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UC Davis - Morris Water Maze

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

The Morris water maze (MWM) is a widely used tool to study spatial learning and memory. It can assess learning, memory, and spatial working memory and 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.

Materials

Reagents and Materials:

- 90-100cm circular tank
- Platforms, one for visible trial and one for testing
- White, non-toxic, liquid paint
- 50mL glass beaker
- Clean cage, lid, water bottle and heat pad/ mouse tested
- Gloves
- Microsoft LifeCam Studio™
- "Smart" software
- 10% Nolvasan cleaning solution
- Lab coat
- Thermometer
- Stir stick
- Paper towels
- Stopwatch
- Log Sheet
- Pen

1 **Study design and scheduling:**

Time of day: Time of day strongly affects performance. For any experiment, testing should take place during the same 2-3 hour period on each day. Morning or afternoon testing at least one hour prior to dark cycle onset are appropriate. Testing after "lights out" in the vivarium is not appropriate. Software will provide information on this, but it should be specified in the protocol for an experiment.

Mixed sex test groups: Males and females should be tested in different test groups (of 4), preferably on different days and after a water change.

Test order: Test order strongly affects performance. The experimental groups (as designated in the design) should be randomized in the test order. A confounded experiment results if all animals in one group are tested before all the animals in another group. If multiple cohorts are used, the randomization should extend across all cohorts.

Blinding: To assure lack of bias, the experimental groups of the animals should not be displayed on the animal housing, the lab schedule, or the data sheets. Experimental group can be added to the datasets after completion of the study.

Group housing: Social interaction can affect performance. The caging used for the study should be standardized and reported with the data. If more than one animal per cage, the cage of each animal should be designated in the data set so cagemates can be identified. If caging changes (animals separated or dying) this should be reported with the data also. In order to randomize test order when 4-5 mice in the same experimental group are housed together, the mice need to be separated into individual cages prior to starting the study.

Test group size: Because the intertrial interval depends on the daily test group size, animals should always be tested in groups of 4. If eight, twelve or sixteen animals are tested per day, one test group of 4 needs to complete testing before the next one starts.

Protocol deviations: A log of protocol deviations should accompany the data sets to help evaluate "outliers" in the dataset.

2 **Protocol:**

Before beginning open SMART 3.003 program on desktop. Be sure that the video cable connecting the camera to the computer is plugged into the rear USB port.

1. Wear disposable gloves and a lab coat.

2. Turn off the overhead fluorescent lights and turn on the side peripheral lights with white bulbs.
3. 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). The tank is placed on a support so that the bottom of the tank is 20- 24" above the floor. Be sure the platform is in the proper position and the tank oriented correctly (check entry points)
4. Opacify water with 25mL of white, non-toxic, powdered or liquid paint. The pool is always maintained in the same position in the room and all visible room cues are consistent across trials. The pool is split into four zones as indicated by marks on the outside of the pool.
NB: proximal cues: small marks, smudges or irregularities on the inside surface of the tub above the water surface can be used as cues by the mice in locating the platform. It is important, especially if hippocampal function or spatial learning is being tested, to wipe this surface before every session to be sure there are no proximal cues.
5. 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). 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, obtain ice from ice machine and add slowly until 21°C is reached. Be sure to stir. You want to be as close to 21°C as possible!
6. A 6×6cm solid plastic platform is placed at a fixed location in the pool as designated in the configuration file. The top of the platform is a non-slip surface to assist the mice in climbing onto and staying on the platform.
7. The top of the platform is transparent and 1cm below water level for the test trials so as to be non-visible to the mouse. It is 1cm above the water level with dark colored tape for the visible trial. The platform is not used for the probe trial.

3 **CALIBRATION:**

1. Click "Configuration" and select "Calibrate"
 - a. Select "Horizontal Calibration" and the green bars will disappear on the screen. Now you can place the horizontal bars on the video image. Place the calibration ruler on the surface of the platform where the mouse will be moving. Put the bars at the inside edges

of the black tape on the calibration ruler. The units should be set to 30 units for the horizontal bars.

b. Repeat these steps for the "Vertical Calibration" and make sure to select "Centimeters" under the "Units" field. The units should be set to 30 units for the vertical bars.

c. Be sure to do this for each experiment because when the experiments are performed in real time the calibration that is set before the experiment is saved to those specific .trs files that are run that day. So, if the wrong calibration is in the program at the time, we can't go back and re-analyze the files with the correct calibration unless we've saved the video (as an .mpeg or .avi).

4 **COMPUTER**

1. Select "New Experiment" from the main menu window.
2. On the line labeled "CODE" enter the session name (e.g. MWM 9-25-13 Cohort 1 Day 1) and click "accept."
3. Click "Image Source" icon on the top menu bar.
4. On the drop down menu that reads "Image Source" choose the "Microsoft Lifecam Studio" (automatically set at 480×640 resolution).
5. 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.
6. Click the "Zones Definition" icon on the top menu.
7. 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.
8. Choose the zone template labeled "MWM Template 10-10-13" and make sure all zone light up with the tub configuration.
9. Click on the "Detection Settings" icon on the top menu.

10. The setting should default to the following configuration:
 - Brightness: 175
 - Contrast: 125
11. Under the "Detection Settings" header, make sure the "Center of Mass" option is selected for "Track Settings."
12. Make sure the box next to "Detection" is checked to apply to all arenas.
13. Make sure the Threshold is set at 60 and erosion is set to 1.
14. Click on the "Accept" button to save settings.
15. Click on the "Time Settings" on the top menu.
16. 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).
17. 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)

5 **Testing**

Test description: The animal's time taken to find a non-visible submerged escape platform in a circular tank of water is measured. This gives a measurement of its cognitive ability as it navigates to the platform by remembered, visible room cues.

Note: This test is usually conducted with two people:

→Person # 1 is responsible for handling the mice i.e. placing them 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.

→Person #2 is responsible for keeping track of the files and making sure they are saved in the appropriate folders.

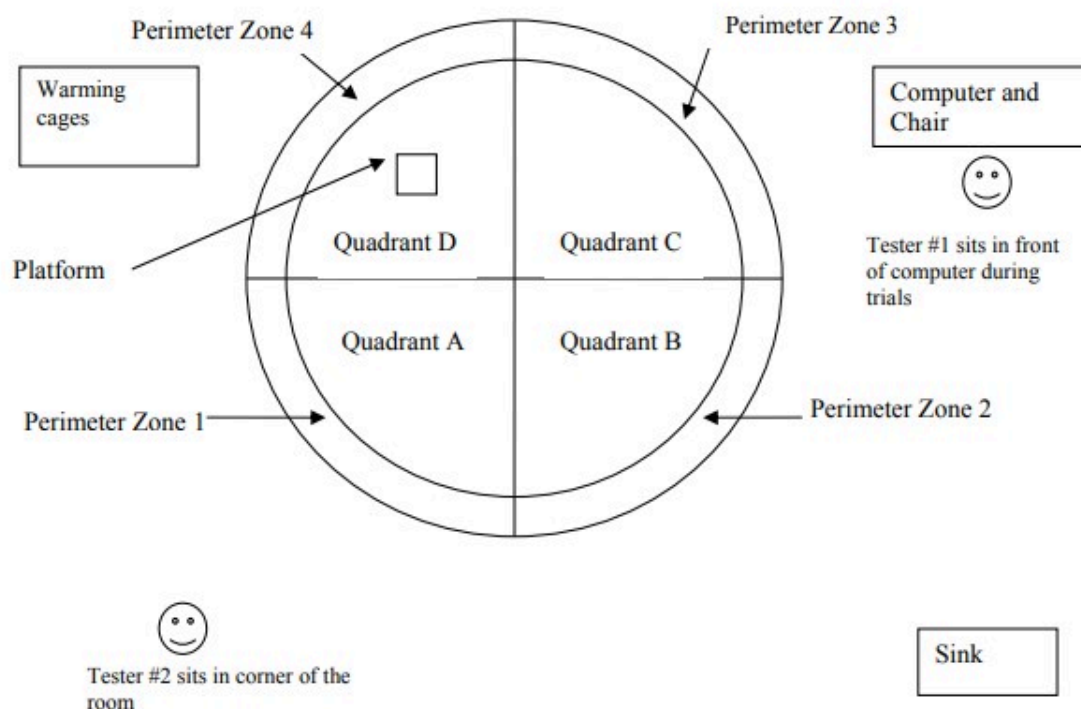
1. The mouse is held at the base of the tail and lowered into the water facing the edge of the pool at its designated departure point. Place the mouse as close to the edge of the pool as possible to make sure it is within the wall zone (check computer screen to verify). There is a departure point on each intersecting line between two zones (A, B, C and D) half way between the center of the pool and the edge (see diagram 1). A different point of departure is used on each trial.

d. At the end of this protocol is the diagram for the positioning of the pool in room (112) and position of numbered zones and lettered departure points.

2. The order of the departure points is as follows:

<u>Males</u>	<u>Females</u>
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)

Departure points A and C are 40cm from the nearest edge of the platform; departure point B is 50 cm from the platform. ***For females, the Day 5 morning trial order is B, C, A, B***



If a reversal is conducted the same procedure is followed during a second series of 5 days conducted 2 days after the first series (skipping the week-end) except that the platform is placed in zone 2.

For **reversal**, different release points are used:

Day 1: D (visible trial), A, D, C, A (test trials)

Day 2: D, C, A, D (test trials)

Day 3: C, D, A, C (test trials)

Day 4: A, D, C, A (test trials)

Day 5: D (probe trial)

3. 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.

4. 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.

5. The mice are to be on the platform for 30 seconds at the end of each trial. Use a stopwatch to verify! 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.

6. 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.

7. Any time the mouse spends not swimming is regarded as "floating." 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 will be designated as "0".

a. Use a stopwatch to accurately count floating time. 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.

Note: The final 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

8. 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 on the social dyadic table across from the computer; room cues such as this must be consistent. The mice should be tested in groups of 4.

9. 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.

6 To Start Recording

1. Click the "Subjects" icon on the top menu. A window labeled "Subjects Database" will pop up.

2. The first line will default to "Subject_01." Click on the edit button (looks like a mouse with a pencil) at the top of the window. An "Edit Subjects" window will appear and you can change the mouse ID, gender, etc.

3. To add additional subjects click on the icon that looks like a mouse with a green plus sign. Add all necessary information (Mouse ID, gender, etc.)

4. Click on the "Scheduler" icon on the top menu.

5. The left hand side labeled "Experiment Subjects" lists all of the subjects previously added.

6. The right hand side labeled "Trial Schedule" will which animals are still scheduled for a run (indicated by a green check mark next to the ID) and which ones have been completed (indicated by a red check mark next to the ID).

7. The Trial schedule is broken down by cohort, day, and trial number. Make sure you are in the correct cohort, day and trial before continuing with the experiment.
8. When you are ready to acquire data, click the appropriate mouse ID in the "Trial Schedule" window. Click on the "Data Acquisition" icon on the top menu and the "Runtime Viewer" window will appear. Verify that the subject code is the correct animal you want to run.\
9. A second window will appear labeled "Time Control." This displays the latency for the run (e.g. 1 minute) and a start/stop button control.
10. When ready to begin data collection, hit the start button. The timer will not start until the program detects an object in the arena to track.
11. Once the trial is over, click the save button at the top of the program screen to save data. A "Save As" window will appear. Save the file with the appropriate name (e.g PI Name MWM Cohort 1 Day 1").
12. After each trial, make sure to click the save icon at the top of the screen. This will save all data currently collected for the session.

7 **Visible Trial (Day 1)**

1. Calibrate using above protocol
2. Set up computer using above protocol
3. The water level for this test must be 1cm below the platform (bottom of the black tape placed around the perimeter of the platform)
4. Departure point B is used
5. Mice that fail to reach the platform on all 3 visible trials should be eliminated from the testing after consultation. They could also be run and then the data not used. This would be preferable if more than 1/cage does not reach the platform on the visible trials
 - a. Alternatively 1 visible trial may be used depending upon study design

8 **PROBE TRIAL- (No platform)**

1. Calibrate using above procedure
2. Make sure water level is 1cm above the platform using the ruler. Once verified, remove the platform from the water and put away

3. Follow computer set-up mentioned earlier:
 - a. Click on the "Time Settings" on the top menu.
4. 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).
5. Under "Stop Conditions", select the "When STOP button is pressed or time expires" instead of the "After '1' visit(s) to: [Zone 5]" for MWM acquisition trial

9 DATASET

1. In the Pan Lab SMARTv3.0 program, open the desired experimental file
2. Open the "Analysis" tab at the top menu bar. On the left side under "Trial Schedule" click 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.
3. 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.
4. 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.
5. Click on the pencil icon to edit selected reports. A new window will open named "Calculations to include in the Summary Report."
6. 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. Target Crossing
6. Resting Time in Zone (%)
7. Resting Time in Zone (sec)
8. Time in Zone (%)
9. Time in Zone (sec)

10. Total Distance
11. Latency to Target
12. Mean Dist. to Target

NOTE: Additional parameters are available and can be included as the study design dictates.

7. Once all parameters have been added to the "Included Calculations" window, click the "accept" button and close the "Report Definitions" window.

8. Highlight all trials in the center window and click the red check mark (next to the "Summary Report" tab) to assign the parameters to the selected trials.

9. Make sure the "Full Trial" option is checked under the "Time Interval" section.

10. Click the "Analyze" button at the bottom of the analysis window. Once the data is generated, a new window will open labeled "Export Preview."

11. Click the "Export" button at the bottom and an excel spreadsheet will be generated. Save this raw data file to the study folder.

12. The float time and float score need to be hand entered into the raw data file.

a. In the raw data file, insert 2 new columns: H and I. This will be between the columns labeled "Trial Duration (HH:MM:SS,00)" and "Distance in Zone – Platform."

b. Hand enter the float time (in sec) and float score for each mouse/trial.

13. Once all float data has been entered, add this excel spreadsheet to the master dataset for the study.