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## Behavior Protocol

In 2 collections

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### ABSTRACT

This protocol details the associative learning assay used to test extinction and conditioning behavior in mice.

### GUIDELINES

#### Mice:

- Surgerized with a headbar for head-fixation
- Singly or pair housed
- Acclimated for 1 week to a 12-hr reverse light/dark cycle; perform all habituation/behavior during the dark portion of the light/dark cycle

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**Protocol status:** Working

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## Habitation day 1

52m

- 1 Begin water restriction: remove ad libitum water from mice.
- 2 Record starting body weight for all mice.
- 3 Calculate allotted daily water for all mice:  50 µL water per gram of weight.
- 4 Calculate goal weight for all mice:
  - 4.1 Within 80-85% starting body weight.
  - 4.2 If mice drop below 77% starting body weight at any point during behavior, supplement the allotted daily water by a few hundred µL until weight is restored.
  - 4.3 If mice drop below 70% starting body weight at any point during behavior, end water restriction and return ad libitum water.

5 Run the Matlab code

```
counterbalance(cohort, numMice)
```

to get the counterbalancing order for all days of habituation and behavior.

6 Headfix habituation outside of behavior apparatus (in today's counterbalance order):


6.1 Headfix for  00:02:00 , short break, then  00:05:00 .

7m

6.2 Put back in home cage for a longer break.

6.3 Headfix for  00:05:00 , short break, then  00:10:00 .

15m

7 Give allotted daily water: give water in a petri dish to the mouse, placed by itself in a mouse cup (without bedding so water does not get absorbed). Leave for  00:30:00 , then record quantity drunk.

30m

## Habituation day 2

20m

8 Weigh mice.

9 Headfix habituation (in today's counterbalance order):

9.1 Outside of behavior apparatus: ⌚ 00:10:00 .

10m

9.2 Put back in home cage for a break

9.3 Inside behavior apparatus, on treadmill but with treadmill prevented from moving: ⌚ 00:10:00 .

10m

10 Give allotted daily water, and record quantity drunk

### Habituation day 3

15m

11 Weigh mice.

12 Headfix habituation (in today's counterbalance order):

12.1 Inside behavior apparatus, on moving treadmill: ⌚ 00:15:00 .

15m

13 Give allotted daily water, and record quantity drunk.

## Days 1 -> 9: Training


14

### Note

- Each mouse should always use the same behavior apparatus across days
- Rewarded cue: 2500 Hz pure frequency tone
- Run behavior using the correct day's counterbalance order

**Calibrate solenoid:** fill the water reservoir with  50  $\mu\text{L}$  of fresh 10% sucrose water, and use the Matlab code

```
calibrateSolenoid_NI
```

to run the solenoid until the sucrose is at the end of the lick port, and to then determine the millisecond solenoid opening needed to deliver  5  $\mu\text{L}$  of reward per click .

### Note

This should be done daily, as the solenoid may get clogged or have some other issue that would change the volume being delivered per activation

15 Weigh mice.

16 Headfix first mouse on treadmill in behavior apparatus.

**16.1** Ensure treadmill can move freely.

**16.2** Ensure mouse can reach the reward delivery spout with its tongue

**Note**

On first day of training, introduce mouse to the reward delivery spout before beginning behavior. You can do this by having the solenoid release 1-2 drops of reward to the end of the spout, and touching this directly to the mouse's mouth until they begin to lick.

**17** Run behavior using the code

```
lickVid_dualConditionExtinction_NI(solOpenDuration)
```

, where

```
solOpenDuration
```

is the ms value calculated in step 14.

**17.1** GUI input: day type = train, probe = no probe, rewarded tone = 2500.

**18** Afterwards:

**18.1** Clean treadmill by wiping it down with Trifectant or a similar veterinary disinfectant.

18.2 Top off sucrose reservoir if it has dropped below  $\text{50 } \mu\text{L}$  .

18.3 Record number of rewarded trials the mouse received.

19 Repeat steps 16-18 until all mice have undergone their daily behavior.

20 Give allotted daily water minus the number of rewarded trials times 5 (as each mouse has received  $\text{5 } \mu\text{L}$  of water per rewarded trial already that day), and record quantity drunk.

21 Clean the solenoid, reservoir, and attached tubing by using the

`calibrateSolenoid_NI`

code to flush MilliQ water through the system.

## Day 10: Training with Probes

22

### Note

- Each mouse should always use the same behavior apparatus across days
- Rewarded cue: 2500 Hz pure frequency tone
- Run behavior using the correct day's counterbalance order

**Calibrate solenoid:** fill the water reservoir with  $\text{50 } \mu\text{L}$  of fresh 10% sucrose water, and ensure that the solenoid is delivering  $\text{5 } \mu\text{L}$  of reward per click.

**23** Weigh mice.

**24** Headfix first mouse on treadmill in behavior apparatus.

**24.1** Ensure treadmill can move freely.

**24.2** Ensure mouse can reach the reward delivery spout with its tongue.

**25** Run behavior using the code

```
lickVid_dualConditionExtinction_NI(solOpenDuration)
```

, where

```
solOpenDuration
```


is the ms value calculated in step 22.

**25.1** GUI input: day type = train, probe = probe, rewarded tone = 2500.

**26** Afterwards:



**26.1** Clean treadmill by wiping it down with Trifectant or a similar veterinary disinfectant.

**26.2** Top off sucrose reservoir if it has dropped below  50 µL .

**26.3** Record number of rewarded trials the mouse received.

**27** Repeat steps 24-26 until all mice have undergone their daily behavior.

**28** Give allotted daily water minus the number of rewarded trials times 5 (as each mouse has received 5uL of water per rewarded trial already that day), and record quantity drunk.

**29** Clean the solenoid, reservoir, and attached tubing by using the

`calibrateSolenoid_NI`


code to flush MilliQ water through the system.

## Day 11: Testing 1


**30**

#### Note

- Each mouse should always use the same behavior apparatus across days
- Run behavior using the correct day's counterbalance order
- If performing a DART infusion, do 2 hours prior to behavior and return mouse to its home cage in between
- Rewarded cue prior to rule change: 2500 Hz pure frequency tone
- Rewarded cue after rule change: 11000 Hz pure frequency tone

**Calibrate solenoid:** fill the water reservoir with  50  $\mu\text{L}$  of fresh 10% sucrose water, and use the Matlab code

```
calibrateSolenoid_NI
```

to run the solenoid until the sucrose is at the end of the lick port, and to then determine the millisecond solenoid opening needed to deliver  5  $\mu\text{L}$  of reward per click.

**31** Weigh mice.

**32** Headfix first mouse on treadmill in behavior apparatus.

**32.1** Ensure treadmill can move freely

**32.2** Ensure mouse can reach the reward delivery spout with its tongue

**33** Run behavior using the code

```
lickVid_dualConditionExtinction_NI(solOpenDuration)
```

, where


```
solOpenDuration
```

is the ms value calculated in step 30.

**33.1** GUI input: day type = flip, rewarded tone = 2500.

**34** Afterwards:

**34.1** Clean treadmill by wiping it down with Trifectant or a similar veterinary disinfectant.

**34.2** Top off sucrose reservoir if it has dropped below  50 µL .

**34.3** Record number of rewarded trials the mouse received.

**35** Repeat steps 32-34 until all mice have undergone their daily behavior.

**36** Give allotted daily water minus the number of rewarded trials times 5 (as each mouse has received 5uL of water per rewarded trial already that day), and record quantity drunk.

**37** Clean the solenoid, reservoir, and attached tubing by using the

```
calibrateSolenoid_NI
```

code to flush MilliQ water through the system.

## Day 12: Testing 2


**38**

### Note

- Each mouse should always use the same behavior apparatus across days
- Do not counterbalance the order of behavior – use the same order as Day 11, so that the amount of time between DART infusion and Testing 2 is consistent across mice
- Rewarded cue: 11000 Hz pure frequency tone

**Calibrate solenoid:** fill the water reservoir with  50 µL of fresh 10% sucrose water, and use the Matlab code

```
calibrateSolenoid_NI
```

to run the solenoid until the sucrose is at the end of the lick port, and to then determine the millisecond solenoid opening needed to deliver  5 µL of reward per click.

**39** Weigh mice.

**40** Headfix first mouse on treadmill in behavior apparatus.

**40.1** Ensure treadmill can move freely.

**40.2** Ensure mouse can reach the reward delivery spout with its tongue.

**41** Run behavior using the code

```
lickVid_dualConditionExtinction_NI(solOpenDuration)
```

, where


```
solOpenDuration
```

is the ms value calculated in step 38.

**41.1** GUI input: day type = test, rewarded tone = 11000.

**42** Afterwards:

**42.1** Clean treadmill by wiping it down with Trifectant or a similar veterinary disinfectant.

**42.2** Top off sucrose reservoir if it has dropped below  50 µL .

**43** Repeat steps 40-42 until all mice have undergone their daily behavior.

**44** Clean the solenoid, reservoir, and attached tubing by using the

```
calibrateSolenoid_NI
```

code to flush MilliQ water through the system.

**45** Perfuse all mice for histology (see histology protocol).