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## Smell discrimination test

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We use this protocol and it's working



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## Abstract

Behavioral test to assess smell discrimination in mice.

## Materials

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

## Troubleshooting

- 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 × 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 FO5301) 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.