



Touchscreen Operant Testing

Version: 2

Replaced by version:

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(note that the following list should be linked to the appropriate location.)

[Summary](#)

[Reagents and Materials](#)

[Protocol](#)

[Reagent Preparation](#)

[Reagent 1](#)

[Reagent 2](#)

[Reagent 3](#)

Summary: *(This area will include a brief description of what the protocol is used for and why someone would need to use it.)*

Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Vendor	Stock Number
Ensure Strawberry Milkshake		
70% Ethanol		
Paper Towels		

Protocol:

**** General notes:**

1. Experimenter is responsible for labeling cages undergoing touchscreen experiment with PI will feed and PI will change cage card indicators. Experimenter responsible for feeding or supplying aliquoted food to vivarium staff every day, as well as changing cages every 2 weeks. Experimenter will sign off on appropriate paperwork.
 - a. Cage subjects' cage after they are done testing for the day.
2. Run mice in the same order, at the same time each day, in the same box throughout the experiment.
3. Always run the same schedule every Friday and Monday for each mouse.
4. Keep feeding schedule as consistent as possible.
5. At each step of Phase 0 and 1, mice will be excluded if their performance does not reach standard to continue to acquisition.

6. Weigh mice daily during Phase 0 and 1; once their weight ‘settles’ during the experiment, can weigh every 2-3 days (recommend M, W, F).
7. Timing: touchscreen is a long experiment, spanning over months.
 - a. Phase 0: ~2 weeks
 - b. Phase 1: ~2-3 weeks
 - c. Phase 2: ~5-6 weeks
 - d. Phase 3: ~6-8 weeks

Phase 0: Food Restriction

Goal: Keep mice at 85% of original free feed weight throughout entire experiment.

Reality: Most mice settle around 90% of their weight but strive for 85%.

1. Weigh animals and calculate 85% of that weight – that is their goal weight.
2. In the afternoon, throw away all of the food in wire cage.
3. Feed 2grams per mouse per day.
 - a. I.E. if there are 4 mice in a cage, you’d weigh out 8g. (I give ± 0.2 g if you’re not using weighted pellets).
 - b. After a while, you can adjust the food amount if the mouse is struggling to lose weight to 1.5g per day and if they’re losing too much, 2.5g per day. Calculate food per mouse and add together for per cage food amount.
4. It takes roughly 2 weeks for animals to go down to 85%.
 - a. Approx 1-2 animals of a cohort of 20 will not be able to reach the weight goal. Animals that after 2-3 weeks haven’t reached 90% of their weight will not move forward in the experiment.

Phase 1: Training

Occurs once mice are at ~85% of free-feed weight

Goal: Mice learn how to associate the initiation of a trial, the touch of the touchpad when lit with the dispense of food reward.

- A. Day 1: Habituation for 20 minutes
 - a. **Purpose:** Mice acclimate to chamber, habituate to drinking milkshake.
 - b. Mice are placed in the chambers with no touchscreen schedule necessary.
 - i. However, you can ‘run’ one to see how many times the mice touch the screen, etc.
 - c. Dispenser dish is pooled with milkshake.
 - d. Note only mice that do not drink the milkshake
- B. Day 2: Habituation for 40 minutes
 - a. **Purpose:** Mice acclimate to chamber for full time, habituate to drinking milkshake.
 - b. Same thing as day 1.
 - c. Note mice that do not drink the milkshake → they may not learn to drink it and would be removed from the cohort.
- C. Day 3: Must Initiate for 45 minutes
 - a. **Purpose:** Mice learn how to initiate a trial
 - b. Record Trial Number, Left vs Right Screen Touches (to rule out side bias)
 - c. Note any milkshake dispensers with milkshake left in them
- D. Day 4-X: Must Touch for 45 minutes
 - a. **Purpose:** Mice learn they must touch the screen for reward.

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- b. Run this schedule daily until each subject reaches 30+ trials for two consecutive days.
- c. Number of days to completion is variable (2-10)
- d. Record number of trials, correct #, incorrect #, left vs right screen touches, % correct.
- e. Note any milkshake dispensers left full → at this point, I would exclude those mice
- E. Day X-Y: Punish Incorrect for 45 minutes
 - a. **Purpose:** Mice learn there is a right and a wrong; an incorrect response leads to no reward, and a 10 second lights on time out.
 - b. Run this schedule daily until each subject reaches 80% accuracy for two consecutive days
 - c. Number of days to completion is variable.
 - i. If a subject does not complete this step within 10 days, recommend excluding them from the study).
 - d. Record number of trials, % correct, correct number of trials, incorrect #, left vs right screen touches.

Phase 2: Pairwise Discrimination Acquisition

Goal: Mice learn to associate one pattern of light as 'correct' to earn a food reward.

- A. Counterbalance the animals getting spider vs. airplane by sex and genotype.
- B. Crucial that each day, each subject gets the appropriate airplane vs spider schedule.
- C. Run schedule every day until subject reaches the criterion of at least 80% accuracy on two consecutive days.
 - a. Time is variable and sessions to criteria is a metric we use to demonstrate learning ability. (~roughly 20-30 sessions)
- D. Record number of trials, % correct, # correct trials, # incorrect trials, # correction trials every day.

Phase 3: Reversal

Goal: Mice re-learn that the other pattern of light is now correct, demonstrating flexibility in learning and memory.

- A. Once each subject reaches the acquisition criterion, you simply change their schedule from airplane to spider or vice versa.
- B. Run schedule every day until subject reaches the criterion of at least 80% accuracy on two consecutive days.
- C. Mice will struggle immensely are forefront, getting often near 0% correct the first few sessions.
- D. Time is variable and again, number of sessions to criterion is a metric of learning and in this case, degree of flexibility (the longer a subject takes, the more they persevere and are unable or unwilling to change their learned behavior)
 - a. Reversal typically takes longer than acquisition because they first have to unlearn the previous correct response and then re-learn the new correct response (~30-40 sessions)

Using Lafayette TS System

1. Dilute milkshake (Ensure Plus nutrition milkshake in strawberry flavor) 1:1. Mixing 1 bottle milkshake with 1 bottle tap water
 - a. Do not refrigerate milkshake

- b. Do not store milkshake for multiple days. Use 1 shake per day.
 - c. Do not use DI water. Use regular, lukewarm tap water.
 - d. Pour diluted milkshake into chamber jars
 - e. You do not need to change jars between mice. Just per day
2. Bring mice to room from the vivarium 30 minutes before starting, allowing time for adjustment from being moved on cart.
3. Computer set up:
 - a. Turn on PC, then box
 - b. Click on Whisker Server, then start ABET III Touch
 - c. Click on Execution Manager tab
 - d. Right click to select "Load Schedule"
 - e. If all mice are on the same schedule, you can click on the top row to right click, load schedule for all the boxes.
 - f. Click on the top row and then on the right side, enter the time for the session (in minutes, press OK, do not press enter to continue) and designate the database for all animals.
 - g. Creating a new database (can do it as you see fit, recommend a new database for each experiment)
 - i. Go to Data Viewer
 - ii. Edit → Preferences
 - iii. Data Saving Options in pull down menu
 - iv. Create New Connection
 1. Input the Connection ID and File name (same thing)
 - v. Click Done at the bottom.
 - h. Click on each box's row and under the "value" column, next to the Animal ID row, fill in the animal ID. Do this for each animal individually
 - i. If the next round of animals are on the same schedule, you can right click on the box or top row and click "reload schedule". Otherwise, select box and "unload schedule" and then load the next one.
4. Load milkshake and set up chambers:
 - a. Make sure correct mask is inserted
 - b. Insert jar into slot and start motor for dispenser
 - c. Watch milkshake and wait until it pools into dispenser. Clean dispenser unless mice are going through habituation.
5. Run mice
 - a. Load all mice into their chambers
 - b. Place tops, clamping boxes shut.
 - c. Close box doors
 - d. Click on top row in execution manager to select all boxes and press the blue play arrow in the ribbon bar to begin.
 - e. Collect mice after the time allotted.
 - f. Weigh mice after their session. Feed appropriately
 - i. Feed the entire cage together. Whether you do it right after they are finished or feed everyone at the same time at the end of the day, up to the experimenter. As long as it is consistent every day.
6. Clean chambers between rounds of mice
 - a. Clean walls and floor of chamber with 70% ethanol
 - b. Do not need to clean the mask or poop tray.
 - c. Make sure dispenser is empty before putting mouse into the chamber.

7. Cleaning at the end of the day.
 - a. Remove jar of milkshake and replace with jar of 70% ethanol.
 - i. Turn on motor
 - b. While motor is running, clean the walls, floor, mask, and screen with 70% ethanol using paper towels
 - c. Dump our poop tray and use 70% ethanol to clean it as well
 - d. Once all milkshake has been pushed through tubing, remove tubing from jar and let the motor run until there is no more liquid in the tube. Clean out the dispenser pool with 70% ethanol.
 - e. *** this is incredibly important. Be absolutely sure that there is no milkshake left in the tubing; it can dry out and crust in the tubing, or dispenser tubes which is very difficult to clean and can lead to extended time troubleshooting.
8. Getting data
 - a. You can write down manually the values and metrics you are interested in.
 - b. You can also export data from the software which is a much more flexible option
 - i. Go to Data Viewer
 1. Can use the date criteria to collect that day's data or any other criteria you might want (if you wanted to find out all an animal's performances, etc)
 - ii. Select then press LOAD at the bottom of the panel on the left
 - iii. Use the right-hand side panel to go to the analysis tab
 - iv. Click subfolder and analysis set
 - v. Then press Execute Analysis
 - vi. Go to Report Editor
 1. Click on Animal ID and press "Add Key"
 - vii. In the Columns, select the parameters you want such as % correct, Trials Completed, Correction Trials, etc.
 - viii. Export to File
 1. Destination can be a folder on the desktop, a USB drive, an external hard drive, whatever you choose.
9. Turn off box THEN PC.
10. Take jar with ethanol and jars with milkshake to a sink and dump them out and clean with water (can use Alconox or mild soap as needed).

