

LAB_079 Active Place Avoidance (APA) for Mice

I. OBJECTIVE

To describe the procedure for measuring hippocampal-dependent spatial learning whereby the animal is expected to learn to utilise visual cues to avoid a stationary aversive shock zone whilst the grid rotates. The APA can be used for measuring spatial learning, reversal learning and memory consolidation.

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

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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 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 APA testing at a similar time each day.

III. EQUIPMENT

- PPE*

Minimum PPE is gloves, gown and mask, 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.
- APA apparatus – The APA apparatus comprises an elevated arena, 80cm in diameter, with a metal grid floor enclosed by a 32 cm-high transparent circular boundary. The metal bars are evenly spaced (0.5 cm apart) and have a 0.3 cm diameter (Figure 1A).
- The arena typically rotates at a speed of 1 rpm. A pre-designated 60° stationary shock zone, defined by the user via a computer program, is set within the rotating arena. A foot shock (500 ms, 60 Hz, 0.5 mA **) is delivered when the animal enters the shock zone (Figure 1B).
- The location of the shock zone remains constant during each phase of testing and is set electronically within the experimental setup. The shock zone can be changed if the testing paradigm requires it, such as with reversal learning.
- Video recording equipment connected to a computer for video capturing.
- Novel visual cues. Cues should be neutral colours* (See Figure 1A).
- Lux meter to measure the light intensity in the testing room.
- A curtain or screened off area for 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.

Conditions:

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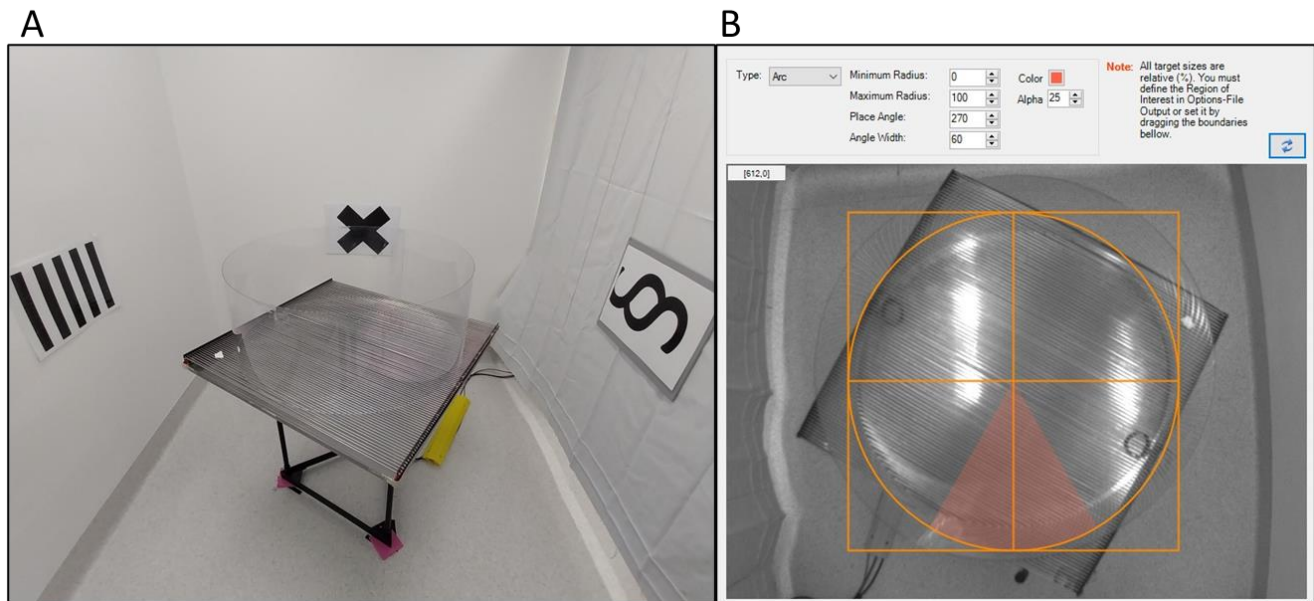


Figure 1: Example of APA arena. (A) This shows a typical APA set up with a clear plastic Arena so that the mice can see the visual cues placed evenly around the room. Note the difference in shapes between each cue. (B) The computer program allows the arena to be tracked during the task. The shaded red area denotes the shock zone.

IV. PREPARATION

- Check AEC approvals to ensure the correct procedure and personnel are approved for the planned work.
- Prepare equipment including disinfecting prior to first use.
- Light intensity should be between 30-70 lux* and remain the same for all animals within an experiment.
- Bring animals 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.
- Four distinct visual cues are placed on the walls of the behavioural room at the same height as the rotating platform, typically 30-50 cm away from the arena.
- 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](#)

V. PROCEDURE

- Each animal is tested individually. It is essential to clean the arena thoroughly after each session.
- Habituate the mice to the APA apparatus 24 hours prior to testing by exposing them to the rotating arena for 5 min without delivering shocks.

Learning paradigm (1–5 days)

1. APA testing can be performed either using a one-day learning paradigm** or a five-day learning paradigm**. For the one-day paradigm a single, 30-minute trial should be conducted. For the five-day learning paradigm, perform one 10 minute** trial, 24 hours apart, each day for 5 days.
2. Ensure that the location of the shock zone and the visual cues remain constant for all trials.
3. Set up the tracking program* as needed and save the configurations.

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4. Gently place the animal onto the APA apparatus opposite the shock zone, along the outer edge of the arena and start a trial.
5. Animals are allowed to move freely around the apparatus.
6. At the end of the trial, remove the animal and return them to the home cage.
7. Save tracking data from the animal.
8. Clean grid thoroughly with paper towelling and disinfectant. Ensure all urine and scat is collected and placed in the sealed bin.
9. Set up the computer for the next animal and repeat steps above.
10. 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.
11. Any noise and odour should be limited during the trial, as this can provide the animal with another cue, affecting their performance.
12. Home cage bedding should remain the same throughout the behavioural testing period as this may provide a new stimulation and affect the behaviour.
13. 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](#)

Reversal acquisition (optional 1 – 6 days)

1. In the reversal test, the shock zone is re-located to a new location, typically 180 degrees from the original shock zone and the mice are tested for their ability to flexibly learn a novel location.
2. The room cues are not changed during reversal learning.

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. Typically, a well-trained animal will avoid entering the shock zone for a prolonged period (>60 secs), showing spatial bias and evidence of spatial learning.
3. Shocks are not delivered for this trial.
4. Animals are allowed to move freely through the maze.
5. The trial is stopped when the animal enters the shock zone area.

Analysis

1. Data is analysed using and tracking program*.
2. Total distance travelled, latency to enter the shock zone, number of shocks received, and maximum time spent avoiding the shock during the trial are usually analysed.

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VI. BIBLIOGRAPHY

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