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Protocol status: Working
We use this protocol and it's working

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Basic Operant Behavioral Training

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ABSTRACT

This protocol includes information about the operant chamber, mouse preparation, initial shaping (training), and instrumental learning. This can be followed by other behavioral tasks, such as probabilistic reversal learning or delay discounting, which are described in other protocols. To promote consistent results, each mouse should be trained in the same operant chamber at the same time of day, five days per week, throughout the study. Male and female mice should be trained in separate operant chambers.

ATTACHMENTS

964-2499.pdf

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Aligning Science Across Parkinson's Disease

MATERIALS

Materials and Operant Box Configuration

- Food-deprived mice (goal 85-90% of ad libitum body weight), aged 3-7 months old, C57Bl/6 background.
- A scale for weighing mice and supplemental food.
- Sweetened condensed milk, diluted to 30% using store-bought sweetened condensed milk (Eagle Brand) and tap water.

Note

Store in 50 mL aliquots (in Falcon tubes) in the freezer.

- A desktop computer for running Arduino software to manage operant procedures and data collection.
- SD cards to record various behavioral events.
- Sound-attenuating cabinets (Coulbourn Instruments).
- Operant test chambers (custom made, 18 x 18 x 26 cm with acrylic walls and floor of stainless steel bars). One side of each chamber was equipped with one yellow LED as a house light, which remained illuminated during all experimental stages unless stated otherwise. The opposite side of the chamber was equipped with left and right side nosepokes 12 cm apart. Each nosepoke contained two yellow LED stimulus lights: one positioned 6 cm above and another located inside the nosepoke. Sweetened condensed milk was delivered to a liquid receptacle from the central port, equidistant from the left and right nosepokes through a solenoid valve (The Lee Company). An infrared detector was mounted horizontally across the center port to detect head entries.

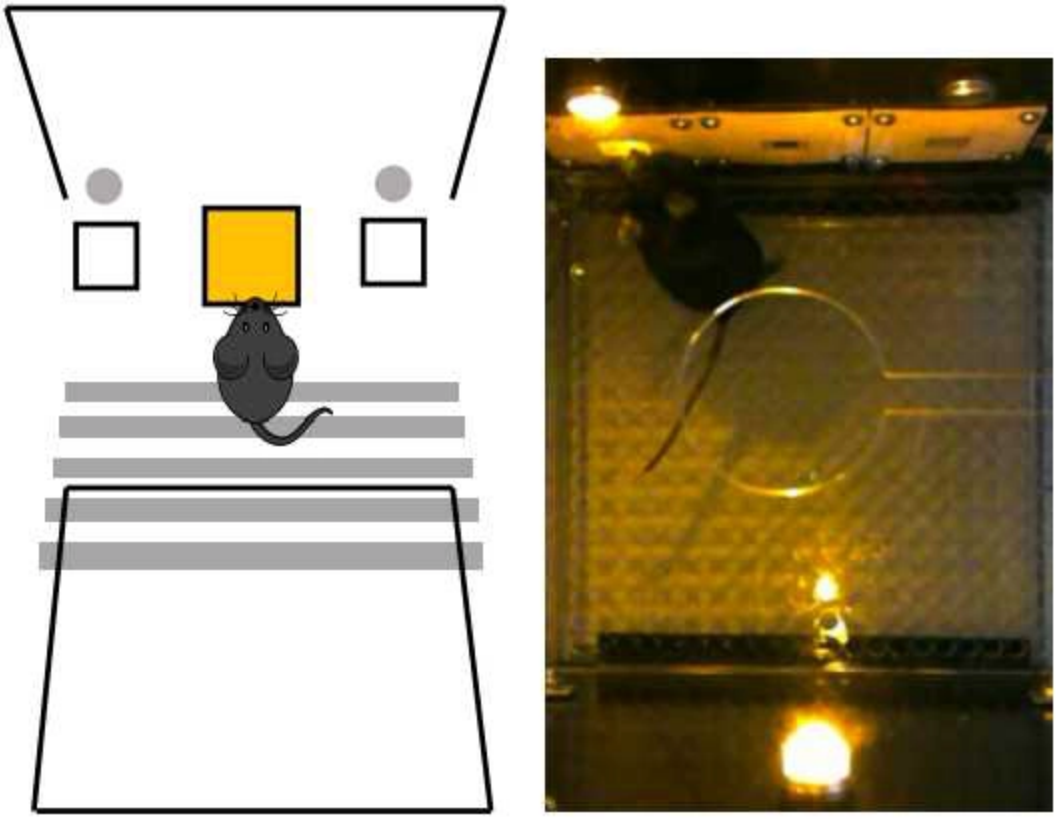

Figure 1: Operant Box Configuration. At the left, the cartoon showing the left and right choice ports, with the central reward port shown in yellow. At right, a top-down photo of the chamber, showing a mouse choosing the cued (left) port.

Operant Box Setup

1 Ensure the correct SD cards are inserted into the logging shield of each chamber.

2 Connect the USB cable of each Arduino to the computer.

3 Turn on the 12-volt power supply.

4 Pour 10 mL diluted milk into each falcon tube for each behavioral box.

5 Connect each solenoid to its behavioral chamber: one end goes into the milk solution, and the other end goes to the chamber.

6 Ensure that milk is pumped into the chamber.

7 Upload the Arduino code to the operant chambers, depending on the training phase of each mouse.

8 In each Arduino window, go to “Tools”, and the select “Port” to choose the correct board number, and then click “Upload”.

9 In each Arduino window, go to “Tools” again, and then select “Serial monitor” to open serial monitor windows.

> **Note**
>
> This allows the experimenter to monitor mouse behavior while the experiments are running.

10 Insert the waste pan and floor into the behavioral chamber.

Starting an Operant Session

11 Place each mouse into their designated box and close acrylic door to the operant box.

12 Allow to acclimate for 1-2 minutes.

13 Turn on 5-volt breadboard power switch and press “Reset” button on the Arduino board.

14 Securely close the door of the sound attenuating box.

Cleanup

15 Replace the falcon tube with clean tap water.

16 Upload the “cleaning” protocol onto each chamber.

17 Allow the water to run through the entire system for at least 00:15:00. 15m

18 Clean the walls of operant chamber, nosepoke ports, and reward port with 75% alcohol.

19 Rinse the waste pan and floor with water, and allow them to air dry Overnight . 50m

20 Turn off the 5-volt breadboard power switch.

21 Turn off the 12-volt power supply.

Training procedure - Preparing the mice with food restriction

22 Three days before the start of Phase 1 (Magazine Training), the free-feeding (ad libitum) weight is recorded by hand in a logbook.

23 Food is removed on the same day, and the food restriction protocol is initiated. This starts with 2.5-3 g of standard food pellets per animal per day for the first few days.

24 Subsequent adjustments may be necessary based on the animals' weights, which must be monitored daily and maintained at approximately 85%-90% of their free-feeding weights.

Note

Food competition by animals within a cage might necessitate separating the mice to house them individually.

Training procedure - Operant Training Phase 1/Magazine Training

25 Three days after starting food restriction, animals can be started on Phase 1. This phase introduces mice to the reward delivery port (1 session).

26 A liquid reward (10 µL of milk) is delivered on a random interval schedule of 40-80 seconds for a total of 40 rewards.

27 Each trial begins with the delivery of milk into the center port, accompanied by a 10-second illumination of the center port light.

Training procedure - Operant Training Phase 2/Blocked Instrumental Learn…

28 In this phase, the side (choice) nosepoke triggers reward delivery (6-10 sessions).

29 Mice are trained to nosepoke at the side ports to obtain a reward on a fixed-ratio 1 (FR1) schedule.

30 Each training session is separated into blocks, separated by a two-minute break period when the house light is turned off.

31 Each block lasts 00:50:00. 50m

32 Only one nosepoke (either the left or the right side) is trained in each block, and the active nosepoke switches during the break period.

33 The initial nosepoke (left or right) is counterbalanced across sessions.

34 Each trial begins with a stimulus cue light above the left or right nosepoke.

35 If the mouse nosepokes that port within 00:00:20, the cue light is extinguished, and a reward (10 µL of milk) is delivered to the center port, which is illuminated for 00:00:10, followed by a random 30-50 second intertrial interval. 30s

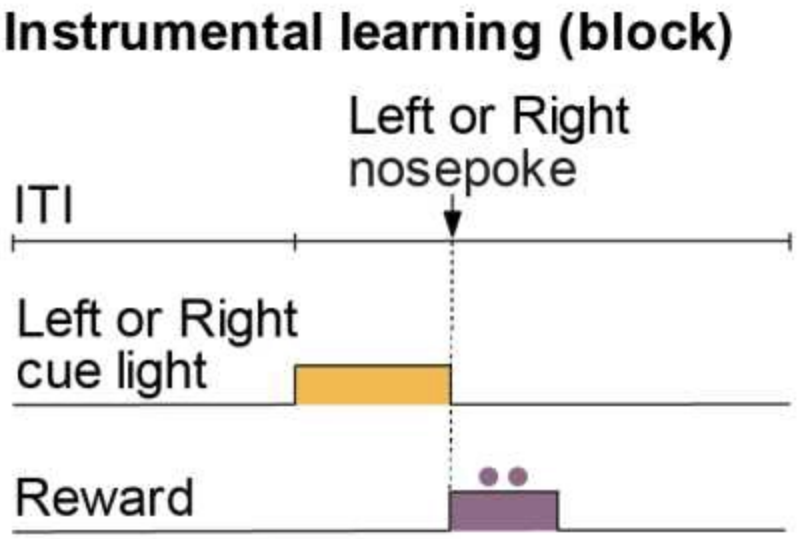

Figure 2: Phase 2/Blocked Instrumental Learning. At the start of each trial, a cue light above the left or right nosepoke is illuminated. The animal has up to 20 seconds to poke at that port. At that time, the central port is illuminated and a liquid reward becomes available there.

36 If the mouse does not perform a nosepoke on the correct side, then the cue lights are extinguished and a timeout period (a random 30-50 second interval) begins.

37 To advance to Phase 3, a mouse must perform a correct response on more than 80% of trials in the last session.

Training procedure - Operant Training Phase 3/Self-initiated Instrumental L...

1h 30m 50s

38 This phase shares some features with Phase 2, but in this phase, the mouse must center nosepoke to trigger trial start (5-8 sessions), and choice trials are presented in a randomized (rather than blocked) fashion.

39 Trials begin with the center port being illuminated for 00:00:10 .

10s

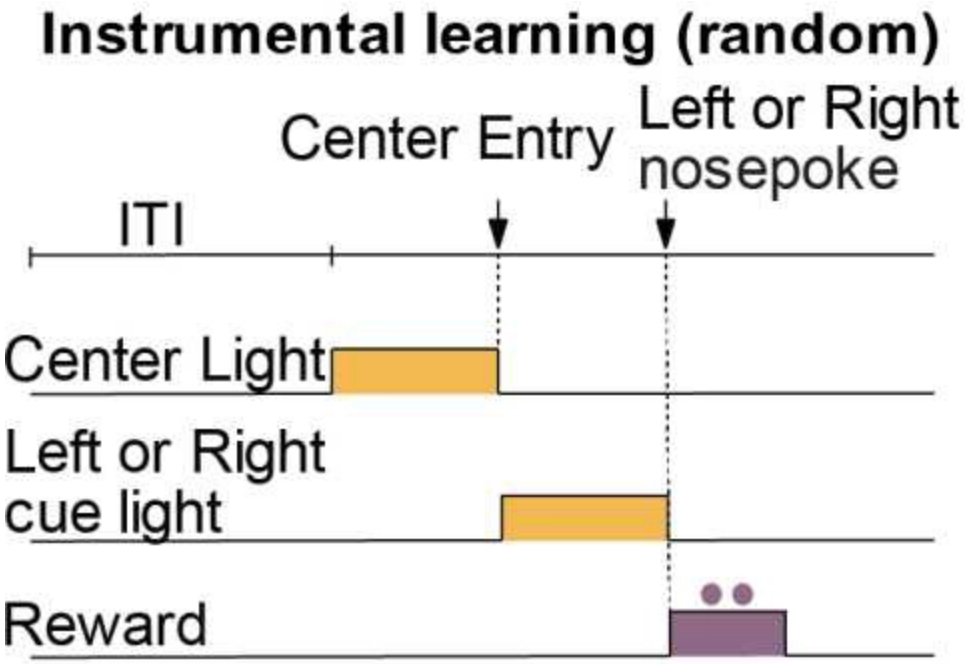

Figure 3: Phase 3/Instrumental Learning (Randomized Trials). At the start of each trial, the center port light is illuminated, and the mouse must poke there to continue the trial. Then a cue light above the left or right nosepoke is illuminated. The animal has up to 20 seconds to poke at that port. At that time, the central port is illuminated and a liquid reward becomes available there.

40 During this period, a trial can be initiated by a center port nosepoke.

41 Failure to make a center nosepoke within this period is considered a center omission and is followed by a timeout period (random 30-50 second interval).

42 Following an initiation nosepoke, the stimulus cue lights at either the left or right port are activated for 10s 00:00:10 .

43 If the mouse nosepokes that port within 00:00:10 , the cue light is extinguished, and a reward (20s 10 µL of milk) is delivered to the center port, which is illuminated for 00:00:10 , followed by a random 30-50 second intertrial interval.

44 Failure to poke the correct side port within 00:00:10 is considered a side omission, and cue lights are 10s extinguished until the next trial.

45 Poking the opposite, uncued side is recorded as an incorrect response and does not trigger punishmen 1h 30m Each session lasts 01:30:00.