

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_087 Barnes Maze for Rodents Institutional author: Queensland Brain Institute AEC Reviewed & Approved: 21/05/2026 SOP Expiry: 21/05/2029	Version #1.1
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LAB_087 Barnes Maze for Rodents (Expiry: May 2029)

OBJECTIVE

To describe the procedure for measuring hippocampal-dependant spatial learning in rodents using Barnes maze.

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

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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 animals 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 animals are already housed within wire top cages or equivalent and both sexes are in the holding room.
- To reduce the circadian cycle's effects on the behaviour, perform behavioural testing at a similar time each day.

SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse/rat bite injury, repetitive task musculoskeletal injury and psychosocial harm.

TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed competent in the procedure.

EQUIPMENT

- PPE*

Minimum PPE is gloves and gown, P2 mask; additional PPE may be required based on facility or additional risk, e.g., working with infectious animals.

- Appropriate trolley for transporting cages.

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- Disinfectant* and paper towel for cleaning equipment.
- Barnes maze apparatus: Barnes maze usually consists of a circular white piece of acrylic plastic (100 cm diameter) with 32 holes* (Figure A).
- Each hole measuring 5 cm in diameter, evenly spaced around the circumference 7.5 cm from the outer edge* (Figure B).
- Start chamber and removable escape tunnel underneath the Barnes maze.
- Video recording equipment connected to a computer for video capturing.
- Novel visual cues. Cues should be neutral colours*.
- Lux meter to measure the light intensity in the testing room*.
- A curtain or screened-off area for the experimenter to be hidden from the animals during testing, if available.

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

PREPARATION

1. Check AEC approvals to ensure the correct procedure and personnel are approved for the planned work.
2. Prepare equipment, including disinfecting, prior to first use.
3. Light intensity should be ~ 1000 lux* and remain the same for all animals within an experiment.
4. Bring animals into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to the start of the experiment.
5. Four distinct visual cues are placed on the walls of the behavioural room at the same height as the maze, typically 30-50 cm away from the arena.
6. Each animal should be handled daily for 30 s to 1 min for at least 2-3 days before the testing. Handling of rodents as per: [LAB_006 Handling and Restraint in Mice and Neonates](#)

PROCEDURE

- Each animal is tested individually. It is essential to clean the maze and escape tunnel thoroughly after each session.
- Habituate the mice to the Barnes maze apparatus at least 1hr prior to testing by exposing them to a Barnes maze for 5-6 min.
- For habituation, attach the escape chamber and place a clean paper towel at the end of the chamber (this will act as bedding and should help to reduce stress).
- Place the animal into the escape tunnel. After 1 minute, gently remove the animal from the chamber and place it in the center of the maze.
- Allow the animal to explore until it enters the escape tunnel or after 5 minutes have elapsed.
- If the time has elapsed, gently move the animal to the tunnel. After 30 seconds, place the animal in the home box.

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ACQUISITION TRAINING (1-10 days)

Barnes maze testing can be performed either using a one-day learning paradigm** or a multiple-day learning paradigm**. There should be at least 1hr between habituation and onset of acquisition. A common training schedule is three acquisition sessions a day (minimum 1hr between trials) per animal.

1. Gently remove the animal from its home cage and place it onto a laminated square of paper – do not let go of its tail.
2. While still holding the tail, carefully lower the starting chamber, which comprises a darkened beaker, making sure the beaker's spout is aligned with the tail to prevent any potential injury (refer to Figure C).
3. Place the laminated square, along with the animal and the chamber, onto the starting position, positioning it at the center of the quadrants opposite to the escape hole (Figure B).
4. Carefully pull the laminated square out without releasing the animal.
5. Wait for 15 s.
6. Lift the start chamber and retreat behind the curtain.
7. The animal is allowed to move freely through the maze.
8. The trial stops when the animal enters the escape tunnel or 3 minutes have elapsed. If the animal fails to enter the escape tunnel, place the animal gently in the escape tunnel for 15 sec.
9. At the end of the trial, remove the animal and return it to the home cage.
10. Click the stop record button for the camera.
11. Clean the maze and escape tunnel thoroughly with paper towelling and disinfectant. Ensure all urine and scat are collected and placed in the sealed bin.
12. Set up the computer for the next animal and repeat the steps above.
13. While the trials are in progress, the researcher generally remains in the testing room (behind the curtain) or anteroom and monitors the animal's performance from the computer screen.
14. Home cage bedding should remain the same throughout the behavioural testing period as this may provide a new stimulation and affect the behaviour.
15. Depending on absorption, drugs/compounds can be given within appropriate times before introducing the animals to the arena. Drug administrations as per the relevant SOP for injection type (examples below):

[LAB_028 Injections - Intra-peritoneal \(IP\) in Mice, Rats and Neonates](#)

[LAB_029 Injections - Intramuscular \(IM\) in Mice and Rats](#)

[LAB_030 Injections - Intravenous \(IV\) tail vein, in Mice and Rats](#)

Probe trial (optional 1 day)

1. The probe trial is optional and is used to test the memory consolidation of the animal after the acquisition/learning phase. Most use a 24-hour delay before initiation of the probe trial.
2. Do not add the escape tunnel to the maze.
3. Place it in the center of the appropriate quadrant on the apparatus.
4. Wait for 15 s and then lift the start chamber.
5. Typically, a well-trained animal will move directly to the escape hole where the tunnel was

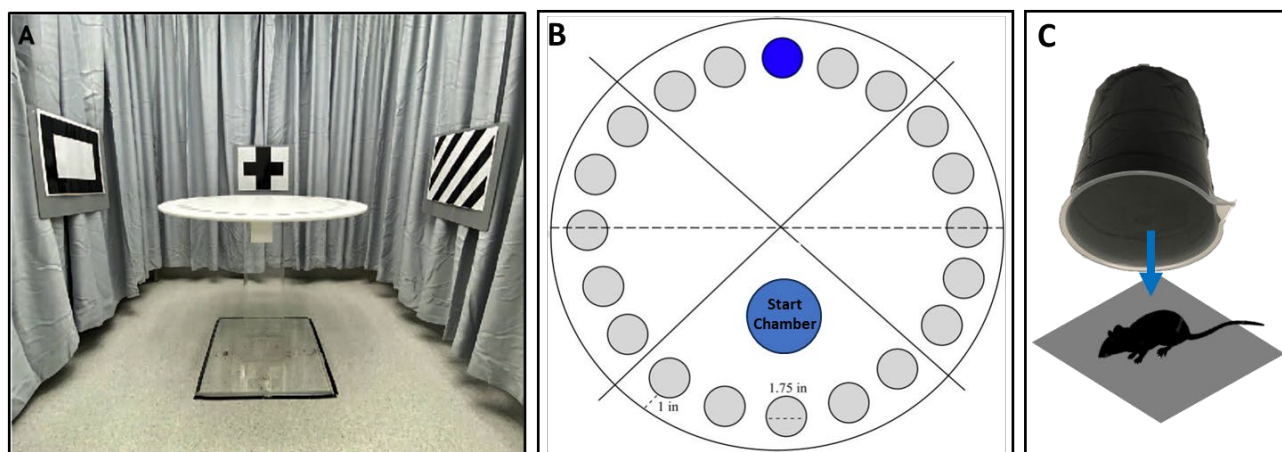
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located, thereby showing spatial bias and evidence of spatial learning.

6. Stop the trial after 1 min.
7. Collect the animal and return it to its home cage.

ANALYSIS

1. Data is analysed using the Ethovision* animal tracking program.
2. During the test, latency to locate (primary latency) and enter (total latency) the escape tunnel is measured.
3. Number of incorrect holes checked (primary errors).
4. Distance travelled prior to locating escape and total distance for each trial.
5. Percent of holes searched.
6. Search strategy- Spatial, serial or random



REFERENCES

1. Blackmore, DG., Brici, D. and Walker, TL. Protocol for three alternative paradigms to test spatial learning and memory in mice. STAR Protocols. 2022; 3(3): 101500. doi: 10.1016/j.xpro.2022.101500.
2. Attar A, Liu T, Chan WTC, Hayes J, Nejad M, et al. (2013) A shortened Barnes maze protocol reveals memory deficits at 4-months of age in the triple-transgenic mouse model of Alzheimer's disease. PLOS ONE 8(11): e80355. <https://doi.org/10.1371/journal.pone.0080355>

The development of this Standard Operating Procedure is duly attributed to Dr. Daniel Blackmore, whose contribution warrants formal acknowledgment. Dr Blackmore is a Research Fellow, an Affiliate of Clem Jones Centre for Ageing and Dementia Research, and the Animal Behaviour Facility Manager at the Queensland Brain Institute (2026).

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Version #	Reviewing AEC	AEC Review Date	Outcome
1.1	Health Sciences AEC	21/05/2026	Approved to 21/05/2029