How to perform the Morris water maze test



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Basic behavioral neuroscience in rodents



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The Morris water maze

Developed by Richard G. Morris in 1984, the Morris water maze is a test of **spatial learning**, and relies on swimming (navigating) from a start location, and locating a submerged escape platform. Usually this is assessed over multiple trials, and spatial learning is ultimately assessed in a trial with the platform removed, which will trigger a preference for the previous location of this platform.

In a similar fashion, this can also be used to measure **cognitive flexibility** (as explained before). In this case the original location of the platform (which the animal has learned) is moved to a different location in the maze. The Morris water maze has proven to be a robust and reliable test that is strongly correlated with hippocampal synaptic plasticity and NMDA receptor function.



Mouse water maze
Diameter is ±120 cm
±55 cm raised walls

Rat water maze
Diameter is ±180 cm
±95 cm raised walls

Escape platform
±8 cm in diameter

Protocol suggestion

Preparation

- Fill up the maze with regular tap water, the temperature should be brought up to approximately 26°C (this can take a while!). Use non-fat dry milk, or non-toxic white paint to make the water opaque. This will make the platform invisible to the animals in the testing phase, but also improve contrast of animal to background for video recording and analysis in EthoVision.
- Place the escape platform in the center of the pool. During the training phase, it must be exposed, one inch above the water. This teaches the animal that there is a platform, and that it is the way to get out of the water.
- Place three or four distal visual cues (length/width ~30 cm) surrounding the arena, visible to the animals while subjected to the test.



A Morris water maze is basically a small pool which is then filled with water. Make sure you can properly drain it after use!

Training phase - Each animal will undergo three consecutive trials

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling and place the animal on the platform in the center for twenty seconds.
- After this period take the animals to one of the 4 quadrants of the maze: north, south, east or west: gently lower the animal into the water, and let the animal search for the platform for a maximum of 60 seconds. If the animal doesn't find the platform withing this timeframe, record the maximum amount of time for the trial (60 seconds), and gently guide the animals to the platform with your hand, and let it sit there for 15 seconds. The animals need to be taught to swim to the platform.
- Repeat the same procedure for two more trials, starting at a different quadrant for each trial. Dry the animals afterwards before returning them to their home cage.

Testing phase - Each animal will undergo up to 12 trials.

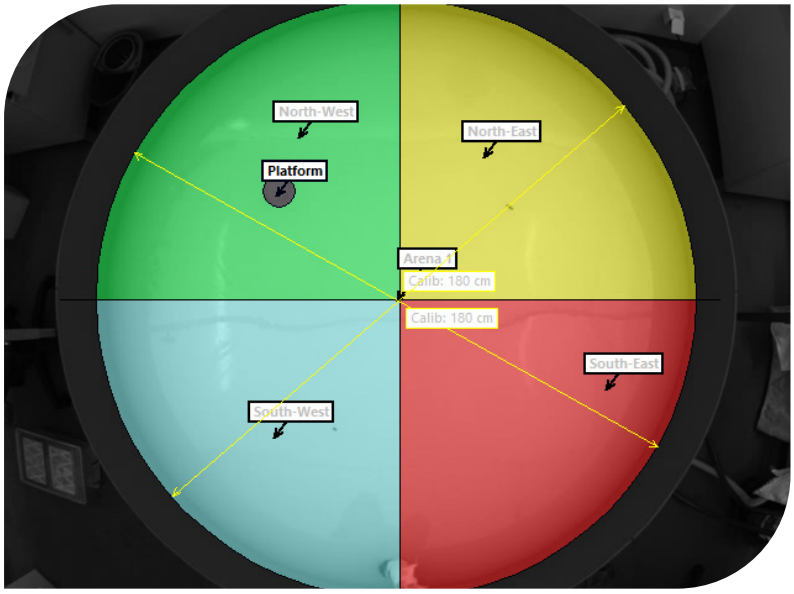
- Fill the water maze with a bit more water, to submerge the platform about an inch below the surface. Make sure the lighting and water temperature are the same as in the training phase.
- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling and place the animal in a quadrant, facing the wall of the maze. Each consecutive trial should begin in a different quadrant (starting direction), but randomize the order! Recording/tracking automatically starts in EthoVision if this option has been selected. Otherwise, do not forget to concurrently activate your video recording.
- Observe the animal until it reaches the platform, and record the time it took. If the animal doesn't reach the platform in 60 seconds, guide it to the platform, as in the training phase. Either way, let the animal sit on the platform for 10 seconds, and then dry it off and return the animal to a holding cage and continue with the next animal.
- Repeat this until all 12 trials are performed this way, while periodically checking the platform, temperature, and cleaning the maze.
- When all trials are complete, dry off the animals, return them to their home cage and housing room and drain the pool.

Optional: reversal learning or probe trial

After these 12 trials you have two options: either do a probe trial with the platform removed entirely, and observing/verifying that the animals understands/remembers the location of the platform. Or move the platform to a different location, and do a reversal learning task. Also observe if the animal indeed remember the previous location, but now also score whether the animal can find the new location of the platform.

Setup in EthoVision

In EthoVision draw 4 main zones, which are the quadrants of the arena. Also draw a zone around/ on the location of the platform. In analysis profiles we want to acquire distance moved, time spent in each quadrant and latency to find platform.



A standard arena setup for the Morris water maze in EthoVision consists of drawing 4 quadrants. Don't forget to draw the area of the escape platform!

Interpretation of the results

The primary and most used outcome of the Morris water maze is the **escape latency**. This is the time it take for the animal find the platform. This can be influences by swimming speed however, which some researchers overcome by (instead of escape latency), take the path length between the starting point and escape platform. With the probe/reversal trial, the time spent around the *old* platform location is measured within a cut-off timeframe (e.g. 60 seconds), or the number of platform (location) crossings.

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