

SOP No: AHT 46

SUBJECT: Morris Water Maze - Rats

POLICY: This technique may only be performed by operators skilled in the technique.

PRECAUTIONS:

EQUIPMENT: The maze itself is a cylindrical black PVC plastic pool of 160cm diameter which is filled to a depth of either 2.5 cm above the hidden platform, or just below the platform disc of the visible platform (see below) with water at $25\pm 1^{\circ}\text{C}$. The depth and temperature of the water is checked at the start of each day's testing and adjusted as required.

The maze is placed directly under a fitted camera, with at least 30cm gap between the maze and all walls, prior to being filled with water from the taps.

Curtains are drawn to separate the computer station from the maze.

Lighting (at the centre of the maze) as measured using the light meter is set at ~90 lux using the three lamps around the room – not the fluorescent lights.

Two platforms are used for the tests:

A white-topped visible platform, which is located always in the North-East position (for each visible trial and each animal), and is situated at the water surface, so that the bottom of the platform disc is on top of the water surface.

A clear square platform (10 cm x 10 cm), which is placed in one randomly assigned quadrant of the pool 2.5 cm below the water surface. The location of this escape platform is kept constant for each animal, but is never designated at the North-East position.

Around the maze on the walls are extravisual cues, consisting of large black and white symbols/shapes, with which the animal uses to orientate itself. These must be kept consistent for all trials.

Water temperature and light readings are made every day and recorded in a lab book, along with the name(s) of experimenter, date of test, age, intervention and ID of animals being tested of each animal before and after each session.

Software and video recording instruments are set up to record the position of the platform and the position of the pool, as well as to record the specific path of each animal – operators should be trained on this software prior to performing experiment.

During the trials, the experimenter is on the other side of one of the curtains where the software system is set up. There is a video link up with a television screen allowing the investigator to watch the animal perform the task, and be on hand if any intervention is required. The water stays in the maze for the duration of the procedure (one week) and any faecal matter removed with the fish net as soon as evident to limit bacterial growth

At the end of the week of testing, the maze is emptied and then cleaned with 70% ethanol.

PROCEDURE:

1. Trial protocol is the same regardless of platform position.
2. Animals are brought up to the room at least 30 mins before the first trial.
3. On the day of testing, only animals which are being tested in the apparatus that day are to be brought into the room.
4. A trial consists of placing a rat in the pool facing the wall in one of four starting locations. The starting location is randomly assigned, but all four locations are used for each session.
5. Experiments consist of 12 trials (3 sessions) with a visible platform, followed by 20 trials (5 sessions) with the submerged platform.
6. Each trial consists of the following: the rat swims around in the pool for up to 90 seconds, or until it finds the platform. If it does not locate the platform, it is gently guided there. If the animal jumps off the platform, it is repeatedly guided to the platform. In all instances, the animal remains on the platform for a total of 30 seconds. The rat is then removed from the maze and thoroughly dried with a towel. The latency, and also the strategic path used by the animal to locate the platform are recorded. After successful training, the mean latency to locate the platform is about 15 seconds, after which the animals consistently remain on the platform for 30 seconds without jumping off.
7. The next trial with the next rat commences with the corresponding platform location.

- 8. Intertrial intervals are set at 30 minutes.**
- 9. A typical week is as follows;**
 - Monday 10 AM – visible platform (in NE) for 4 trials, with 30 mins between trials for each animal – should finish at 12PM**
 - Monday 2PM (starting two hours after the completion of morning session) – 4 trials with visible platform (in NE) as above.**
 - Tuesday 10 AM – see Monday AM.**
 - Tuesday 2 PM – 4 trials with submerged invisible platform (which changes for each animal, but is kept constant for the individual animal). You may need to change the depth of water at this point.**
 - Wednesday 10AM – 4 trials with submerged platform, with 30 mins inter-trial interval**
 - Wednesday PM – see Tuesday PM**
 - Thursday AM – see Wednesday AM**
 - Thursday PM – see Wednesday PM**
 - Friday AM – probe trial. One trial (90 sec) with no platform. Swimming speed and the measure the amount of time the rat spends swimming in the quadrant previously associated with the platform is measured from this trial.**
- 10. At the completion of each session, each rat is given a reward (condensed milk or other) and returned to the home cage and animal house. If animals appear to be chilled (noted by shivering) at the end of testing, they will be housed on a heat pad overnight.**

RECOMMENDATIONS

Special Circumstances;

The stress status of each subject should be monitored throughout the test session. Testing will be terminated for a subject:

If excessive vocalisation occurs when the experimenter is about to pick the animal during the testing times, including for the first trial.

If excessive aggressive behaviour towards the experimenter occurs during the testing times, including for the first trial.

If an animal fails to swim (ie: floats) in four consecutive (ie for all trials conducted in one day).

Also, if an animal fails to recover its body heat between trials (ie: if it is shivering or cold to touch), the animal will remain in its cage for a further five minutes. If there is no improvement after this time, water maze testing for this animal will be terminated.

Variations to this protocol do exist, particularly when pharmacological intervention is required (depending on the specific kinetics [i.e. half-life] of the compound in question), and must be arranged with the behavioural testing facility manager/AEC committees.

Note; Protocol for mice essentially the same, except the dimensions of the bath may vary (i.e. smaller).

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REVISED:

REFERENCES

1. Morris R (1984). "Developments of a water-maze procedure for studying spatial learning in the rat". *J Neurosci Methods* 11 (1): 47-60.