



STANDARD OPERATING PROCEDURE

TITLE: Morris Water Maze

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Morris Water Maze. The test evaluates spatial 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 below 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.

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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).

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 4 days of Hidden Platform, Probe 1, 4 days of Reversal Hidden Platform, and Probe 2. 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 Quadrant 2 approximately 1cm beneath water surface.
- 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 3sec. If animal cannot find platform within 60sec, animal should be led to platform and allowed to rest on platform for 10sec, after which animal should be removed and housed individually.
- Trial 1 is run for all animals before beginning trial 2, using same platform location but new specified drop location. Total of four trials is run for each animal, with at least 30min break between trials.

2. Hidden Platform (Days 2-4)

- Four trials are run per animal per day, using drop locations indicated in Table 1.

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- Procedure is repeated for total of 4 days. Number of days may vary based on performance of subjects. Ideally probe trial is inserted after Hidden Platform Day 4 when escape latency reaches 15-20s and subjects display searching patterns in platform quadrant.
- 3. Probe 1 & Reversal Hidden Platform (Day 1)**
- Platform is removed and animal is placed in drop location farthest from original platform location. Trial begins immediately and ends after 45s, when animal is removed and returned to cage.
 - Platform is placed in quadrant opposite original platform quadrant and four trials are run per animal as in Hidden Platform Days 1-4, using drop locations indicated in Table 2.
- 4. Reversal Hidden Platform (Days 2-4)**
- Four trials are run per animal as in Reversal Hidden Platform Day 1, using same platform location as Reversal Hidden Platform Day 1 and drop locations indicated in Table 2.
 - Procedure is repeated for total of 4 days of reversal. Number of days may vary based on performance of subjects. Ideally probe trial is inserted after Reversal Hidden Platform Day 4 when escape latency reaches 15-20s and subjects display searching patterns in platform quadrant.
- 5. Probe 2**
- Platform is removed and animal is placed in drop location farthest from last platform location. Trial begins immediately and ends after 45s, as in Probe 1.
- 6. Visible Platform Training**
- If results of Probe 2 are acceptable, platform is placed in Quadrant 1 and platform visual cue is attached to platform.
 - Four trials are run per animal, as in Hidden Platform and Reversal Hidden Platform, using drop locations indicated in Table 3.

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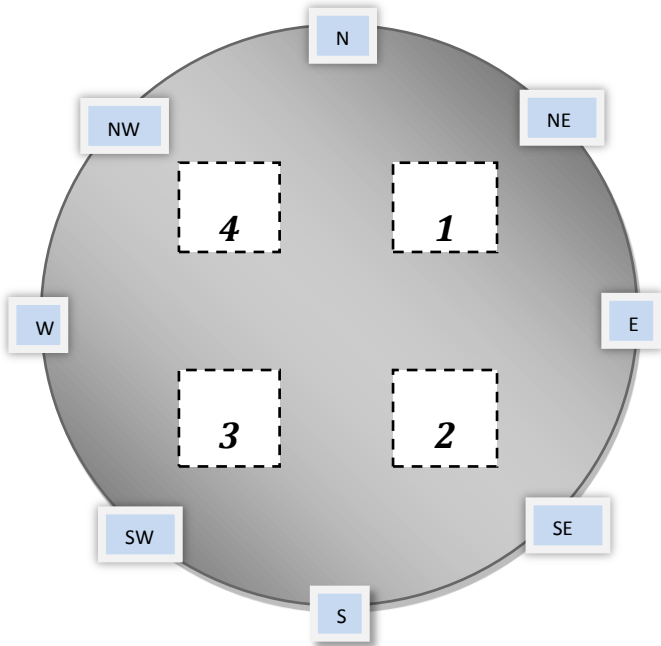


Figure 1. Platform & Drop Location Map

Table 1. Drop Locations

		Trial 1	Trial 2	Trial 3	Trial 4
Hidden Platform (Quadrant 2)	<i>Day 1</i>	N	W	NE	NW
	<i>Day 2</i>	W	NE	SW	NE
	<i>Day 3</i>	N	NW	SW	W
	<i>Day 4</i>	NE	W	N	SW
Probe 1 (no platform)		NW			
Reversal Hidden Platform (Quadrant 4)	<i>Day 1</i>	SE	SW	E	S
	<i>Day 2</i>	S	E	NE	SE
	<i>Day 3</i>	E	SW	SE	S
	<i>Day 4</i>	SE	S	NE	E
Probe 2 (no platform)		SW			
Visible Platform Training (Quadrant 1)		SW	W	S	NW

Data Analysis

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