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Working

UC Davis - Radial Arm Water Maze

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ABSTRACT

Summary:

The radial arm water maze (RAWM) measures spatial learning by combining both a water maze and radial arm maze (RAM). This test measures both working and reference memory through a reward system, in this case escape from water. This method is advantageous to other RAM which use food as a reward in that it may be utilized with mice on specialized diets.

Reference: Behavioral consequences of ovarian atrophy and estrogen replacement in the APPsswe mouse. Golub et al. Neurobiol Aging (2008) 29(10): 1512-1523.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=277&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
RAWM apparatus		
Nolvasan		
Tempura paint		Jazz

1 Scheduling and Design:

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.

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.

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.

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).

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 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.

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.

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

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

2 Protocol:

RAWM Apparatus and Set Up:

RAWM Apparatus: Six arm apparatus (30.5 “diameter, 8” high, 10” long, 5 5/8” wide) in swim pool. 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.

1. Place on table in maze room (MBP Rm112). Level the apparatus by adjusting the legs of the table. Tempura paint is added to the water. (platform must not be visible). Room cues include room furniture, poster, ceiling mounted cameras, circular wall clock (10 inch diameter), paper towel dispenser (8 x 10 inch) and the investigator.

2. Adjust water temperature to 21 degrees centigrade. Use lights on side of room and no overhead room lights.

3. Platform is placed at the end of the designated arm with the base touching the end of the arm.

Position of maze in the room:
Arm 1 is perpendicular to the back wall.
Arm 4 is perpendicular to the front wall (with door)
Arm 2 is adjacent to seated scorer and computer table.
Tester stands between arms 3 and 4.

Trial Procedure:

1. 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. The animal is dried off and placed in the warmed home cage for the 30 minutes.

2. Animal home cages are placed on the heating pad in the testing room. No need to acclimate to room. Animals are removed from home cage by the tail (Do not place in palm or carry any other way).

3. The animal is released in the apparatus facing the middle at the end of the start arm. The animal is required to find the platform.

4. Investigator 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. Do not remove animal from water when re-placing.

Scoring of Errors: (record arm number if incorrect, use check marks for other errors)

- If the animal does not swim to the correct (platform) arm, it is pulled back 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 animal does not leave the start arm for 10s, score as error and re-place mouse by lifting the animal by the tail and facing it center.
- If the animal does not leave the center area for 10s, score as error and re-place mouse in start arm facing center.
- If animal 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.

5. Record with Y or N if animal circles or floats.

6. The number of errors are counted for the sixty second trial period or until the animal finds the platform. Record the number of errors (3mark for each error) and the latency to reach the platform.

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

8. Start next trial (1-4) immediately after 30s on platform.

PLATFORM/START ARM SEQUENCE (NO ADJACENT ARM START)

SESSION (Day)	PLATFORM ARM	TRIAL START 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

3 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.