify that the GoCue is being delivered throughout the session.

Note

If the experimenter suspects that the GoCue is not within the correct range, it should be measured using a decibel meter to ensure that it is within the range of 73+/- 2 dB at 7.5 kHz.

- 20 Visually verify that all mouse licks are being registered by ensuring that lines appear in the LickVisualizer window of the GUI at the same time that the mouse's tongue touches the lickspout.

Note

If mouse licks are not being registered, the lick detection board must be tuned such that each mouse lick registers.

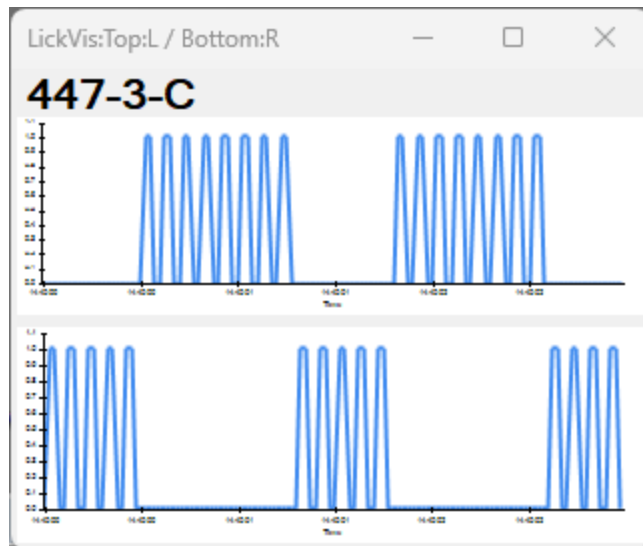


Figure 10: Dynamic Foraging GUI LickVisualizer window

- 21 Visually verify that water is being delivered to each lickspout during the session, by ensuring that a drop of water can be seen at the tip of the lickspout after reward delivery.
- 22 Throughout the session, monitor the mouse's behavioral performance through the behavioral visualization in the GUI. Depending on bias, engagement, and licking ability, the experimenter may need to intervene to help shape the mouse's behavior and aid their learning of the task. Such manipulations can include lickspout adjustment and manual water delivery.

Note

The experimenter should monitor for bias via the bias graph (see Figure 11). If the bias becomes severe (> 0.7), the user should deliver manual water to the unchosen lickspout when the reward probability on that side is high. If the mouse does not respond to manual water, the user should move the lickspout towards the biased side by 200 μ m, which will make it harder for the mouse to reach, allowing the mouse easier access to the lickspout that was previously disfavored.

The user should continue with this pattern until bias improves. Once the bias value reaches 0.3, the user should begin to move the lickspout back towards center in increments of 200 μ m.

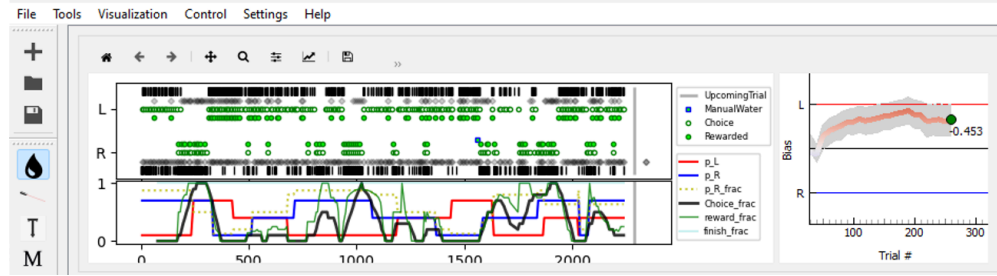


Figure 11: Behavior and bias graph during dynamic foraging behavior

- 23 Ensure throughout the session that the mouse is not displaying signs of distress such as vocalization or eye foam. If the mouse displays signs of stress, end the session.

End the Behavior Session & Takedown

- 24 When the mouse has completed the behavior session, stop the session through the GUI by unclicking the Start button (see **Figure 1:** part 7). Refer to pre-determined endpoint criteria ([go to step #17](#)) to evaluate when to end a session.

The end of a session can be defined by a pre-determined trial number or session length, a loss of engagement by the mouse, or other endpoint criteria being met.

Note

Mice in early training (<7 days) often stop responding after 30 min in the box. If mouse engagement is low (finish rate < 30%), it is best to remove the mouse from the box and resume training the following day.

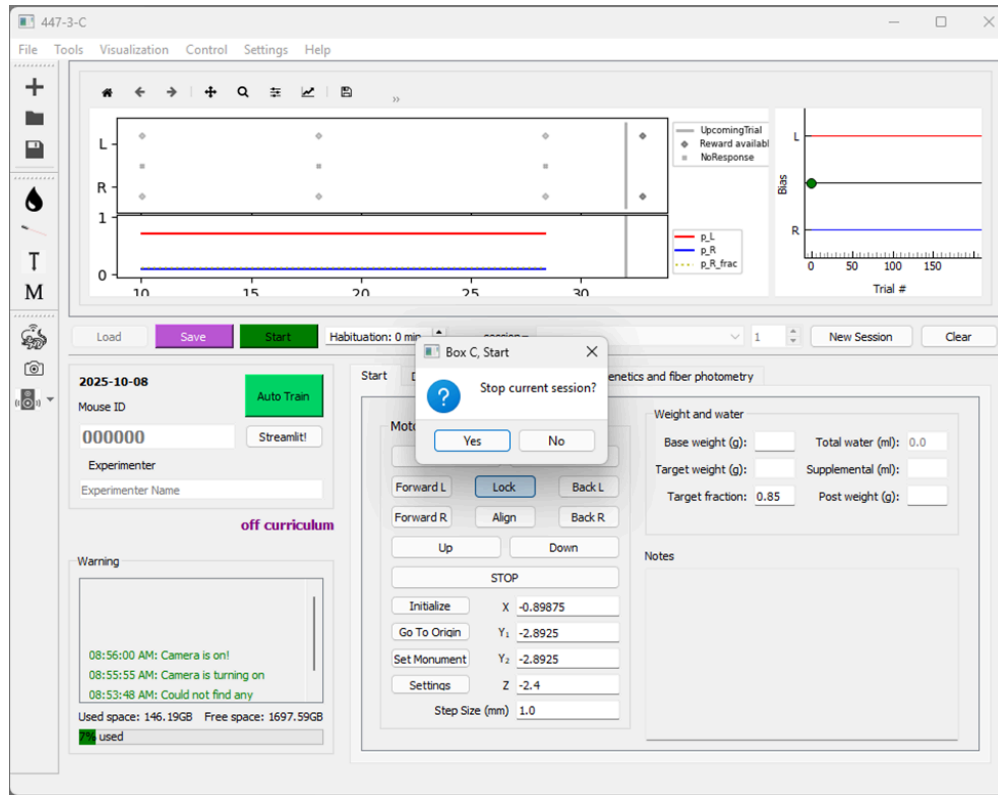


Figure 12: Dynamic Foraging GUI as the user stops the behavior session

- 25 Remove the alligator clip from the mouse tube (**Figure 5:** part 5), disengage the magnet securing the mouse within the box (**Figure 5:** part 10), and remove the mouse tube from the behavior apparatus.
- 26 Carefully release the mouse from the head-fixed position by releasing the headfixing screws (**Figure 5:** part 4) with a 1/16" hex driver, and allow the mouse to crawl from the tube into your hand.
- 27 Weigh the mouse, record the weight, and return the mouse to its home cage.

Calculate and Deliver Supplemental Water

- 28 Calculate supplemental water.
- 28.1 Using the following equation, calculate the amount of supplemental water to deliver to the mouse. If using the Dynamic Foraging GUI, total and supplemental water can be calculated using the GUI (see **Figure 1:** part 8).



Target Weight - Post Session Weight = Supplemental Water

(Target Weight = 85% of Baseline Weight)

(Baseline Weight = average of 3 weights taken the 3 days before the animal is water restricted)

Note

In this protocol, mice must receive a minimum of 1 mL of water per day, regardless of their body weight.

Example - Baseline Weight Calculation:

Day 1 weight: 25.43 g

Day 2 weight: 25.87 g

Day 3 weight: 25.64 g

Baseline weight = (25.43 g + 25.87 g + 25.64 g)/3 = 25.65 g

Example - Target Weight Calculation:

Baseline weight = 25.65 g

Water restriction at 85% of baseline weight

$25.65 \text{ g} \times 0.85 = 21.8 \text{ g}$

Maintain mouse at target weight of 21.8 g

Example - Supplemental Water Calculation:

Post Session weight = 20.5 g

Target weight = 21.8 g

$21.8 \text{ g} - 20.5 \text{ g} = 1.3 \text{ g} = 1.3 \text{ mL water}$

- 29 Using the value calculated above (or the value recommended by your institutional veterinary staff) record the date, time, total volume of water ( 1.3 mL in the example above), and experimenter initials on the water restriction card, and deliver supplemental water in the mouse's water bowl (**Materials - Hardware:** Figure 2) with a  1 mL syringe.
- 30 Save all behavior data through the GUI (see **Figure 1:** part 9).

Rig Cleanup and Maintenance

- 31 Remove all excrement from the box and sanitize equipment with 70% EtOH after each session or mouse that is trained, including:
- Behavior box
 - Mouse tube



- Lickspouts
- Weigh station

31.1 Once per week, perform spot checks and water calibration as described in the protocol below.

Protocol



NAME

Solenoid Valve Calibration for Behavior Rigs Utilizing Water Reward

CREATED BY

Ella Hilton-VanOsdall

Preview

Protocol references

Grossman, C. D., Bari, B. A., & Cohen, J. Y. (2022). Serotonin neurons modulate learning rate through uncertainty. *Current Biology*, 32(3), 586–599.e7. <https://doi.org/10.1016/j.cub.2021.12.006>

Bari, B. A., Grossman, C. D., Lubin, E. E., Rajagopalan, A. E., Cressy, J. I., & Cohen, J. Y. (2019). Stable Representations of Decision Variables for Flexible Behavior. *Neuron*, 103(5), 922–933.e7. <https://doi.org/10.1016/j.neuron.2019.06.001>