Building Instruction for head-fixed stage:

1. Attach four 3-inch-thick posts onto the right end of an optical breadboard with ¼”-20 x ¾” setscrews to create a 6-inch by 4-inch rectangle.
2. Place aluminum head-fixed stage (CAD file available) on the four posts and stabilize with four 8/32 x ¾” cap screws.

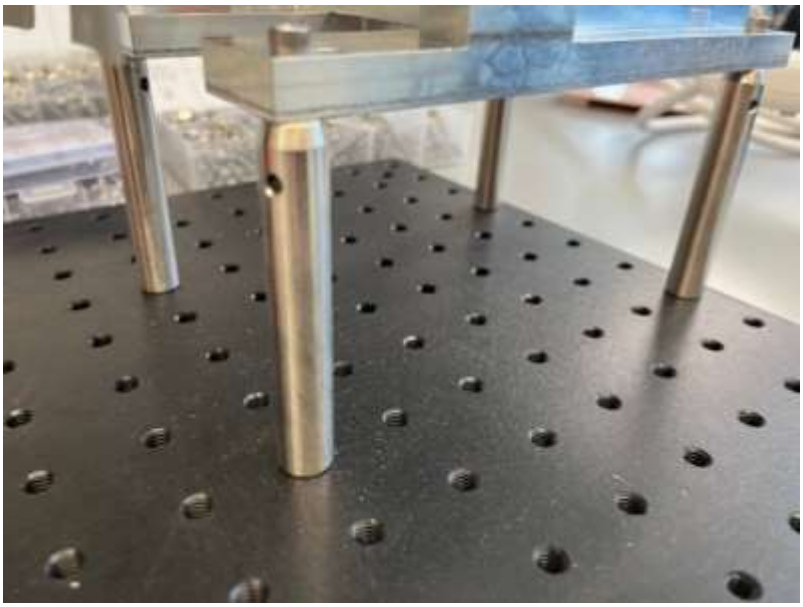


Figure 1 Head-fixed stage supported by four posts on breadboard

3. Place head ring holder bottom (CAD file available) onto the head stage and stabilize with two M3*12 cap screws. Then, stabilize the head ring holder top (CAD file available) with two M3*8 cap screws. Finished look below.

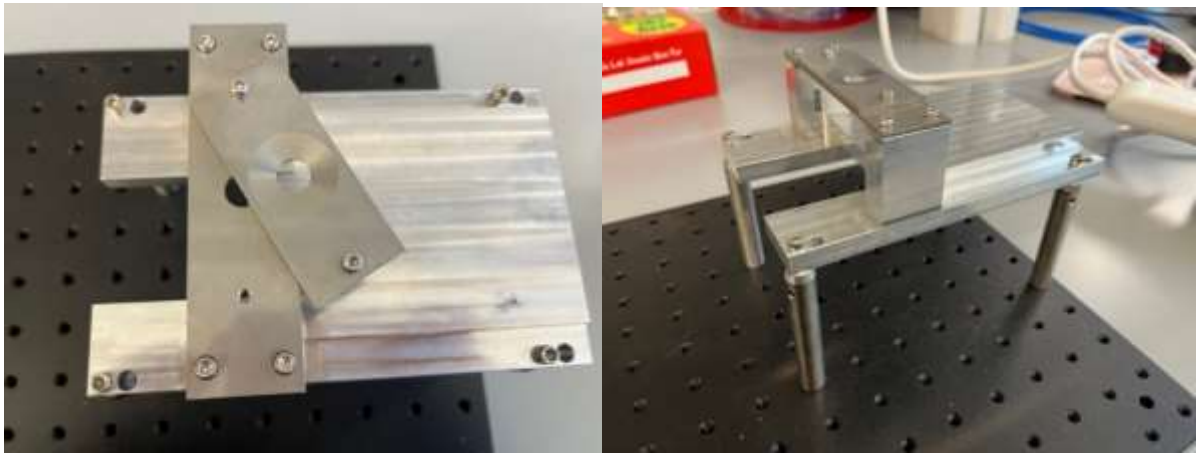


Figure 2 Head ring holder top and bottom setup on the stage

4. Place a clamping fork on the left side of the stage and clamp a pedestal base inside for holding the translation stages.

Figure 3 Translation stage base

5. Connect two translation stages together with a 8-32 x 5/8” setscrew to make the X and Y stages.
6. Use two 8-32 x 3/8” cap screws to connect the Y and Z stages with an angle bracket in between (see photos attached).

Figure 4 Translation stages assemble

7. Place a 1-inch post holder onto the pedestal base and a 1.5-inch post inside of the tube holder. Stabilize the post holder and post.
8. Attach the XZY stages onto the 1.5-inch post with an 8-32 x 5/8” setscrew. The correct orientation of the XYZ stages is shown below. Make sure to stabilize the system.

Figure 5 Translation stages placement

9. Connect two 1-inch-thick posts together with a ¼”-20 x ¾” setscrew, and connect one end of this combined 2-inch post to the Z stage with a 8-32 x ½” setscrew.
10. Connect the other end of the combined 2-inch post with a thin 1-inch post with a 8-32 to 4-40 inch adapter.

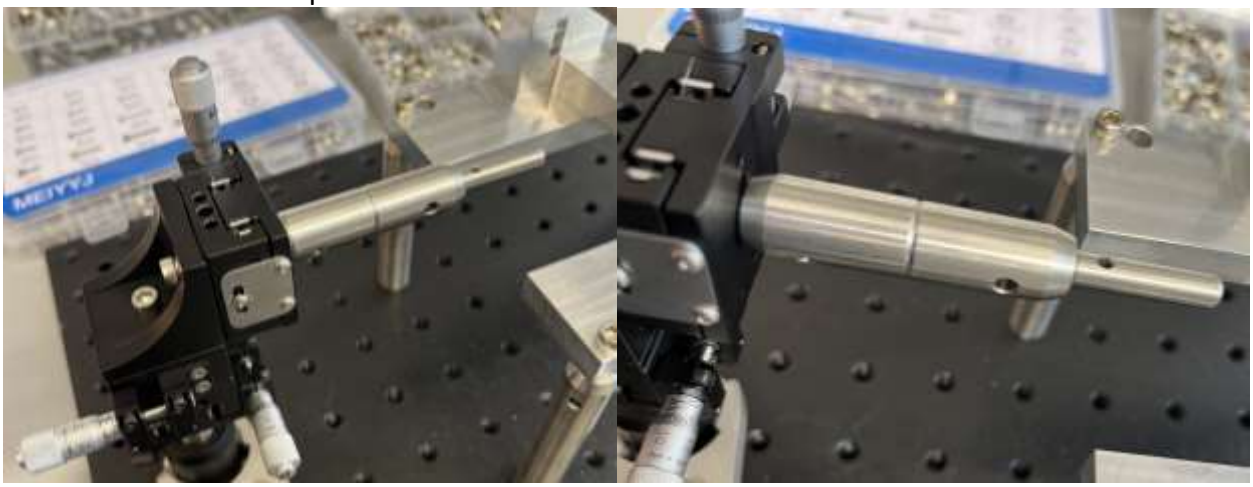


Figure 6 Tube extension for holding lick tube holders

11. Place the 3D printed linear retraction solenoid wing holder onto the combined 2-inch posts.
12. Place the right-angle post clamp onto the 1-inch thin post to hold the other lick tube holder.
13. Place the lick tube holder inside of the right-angle post clamp. Finished look below.

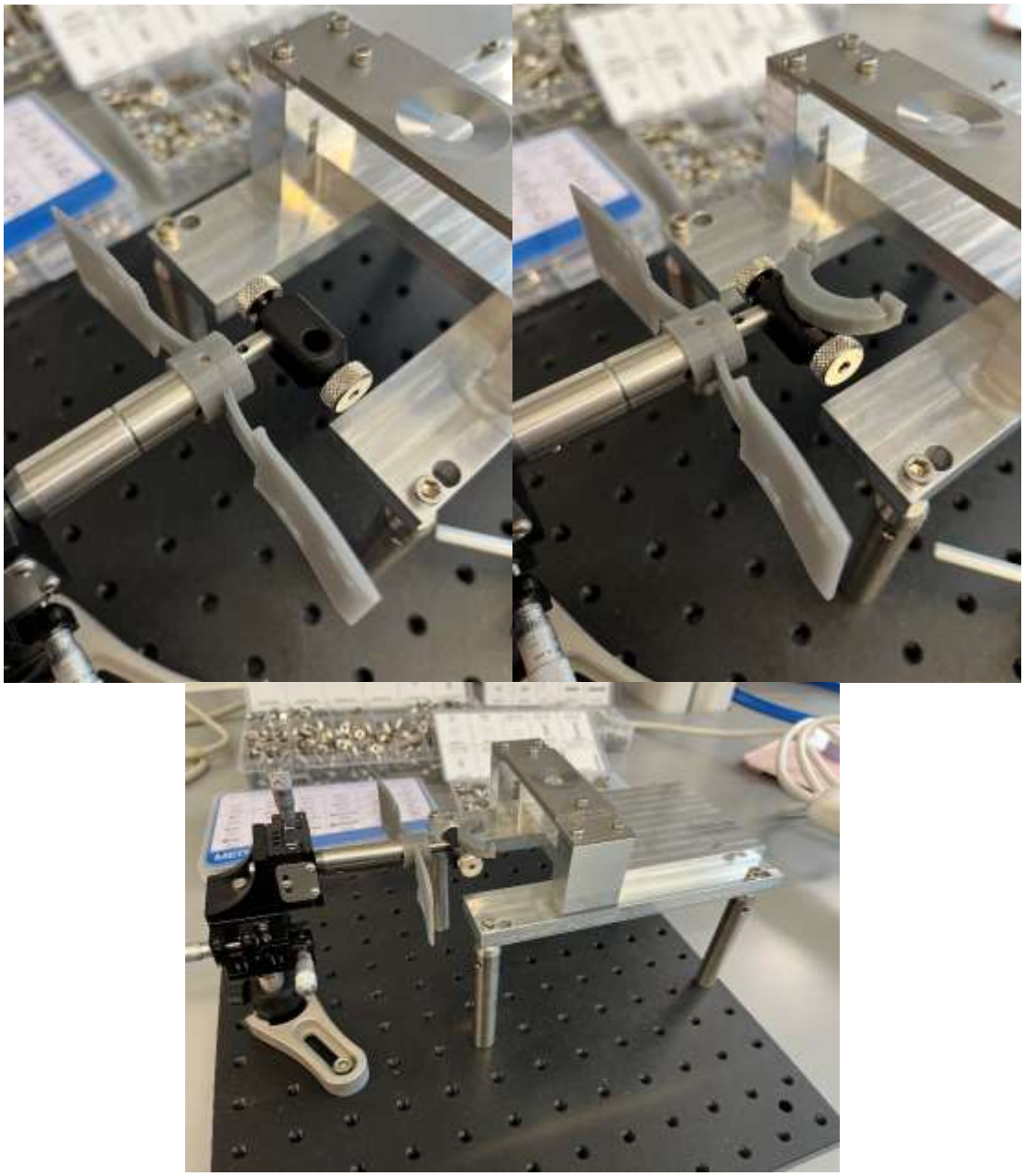


Figure 7 Lick tube holders placement

14. Attach two linear actuator lick retraction solenoids on the wing holder with two M2.5*6 cap screws and nuts.
15. Use an 18-guage cannula to solder four 4-to-5-inch cannulas as lick tubes. Fit two of these lick tubes into the linear actuator solenoid attachment piece's small holes (see below).

Figure 8 Soldered lick tubes and placement in the linear retraction solenoid attachment piece

16. Attach the two attachment pieces onto the retraction solenoids. Make sure both lick tubes fit in the side opening slots on the middle tube holder.

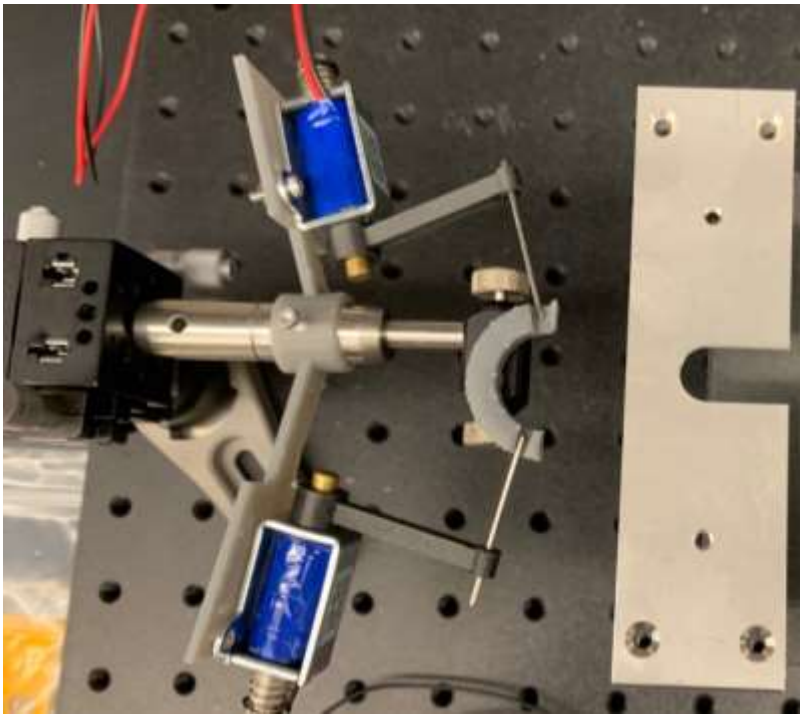


Figure 9 Adding lick retraction solenoids and lick tubes via 3D printed attachments

17. Connect the third soldered lick tube to one end of a 4-feet long 1/16" ID x 1/8" OD Ester tubing with a 1/8" x 1/16" coupler adapter.

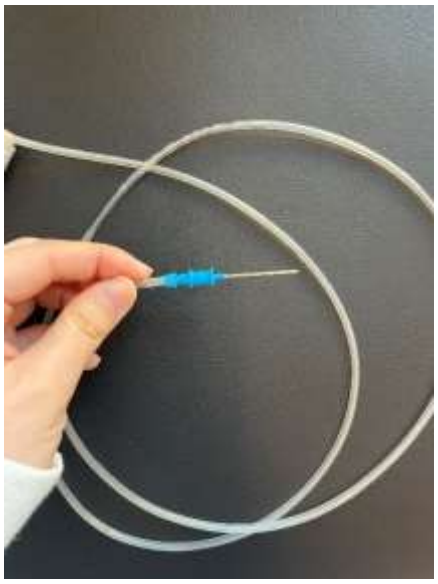


Figure 10 Connect middle lick tube with reward delivery tubing

18. Connect the other end of the tubing to an opening of the Parker solenoid. Next, connect the other opening of the solenoid to a half ft long 1/16" ID x 1/8" OD Ester tubing. Place another 1/8" x 1/16" coupler adapter at the end of that tubing.

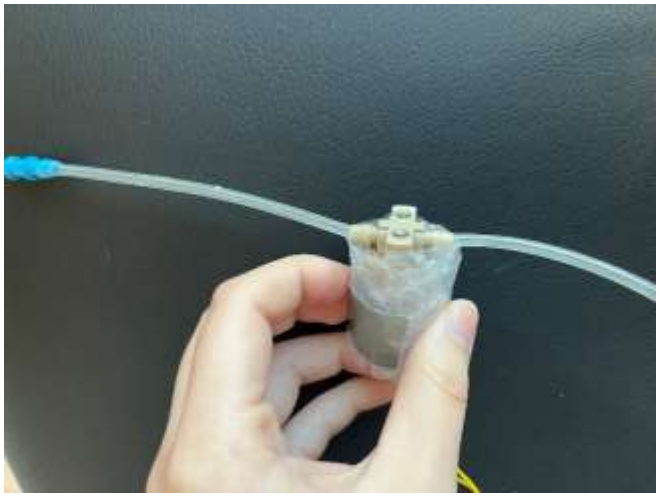


Figure 11 Connect reward delivery tubing onto both sides of the solenoid

19. Connect the other end of the 1/8" x 1/16" coupler adapter to a 10ml syringe with an 18-gauge blunt needle. Attach the solenoid and syringe system on the outside of the behavior box with hook and loop tapes. Fluid reward will be added to the syringe during experiments.

Figure 12 Reward delivery system view from outside of the behavioral box

20. Place the middle lick tube into the middle hole of the middle lick tube holder. In our lab, we place two lick tubes for stability purposes. At the moment, only one lick tube is connected to the fluid delivery system, but both lick tubes can be connected to different solenoids if needed.

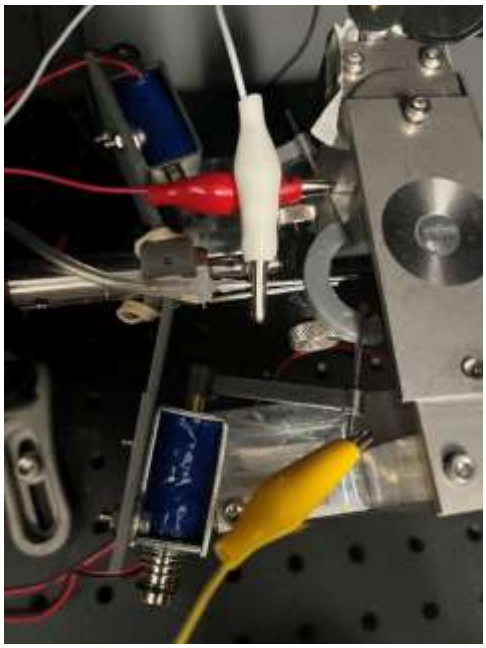


Figure 13 Lick tube placement complete view

21. Using alligator clips, connect lick detectors with lick tube to desired pins on the plastic box terminal blocks (exterior side). Place all hardware components to desired locations

around the head-fixed stage. For example, our lab taped two speakers on the side of the post next to the head-fixed holders.

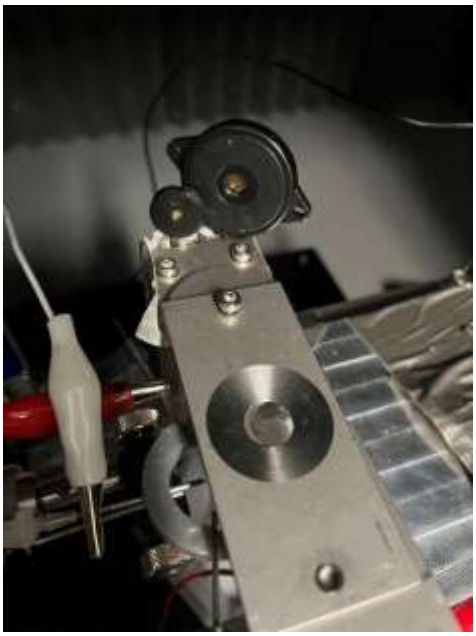


Figure 14 Example of speakers placement on the head-fixed stage

22. Place the head-fixed stage inside of the aluminum behavioral box with the complete circuit board and white noise machine.
23. Use adhesive spray to attach the acoustic foams on the walls inside of the box for sound-proofing purposes.

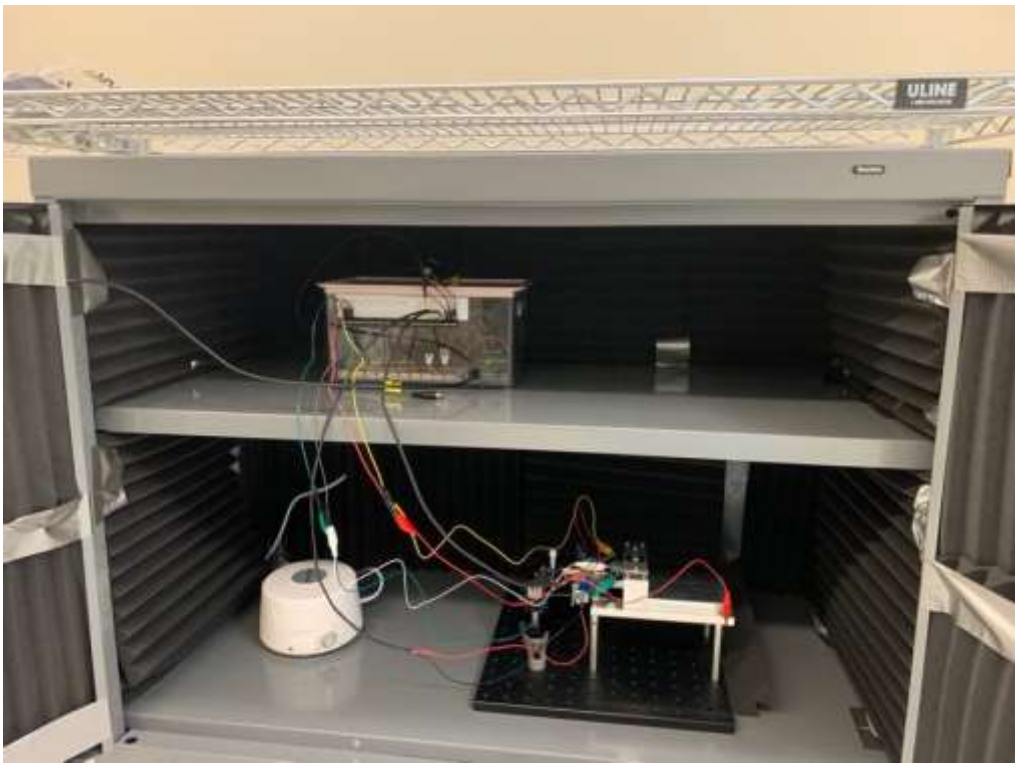
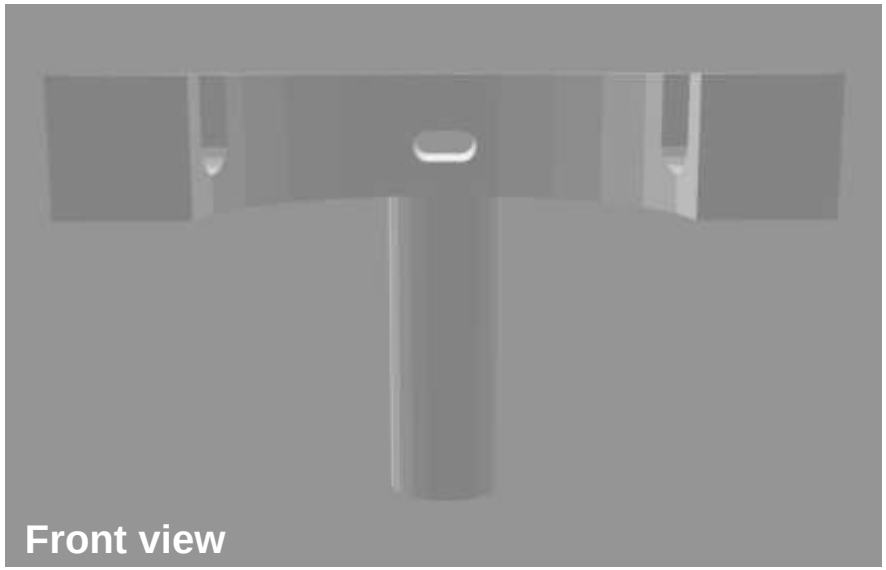


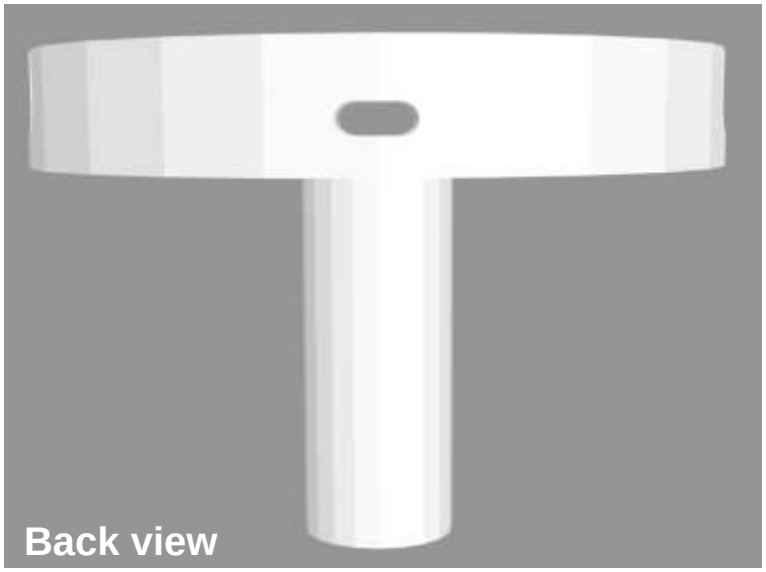
Figure 15 Final look of behavioral box setup

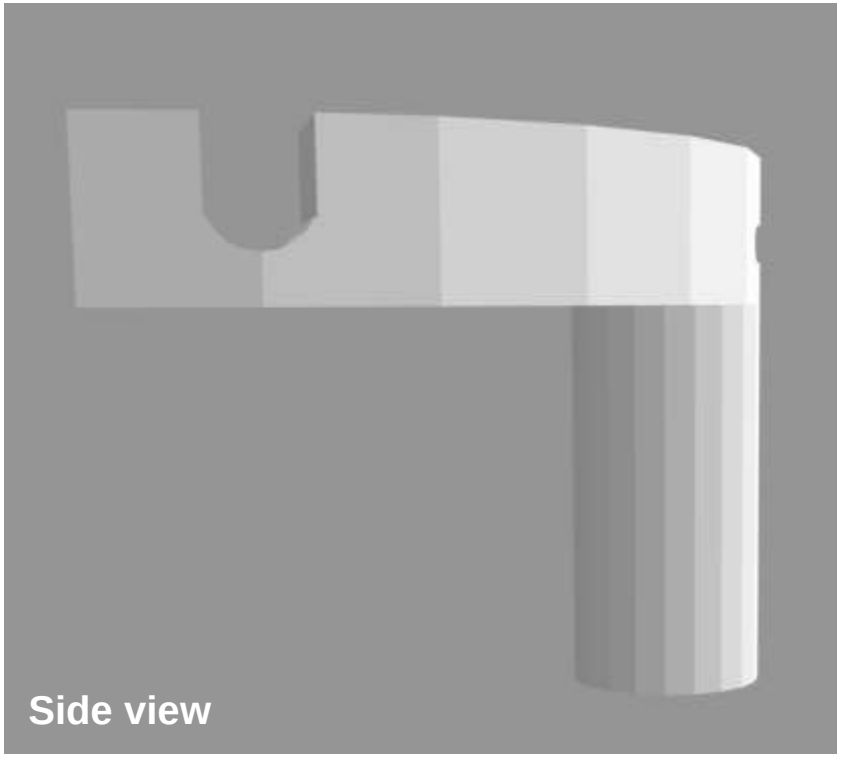
Appendix

A. 3D printing files overview

Middle lick tube holder

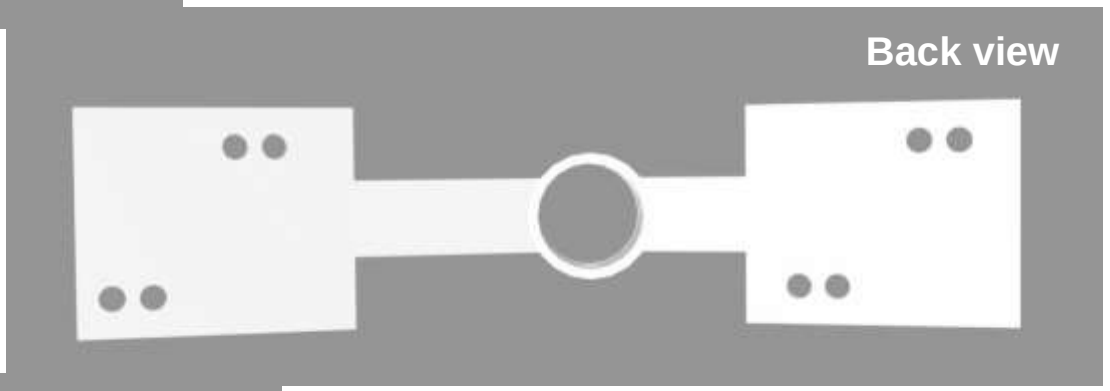


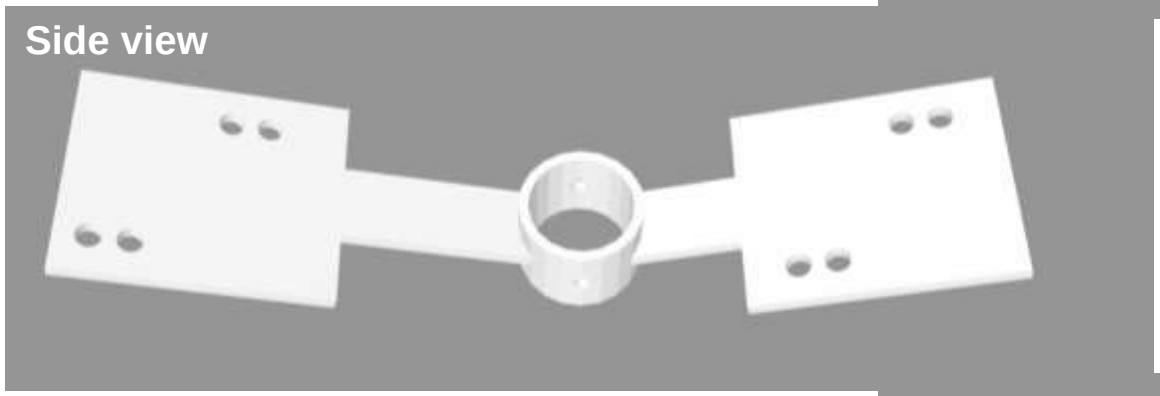


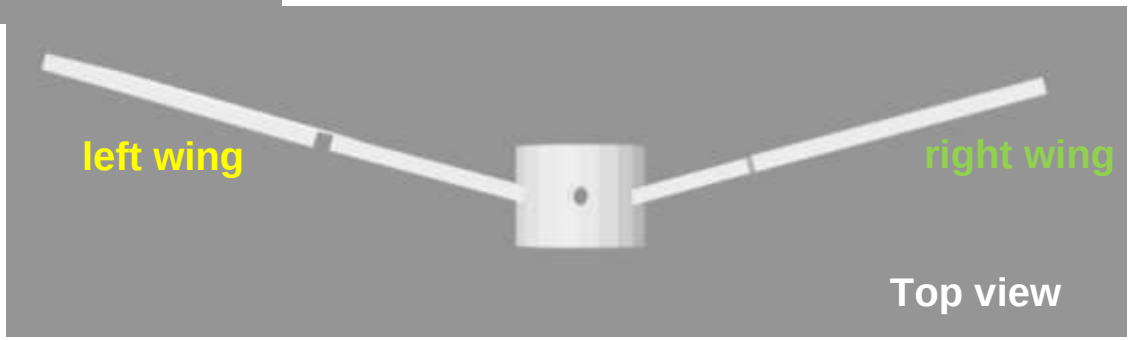


Side lick tube holder

Linear actuator solenoid wing holder







B. Lick tube position placement instructions

Instructions of setting up lick tube positions & training animals to lick prior to experiments

1. Before each task starts, we train animals sitting in head-fixed stages to lick for random sucrose rewards.
    a. On the first day, animals are not familiar with the position or existence of the lick tube so we initially position the lick tube just a little underneath and close to the mouse's mouth to let the animal know that there is a lick tube delivering sucrose drops (stage 1).
    b. As the animal starts actively licking, we slowly retract the lick tube position away from the animal's mouth to allow tongue extension to consume the sucrose at a more comfortable position (**stage 2**). The front half of the animal's tongue should reach the lick tube. This process is typically carried out over 5-10 drops of sucrose delivery.

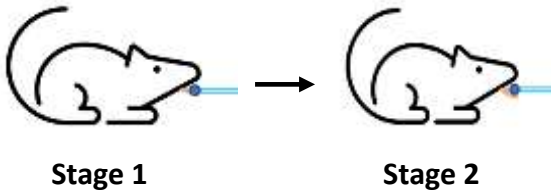


Figure 16 Mouse lick position transition

    c. We then train animals on 100 random rewards trials for about 2-3 days depending on the animals' licking. We check for the animals to reach at minimum 1000 licks over the random rewards session (20 minutes) to demonstrate that this animal is motivated enough, knows lick tube position, and can be moved to the actual experiment.
2. For the cue-action-reward task, it's important to position the side lick tube at an appropriate distance for the animal to perform an action and then consume rewards in the middle lick tube. This is to ensure that the animal isn't accidentally licking both tubes at the same time. We train the animals to lick the side lick tube starting at position 1 for 3 days and move to position 2, etc. (see below). If animals fail to lick on advanced positions, we move back to the previous position for longer training.
3. For the operant task and choice task, we conduct the random rewards sessions first and then place the side lick tubes at around position 2-3 for animals to lick.

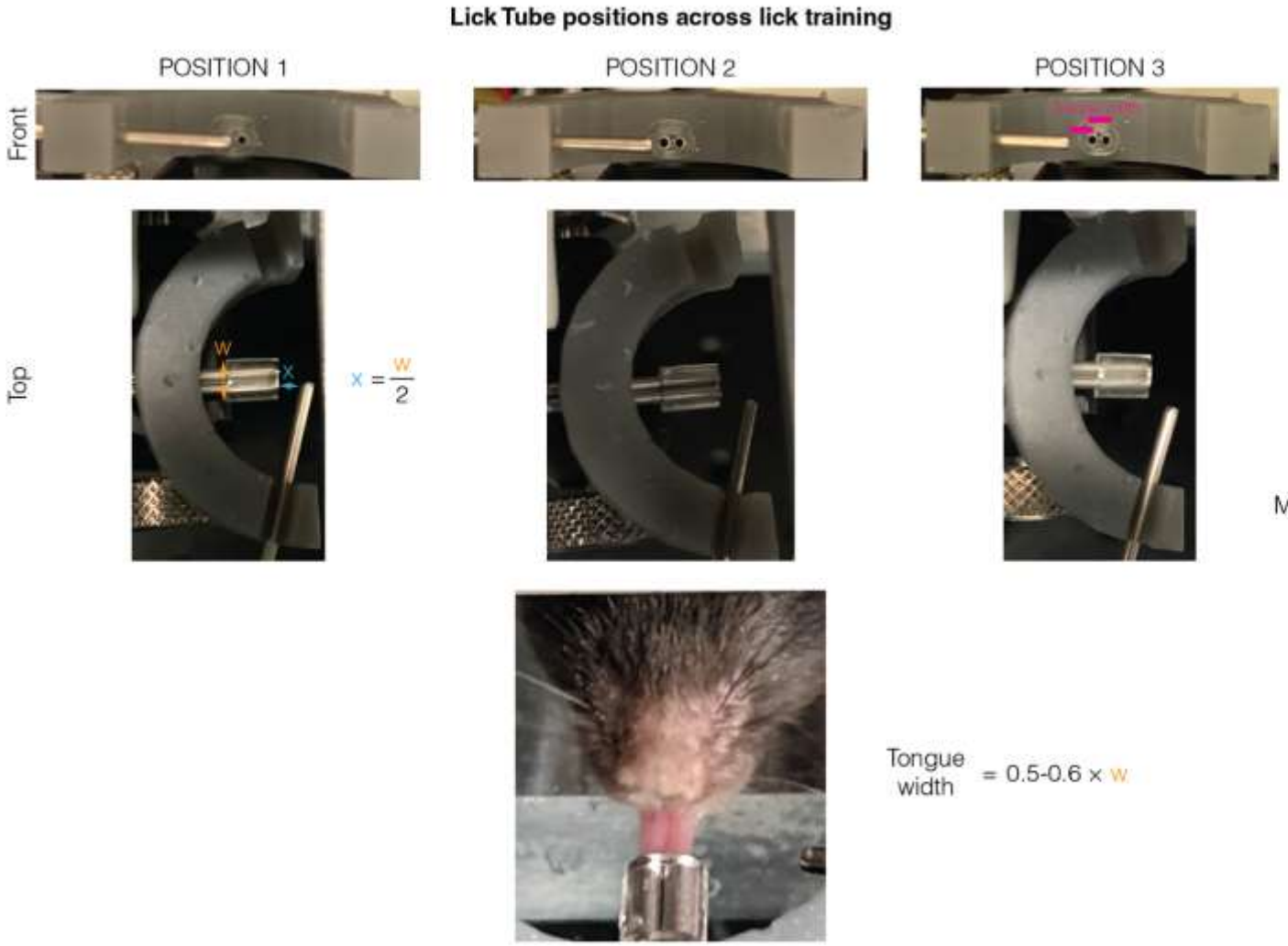


Figure 17 Lick tube positions across training (both lick tubes in the middle are used)

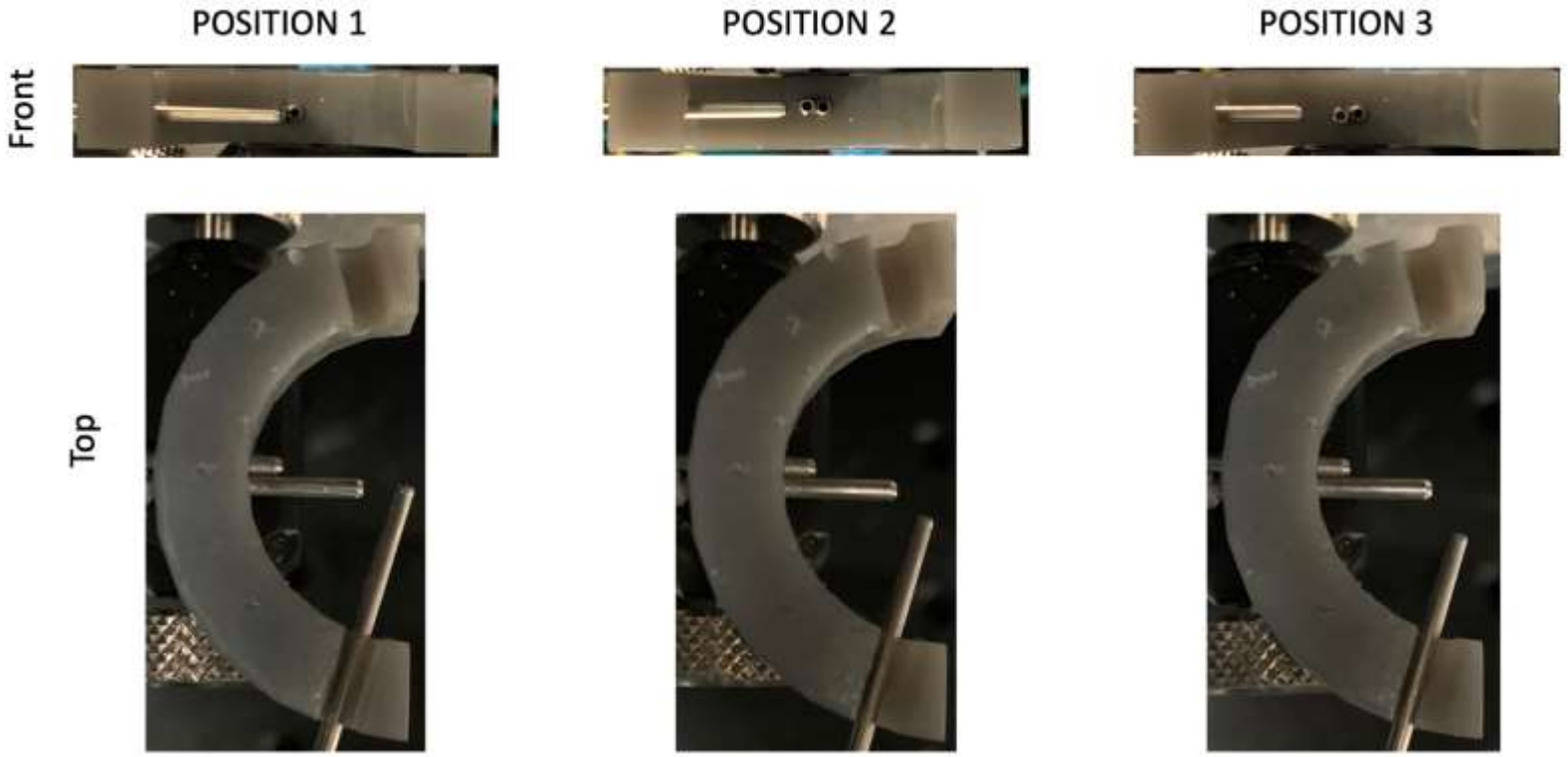


Figure 18 Lick tube positions across training (only one lick tube in the middle is used)

C. Head-fixed stage parts purchase information

	Item Name	Function	Vendor/ manufacturer part #	Quantity per system	Unit Cost ($)	Link
Head-fixed stage setup	Optical breadboard	To place all the head-fixed set up	Thorlabs MB12	1	155.53	https://www.thorlabs.com/thorproduct.cfm?partnumber=MB12
	3 inch thick post	Hold heat-fixed stage up	Thorlabs TR3-P5	4	5.02	https://www.thorlabs.com/thorproduct.cfm?partnumber=TR3-P5
	Clamping fork	Clamp the post onto breadboard	Thorlabs CF125C-P5	1	11.13 (5 pack)	https://www.thorlabs.com/thorproduct.cfm?partnumber=CF125C-P5
	1.5 inch thick post	Hold translation stages	Thorlabs TR1.5-P5	1	4.6	https://www.thorlabs.com/thorproduct.cfm?partnumber=TR1.5-P5
	1 inch post holder	To hold the 1.5 in post	Thorlabs PH1-P5	1	7.24	https://www.thorlabs.com/thorproduct.cfm?partnumber=PH1-P5#ad-image-0
	Pedestal base	Base for the above post and post holder	Thorlabs BE1	1	10.06	https://www.thorlabs.com/thorproduct.cfm?partnumber=BE1#ad-image-0
	Translation stage	Linear stages for adjusting licktube positions	Thorlabs MS1S	3	227.25	https://www.thorlabs.com/thorproduct.cfm?partnumber=MS1S
	Angle bracket for MS translation stages	To make XZY translation stage mounts	Thorlabs MS102	1	33.83	https://www.thorlabs.com/thorproduct.cfm?partnumber=MS102
	1 inch thick post	To extend and hold licktube holders	Thorlabs TR1-P5	2	4.4	https://www.thorlabs.com/thorproduct.cfm?partnumber=TR1-P5
	8-32 to 4-40 adapter	To hold the above two 1 inch thick posts together	Thorlabs AP8E4E	1	2.1	https://www.thorlabs.com/thorproduct.cfm?partnumber=AP8E4E#ad-image-0
	1 inch thin post	To hold licktube holders	Thorlabs MS1R	1	6.77	https://www.thorlabs.com/thorproduct.cfm?partnumber=MS1R
	Right angel post clamp	To hold lick tube holder onto the post	Thorlabs MSRA90	1	17.85	https://www.thorlabs.com/thorproduct.cfm?partnumber=MSRA90
	8-32 Cap Screw and Hardware Kit	Used for connecting 3 inch posts to the head-fixed base and connecting translational parts with angle bracket	Thorlabs HW-KIT1	1	61.14	https://www.thorlabs.com/thorproduct.cfm?partnumber=HW-KIT1
	1/4“ -20 Cap Screw and Hardware kit	To hold posts to optical breadboard and other parts	Thorlabs HW-KIT2	1	121.1	https://www.thorlabs.com/thorproduct.cfm?partnumber=HW-KIT2
	8-32 Setscrew and	To hold translational stages together and other parts	Thorlabs HW-KIT3	1	121.2	https://www.thorlabs.com/thorproduct.cfm?partnumber=HW-KIT3

	Hardware Kit					
	M2-M4 head cap screws set	To hold head ring stable between top and bottom ring attachment piece	MEIYYJ 0759337318592	1	29.99	https://www.amazon.com/MEIYYJ-Stainless-Machine-Assortment-Tweezers/dp/B08FT933MM/ref=sr_1_12?dchild=1&keywords=m2+m3+m4+cap+screws&qid=1608684312&s=hi&sr=1-12
Fluid delivery system	1/16" ID x 1/8" OD x 1/32" Wall Superthane ® Ester-Based Tubing	Delivers fluids	US Plastic 56400	4 ft	0.16/ft	https://www.usplastic.com/catalog/item.aspx?catid=717&itemid=66285
	1/8" x 1/16" Eldon James™ Anti microbial HDPE Reducing Coupler	Connects the above tubing to 18G needle and lick tube	US Plastic 65611	2 per lick spout / fluid delivery	0.59	https://www.usplastic.com/catalog/item.aspx?itemid=114542
	10 ml Syringe	Contains fluid for delivery	Fisher Scientific 14-955-459	1	2.05	https://www.fishersci.com/shop/products/sterile-syringes-single-use-12/14955459
	Blunt tip 18-gauge needle	Connects syringe to tubing for liquid delivery	BD 305181	1	0.15	https://www.esutures.com/product/0-in-date/119-bd/667-needles/46340600-bd-blunt-fill-needle-18g-x-1-305181/?gclid=CjwKCAiA57D_BRAZEiwAZcfCxfhDyO5ifpblReX979qkez9b-uBHgSXSA6Lhb40ODROrGEr5bz7d7BoCxioQAvD_BwE
	18-gauge cannula	Soldered as lick tubes	Component supply HTX-18R	8 in	0.2/ in	https://componentsupplycompany.com/product-pages/hypodermic-metric.php
Behavioral box setup	Behavioral box	Box to store head-fixed setup	Global industrial WGB2236106	1	313.95	https://www.globalindustrial.com/p/standard-kd-desk-height-storage-cabinet-36-w-x-18-d-x-30-h-medium-grey?PicGroupKey=75074
	White noise generator	To produce white noise inside of behavioral box	Marpac M1DSUSWH	1	44.99	https://www.amazon.com/Marpac-Classic-White-Noise-Machine/dp/B00HD0ELFK?th=1
	Acoustic foam	Sound-proof and absorption acoustic panels for behavioral ox walls	Acoustic Panels B091CD4KYB	1 pack	17.99	https://www.amazon.com/gp/product/B091CD4KYB/ref=ppx_yo_dt_b_asin_title_o00_s00?ie=UTF8&th=1
	Adhesive spray	To attach acoustic foams on box walls	The Gorilla Glue Company 6314410	1	18	https://www.amazon.com/gp/product/B07MY28DYX/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&th=1

Figure 19 Screwset reference