LAB_066 T or Y Maze Testing for Rodents

I. OBJECTIVE

To describe the procedure for measuring spatial learning through spontaneous alternation and/or reference memory testing in a T or Y maze.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. COMMENTS / RECOMMENDATIONS

- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour such as human traffic, unnecessary noise, and intense lighting.
- Male and female rodents should be tested separately, with one sex in the room at a time. Where possible males should be tested first, preferably on separate days but with at least thorough cleaning between the sexes. This is unless rodents are already housed within wire top cages or equivalent and both sexes are present in the home room.
- The T maze or Y maze can be used for measuring both spontaneous alternation and recognition memory, depending on what is available for your laboratory.

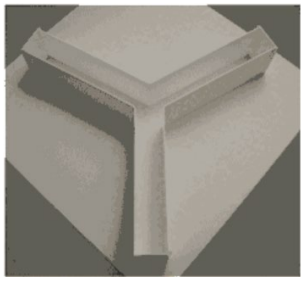
III. EQUIPMENT

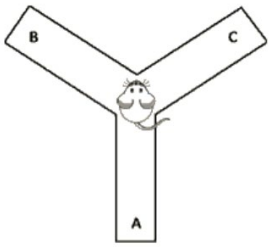
- PPE*

Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

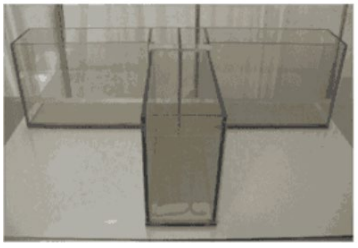
- Appropriate trolley for transporting cages.
- Disinfectant* and paper towel for cleaning equipment.
- Arena – the T and Y maze can be made of any suitable non-porous material and can be either opaque or clear and consists of three arms (a start arm; and left and right arms). Label the arms for ease of reference. The recommended dimensions are specified in the table below. In the Y maze (A) all arms are identical at 120° from each other. In the T maze (B) the start arm is 90° from the other arms.

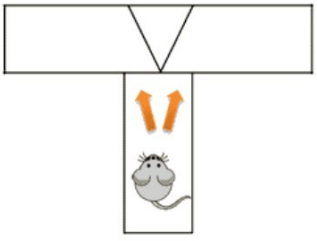
(A)





(B)





Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

Arena dimensions	Mouse	Rats
The length of the arms (cm)	25-35	40-50
The stem length – long side (T maze only)	35-45	50-60
The height of the walls (cm)	10	20

- Video recording equipment connected to a computer for video capturing.

To facilitate automatic tracking with video recording equipment, use diffuse lighting to minimise reflections.

- A curtain or screened off area for experimenter to be hidden from the rodents during testing, if available.

IV. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
2. Prepare equipment items including disinfecting prior to first use.
3. Bring rodents into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to start of experiment.

Length of habituation time in the testing room should be consistent for all rodents within an experiment.

4. Prepare the maze based on type of testing required. Opaque walls are used for spontaneous alternation where spatial cues are not necessary, clear walls are used for spatial reference memory. Distinct visual cues are placed either on the walls of the behavioural room or for easier reference, can be placed on the walls of the maze.

V. PROCEDURE

1. Record light levels in the middle of the arena, for reproducibility and consistency.

Lux range should be between 20-900 LUX and should remain the same for all rodents within an experiment.

2. Start recording and identify subject/s within the camera view.
3. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates
   LAB_039 Handling and Restraint in Rats and Neonates

Spontaneous alternation

4. Place rodent in the distal part of the start arm, facing away from the centre of the maze.
5. Rodents are allowed to move freely through the maze.
6. Trials usually last for 5-10 mins.
7. At the end of the trial, remove the rodent and return them to the home cage.
8. Stop recording and make sure to save the video file.
9. Remove scat and thoroughly disinfect the maze and allow to dry completely.
10. Analysis can be made manually or using animal tracking software.

Spatial reference memory

11. In the initial trial, either the left or right arm is closed off so that the rodent can only access 2 of the 3 arms. The arm to be closed off should be alternated between runs and test rodents to avoid arm bias.
12. Place rodent in the distal part of the start arm, facing away from the centre of the maze.
13. Rodents are allowed to move freely within the two open arms of the maze.
14. The initial trial usually lasts for 15 mins.
15. After a 1 hour intertrial interval, rodents are returned to the start arm of the maze and allowed to explore all three arms freely for 5 mins.

16. Repeat steps 7 to 10.

VI. ANALYSIS

Spontaneous alternation

- Examine the video and record the number of all arm entries and alternations. An alternation is defined as consecutive entries into all three arms.
- Calculate the percent (%) alternation = (Number of alterations/[Total arm entries – 2]) x 100

Spatial reference memory

- Analyse the total distance travelled during testing and the time spent in each arm
- Record the number of entries into each arm
- Rodents with intact spatial reference memory will spend more time in the novel arm

VII. REFERENCES

1. Kraeuter AK., Guest P.C., Sarnyai Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. In: Guest P. (eds) Pre-Clinical Models. Methods in Molecular Biology, vol 1916. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-8994-2_10
2. Prieur, E., & Jadavji, N. M. (2019). Assessing Spatial Working Memory Using the Spontaneous Alternation Y-maze Test in Aged Male Mice. *Bio-protocol*, 9(3), e3162. https://doi.org/10.21769/BioProtoc.3162
3. Sarnyai, Z., Sibille, E.L., Pavlides, C. *et al*. (2000). Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin$_{1A}$ receptors. *Proceedings of the National Academy of Sciences,* 97 (26) 14731-14736; DOI: 10.1073/pnas.97.26.14731

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
#1	LBM	07/04/2022	07/04/2025