Radial Arm Water Maze

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Summary:

The Radial Arm Water Maze (RAWM) investigates learning and memory performance in neurodegenerative disorders (such as Alzheimer's), models of aging, and drug testing. The test combines the measures of the dry Radial Arm Maze with the rapid learning and aversive aspect of the Morris Water Maze. Rodents (mice or rats) are placed in the center compartment and allowed to explore the arms in search of this reward (the escape platform). The mice must remember which arms have been visited in order to ensure that they do not repeatedly enter arms that do not have the escape platform. The test measures both working memory (ability to recall to avoid the arms previously used for escape during each testing day temporary information), and reference memory (ability to recall valued information, the arm that contain the platform).

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
A 90-100 cm circular tank w. 6 arm radial insert	Conduct Science	
Escape platform (2.5 cm round)		
White non toxic liquid paint		
Camera	Basler or Microsoft	
Tracking Software	Ethovision or Panlab/Smart	
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.** Placed tank is placed on a support so that the bottom of the tank is 20-24" above the floor.

d. Fill tank with water to a depth of 2cm below the top of the tank with water level 1cm above top of platform (the platform should not be visible).
e. Platform is placed at the end of the designated arm with the base touching the end of the arm.
f. 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.
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.

APPARATUS SET UP NOTES:

- RAWM Apparatus: Six arm apparatus (30.5 "diameter, 8" high, 10" long, 5 5/8" wide) in swim pool (100 cm wide). This creates a middle free area of 11" diameter. The escape platform is a 2.5" round platform, 1.0 cm below the water surface.
- Position of the maze in the room:
    - Arm 1 is perpendicular to the back wall.
    - Arm 4 is perpendicular to the front wall.
    - Arm2 is adjacent to a seater score and computer.
- The tester stands between arms 3 and 4.

2. SCHEDULING & DESIGN RECOMMENDATIONS

a. 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***. If a study requires multiple cohorts for testing, each 9-day trial will begin on the same day of the week.
b. Mixed sex test groups: Males and females should be tested in different test groups (of 8 or 16), preferably in different cohorts and ***after a water change***.
c. 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, randomization should extend across all cohorts.
d. Trial Timing: Mice undergo 4 consecutive training trials (i.e. mice remain the maze, either swimming or on the platform). The fifth trial (retention trial) is completed 30-40 minutes following the end of the fourth training trial. The data recorder will keep track of the time the 4th trial ended and when 30 minutes have passed (i.e. with a timer). The training trials for subsequent mice will continue during this 30-40 minute retention period. After 30 minutes, the fifth trial will be conducted on a previous mouse following the completion of any current training trials on a new mouse. All 4 training trials for a new mouse must be completed ***prior to starting*** a fifth trial on previous mouse (i.e. there is no pausing between the 4 training trials).
e. 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.

f. 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 each day of testing. After the testing has completed for the day, mice will be recombined in their original home cage with the same cagemates.

g. Water maze maintenance: Prior to the testing on each day, stir the water in the maze to ensure even opacity of the paint. The maze ***is to be cleaned on day 5***. This includes draining and rinsing the maze and tub with clean water, disinfecting all surfaces with 10% nolvasan solution and re-rinsing with fresh water. After cleaning, the maze will be refilled with fresh water and paint to the correct opacity. On day 9, repeat the cleaning process but do not refill with fresh water/paint if no further testing will be conducted on study.

h. Test group size: Because of the 30-40minute intertribal interval, animals should be tested in groups of 8 or 16.

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

3. PROCEDURE

This test is conducted with two people. The tester is standing between arms 3 and 4 at the tub and reaches to place mouse without moving. The scorer is sitting back by the tabletop by arm 2.

There are 9 daily sessions of 5 trials each.

a. The first four trials are consecutive with the only inter-trial interval being the 30s platform familiarization after each trial (no drying off).

b. The fifth (retention) trial is 30-40 minutes following the fourth trial.

Home cages are placed on the heating pad in the testing room. At the end of the each day, the mice are dried off and placed in the warmed home cage for the 30 minutes.

Mice do not need to acclimate to testing room.

a. The mouse is held at the base of the tail and released in the apparatus facing the middle, at the end of the start arm (see table: **Designated platform start arm sequence)**.

b. The mouse is required to find the platform. If the mouse does not swim to the correct arm, it is pulled back to the start arm by the tail and released again. Do not remove the mouse from water when replacing.

c. Record with Y or N if mouse circles or floats.

d. Count the number of errors (see table: **Scoring of Errors**). The number of errors are counted for the sixty second trial period or until the animal finds theplatform. Record the number of errors (·mark for each error) and the latency to reach the platform.

e. At the conclusion of a trial (60s or finds platform), the mouse is allowed 30s on the platform. If the mouse did not find it, guide the animal across the water to the platform.

f. Proceed to next trials (2 to 5) after the 30s platform time.

Designated Platform Arm Sequence:

Session (day)	Platform Arm	Trial start arm/sequence (arm #)
1	5	3,2,3,1,1
2	3	5,1,5,6,6
3	1	4,5,4,3,3
4	6	2,3,2,4,4
5	2	4,6,4,5,5
6	4	2,1,2,6,6
7	5	2,3,2,1,1
8	3	1,6,1,5,5
9	1	5,3,5,4,4

Scoring of Errors:

If mouse does not swim to the correct platform arm, it is pulled back to the to the start arm by the tail and released again. The animal should reach the end of the arm before it is pulled back (incorrect arm). Do not remove mouse from water.
If the mouse does not leave the start arm for 10s, score as error and replace mouse by lifting the animal by the tail and facing it center.
If the mouse does not leave the center area for 10s, score as error and re-place mouse in start arm facing center.
If mouse somehow clings to any part of the maze, score as error and re-place mouse in start arm facing center.
If animal enters platform arm but does not directly proceed to end where platform is (10 s), score as error and re-place mouse in start arm facing center.

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. DATA MANAGEMENT

All data is recorded on data log sheets and transcribed into the study dataset. Each mouse will have 1 row of data that contains all parameters transposed across columns for the 9-day testing.

1) Recorded parameters:
    a. Test order
    b. Number of total errors per trail
    c. Latency (in sec) to reach the platform for all 5 trials
    d. Floating behavior (y/n) per trial
    e. Circling behavior (y/n) per trial

2) Calculated parameters:
    a. Average latency (in sec) for all 5 trials per day
    b. Average time from start of trial until animal reaches platform, all days
    c. Average number of errors, all Trial 4
    d. Average number of errors Days 8 and 9, Trial 4
    e. Average number of errors Days 8 and 9, Trial 5
    f. Average number of errors, all Trial 1-4
    g. Average number of errors, all Trial 5
    h. Average number of errors, all Trial 5 minus all Trial 4
    i. Average number of errors Days 8 and 9, Trial 5 minus Days 8 and 9, Trial 4

The log sheets will also capture the start time for the first 4 consecutive trials, the end time for the fourth trial and the start time for the fifth (retention trial). The radial arm water maze codebook (Excel spreadsheet) will be included in the dataset. The codebook defines the order of entry for all parameters per mouse.

Ethovision:

Open Ethovision software and the configuration file for the Radial maze test and set the maze so that the arms correspond (1, 2, 3, 4, 5, 6) to the software map file. Activate Ethovision tracking and recording. Data analysis include:

- Number of total arm entries (the animal places all four paws in an arm)
- Number of correct arm entries (the animal enters a novel arm not previously entered)
- Number of error arm entries (the animal enters an arm previously entered or enters a platform-free arm)

$$Memory\ score = \frac{(correct\ arm\ entries) - (incorrect\ arm\ entries)}{(correct\ arm\ entries) + (incorrect\ arm\ entries)}$$