

STANDARD OPERATING PROCEDURE

TITLE: Delayed Matching-to-Place (DMP) Water Maze

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Delayed Matching-to-Place (DMP) Water Maze. The DMP Water Maze evaluates spatial memory and working memory, and parameters collected include escape latency, thigmotaxis, distance moved, and velocity. This protocol is designed for mice but may be modified to fit rats. As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials

- *Subjects:* any strain of mice. Subjects require training and should be acclimated to testing environment and experimenter before testing.
- *Apparatus:* circular water tank with 6ft diameter and 2ft depth.
- *Platform:* plastic platform (1'6" height) with circular podium (diameter 1/10 of tank diameter) with rough surface to provide enough friction for subjects to walk/run/rest, placed 1/3 length of tank diameter away from tank wall. Tank should be filled with clean water until platform is submerged 1cm beneath surface.
- *Tempera paint:* at least 32fl oz. poured into empty tank until platform is invisible when viewing from water surface. Used to make water opaque and create detectable background image for tracking software.
- *Overhead camera:* mounted to ceiling directly above apparatus.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blinds:* placed around apparatus at least 1ft from perimeter of tank.
- *Visual cues:*
 - *Platform visual cue:* ping pong ball with black stripes, used to indicate platform location during Visible Platform Training.
 - *Privacy blind visual cues (at least 4):* paper printouts of shapes, one attached to center of each privacy blind.
- *Standing lamps (4):* spaced evenly around apparatus.
- *Light meter:* used to measure illumination in arms of apparatus.

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- *Aquarium heaters*: used to keep up to 450gal water within 1° of 25°C (only necessary if testing room is not equipped with thermostat).
- *Aquarium net*: used to collect debris and other particles from water.
- *Water pump*: used to drain tank after each colony or maximum of 16 days.
- *Bleach*: diluted 1:32, used to disinfect tank after draining.
- *Radio*: used to create background noise (only necessary if external noise outside testing room interferes with testing).
- *20% ethanol*: used between trials to eliminate visual and olfactory residue in arena.

Test Settings

- *Temperature*: room and water should be approximately 22°C.
- *Lighting*: consistent illumination throughout apparatus, as dim as possible to reduce stress and platform visibility for subjects while providing adequate illumination for detection via tracking software (approximately 75lux).

Detailed Standard Operating Procedure

Before testing:

- *Acclimation*: subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
- *Subject training*: Visible Platform Training follows 7-9 days of Hidden Platform. Animals with failure to learn during Visible Platform Training should be considered for exclusion.

Testing procedures:

1. Hidden Platform (Day 1)

- Hidden platform is placed in Day 1 position as indicated in Figure 1.
- Tempera paint is stirred until platform is invisible when viewed at water level. If platform is visible, more paint should be added.
- Debris is removed from tank using fish net and water temperature is verified to lie +/- 1°C from 22°C.
- Animal is placed at first specified drop location (see Table 1 for recommended drop locations). Trial begins immediately and ends when animal rests on platform for 3s. If animal cannot find platform within 90s, animal should be led to platform and allowed to rest on platform for 10s, after which animal should be removed and housed individually in temporary holding cage with paper towel.
- Animals are grouped in fours; after trial 1 is run for each of the four animals, trial 2 is run for each of the four, using same platform location but new specified drop location, then trial 3, then 4. Each animal should have at least 8-12min break between trials.
- At end of trial 4, animal is removed from apparatus and returned to home cage. Once entire group of four animals has completed trial 4, temporary holding cages are cleaned with 20% ethanol and paper towel is replaced.

2. Hidden Platform (Days 2-7)

- Hidden platform is placed in Day 2 position as indicated in Figure 1. Four trials are run per animal, as in Hidden Platform Day 1, using drop locations indicated in Table 1.
- Procedure is repeated for total of 7 days, though testing can continue for additional 2 days if necessary.

3. Visible Platform Training

- Platform is placed in position V as indicated in Figure 1, and platform visual cue is attached to platform.
- Four trials are run per animal, as in Hidden Platform Days 1-7.

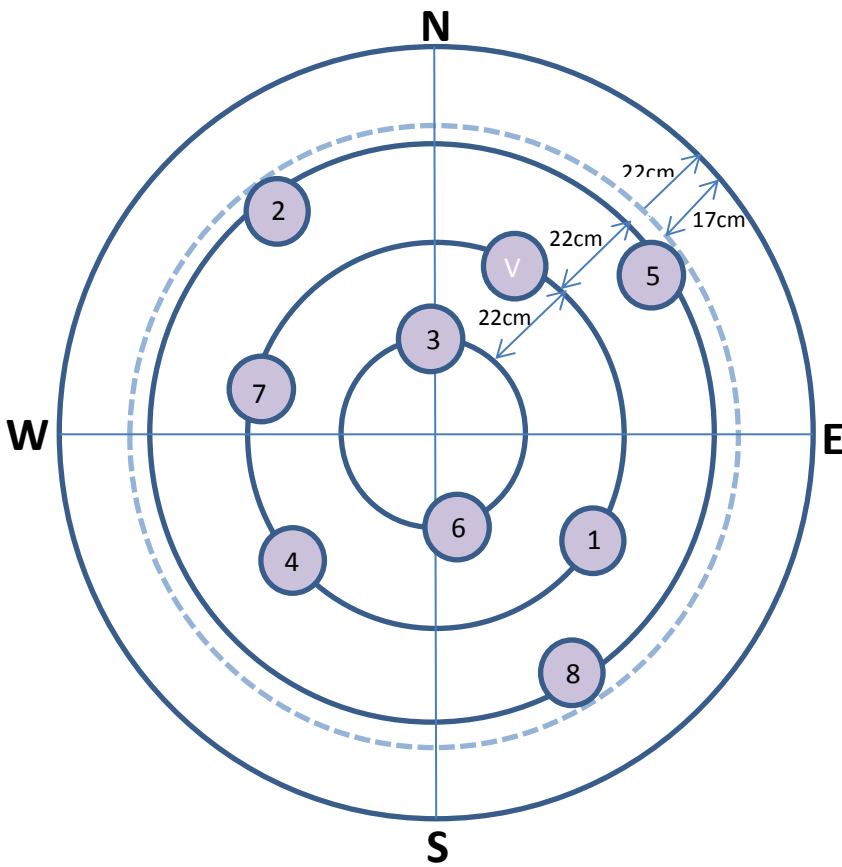


Table 1. Drop Locations

	Trial 1	Trial 2	Trial 3	Trial 4
Day 1	SW	NW	W	N
Day 2	SE	SW	NE	S
Day 3	S	E	W	SE
Day 4	SE	N	E	NW
Day 5	NW	S	W	SW
Day 6	NE	W	N	SW
Day 7	SW	NE	S	E
Day 8	N	W	NE	NW
VPT	S	W	SW	SE

Figure 1. Hidden Platform Locations

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Data Analysis

- The following parameters are collected for analysis:
 - Distance moved
 - Mean velocity
 - Time in each zone
 - Escape latency
 - Thigmotaxis