Aug 19, 2020

7-trial RAWM protocol

PLOS One

Teresa Macheda[1], Kelly Roberts[1], Adam D Bachstetter[2]

[1]Spinal Cord & Brain Injury Research Center, University of Kentucky, Lexington KY, USA, Department of Neuroscience, University of Kentucky, Lexington KY, USA;

[2]Spinal Cord & Brain Injury Research Center, University of Kentucky, Lexington KY, USA, Department of Neuroscience, University of Kentucky, Lexington KY, USA, Sanders-Brown Center on Aging, University of Kentucky, Lexington KY, USA

1 Works for me dx.doi.org/10.17504/protocols.io.bb84iryw

Teresa Macheda

ABSTRACT

Cognitive impairments can be a significant problem after a traumatic brain injury (TBI), which affects millions worldwide each year. There is a need for establish reproducible cognitive assays in rodents to better understand disease mechanisms and to develop therapeutic interventions towards treating TBI-induced impairments. Our goal was to validate and standardize the radial arm water maze (RAWM) test as an assay to screen for cognitive impairments caused by TBI. RAWM is a visuo-spatial learning test, originally designed for use with rats, and later adapted for mice. The present study investigates whether test procedures, such us the presence of extra-maze cues influences learning and memory performance. C57BL/6 mice were tested in an 8-arm RAWM using a four-day protocol. We demonstrated that two days of training, exposing the mice to extra-maze cues and a visible platform, influenced learning and memory performance. Mice that did not receive training performed poorer compared to mice trained. To further validate our RAWM protocol, we used scopolamine. We, also, demonstrated that a single mild closed head injury (CHI) caused deficits in this task at two weeks post-CHI. Our data supported the use of 7 trials per day and a spaced training protocol as key factor to unmask memory impairment following CHI. Here, we provide a detailed standard operating procedure for RAWM test, which can be applied to a variety of mouse models including neurodegenerative diseases and pathology, as well as when pharmacological approaches are used.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0232862

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Optimization and validation of a modified radial-arm water maze protocol using a murine model of mild closed head traumatic brain injury

DOI

dx.doi.org/10.17504/protocols.io.bb84iryw

PROTOCOL CITATION

Teresa Macheda, Kelly Roberts, Adam D Bachstetter 2020. 7-trial RAWM protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bb84iryw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Optimization and validation of a modified radial-arm water maze protocol using a murine model of mild closed head traumatic brain injury

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0232862

and source are credited

CREATED

Feb 05, 2020

LAST MODIFIED

Aug 19, 2020

PROTOCOL INTEGER ID

32764

MATERIALS TEXT

- RAWM apparatus (pool, inserts, cues, platform)
- Dimmable lights
- Non-toxic liquid paint
- Stick for stirring the paint in the pool
- Recovery cages
- paper towels
- Heating pads
- Thermometer
- Humidity/temperature monitor
- Light meter
- Computer connected to a video tracking system

1 Experimental Design and Recommendations:

Housing: Behavior is affected by the housing mode. Singly housed mice could perform differently than group housed mice.

Blinding: Experimental groups of mice should be coded while the operator is testing the mice.

Test order: Mice should be randomized in test order, to avoid confounded results if all the mice from the same experimental group are tested before all the mice from a different group.

Trial timing: Inter-trial time in between trials for the same animal is 10-15 minutes, so 10-15 mice could be tested in the same experiment. Mice are tested in 7 trials divided in two blocks (block 1: trials 1-3, block 2: trials 4-7), since a staggered design is used and time in between blocks is in between 50-60 minutes, a second group of mice could be tested during this time break. Mice are tested in cohort of 10-15 mice.

Time of testing: Time of day strongly affects performance. Testing should be conducted during the same time between days and experiments. If a study requires more animals, several cohorts should be tested. It is recommended that each cohort of mice is tested starting the same day of the week and by the same personnel to reduce the introduction of new variability in the task.

Sex: Male and female mice should be tested in separate groups, in particular in different cohorts and after the RAWM equipment has been clean and water changed.

Pool maintenance:

Citation: Teresa Macheda, Kelly Roberts, Adam D Bachstetter (08/19/2020). 7-trial RAWM protocol. https://dx.doi.org/10.17504/protocols.io.bb84iryw

2

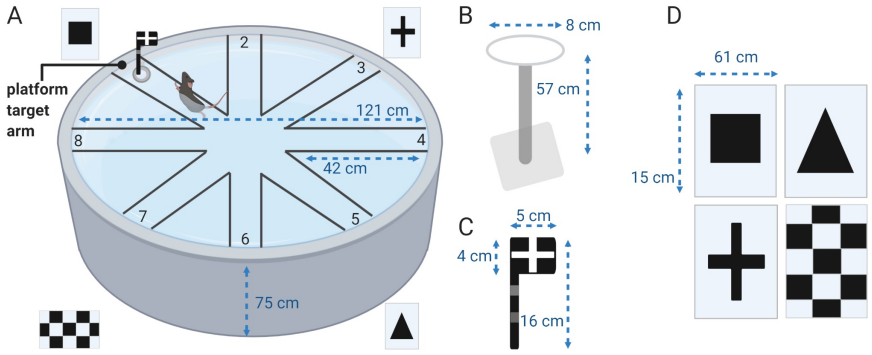
The day or few days before RAWM is performed, the pool should be inspected. Each day prior to the testing, stir water to ensure even opacity of the paint. Pool needs to be cleaned at the end of the experiment: empty the pool and clean it with clean water first, then a disinfecting solution should be used. Arms and platform should be washed and disinfected as well.

3 **RAWM APPARATUS AND SET UP:**

The apparatus is isolated from the rest of the room by double black black-out curtains. Light intensity in each arm and center is 4-6 lux.

Apparatus: Circular platform (diameter 121 cm, depth 75 cm), a 9 cm base and a 8-shaped platform are inserted in the pool. Then eight identical metal inserts (65 cm high by 42 cm long) are placed in the pool to make an 8-arm RAWM. The escape platform is 8 cm diameter and 57 cm high and seats 1 cm below the water surface.

Spatial Cues: Four extra-maze cues (triangle, square, checkboard pattern and cross) are made of white corrugated plastic and black vinyl material and located around the maze. Platform is made visible by a 16 cm tall flag. Cues should remain constant during the entire length of the test, in particular if multiple groups of mice need to be tested over time and data combined for statistical purposes.



Pool and cues used for RAWM test. (A) RAWM pool with the four cues are shown with a mouse swimming to reach the visible platform. **(B)** Pool and arm dimensions are shown, as well as each cues distance from the floor. **(C)** Dimensions of platform, **(D)** flag and **(E)** cues are reported.

Set up:

1. Mice need to be handled for at least 3 days before the test and tails marked for an easy identification during testing.
2. A day or two before the test starts, the pool needs to be inspected for any damage or leaks and the overhead lights need to be tested as well.
3. Add to the pool in order: 9-cm base, 8-shaped platform and inserts to make a 8-arm maze. Inserts could be numbered, so same inserts will be used to make the same arm across experiments.
4. Add liquid paint and refill the pool with water. Arms need to be risen 9-cm above the level of water to discourage mice from escaping.
5. Prepare the experimental file using the tracking software available.
6. Prepare a recording sheet to record room and water temperature, humidity and animal's notes.
7. Set up heating pads and clean recovery cages. Make sure to put only half of the recovery cage on the heating pad, and place paper towels in it. Paper towels should be promptly changed when wet.

4 **Data Collection:**

All data (number of errors, distance, latency, speed) are recorded using the tracking software of your choice.

Citation: Teresa Macheda, Kelly Roberts, Adam D Bachstetter (08/19/2020). 7-trial RAWM protocol. https://dx.doi.org/10.17504/protocols.io.bb84iryw

Scoring of Errors:
An error is scored when the mouse enters an arm that is not designed as goal arm, or when it enters the goal arm without escaping. Also, when the subject spends ≥ 10 seconds in the same arm and/or center an error is added to the scoring.

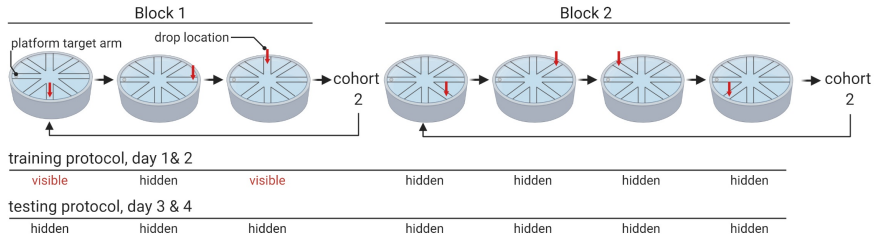
5 Procedure

Mice will be tested in 7 trials per day.

- Acclimation: cages are moved in the testing room and subjects are placed in clean bedding-free recovery cages for at least 30 minutes before testing to minimize effects of stress on behavior during testing. Recovery cages are equipped with paper towels and placed ½ on a heating pad to facilitate the drying process. This allows the mouse to self-regulate. During the test the wet paper towels should be promptly replaced, so mice could have a faster recovery in a dry and warm recovery cage.

- Check pool temperature (21 ± 1° C) before starting with testing procedure.

Testing procedure
Mice are divided in two cohorts of 10-15 mice, each cohort will be tested in block 1 first, then in block 2. Block 1 has a total of 3 trials (trial 1 and 3 visible platform during training, hidden during testing days), instead block 2 consists of 4 hidden trials. Briefly, cohort 1 will be tested in trials 1-3 (block 1), then cohort 2 tested in trials 1-3 (block 1). Cohort 1 will be tested in trials 4-7 (block 2), next cohort 2.



Step-by-Step procedure of RAWM test during training and testing days. During training days (day 1 and 2), the platform is made visible by a flag during block 1 (trial 1 and 3). During the testing days (day 3 and 4), the platform is hidden during all trials. The drop location of the mice varies in a semi-random fashion as shown by the red arrow.To reduce fatigue and learning limitation, a staggered design is used in RAWM test, with one cohort (10-15 mice) tested in a block before and then a second cohort is tested.

Day 1 and Day 2
Block 1: trials 1-2-3
Trial 1 (visible platform):
1. Put the flag on the platform.
2. Gently place the mouse into the first arm facing the pool wall.
3. The trial should last up to 60 s.
4. The software automatically will count the number of errors. Incorrect arm entries occur when the mouse visits an arm that is not a goal arm and when entries in the goal arm are made without escaping. The operator will record if the mouse spend more than 15 s in one place and counts it as an error. Latency, distance and speed will be also recorded.
5. After 1 min, if the platform has not been located, the mouse is gently guided to it.
6. The mouse is allowed to stay 15 s on the platform in both cases when the mouse is guided or if the mouse is able to escape with no help.
7. Remove the mouse from the platform and gently dry him before placing back into its recovery cage.
8. Continue with the other mice in cohort 1.

Trial 2 (hidden platform):
1. After all mice from cohort 1 have had a trial, switch platform from visible to hidden.
2. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

Citation: Teresa Macheda, Kelly Roberts, Adam D Bachstetter (08/19/2020). 7-trial RAWM protocol. https://dx.doi.org/10.17504/protocols.io.bb84iryw

Trial 3 (visible platform):

1. After all mice from cohort 1 have had a trial, switch platform from hidden to visible.
2. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

At this point mice had 3 trials, cohort 2 will start block 1.

1. Repeat steps from *Block 1* for cohort 2.

Mice from cohort 2 will rest at this point, while cohort 1 will be tested in block 2.

Block 2: trials 4-5-6-7

Trial 4 (hidden platform):

1. After all mice from cohort 2 have had a trial, switch platform from visible to hidden and mice from cohort 1 will be tested.
2. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

Trial 5 (hidden platform):

1. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

Trial 6 (hidden platform):

1. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

Trial 7 (hidden platform):

1. Repeat steps 2-8 from trial 1, but the *drop arm* this time will be different.

At this point cohort 1 had 4 trials, cohort 2 will start block 2.

1. Repeat steps from *Block 2* for cohort 2.

Allow mice to dry off before placing them back to the home cage and housing room.

If less than 10 mice/ cohort are tested, more resting time between blocks is required.

Testing day:

Repeat procedure from "training day", but all the trials will be performed with the hidden platform.

Allow mice to dry off before placing them back to the home cage and housing room.

Day 3 and Day 4:

Repeat steps from Day 1 and 2, but this time during block 1 (Trials 1, 2 and 3) the platform will be hidden.

Citation: Teresa Macheda, Kelly Roberts, Adam D Bachstetter (08/19/2020). 7-trial RAWM protocol. https://dx.doi.org/10.17504/protocols.io.bb84iryw