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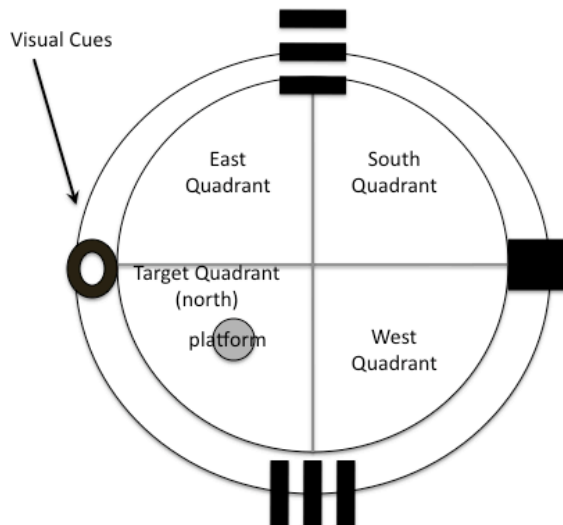
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Morris Water Maze

The Morris Water Maze task involves placing the rodent in a pool of water where it must use visual cues to remember the location of a hidden platform just below the water's surface. Probe trials (transfer tests) are also used to assess the rodent's ability to retrieve information learned in previous hidden platform tests. The water is made opaque by adding nontoxic white paint and must be periodically drained for cleaning and disinfection. The 10-cm circular escape platform is made from water-resistant material. Animals are dried with towels, provided with heating pads and have their body temperature monitored to prevent hypothermia. Any subject not locating the platform within 3 mins will manually guided to the platform. Total swim time, swim distance, amount of time spent in each quadrant, and swim pattern are quantified by the means of software or are hand-scored from video-tapes. Typical water maze protocols consist of visible platform trial(s), hidden platform trials and a probe trial, the order of which varies [1-3].

There are three basic types of trials in the water maze escape task:

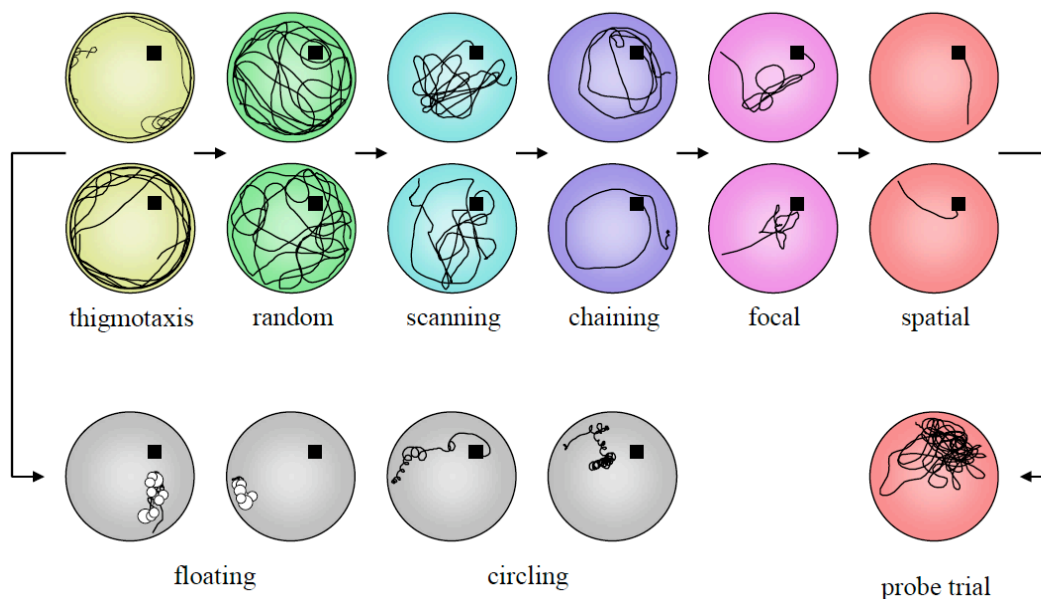
- 1) In the **cued version or visible platform trials** the platform is visible to the animal.
- 2) In the **non-cued or hidden platform trials** version the platform is not visible.
- 3) **Probe Trial** – there is no platform



High contrast visual cues should be used. Rodents can not see color and do not have good visual acuity.

Some common confounds in the water maze

- 1) The treatment condition (drug, genotype age etc) impairs the animals' vision, search strategy, stamina etc.
- 2) The treatments alter the motivation to escape the water.
- 3) Behavioral despair or anxiety is induced
- 4) Some animals have more effective or efficient search strategies
- 5) Hypothermia may alter behavior differentially in different treatment groups (i.e. serotonergic drugs).
- 6) Motor coordination or stamina may be differentially affected in different treatment groups.
- 7) Different groups of animals do not have the same baseline behavior (i.e. do not have the same final learning times, the same rate of learning or the same performance in visible platform trials)
- 8) The variability within subjects is high – often enough to lose all statistical power. Cohort to cohort variability is also high
- 9) Water temperature can affect performance



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How to prevent confounds in the water maze

Visible platform

It is essential to score behavior in the visible platform in order to accurately interpret behavior in the hidden platform and probe trials. Adjunct tests for activity levels (such as open field) for motor coordination (such as balance beam) and muscle strength (such as grip strength) should be, but are rarely, performed. In some cases, tests of visual acuity are also needed, as loss of visual acuity can prevent the animals from being able to use the visual cues.

Multiple measures

If possible, the time spent freezing or floating and anxiety-like behavior (thigmotaxis when the animal is not actively trying to escape by climbing) should be scored to guard against the possibility of behavioral despair induction and anxiety.

Swim speed should be assessed in the visible and in the hidden platform versions of the test.

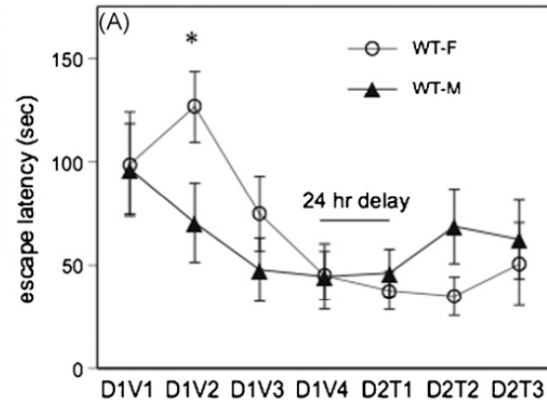
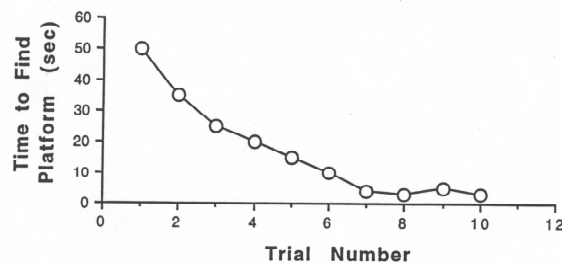
Time spent in the outer edges of the pool (thigmotaxis) should ideally also be measured. This is not always an anxiety measure. The instinct of the animals is to try to escape the pool by trying to climb out the edges. Extinguishing this behavior takes some time.

Time spent in each quadrant is also a useful adjunct measure.

Data Analysis and Presentation

One of the most common measures is the latency to find the platform. The data below would be typical of an animal who "learned" the location of the platform.

However, it should be noted that a similar "learning curve" is evident when animals are tested in a series of visible platform trials [4]. The reasons for this are discussed below.



Measures

Escape latency (visible and hidden) – scored with stopwatch (all other measures are scored with the Viewer tracking system using the Water Maze Plug-in)

Swim distance

Average Swim speed

Latency to target quadrant

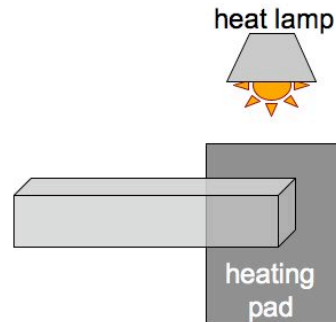
% Time spent in target quadrant

Differences between mice and rats

It is important to note that although rats are excellent swimmers, mice are not. There is some danger of hypothermia in rats but this is a more of a risk in mice. Rats may also dive, which complicates matters if you are using a tracking system.

Maintaining Body Temperature

Heating pads and heat lamps can prevent hypothermia, but they can also induce hyperthermia. You can either get an radiant heating pad, such as is used for reptiles or you can set up a thermal gradient. The animal can then regulate it's body temperature by choosing he correct temperature zone.



1-2 day Water Maze Protocol

Baseline performance and training - Visible platform trials

The primary measure in the visible platform trials is the latency to mount the platform (escape latency). Escape latency is the primary measure. The animal's normal behavior is to search the edges of the maze in order to try to escape. The first few trials the animals are not searching for a platform, and in fact will regularly bump into a highly visible platform without trying to mount it. In the first visible platform trial (V1), the majority (60-80%) of mice and rats fail to voluntarily escape onto the visible platform within a typical trial length of 1min. More than half (50-60%) of the animals still fail to escape within 2 min and 40-50% of the animals still fail to voluntarily escape within 3min.

This phenomenon is clearly detailed in the original papers by Morris, and in studies from around this time (see for example [5-7] When the animals are trained that the only way out of the pool is to go to the platform, they will eventually (usually within 3-5 trials) immediately swim to the platform. Females and males are likely to have a different rate of habituation and differential stress responses [4, 8, 9]. After baseline performance is attained, each visible trial is also a training trial as to the location of the platform. Thus, there are several purposes of the visible platform trials.

1. Habituation to the stressful environment
2. Training to reverse the instinctual behavior to try to escape from the edges of the pool
3. Training as to the location of the platform
4. To provide a baseline for the subject's performance to compare with the testing trials
5. Providing equivalent baseline performance between experimental and control groups

The number of visible platform trials required for your specific subjects can only be determined empirically. The criteria are:

1. 90-100% of the animals should swim directly to the platform (under 20-30 sec) and do so reliably (i.e. with the same escape latency in the next trial). This usually takes 3-4 trials. However, this depends on many factors (sex, species, age, strain, treatments). Additionally, your experimental groups and your control groups should have the same escape latencies.

2. You must have a sufficient number of training trials so that the animals can perform at the requisite delay. This can only be determined empirically, but about 4-6 trials is needed for adult, male Long-Evans rats to reliably perform a 6 hr delay.
3. Increasing the number of training (visible) trials makes the task easier. This will make control performance more reliable and less variable, but may also obscure deficits because of over-training.
4. Decreasing the delay time makes the task easier. Again, this tends to result in more reliable and less variable control performance, but may also obscure deficits because of a loss of sensitivity

Memory measures – hidden platform trials

The delay time is measured from the time of the final visible platform trial. Long –term memory deficits can usually be assessed within about a 6-24 hr train-test interval. The primary measure is escape latency. There are 2 primary ways to measure “success”.

1. The animals perform at or near their own visible platform baseline (assessed by a difference score or ratio [4])
2. The animals find the hidden platform in under 30-45 sec (cut-off determined empirically).

Additionally, animals remembering where the platform was should have shorter swim distances, a shorter latency to the target quadrant and a higher % of time in the target quadrant. Swim speed should be similar between your experimental and control groups. If it is not the case, this indicates a potential confound and additional testing may be needed.

If the animals have a long term memory deficit (i.e. are not successful at finding the platform in the 1st hidden trial), the 2nd and 3rd trials can assess shorter-term memory.

Probe Trials

Despite the many interpretational and logistical issues associated with probe trials (see below), there is an almost religious devotion to the belief in the utility of a probe trial as a standard assessment of memory. Reviewers are likely to ask for one, so it is a wise idea to include one in your experimental protocol if possible. In the probe trial, the platform is removed. The latency to the target zone (where the platform was) can now only be scored with Viewer. Other measure of performance include the latency to the target quadrant, % time in the target quadrant, % swim distance in the target quadrant and swim distance to the target zone.

Alternative Protocols

In addition to the basic protocol detailed above, there are other trials which may prove useful.

Short-term memory and reference memory

After the last hidden platform, (usually 24 hr later) the platform may be moved to a different location. It would be predicted that in the first of the trials, animals remembering where the platform used to be would take a long time to find the new platform location initially. However, if they have normal short term memory, they should find the new platform location successively more quickly with subsequent trials.

Probe trials – problems and logistical issues

Long-term memory is typically assessed in a probe trial in which the platform has been removed. It is widely assumed (and perhaps erroneously so), that subjects remembering where the platform

was would have a shorter latency to the target quadrant (where the platform was), and a shorter latency to the target zone (the circular region where the platform was) and would spend more time in the target quadrant than predicted by chance (25%). In fact, it has also been suggested that probe trials may be more accurately described as extinction procedures, and furthermore that extinction may affect interpretation of the probe trial as being indicative of spatial memory performance [10-12]. Tests such as the open space swim test, in which the animals can swim but have no escape, are used to induce and/or assess depression in rodents [13]. The similar Porsolt forced swim test is also used for that purpose [14], albeit in a smaller arena.

Logistical issues regarding the probe trial may also limit its utility. A typical measure of probe trial performance is the latency to find the target zone (where the platform was). Although this intuitively seems to be a very reasonable measure, there are several practical concerns that affect this measure. Firstly, differences in apparent memory performance in this task are hard to determine if there are differences in acquisition during the typical hidden platform training trials i.e. if all the animals have not learned the task to the same criterion. The platform itself is typically small (10-20 cm diam) and the subject can sometimes swim almost directly to the platform location without sufficiently entering the zone (either with enough of the body or for enough time) for the tracking software to record this, making interpretation difficult. Several groups address this problem by making the target zone larger than the actual diameter of the platform and/or by decreasing the time threshold criteria for entry into the target zone. It is thus often a more equivocal measure than is generally appreciated. A further issue with the probe trial as an assessment of long-term memory is that the measures used are inherently variable and intrinsically lack power. Time spent in the quadrant zone is rarely greater than 40-60% and chance performance is 25 % [15, 16], thus, there is a very small window within which differences can be seen.

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