Barnes Maze Protocol

This test is a hippocampal-dependent cognitive test designed to measure spatial learning and memory performed over a total of 11 consecutive days with three phases: habituation, acquisition learning, and reversal learning. The reversal learning phase can be removed if necessary. This test is moderately stressful (white noise, bright lights) and no food deprivation.

Before you begin:

- Place two plastic bags on the bottom of the pool. This will make it easier to clean at the end of the test.
- Place the tripod in the pool and secure the maze. Place the white top on the maze (if you are using white mice, there is a black top that can be used for better tracking).
- Position 1 should be placed underneath the cue nearest you (the *I*) and position 19 (the acquisition target) should be midway between the *I* and the *star*. You will adjust the positions once you open the program. Your target platform should not be directly underneath a cue as this would make the test too easy for the animal.
- Place the speakers on the floor of the room and open the Audacity program. Select *generate* from the toolbar and then *white noise* from the drop-down menu. Select OK. The speaker volume is taped on the remote and the computer should be set at 75%. Once the white noise is selected, use the *shift* + *spacebar* to begin the noise on a loop. The button on the speaker remote will allow you to start and stop the noise with ease.
- Open WaterMaze program and open a new protocol (*.wmpf* file) from the toolbar. From the *Barnes Maze Templates* select the corresponding day. Adjust the number of animals you will be using and ***Save As*** *a new file on the desktop in your folder*. Do not save over the template! (If you do happen to save over the template, they are saved in another folder on the server.) Enter your animal IDs. From the *Design* menu, select *clone trials*. Select all animals and clone trials. This will apply the protocol to all animals. From here on you can save over your file.
- Ensure that the maze and corresponding platforms (1-20) are in position. Each platform should be 14cm in diameter. This is larger than the escape, but since the program tracks the animal body it allows for more accurate tracking when the animal is exploring each hole. Save and exit. You are now ready to begin the test.
- The program will automatically save your data and video files in your folder on the desktop. These files become large, so it is best to transfer all files and corresponding folders off of the desktop to your folder on the server after each day.
- **PLACE THE MICE IN THE GOAL BEFORE EACH DAY OF TESTING TO GET THEM FAMILIAR WITH THE SAFE SPACE.**

Day 0: Habituation (shaping) Trial

There is only one trial per animal today. There are no spatial cues on the wall, as the animals are only being trained to understand that there is an escape box. Each trial is 120 seconds.

1. Place starting cylinder in the center of the platform. Take a **reference** frame.
2. Place the animal in the cylinder. Begin the white noise and after **15 seconds**, remove cylinder and **start**.
3. Animal will explore platform for up to 120 seconds (stop trial if they find and enter the escape box).
4. If the animal does not find and enter the escape box after the trial time, gently guide the animal to the box and allow animal to stay in box for **60 seconds**.
5. Remove animal and clean platform, escape box, and cylinder with 70% ethanol.
    a. Make sure that all of the false boxes are fully in place before starting next animal trial.

Day 1 – 5: Acquisition Learning
There are two trials per animal each day (total of 10 trials). Spatial cues are placed on each wall. Each trial is up to 300 seconds. Target is Platform 19.

1. Place starting cylinder in the center of the platform. Take a **reference** frame.
2. Place the animal in the cylinder. Begin the white noise and after **15 seconds**, remove cylinder and **start**.
3. Animal will explore platform for up to 300 seconds (stop trial if they find and enter the escape box). If animal enters the escape box, stop white noise upon entry and wait for 60 seconds before removing animal.
    a. Time to locate the escape box is measured as *primary latency*
    b. Time to enter the escape box is measured as *total latency*
4. If the animal does not find and enter the escape box after the trial time, gently guide the animal to the box and allow animal to stay in box for **60 seconds**.
5. Remove animal and clean platform, escape box, and cylinder with 70% ethanol.

Day 6: Acquisition Probe
There is one trial per animal. Spatial cues remain on each wall and the escape box is replaced with a false box. Each trial is 180 seconds.

1. Place starting cylinder in the center of the platform. Take a **reference** frame.
2. Place the animal in the cylinder. Begin the white noise and after **15 seconds**, remove cylinder and **start**.
3. Animal will explore platform for 180 seconds.
4. Remove animal and clean platform and cylinder with 70% ethanol.

Day 7 – 9: Reversal Learning (Optional)
There are two trials per animal per day (total of 6 trials). Spatial cues remain in position, but the escape box is moved 180° from the original position. Target is Platform 9. Each trial is 180 seconds.

1. Place starting cylinder in the center of the platform. Take a **reference** frame.
2. Place the animal in the cylinder. Begin the white noise and after **15 seconds**, remove cylinder and **start**.
3. Animal will explore platform for up to 180 seconds (stop trial if they find and enter the escape box). If animal enters the escape box, stop white noise upon entry and wait for 60 seconds before removing animal.
4. If the animal does not find and enter the escape box after the trial time, gently guide the animal to the box and allow animal to stay in box for **60 seconds**.
5. Remove animal and clean platform, escape box, and cylinder with 70% ethanol.

Day 10: Reversal Learning Probe (Optional)
There is one trial per animal. Spatial cues remain on each wall and the escape box is replaced with a false box. Each trial is 90 seconds.

1. Place starting cylinder in the center of the platform. Take a **reference** frame.
2. Place the animal in the cylinder. Begin the white noise and after **15 seconds**, remove cylinder and **start**.
3. Animal will explore platform for 90 seconds.
4. Remove animal and clean platform and cylinder with 70% ethanol.

Analysis

- Open the WaterMaze program and open your "*.wmpf*" data file (can be done in the common office area and will need to be done for each day).
- Select *Analysis* → *Export*
- Select *All animals + All Trials*
    - Make sure that *Time to Platform, Trial Duration, Distance Travelled, and Zone analysis* are selected (should be highlighted by default)
- Export to file (will save as an excel file)

Shortcut

1. Ctrl + O (opens your file)
2. Ctrl + E (export)
3. Ctrl + Shift + A (selects all animals & all trials)
4. Ctrl + E (exports to file)

- From each excel file, you will average each trial. Your first measures will be 1. Time to platform = Primary Latency and 2. Trial Duration = Total Latency. These measures should be plotted against time and analyzed with a Two-Way ANOVA.
    - On the probe days there is only one trial, so each group will be compared using an Unpaired t-test.
- Search strategy is also an important measure. To do this, you will need to watch each trial (can select fast-forward to make it play faster) and determine what strategy the animal used and score as 2, 1, or 0. Analyze with Unpaired t-test.
    - 2: Direct (less than 3 errors)
    - 1: Serial/Random (More than 3 errors. Animal begins at one hole and travels along perimeter to check each hole)
    - 0: Perseveration (Animal either does not explore or find the escape)
- To measure time spent in each quadrant, you will need to add the time spent at the platforms that correspond to each quadrant. This in not ideal since we cannot change the quadrants in the WaterMaze Program as we are running the test, however is still accurate.
    - Quadrant 1 (target): Platforms 17, 18, 19, 20, 1
    - Quadrant 2: Platforms 2, 3, 4, 5, 6
    - Quadrant 3: Platforms 7, 8, 9, 10, 11
    - Quadrant 4: Platforms 12, 13, 14, 15, 16
- Other analyses that can be completed:
    - Primary Errors: Number of incorrect holes checked prior to locating escape
    - Total Errors: Number of incorrect holes checked prior to entering escape
    - Path Length: Distance Traveled (to locating and entering the escape hole)
    - Number of Holes Checked

References

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2. Attar, A.*, et al.* A shortened Barnes maze protocol reveals memory deficits at 4-months of age in the triple-transgenic mouse model of Alzheimer's disease. *PLoS One* **8**, e80355 (2013).
3. Patil, S.S., Sunyer, B., Hoger, H. & Lubec, G. Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. *Behav Brain Res* **198**, 58-68 (2009).