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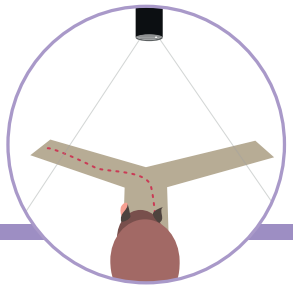
How to do T/Y maze testing

This is a chapter from the e-book:

Basic behavioral neuroscience in rodents.

THE T/Y MAZE



The Y-maze and T-maze look and function very similarly to each other, although some differences in readouts exist. The T-maze is constructed in the shape of a *T* and presents the animals with two choice options: a 90-degree left or right turn. Using different motivational cues, either **working memory** or **short term spatial memory** can be measured. Internal and/or external visual cues can be used to probe spatial memory, while positive/negative reinforcement in one of the choice arms can be utilized to test intact working memory.

MOUSE T/Y-MAZE

3 Equal arms: 35 cm length
15 cm raised walls
3 doors between the arms
manual operation

RAT T/Y-MAZE

3 Equal arms: 52 cm length
30 cm raised walls
3 doors between the arms
manual operation

IMPORTANT

The T-maze has arms set at 90 degrees of each other, the Y-maze at 120 degrees (equal corners)

The Y-maze is very similar to the T-maze. The name already gives it away considering the construction/layout of this maze compared to the T-Maze: the Y-maze is shaped in a *Y*. This minimal distinction does change the *arm-choice* component of the test, since in this setup there are three equal arm choices at 120-degrees. Popular paradigms in the Y-maze thus do not consist of baiting/motivating the arm choice, but rather relying on the animal's innate preference to explore previously unexplored areas.

Short-term memory can be tested by blocking access to one of the arms in the first phase of the test and observing the time spent in that arm in the second phase where all three arms can be accessed. This novel arm preference task is a test for **allocentric spatial memory** as the animals use cues from both inside and outside of the maze to remember the location of this unexplored arm.

Spontaneous alternation is a unique readout that can be measured with the Y-maze (which can also in theory be measured with the T-maze, although requiring constant investigator interaction). This is a paradigm that tests **working memory**, and involves the animals freely exploring all three arms and observing if they chose to enter the arm most recently explored or they alternate and enter the more novel arm.

PROTOCOL SUGGESTION

Spontaneous alternation in the Y-maze

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling. Place the animal in at the same end of one arm and allow the to move freely through the maze during an 8-minute session. Recording/tracking automatically starts in EthoVision XT if this option has been selected. Otherwise, do not forget to concurrently activate your video recording.
- Preferably leave the testing room to allow free and uninterrupted movement of the subject animal.
- After the testing time is finished, gently pick up the animal, again using your preferred handling technique, and return it to its home cage.
- Before cleaning the arena, visually count the faecal pellets present and manually record the numbers for further analysis.
- Remove all faecal pellets and wipe up all spots of urination. Spray the entire maze with 30-70% ethanol and wipe down with a clean paper towel. Allow the ethanol solution to completely dry prior to testing other animals.

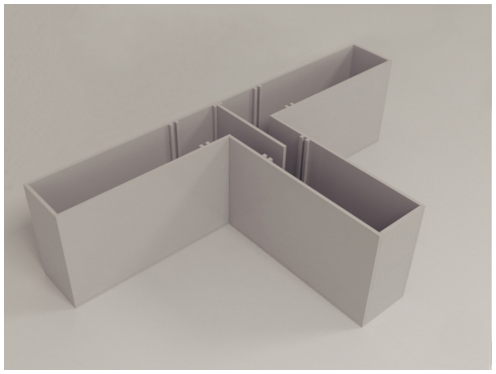
T-maze or Y-maze?

It depends on what you would like to measure. As a test for spatial learning and memory retrieval, the T-maze presents a clear left-or-right choice, in which this choice could be motivated by placing a bait.

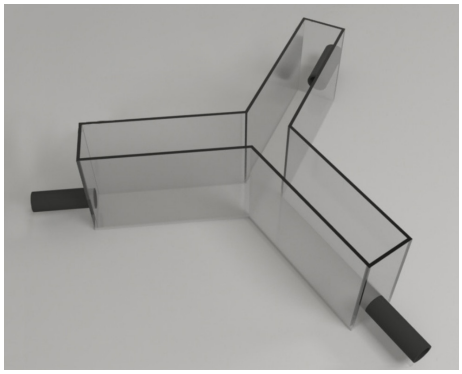
When opting for spontaneous alternation, the Y-maze is the more common choice, since all three arms present an equal choice (equal 120 degree corners).

Suggested protocol for the T-maze

A T-maze protocol is very similar to the Y-maze. A crucial difference is the arm choices. With spontaneous alternation is the Y-maze, free moving arm choices is of utmost importance. However in a T-maze often a left-or-right choice is preferred towards a baited arm (or with another form of reward/learning). Thus the animal is placed in a starting arm, and makes a left or right choice. This is repeated multiple times to obtain a score.



A standard T-maze with sliding doors.
Credits: He, S. and Corscadden, L. (2022). Maze Engineers.



A standard see-through Y-maze.
Credits: He, S. and Corscadden, L. (2022). Maze Engineers.

SETUP IN ETHOVISION XT

In EthoVision XT three main zones should be drawn, representing the three arm choices, let's call the arms a, b, and c.

INTERPRETATION OF THE RESULTS

In the analysis profile the **total number of arm entries** must be counted, which is a measurement of activity and locomotion during the testing session and is also be used to calculate the percentage of alternation. Alternation is defined as successive entries into the three arms, of which the order does not matter per se, but that an alternation is only achieved in a *set* of three different arm entries. For example, successive entries into arms a, c and b is considered an alternation, while entries into arms a, b, and a again is not considered an alternation. This measure of **spontaneous alternation** can be expressed as an absolute number, or as a percentage, and is a measure of short term spatial memory.

$$\text{Spontaneous alternation \%} = \frac{\text{\# spontaneous alternations}}{\text{total number of arm entries - 2}} \times 100$$