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

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Barnes Maze Protocol

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ABSTRACT

The Barnes Maze test is a test of rodent's spatial learning and memory abilities. In this test, animals are placed in an aversive environment (with a loud noise and bright lights) and trained to locate a hidden escape box. This particular protocol was adapted from a protocol by Attar et al. (2013) that allows for memory testing after only five training trials. The entire protocol takes 4 days:Protocol modified from: https://pubmed.ncbi.nlm.nih.gov/24236177/.
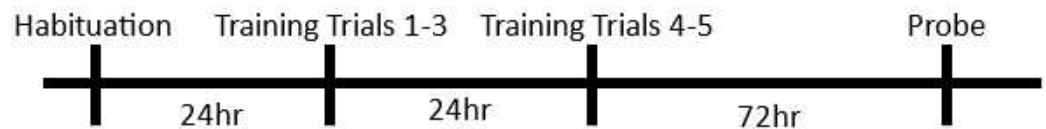
Figure 1. Schematic of timing for Barnes Maze Assessment.

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MATERIALS

1. Barnes Maze
2. 70% ethanol
3. Camera and Noldus EthoVision software
4. Clean rodent bedding
5. Loud white noise maker with remote control (we use this one)
6. Extra bright overhead lights (we use two 3,000 lumen overhead lamps)
7. Plastic beaker

PROTOCOL MATERIALS

⊗ 70% EtOH   In 3 steps

Set-up

1 Set up extra bright overhead lamps directly above maze so there is no difference in the brightness across the maze.

2 Set white noise maker to highest volume setting (butkeep off for now).

3 Make sure there are landmarks around the room that the animals can use to orient, everything in the room must be kept in the exact same spot throughout all training and testing days.

Habituation   1h 3m

4 Bring mice up to acclimate *just outside* the behavior room for at least ⏱ 01:00:00 .   1h

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5   Place fresh bedding in escape box and clean maze with ⊗ **70% EtOH** **Contributed by users** .

6   Place mouse in a clear plastic beaker and bring into behavior room.

7   Place mouse in middle of maze by flipping over the beaker and turn on the white noise maker.

8   Let mouse explore the maze for ⏱ 00:00:30 (stay in the room).   `30s`

9   After ⏱ 00:00:30 , gently guide the mouse towards the escape hole until they enter. To get them to enter, `30s` it is often helpful to gently pull their tail in the opposite direction.

10   Once the mouse enters the escape hole, turn off the white noise maker, and leave the room.

11   Wait ⏱ 00:02:00 before removing the mouse, placing them back in the plastic beaker and bringing them `2m` out to their cage.

12   Clean maze (including the sides and inside of each hole), spin top table (leaving escape hole in same location), and replace escape hole bedding before beginning habituation for each mouse.

Training

13 Each mouse undergoes a total of 5 training trials spread out over two days. Typically, this means 2 trials on day 1 and 3 on day 2. Training trials for each mouse should not be run back-to-back. Instead, all mice should undergo training trial 1 before training trial 2 starts. During each trial:

13.1

Place fresh bedding in escape box and clean maze with ⊗ 70% EtOH **Contributed by users** .

13.2

Start Ethovison (https://www.noldus.com/ethovision-xt) recording, place mouse in a clear plastic beaker and bring into behavior room.
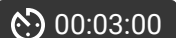
13.3

Place mouse in middle of maze by flipping over the beaker, turn on the noise maker, and exit the room.

13.4 Watch the mouse and record:
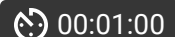   a. The time it takes the mouse to first look at the escape hole.
   b. The time it take the mouse to enter the escape hole (primary latency)

13.5   `3m`

Once the mouse enters the hole turn off the noise maker. If the mouse doesn't enter the escape hole after 🕐 00:03:00 , gently guide them to the hole.

13.6   `1m`

Allow the mouse to remain in the escape hole for 🕐 00:01:00 before returning to it's cage.

13.7

Clean maze, spin, and replace bedding before starting next mouse.

Data Analysis 1 `30s`

14 Graph up the first look and first entry for each trial. If the mice learned where the hole was, you'd expect to `30s` see a decrease in the values over time. Typically, the average latency for mice that have learned where the hole is is around ⏱ **00:00:30** . Controls for this test are distance traveled and velocity.

Probe Trial

15 The probe trial must be run 72 hours after the last training day. During this phase, the escape box is removed so there is no chance that the mouse can locate the escape hole by sight or smell.

15.1 Clean maze with ⚛ 70% EtOH **Contributed by users**

15.2

Start Ethovison recording, place mouse in a clear plastic beaker and bring into behavior room.

15.3

Place mouse in middle of maze by flipping over the beaker, turn on the noise maker (it will remain on throughout the duration of the trial), and exit the room.

15.4 1. Watch the mouse and record in your notebook:
    a. The amount of incorrect hole checks before the mouse checks the target hole
    b. The time it takes the mouse to first look at the escape hole.

15.5   The trial ends after 90 seconds, the noise maker can be turned off and the mouse returned to their cage.

15.6   Clean and spin maze before running next mouse.

Data Analysis 2

16   Graph up latency, number of incorrect hole checks, and percent of time in target quadrant (this can be extracted from the EthoVison data). Distance traveled and velocity can serve as controls.