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

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Smell discrimination test

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ABSTRACT

Behavioral test to assess smell discrimination in mice.

MATERIALS

- Chocolate pellets (Bio Serv FO5301)
- Trimethylthaizoline (TMT) (Sigma Aldrich 13623-11-5)

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1	Transfer the cage into the experimental room at least 1h before testing. Always maintain the cage in a ventilated cage rack to avoid smell contamination.
2	Set the open field before starting experiment. The open field is a dark-grey box of 40 x 40 cm with clean floor and a light suspended above the center. A maximum of 150 lux is display to avoid excessive stress. The experiment need to be done in a highly ventilated room.
3	At a randomly selected corner, place the 8cm plexiglass cylinder which has holes on the side.

- 4 Always start with the positive smell as the TMT smell might affect further experiments.
- 5 Before the test place ~10 chocolate flavor pellets (Bio Serv F05301) into the plexiglass cylinder.
- **6** Transfer the mice into the center of the open field using a paper tube.

7	Record the mice's positions for 20 minutes using tracking software, such as Ethovision XT or AnyMaze.
8	At the end of the test, remove the mice from the apparatus. Remove the cylinder and clean the apparatus with 50% ethanol before proceeding to the next animals.
9	On the following day, repeat the experiment but this time the cylinder needs to be placed in a different corner.
10	In the cylinder place a cotton ball that was covered with ~20µl of 5% TMT solution (Sigma Aldrich 13623-11-5), in water.
11	Place the mice in the center of the open field and record positions for 20 min.
12	Clean thoroughly the cage after each experiment with 50% ethanol.
13	Using the tracking software extract: a) distance travelled for 20 min b) average speed c) time in the center of the apparatus d) time in the corner paired with the smell
14	Using each value, compare the time spent in chocolate or TMT corner and the ratio correspond to the olfactory discrimination index.

