

Morris Water Maze 莫氏水迷津

1. Purpose

- 1.1 The Morris water maze can be used to investigate the spatial learning and memory in testing mice by measuring the latency that animal learn to escape the aversive water tank with some spatial cues around it. This task can be used to investigate the various issues of learning and memory and memory-related neurodegenerative disorders.

2. Safety Requirements

- 2.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

3. Notes

- 3.1 The validity of results obtained from behavioral phenotyping is largely dependent on methods of animal husbandry. It is important that individuals following this procedure are experienced and aware of the animal's welfare, and be familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.
- 3.2 The majority of mouse behavioral studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.
- 3.3 Environmental factors may contribute to the levels of mouse anxiety. The water temperature, humidity, ventilation, noise intensity and light intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained. In particular, background noise and illumination levels should be measured and documented for each room. Ideally, all mice should be exposed to the same illumination levels in the holding room. For example, in conventional housing, mice housed on the top of the racks may have up to 10 times more Lux than mice on the bottom of racks. No additional experiments which are either noisy or emit odors should be performed during acclimation and testing in the antechamber and the testing room. Ensure that during the test animals are not exposed to any distracting visual signals.
- 3.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day. The ideal testing time for all animals is during the first half of the lights-on period (in the morning until early afternoon). If animal groups are tested at different times of the day it is necessary to perform subsequent tests analogous to the initial test in the experimental design.
- 3.5 Light is an important anxiogenic factor that will strongly influence the ambulation in a U-shaped way. Experiments under various illumination intensities and factor-analysis have shown that locomotion under dim light is a measure of activity rather than of fear (e.g. Trullas & Skolnik 1993). Most pharmacological studies have shown that anxiolytic agents are more likely to

have an effect on the area of the activity rather than on the amount of activity itself (Crawley & Paylor 1997; Choleris et al. 2001).

4. Quality Control

- 4.1 Before each experiment, measure illumination with a Luxmeter and make a record. To obtain evenly distributed light, indirect lighting should be employed. Alternatively, an illumination system for simple and even illumination can be employed. It is most important that light is not significantly darker in the corners (avoid shadows), to prevent bias in the locomotor activity of the animal.
- 4.2 The water temperature should be maintained consistently to avoid any unnecessary confounding.

5. Equipment

- 5.1 Illumination system, thermometer, EthoVision video tracking system.
- 5.2 The test arena is constructed as following:
 - 5.2.1 The water tank is 120 cm in diameter and the circular target platform is 10 cm in diameter and submerged 1 cm under the water surface. Water level in the tank is 30 cm in height from the base.
 - 5.2.2 The water temperature is maintained at $22 \pm 2^{\circ}\text{C}$.
 - 5.2.3 The base color of the water tank is white when C57BL/6 mice are the subjects when a video-tracking system is used.
- 5.3 Video-tracking system. The EthoVision video tracking system is used to analyze data with different zones/parameters.

6. Supplies

- 6.1 Pens
- 6.2 Marker pen
- 6.3 Datasheet
- 6.4 Gloves
- 6.5 Facial mask
- 6.6 Ethanol 70%
- 6.7 Hand towels
- 6.8 Kimwipes
- 6.9 Color paper
- 6.10 Battery

7. Procedures

- 7.1 Minimum $n=6$ mice per experimental group.
- 7.2 On the day before testing mice should be individually marked to be easily identified on the test day. One suggestion is to mark their tails.
- 7.3 Transport animals for testing and leave undisturbed for 30 minutes before the test in the temporary individual cage in the testing room.
- 7.4 Ensure that the light conditions, water temperature are set appropriately and that all equipment is working correctly.
- 7.5 Switch on the computer and record mouse activity by the EthoVision video tracking system for a maximum of 60 seconds.
- 7.6 Remove a mouse from its temporary holding cage, gripping the tail between

the thumb and the forefinger and place it in the desired starting point by facing toward the center of water tank. It is important to ensure that the tracking of each mouse starts as soon as the mouse is released to make data comparable.

- 7.7 Following the experimental session, remove the mouse carefully from the apparatus, gripping the tail between the thumb and the forefinger. Drying the mouse before placing it back to the temporary individual cage in the testing room.
- 7.8 Cleaning the water tank if there are any feces in the tank.
- 7.9 Set up the appropriate database and arena range in the program so that mouse tracking can be analyzed by EthoVision system software.
- 7.10 Save data from the experimental sessions onto a disc and analyses.
- 7.11 The results are presented in 1 excel file and 1 track image.

