The Morris Water Maze

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Purpose: To interact with changing environments, animals must process new information provided to them by those environments. To succeed in those situations if encountered again, animals must be able to recall information gained during prior interactions with their environments. In other words, animals must be able to learn, and they must be able to retain what they learn; they must have memory. Learning and memory as processes cannot be directly observed, however, and their presence must be tested through indirect methods. The neurologic systems involved in learning and memory can be disrupted in many ways, most of which are not fully understood. Some of the ways in which learning and memory may be impaired, however, are lesions in the hippocampus, several areas of the cerebrum, the neocortex, striatum, basal forebrain, and cerebellum.

There are many testing paradigms available to assess learning and memory in rodents. One of the most elegant and simple is the Morris water maze (MWM)—also known as the Morris water task and Morris water test—a simple spatial task based on visual cues, in which a rodent swims in a tank to find a hidden platform. This behavioral test was first described in 1981 (1) to assess learning and memory in rats. It has since been adapted for use in laboratory mice, deer mice (*Peromyscus maniculatus*), meadow voles (*Microtus pennsylvanicus*), Mongolian gerbils (*Meriones unguiculatus*), and the gray short-tailed opossum (*Monodelphis domestica*) (2, 3). The MWM is based on the idea that most rodents find swimming aversive and will view escape from water as a positive reinforcer. The test is useful for the study of rodent models of neurodegenerative disease, such as Alzheimer's disease and Parkinson's disease, as well as the effects of pharmacologic compounds on learning and memory. The MWM also is a recommended part of a functional observational battery to assess genetically altered mice (4-6).

Methods: The tank used to conduct the MWM is made of a water-tight material, often plastic or galvanized metal (Fig. 1). Tank configuration may vary but typically is circular and measures 80 to 180 cm in diameter and 30 to 60 cm in height. Smaller-diameter tanks are more appropriate for testing mice. An escape platform is necessary to perform the test. The platform may be clear or opaque (white or dark, depending on the color of the water) and 5 to 12 cm in diameter, with smaller platforms more appropriate for testing mice. Before the trials begin, the tank is filled with enough water so that the animal cannot reach the bottom with either its limbs or tail. To obscure the platform, the water may be rendered opaque with milk powder or white paint (for pigmented animals) or ink (for albino animals), or the tank may be colored black and the water left untreated. Because the path taken by the animals to the platform is often recorded by video camera, contrast between the rodent and the water is necessary for imaging purposes.

The room in which the testing apparatus is located contains environmental cues, which aid the rodent in spatial learning. These cues may be high-contrast geometric patterns placed on the walls of the room, posters, or anything visible to the animal from the water and platform, such as room dividers, equipment, furniture, and door frames. Consequently, great care should be taken that such cues remain fixed throughout the duration of the experiment.

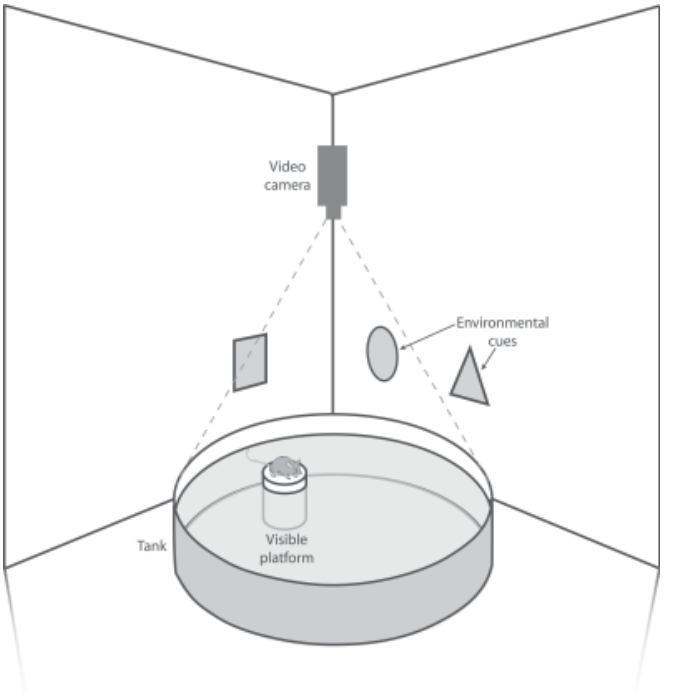

Figure 1. An example of a typical Morris water task configuration. Note the presence of a video camera to record the path taken by the animal and the prominent environmental cues on the wall.

The first step of performing the test is pretraining. Pretraining decreases the confounding effects of stress on the animal and screens for problems that might make the animal unsuitable for further work, such as an inability to swim. In pretraining, an animal is introduced to the pool and learns the location of the platform. There are two basic trials, one utilizing a visible and the other a hidden platform, both of which require pretraining. Visible platform trials test an animal's ability to perform the task and provide an assessment of visual and motor ability. In the visible platform test, the platform protrudes above the surface of the water so the rodent can see it while swimming. Alternatively, the platform may be submerged and a prominent marker, one which extends above the surface of the water, is placed on the platform. An animal is placed on the platform and encouraged to enter the water. After entering the water, the swimming animal is gently guided back to the platform. Once a pretraining period is complete, the animal is not guided back to the platform; this scenario allows testing of the processes of memory and learning. This process is repeated several times during a trial, with the animal returned to the home cage between trials.

For the hidden platform test, pretraining entails placing an animal in the water tank and allowing it a set period of time, usually between 60 and 120 sec, to reach a submerged (hidden) platform and climb out of the water. Generally, t he animal is placed facing the wall near the edge of the tank so that it must swim to find the platform. The animal is left on the platform for

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a brief period (e.g., 10 to 30 sec), and then another training session is performed by placing the animal in a different quadrant of the tank.

Pretraining trials are performed several times per day over a period of days (e.g., two trials daily for 10 days, four trials daily for 4 days, etc.). At the completion of either the visible or hidden platform pretraining, a probe trial typically is conducted. In a probe trial, the platform is removed entirely. When the rodent is placed in the water, a direct path to the quadrant that formerly contained the platform indicates successful learning.

As animals learn the location of the platform, the time spent swimming (latency) decreases, and the path taken to the platform becomes more direct. Videotracking and data software systems are available which automatically record the location of animals within the tank and calculate the pathway of swimming, time spent in each quadrant of a tank, average swim speed, distance traveled, and latency to reach the platform. Statistical packages also are available for analysis of the data from individual animals and treatment groups for consecutive sessions.

Variations of the MWM: There are few structural variations of the MWM. A difference present in every laboratory conducting the test, however, is the environment. Environmental differences may include the presence of an investigator or others, cues painted or posted (i.e., posters or cardboard cutouts) on the walls of the room, cues painted on the sides of the tank, equipment present in the room, water temperature, and changes in room lighting. Variations in the test method mainly are concerned with actual testing paradigms (7).

After successful pretraining, animals may be tested further through retraining, in which the platform is moved to a new location in the pool and the animal's ability to learn a new location for the platform is examined. Persistence of spatial memory may be examined by cessation of testing for a certain time interval (e.g., 1 week) and then monitoring latency to find the platform, which is kept in the original testing location. In rats, platform discrimination tests may be performed. In this procedure, two visible platforms are used: one that is supported and can bear the weight of an animal and one that floats and cannot support an animal. This test is further subdivided into spatial and nonspatial discrimination tasks. For spatial discrimination, the two platforms appear identical, and discrimination between the platforms is based on an animal's learning the location of the supported platform relative to distant visual cues in the room. For the nonspatial version of the task, rats learn to discriminate between the two platforms based on platform shape, color, brightness, etc., and distant visual cues can be removed or blocked from view by use of a curtain.

Animal welfare concerns: Animal welfare concerns are of paramount importance with the MWM. Rodents should be placed gently in the water hindfeet-first, as dunking the animals causes great stress. Animals must be supervised in some fashion while they are performing the task. Rodents may not attempt to swim (or may be unable to swim) and could sink to the bottom of the tank immediately (8). Some strains become so agitated upon immersion that they must be rescued (9) . In contrast, other strains of rodents seem to find swimming pleasant and routinely will take longer to reach the platform (5). Variations in the performance of inbred strains of mice and rats are common and specific to each strain (10-13). Animals with impaired motor function, advanced age, or sensory input issues may also need special supervision. The time spent in the water must be strictly limited to prevent overtiring or hypothermia, especially with multiple trials during the acquisition portion of the task. The water should be warmed to at least room temperature before rodents are placed in it, and rodents should be dried upon completion of the task before their return to the home cage. In addition, the water must be changed daily to prevent growth of pathogenic organisms, particularly when the water is rendered opaque with substances which might encourage bacterial growth. Trials using animals of different health statuses may expose "clean" animals to unwanted pathogens.

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