Morris Water Maze

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Summary:

The Morris Water Maze (MWM) test assesses hippocampal-dependent spatial working memory, learning and memory using a task in which mice swim to a hidden platform using distant (outside the tank), visual landmarks. It can also be indicative of damage to cortical regions of the brain. It can measure the effect of neurocognitive disorders on spatial learning and possible neural treatments, to test the effect of lesions to the brain in areas focused on memory, and to study how age influences cognitive function and spatial learning.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
A 90-100 cm circular tank		
White non toxic liquid paint		
Camera	Basler or Microsoft	
Tracking Software	Ethovision or Panlab/Smart	
Platforms for visible/trial tests		
Thermometer		
Gloves, Lab coat/PPE		
Paper towels		
Clean holding cage/heating pad		
Disinfectant	10% Nolvasan	

Protocol:

1. SET-UP

a. Turn off the overhead fluorescent lights and turn on the side peripheral lights with white bulbs.
b. Check the light level with the light meter, the reading should be around 20-30 Lux. Lights should be dim enough that there is little to no reflection of the water.
c. Fill tank with water to a depth of 2cm below the top of the tank with water level 1cm above top of platform if performing a test trial and 1 cm below the top of the platform if performing a visible trial (bottom of black tape).
d. The tank is placed on a support so that the bottom of the tank is 20-24” above the floor.
e. Opacify water with 25mL of white, non-toxic, powdered or liquid paint. The pool should always be set in the same position in the room and all visible room cues are consistent across trials.

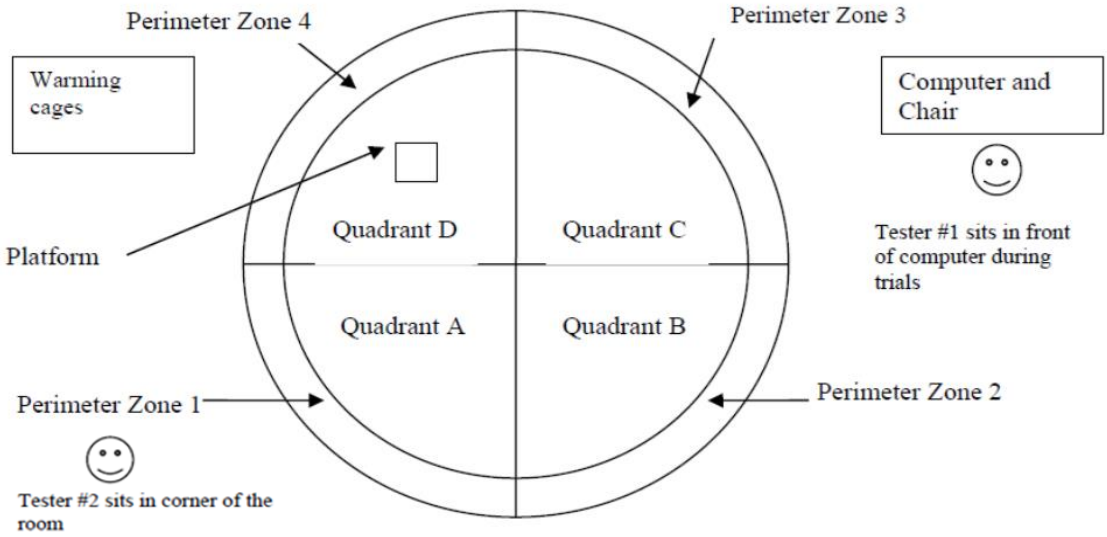
f. The pool is split into four zones as indicated by marks on the outside of the pool.
g. Check the water temperature is 21°C (record on data sheet), water height (should be 2cm below top of tank), and water transparency (stir the water).
h. If water temperature needs to be raised, scoop out a tub full of water and replace with hot water. If water temperature needs to be lowered, add cold water until 21°C is reached.
i. A 6x6cm solid plastic platform is placed at a fixed location in the pool as designated in the
j. configuration file. The top of the platform is a non-slip surface to assist the mice in climbing onto and staying on the platform.
k. For the test trials, the top of the platform is transparent and 1cm below water level so as to not be visible trial. For the visible trial, the platform is 1cm above the water level with dark colored.

NOTE: No platform is used during the probe trial.

2. PROCEDURE

Overview of the test:

The maze is a circular tank divided in 4 quadrants (A to D) with the target platform in quadrant D (see DIAGRAM). The test has three trials (**Visible, Training and Probe**) performed over 4 (males), or 5 (females) consecutive days. Day 1 is the visible trial when mice attempt to swim from quadrant C to the platform, which is visible; this controls for visual acuity. On Day 1, (following the visible trial), training trials begin. These are a series of learning trials in which mice attempt to swim from each of the 4 quadrants to the platform which is now submerged (non-visible). Mice perform 4 trials per day for 4 (males) or 5 (females) consecutive days. For each of the 4 (or 5) daily trials, mice depart from a different quadrant in a fixed order. On the last learning trial day, mice perform the probe trial, which tests the mice ability to reach the target quadrant (platform removed) departing from quadrant B, a measure of spatial reference memory. Distance, time, and entries in each of the 4 quadrants, and time to reach the platform (escape latency) are captured by a videotracking system. The time to reach the platform is an index of the subject's cognitive ability as the mouse will reach to platform faster if it uses and remembers the visible room cues. **NOTE: Males and females use different protocols as described above.**



This test is usually conducted with two people: A handler: responsible for placing mice in the tub, guiding them to the platform, and returning the mice to their home cages after each trial. He or she is also responsible for handling the stopwatch to measure floating time and ensuring mouse is on platform for 30 seconds. A recorder: responsible for keeping track of the files and making sure they are saved in the appropriate folders.

Testing mice:

a. The mouse is held at the base of the tail and lowered into the water facing the edge of the pool at its designated departure point (SEE TABLE below). Place the mouse as close to the edge of the pool as possible to make sure it is within the wall zone (check computer screen to verify). There is a departure point on each intersecting line between two zones (A, B, C and D) half way between the center of the pool and the edge (see diagram). A different point of departure is used on each trial. The order of the departure points is as follows:

Males	Females
Day 1: C (visible trial), A, B, C, A (test trials)	Day 1: C (visible trial), A, B, C, A (test trials)
Day 2: B, C, A, B (test trials)	Day 2: B, C, A, B (test trials)
Day 3: C, B, A, C (test trials)	Day 3: C, B, A, C (test trials)
Day 4: A, B, C, A (test trials)	Day 4: A, B, C, A (test trials)
Day 5: B (probe trial)	Day 5: B, C, A, B (test trials) / B: (probe trial)

Departure points A and C are 40cm from the nearest edge of the platform; departure point B is 50 cm from the platform.

b. The technician retreats to the seat in front of the computer and observes the animal on the computer screen until the animal's trial is finished.

c. On each test trial, the mouse is required to attempt to swim to the submerged platform. The time taken to find the hidden platform is recorded as latency.

d. The mice are to be on the platform for 30 seconds at the end of each trial. A stopwatch is used to measure the time. If the mouse jumps off the platform, time is stopped, the animal is replaced on the platform and the 30 seconds continue (total time on platform is to be 30 seconds). If an animal immediately jumps off the platform twice it is held on the platform by the tail, facing away from the investigator.

e. Animals failing to reach the platform within 90 seconds are held by the tail, turned to face the direction of the platform and guided to the platform while remaining in the water. They are then pushed face forward to climb onto the platform facing the center of the pool.

f. Any time the mouse spends not swimming is regarded as "floating." Floating time is measured using a stopwatch. Stop the stopwatch when the mouse swims and continue the stopwatch

when the mouse starts to float. You may have to do this multiple times if the mouse floats and swims multiple times throughout the trial. The amount of time spent floating shall be scored on a scale of 1-9 with a score of 1 given for less than 10 seconds floating, 11-20 seconds given a score of 2 and so on with a score of 9 given to any animal floating for the full 90 seconds. No floating is designated as "0".

ADDITIONAL NOTE: Floating Score: The float score is the TOTAL TIME the mouse spent floating during the entire trial irrespective of whether the mouse floated consecutively or varied between floating and swimming. For example, if a mouse floated for 11 seconds, swam, and then floated for another 11 seconds, its total float time (float score) is recorded as a 3. Both float time and float score are reported.

No Floating	0
1-10 sec	1
11-20 sec	2
21-30 sec	3
31-40 sec	4
41-50 sec	5
51-60 sec	6
61-70 sec	7
71-80 sec	8
81-90 sec	9

g. The mouse is dried with a towel between trials and rested alone in a clean, empty cage with a wire bar lid placed on a heating pad. The warming cage(s) are kept at a given location so that the room cues remain consistent. The mice should be tested in groups of 4.

h. On the fifth day spatial bias is assessed in each mouse in a "probe trial" in which the platform is removed, and the animals are allowed to swim freely for 90 seconds. This gives an index of spatial memory. The animal is released at the center of the zone opposite where the platform was located.

3. CLEAN UP

a. Return the animals to home cage after time spent in both holding cages. The mouse should be completely dry.

b. At the end of the day clean all surfaces with Nolvasan.

c. After completing the MWM test, empty the pool and scrub with CoveragePlus.

4. SUPPLEMENT INFO-ETHOVISION COMPUTER SET UP:

a. Experimental settings:
- Video source–chose "live tracking"
- Number of arenas–chose "2"
- Tracked features–chose"center-point, nose-point, and tail-based detection"
- Units 1.Unit of distance–cm
  2.Unit of time–sec
  3.Unit of rotation-deg

b. Arena settings:
To align platform in the water maze, use a ruler to make sure that the platform is always the same distance away from the wall.
When setting up the platform zones place the black wire cup on top of the platform to assist in visualizing the location of the platform. There is an entire arena zone, zones for each quadrant, and a target zone for the platform.

1. NE (C): Align circular arena settings to entire pool, align smaller circular zone to platform location (NE), make sure the calibration distance is set to 122 cm and covers the diameter of the pool. Validate arena settings
2. NW (D): Align circular arena settings to entire pool, align smaller circular zone to platform location (NW), make sure the calibration distance is set to 122 cm and covers the diameter of the pool. Validate arena settings
3. SE (B): Align circular arena settings to entire pool, align smaller circular zone to platform location (SE), make sure the calibration distance is set to 122 cm and covers the diameter of the pool. Validate arena settings
4. SW (A): Align circular arena settings to entire pool, align smaller circular zone to platform location (SW), make sure the calibration distance is set to 122 cm and covers the diameter of the pool. Validate arena settings
5. Probe: Align circular arena settings to entire pool, align smaller circular zone to platform location (NE, NW, SE, and SW), make sure the calibration distance is set to 122 cm and covers the diameter of the pool. Validate arena settings

c. <u>Detection settings</u>
   i. Method
      1. Chose “dynamic substraction”
      2. Chose “model-based (XT 5)
   ii. Video
      1. Chose sample rate “30” per sec
   iii. Detection
      1. Chose “subject is darker”
      2. Dark contrast “40-205”
      3. Current frame weight “1”
   iv. Subject size
      1. “min 0 – max 125000”
   v. Subject contour
      1. Check “contour erosion” at 1 pixel
      2. Check “contour dilation” at 1 pixel
   vi. Chose “erode first, then dilate

5. SUPPLEMENT INFO-SMART/PANLAB COMPUTER SET UP:

<u>Experimental settings:</u>

- Select “New Experiment” from the main menu window.
- On the line labeled “CODE” enter the session name (e.g. MWM 9-25-13 Cohort 1 Day 1) and
- click “accept.”
- Click “Image Source” icon on the top menu bar.
- On the drop down menu that reads “Image Source” choose the “Microsoft Lifecam Studio”
- (automatically set at 480x640 resolution).

<u>Arena settings:</u>

- Click the “Configuration” icon on the top menu. This will bring up a frozen image of the water maze with red vertical lines and green horizontal lines. Place the non-visible platform

in the center of the tub and put the calibration ruler on top of the platform. Make sure the units are in cm and put the red and green bars at the inside edges of the red tape on the calibration ruler for both vertical and horizontal calibration. Click "accept." This must be done before the program will allow you to run any experiments.

- Click the "Zones Definition" icon on the top menu. Under the first header labeled "Zones Definition," click the icon with the green arrow pointing right. This will allow you to load the zone template. Choose the zone template labeled "MWM Template 10-10-13" and make sure all zone light up with the tub configuration.

Detection settings:

- Click on the "Detection Settings" icon on the top menu. Default configuration:
  Brightness: 175
  Contrast: 125
- Under the "Detection Settings" header, make sure the "Center of Mass" option is selected for "Track Settings."
- Make sure the box next to "Detection" is checked to apply to all arenas.
- Make sure the Threshold is set at 60 and erosion is set to 1.
- Click on the "Accept" button to save settings.
- Click on the "Time Settings" on the top menu.
- Verify that "Pre-set time" is selected, that "Latency Period" is set to zero, and that "Acquisition period" is set at the correct timing for the experiment (e.g. 1 minute).
- Under "Stop Conditions", select the proper stopping parameter for the experiment (e.g. "After '1' visit(s) to: [Platform]" for MWM acquisition and visible trial).

Data Analysis:

a. In the Pan Lab SMARTv3.0 program, open the desired experimental files.
b. Open the "Analysis" tab at the top menu bar. On the left side under "Trial Schedule" click
c. and drag the trial(s) to be exported into the center window. Each trial will show up as a line of data in the center window.
d. Under the "General Configuration" on the right side, click on the "Zone Definition" tab and find the exact .SMZ file used for testing (i.e. "MWM Template 10-10-13.SMZ"). Highlight all trials in the center window and click the red check mark (next to the "zone definition") to assign the selected zones to the selected trials.
e. Under "Report Definition," check the "Summary Report" tab and click on the icon on the right with 3 dots to manage report definitions. A new window named "Report Definitions" will open.
f. Click on the pencil icon to edit selected reports. A new window will open named "Calculations to include in the Summary Report." On the left side under "Available Calculations" there are several tabs of parameters to include in the data report. Under each of the tabs (listed below), highlight the parameters and click on the arrow icon (in the center) to move them to the "Included Calculations" window on the right side.

NOTE: Make sure the exact order is followed for each of the parameters below. The report is generated in the order each parameter is listed in the "Included Calculations" window.

VISIBLE TRIAL

1. Under the "Subject" tab:
    A. Subject Info
        1. Subject name
2. Under the "Session" tab:
    A. Experimental Info
        1. Exp. File Name
        2. Exp. File Date
    B. Trial Info
        1. Trial Date
        2. Trial Duration (HH:MM:SS,00)
        3. Trial Name
        4. Trial Session
        5. Trial Time
3. Under the "Data" tab:
    A. Tracking Info
        1. Distance in Zone (%)
        2. Distance in Zone (sec)
        3. Entries in Zone
        4. Latency 1st entry into Zone (sec)
        5. Resting Time in Zone (%)
        6. Resting Time in Zone (sec)
        7. Time in Zone (%)
        8. Time in Zone (sec)
        9. Total Distance
        10. Dist. to Target
        11. Latency to Target
        12. Mean Dist. to Target

TEST TRIAL

1. Under the "Subject" tab:
    A. Subject Info
        1. Subject name
2. Under the "Session" tab:
    A. Experimental Info
        1. Exp. File Name
        2. Exp. File Date
    B. Trial Info
        1. Trial Date
        2. Trial Duration (HH:MM:SS,00)
        3. Trial Name
        4. Trial Session
        5. Trial Time
3. Under the "Data" tab:
    A. Tracking Info
        1. Distance in Zone (%)
        2. Distance in Zone (sec)
        3. Entries in Zone
        4. Latency 1st entry into Zone (sec)

5. Resting Time in Zone (%)
6. Resting Time in Zone (sec)
7. Time in Zone (%)
8. Time in Zone (sec)
9. Total Distance
10. Dist. to Target
11. Latency to Target
12. Mean Dist. to Target

PROBE TRIAL

1. Under the "Subject" tab:
    A. Subject Info
        1. Subject name
2. Under the "Session" tab:
    A. Experimental Info
        1. Exp. File Name
        2. Exp. File Date
    B. Trial Info
        1. Trial Date
        2. Trial Duration (HH:MM:SS,00)
        3. Trial Name
        4. Trial Session
        5. Trial Time
3. Under the "Data" tab:
    A. Tracking Info
        1. Distance in Zone (%)
        2. Distance in Zone (sec)
        3. Entries in Zone
        4. Latency 1st entry into Zone (sec)
        5. Resting Time in Zone (%)
        6. Resting Time in Zone (sec)
        7. Time in Zone (%)
        8. Time in Zone (sec)
        9. Total Distance
        10. Dist. to Target
        11. Latency to Target
        12. Mean Dist. to Target