Instructions for 9/21/2024:

**Checking the BAT and preparing solutions.**

1. Check the BAT rig to make sure it’s clean and that there are clean bottles for you to use.
   1. If the BAT rig is dirty spray some ethanol on the floor of the cage and wipe it down with a paper towel. Make sure the cage bottom, the grate part is resting precisely in between the little plastic holders on the bottle. If these are shifted such that it’s not evenly placed the rat can sometimes escape.
   2. Make sure the fan is off for TG63,64,65, and 66. It should only be on for ORX128.
   3. If there are no clean/empty bottles, you can take them all over to the sink in the vivarium and rinse them out with warm water and let them dry a little on some paper towels or wipe down the outside of them to make them dry.
2. While you’re in the BAT make sure you have the correct solutions for the day. For example: Today you will need 0.01% Butyl Acetate and 0.01% 2-Hexanone for TG63 & TG64’s day 6 of conditioning.
   1. If you see that there is not enough of each prepared solution for the two bottles or if the prepared solutions are mislabeled/not labeled at all, remake the solution in Main Lab.
   2. To remake the solution, take a new 50/100mL media container/bottle and fill it with 50mL of miliQ water. Then grab one of the pipettes from either of the surgery rooms and make sure it’s set to 5 microliters. Then take some tape and label each bottle. After that in the hood there should be all the odors. Carefully pipette 5 microliters of each solution into the water and close the bottles. Be careful to completely close all the odor solutions and bottles.

**Preparing the Rig.**

1. Since TG63 is an ortho animal to the left of the fume hood in a tray are all the ortho odor disks. Some might not be completely smooth on the end so you can rub them against some of the blackish sandpaper near the hotplate on the main lab bench/table behind you. You’ll only need 2 per animal per day for conditioning.
2. You can start with either animal but let’s say you start with TG63. Click the mouse on the computer so that it’s on then click on the Davis Collect Data icon with the mouse. Go to the top right of the panel and click test hardware and then click position 10. This should move the bottle rack/carriage to the left enough so that you can put the odor disks on.
3. Fill up the two bottles with water and charge/shake them so that there’s no air in the metal spout. Weigh this horizontally on the scale and them carry it gently and horizontally into the holes for the bottles in the rig.
4. Using the disk cut up a section of clean napkin and roll it up and fold it in half. This should fit neatly in the disk. Then using the plastic disposable transfer pipettes in the white box on the table near the door, douse the napkin with the Butyl Acetate odor.
5. Now put the disks onto the bottle spouts so that the disk fits (such that the napkin is facing the rack, and the holes are facing the rat) flush next to the white PTFE block of the tube rack. Since the tight fit varies you can carefully add some tape such that the disks still press flush against the bottle rack. This will ensure they don’t accidentally fall off.
6. For TG64 just put the 2-Hexanone solution into the bottles themselves, just remember to clean them out with warm water, and take off the odors disks from before and toss them in the trash.

**Running the rat**

1. Now exit the test hardware and go to select program and scroll to “stink\_con\_un3” this will be the same program for TG63 & TG64.
2. Bring the rat into the room and weigh her and write it on the back of the cage card. Then carefully put the rat into the rig and close the top such that the end facing you fits under the metal and the other side rests on the white plastic holders. Now attach the Velcro on the back to keep the lid shut.
3. Then you can click run and head out of them room and observe the rat from the VNC and check to make sure it doesn’t do anything strange.

**Saving the Data**

1. After the animal is done running a popup to save the data should appear click save and then click through the folders to get to the right folder. If these folders don’t exist, you can just save the data to the desktop, and I can look at it later. Or you can create the folder. Make sure you write down the bottle weights after as well.

TG63

├─ Isaac/

│ ├─ Ortho/

│ │ ├─ Enriched/

│ │ └─ Unenriched/

│ │ ├─ TG63\_Training/

│ │ ├─ TG63\_Hab/

│ │ └─ TG63\_Tests/

TG64

├─ Isaac/

│ ├─ Combined\_6D/

│ │ ├─ Enriched/

│ │ └─ Unenriched/

│ │ ├─ TG64\_Training/

│ │ ├─ TG64\_Hab/

│ │ └─ TG64\_Tests/

**TG65/65**

1. These rats repeat the same process but without odors. They should both get two bottles of water and instead of “stink\_con\_un3” the program should be “stink\_hab5”.
2. No need to record the bottle weights for these but make a note if they’re not licking at all.

**ORX128 (He is on the opposite side of the rack)**

1. For ORX128, he will also get just two bottles of plain water but there are a few differences.
   1. Change the program to “orx\_bat\_hab5”
   2. The name of the animal can only be saved as OX128 not ORX128
   3. The fan should be on for this animal

**After running the animals**

1. After running all of the animals, but not before you finish running ORX128 you can give them all about 10mL of water afterwards.
2. Also remember to wipe down the rig with ethanol and clean up any poop/pee.
3. If you have time you can also rinse out the bottles.