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Mouse reach-to-grab task training protocol

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We use this protocol and it's working

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Abstract

This protocol trains mice in a reach-to-grab task through structured habituation, shaping, and training phases.

Materials

The behavior apparatus consists of:

- a rectangular plexiglass box 25 cm x 82 cm x 12 cm.
 - The front wall has a vertical slit in the middle of 5mm width.
 - The floor is a grid so that the mice can't store food.
 - There is an elevated front rod on the inside of the box, 3mm away from the front wall, at 7 mm height. It is used as a starting point for animal to reach and a 'perch' where animals keep their paws in preparation for the reach.
- a platform to place the food pellets, outside the front slit. We use a rotating disk of size ... but any platform of the same height can be used.

Troubleshooting

Problem

It is quite often that animal training may not go as smoothly as described here or in literature. Mice might naturally lick off pellets instead of reaching for them, or not go to the back of the box or stay there. Some would simply exclude the mice from the protocol, but we chose to control the protocol manually and modify the protocol based on observed behaviors.

Solution

Training environment is key to a successful training: • Behavioral room should be quiet with proper temperature and lighting. • There should be only the one experimenter in the room. The experimenter shouldn't wear any kind of perfume. • If a mouse jumps out of the hand during habituation to the experimenter, it can be returned to the home cage. The habituation can be tried again after habituating the rest of the mice.

Problem

Tips to engage animals into training:

Solution

- On shaping day 1, if the mouse shows no interest in getting food (no licks, no reaches), you can try to provide sugar pellets in the home cage with regular chow at the end of day, but still maintain the same total amount of food provided (e.g. 2g of chow + 2g of sugar pellets can be provided for calculated 4 g food daily. Make sure to add an additional shaping day.
- During the initial days of training, if the mouse starts biting on the slit or performs more than 20 consecutive in-vain reaches, a brief noise can be introduced by gently scratching the top corner of the side wall. This will distract the mouse and move it to the rear of the reaching box. We have noticed that the mouse would then come forward again after hearing the rotating disk.
- If the mouse stays in the back of the test box for more than 5 seconds, rotate the disk again. The sound of the rotating disk might encourage the mouse to come forward.
- At the beginning of single-pellet training, if the mouse sniffs through the slit but does not reach, adding a small pile of pellets in the center of the disk encourages the mouse to reach.

Problem

Tips to encourage reaching and prevent licking:

Solution

- If the mouse attempts to lick off the pellets during the early days of training, move the disk slightly away from the slit. This will prevent the pellet from being reachable by tongue but still reachable by paw. In case the mouse stops licking for a full session, move the disk closer to the slit in the next session. Move 1 to 2 mm closer every 5 minutes, as long as the mouse keeps reaching, until you can place it at the standard 5 mm distance from the front wall.
- If the mouse loses too much weight (i.e. < 75% of baseline body weight), it has a tendency to start licking, even after the shaping phase. If the previous solution to licking does not work, we



found it helpful to increase the food portion after the session and move back to the shaping setup on the next session.

Problem

Tips to increase success rate:

Solution

- We strongly encourage a single experimenter to conduct the habituation and behavioral shaping and training sessions. We noted that animals can stop or reduce reaching when a new experimenter is involved at any stages of the task. For instance, even well trained (i.e., "experts") mice could stop reaching when additional experimenter assisted with the training.
- Shaping and training sessions are expected to be performed at the same time of the day to reduce variabilities due to circadian rhythm.
- Successful acquisition of this reaching skills requires daily training for at least consecutive 4 days after the completion of shaping.
- We were using a rotating disk for pellet delivery. Some modifications may be considered and can be helpful to increase success rate of reaches, such as slightly increasing depth of wells, adjusting the height of disk, and precisely positioning the food dispenser.

Before start

The behavioral protocol consists of 5 days of habituation and 2 to 3 days of shaping followed by 7 days of training. Each of these stages will be detailed hereafter. All sessions are done during the light phase of the light/dark cycle.

Note

We noticed that mice are most motivated in the afternoon, when fed approx. 1 hour before the start of the dark phase of the light/dark cycle (16-hour food deprivation). Therefore, we planned all the experiments to end approx. 1 hour before the start of the dark phase. The sugar pellets used in the protocol were 5 mg spheres of approx. 2 mm in diameter (1811327 (5TUT, TestDiet, Richmond, IN, USA))

Habituation - Habituation to the experimenter (days 1–2).

- 1 We started each training session with 5 days of habituation that allows mice gradually habituate to the experimenter and the testing environment (e.g., room and behavior apparatus).

Note

During habituation, mice were food restricted. Throughout the experiment, make sure that the mice will be handled by the same experimenter until the end of training (e.g., for the weekly cage change).

- 2 By the end of day 2 and to proceed to day 3, mice should get acclimated to the experimenter and handling (i.e., do not try to bite and circulate around experimenter's hand in home cage, do not jump off experimenter's hands/arms nor defecate and urinate excessively). If needed, extra days of habituation can be added, or the mice should be excluded from the study.

Habituation - Habituation to the test setup I (day 3):

- 3 The purpose of this session is to acclimate the mice to the test box. To reduce stress, we propose that their first contact with the box be with a cage mate.
- 4 Transfer cage to the behavior room.
- 5 Allow mice to acclimate to the room for  00:05:00 .
- 6 Place two mice from the same cage in the middle of the reaching box by holding tails.
- 7 Let the mice explore the box for  00:20:00 .
- 8 Put animals back to their cage and add a few sugar pellets on the floor for consumption.
- 9 Clean the box with 70% ethanol between mice.



Habituation - Habituation to the test setup II (days 4–5):

- 10 The purpose of the last two sessions of habituation is to allow animals get acclimated to the box individually, in the presence of sugar pellets. Recording the mouse behavior on habituation day 5 allows to assess this acclimation.
- 11 Transfer cage to the behavior room.
- 12 Allow mice to acclimate to the room for  00:05:00 .
- 13 Place the feeder with a pile of sugar pellets 5 mm away from the front wall.
- 14 Place a plastic tray with 10 sugar pellets inside the box against the front wall.

Note

No need to set up the elevated front rod at this stage.

- 15 Place a mouse in the center of the box.
- 16 Let the mouse explore the box for  00:20:00 .
- 17 Move the mouse back to its cage.
- 18 Clean the box, the tray, and the disk with 70% ethanol between mice.
- 19 By the end of day 5, animals should show free exploration of the reaching box, interest in the slit and the pellets.

**Note**

Animals may not eat any pellets at this stage as they are not hungry but should be spending enough time near the slit and sniff it.

Shaping

20 Overall procedure

Shaping stage is included to initiate reaching in simpler setting and determine the paw dominance before starting the actual training.

During the training animals should be food-restricted

Note

In our lab, all animals were food-restricted throughout the shaping and training periods at the same level, i.e., around 80% of baseline bodyweight. The mice were housed in groups of 4 and food was placed on the cage floor. Daily food provided is the equivalent of 8% of animals' baseline bodyweight.

21 Transfer the cage to the behavior room.

22 Allow mice to acclimate to the room for  00:05:00 .

23 Place the feeder with a pile of sugar pellets 5 mm away from the front wall.

Note

The disk can be placed closer to the slit and gradually moved away during the session. Also, the elevated front rod should not be set up during the shaping phase.

24 Place the mouse in the middle of the box.



25 The mouse may retrieve sugar pellets by licking or reaching and the following numbers should be recorded:

- failed and successful licks
- failed and successful reaches with the right and left paws

Note

When there are no sugar pellets remaining on the disk within the reaching distance from the slit, provide more pellets.

26 Move the mouse back to the home cage at the end of the session.

27 Clean the box and the disk with 70% ethanol between mice.

28 For each mouse, calculate total number of reaches (**equation 1**) and percentage of reaches (**equation 2**) with right paw to determine paw dominance. The dominant paw is the paw used for more than 70% of all reaches (successful and failed).

Equation 1

$$\text{total reaches} = \text{failed right reaches} + \text{failed left reaches} + \text{successful right reaches} + \text{successful left reaches}$$

Equation 2

$$\text{Percentage of right reaches} = \frac{\text{failed right reaches} + \text{successful right reaches}}{\text{total reaches}} \times 100$$

**Note**

- Shaping stage takes 2 to 3 days. By the end of this stage, mice should be able to perform at least 20 reaches within 20 min and show paw dominance. Even if mice reach these criteria in shaping day 1, we strongly recommend keeping shaping day 2. Shaping day 3 is optional. If mice don't meet this criterion, they are excluded from the study.
- Mice might start using their tongues to get sugar pellets (licking) before using their paws (reaching). In that case you can try moving the disc even further from the slit (>9mm).
- Shaping day 3 should be included if (i) the mouse still predominantly retrieves food pellets by licking at the end of shaping day 2, even if the mice have performed more than 20 reaches within the session, or (ii) if paw dominance can't be determined at the end of shaping day 2.

Training

1w

29 Overall procedure.

Training takes place after the shaping stage and requires at least 7 sessions (T1 to T7).

Note

Online observations are helpful to obtain preliminary results of each session (e.g., success rate and number of reaches), which can be used to optimize the training protocol whenever needed (see Troubleshooting section).

30 Transfer the cage to the behavior room.

31 Allow mice to acclimate to the room for  00:05:00 .

32 Fill the slots of the feeder with sugar pellets.

33 Place the disk such as:

- Its edge is at 7 mm distance from the front wall.
- The sugar pellet is aligned with the left or right edge of the slit for mice showing right or left paw dominance, respectively.



- 34 Set up the elevated front rod.
 - 35 Place the mouse in the center of the box.
 - 36 Monitor the activity of the mouse for  00:20:00 .
 - 37 Rotate the feeder using the buttons when the slot in front of the slit becomes empty.
 - 38 Food pellets should be delivered:
 - 39 After successful reaches, when the mouse moves away from the slit to consume the pellet.
- Note**
- After food consumption, if the mouse stays at the slit and keeps performing in vain reaches, no pellet should be delivered until the animal goes to the back of the reaching box and returns to the slit again.
- 40 After failed reaches, when the animal goes to the back of the reaching box and comes to slit again.
 - 41 At the end of the session, put the mice back to its home cage.
 - 42 Clean the box and the disk with 70% ethanol between mice.
 - 43 For each mouse calculate the success rate (**equation 3**).

Equation 3



$$\text{success rate} = \frac{\text{successful reaches}}{\text{sum of failed and successful reaches}}$$

- 44 By the end of training, mice persistently reached for the pellets and conducted 100–300 reaches within  00:20:00 . Around 70% of the animals trained using this protocol showed a success rate of 30–40% (Chen et al., 2014).

Protocol references

1. Chen et al., 2014