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Behavioral Model for Assessing Cognitive Decline

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Abstract

The water maze task can be used to assess sensory motor and cognitive function in rodents. When properly employed, this task can behaviorally assess acquisition of a spatial search strategy, as well as working and reference memory. The following section uses research on age-related, cognitive decline to illustrate the methods employed and highlight areas that can, if not properly controlled, confound a study.

Keywords

Spatial water maze; Hippocampus; Memory; Sensory motor; Stress

1. Introduction

The spatial version of the water maze (SWM) is commonly employed for examining cognitive ability in rats and mice and is considered the “gold standard” for testing hippocampal function. When employing the SWM, proper care must be used to insure that differences are specific to cognitive process of interest and not confounded by other relevant variables. This article focuses on studies of aged animals to illustrate the important aspects of SWM paradigm. However, the suggestions may be applied to any study, since genetic manipulations or drug treatments are likely to be influenced in a like manner.

Senescence is associated with selective changes in specific cognitive functions (1–5). Memory capabilities that depend on proper hippocampal function appear to be of particular vulnerability to the aging process (6, 7). Rodents provide a good aging model since they exhibit enormous variability in cognitive function and are not subject to other cognitive debilitating diseases, such as Alzheimer’s disease. However, aging is associated with diverse physiological changes that could contribute to behavioral differences on the water maze. One critical factor is the stress associated with water maze training, since aged animals are more sensitive to stress (see Notes 1 and 2). Thus, performance in older animals deteriorates dramatically when training is performed at colder water temperatures, including room temperature (8–11). Based on previous experience, we maintain the water temperature at $28 \pm 2^\circ\text{C}$. Colder temperatures increase the level of stress, and temperatures above this are not motivating such that animals tend to float rather than swim to escape. In addition, we dry the

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⁶Stir the water occasionally to remove any olfactory cues left by the animals, including the water on top of the platform. Remember that rodents have a superior sense of smell.

⁷To help remove water from the pool when behavioral testing is complete, consider using a submersible water pump. Most pumps can be fitted with a normal garden hose to lead the extracted water to a nearby sink or drain.

¹Decreasing the amount of stress experienced by the animal is a crucial component for properly conducting this experiment. Essential aspects of the task that can cause stress include water temperature, physical handling, and swimming duration.

²Avoid talking or other noise production during the trials as much as possible as it may startle and stress the animal, affecting performance.

animals after every block of trials and they are maintained under a constant source of heat as per a heating lamp or fan.

Environmental conditions outside the training environment can also influence behavior. Aged animals are impaired to a greater extent when a restraint stress is interposed between learning and recall (12). However, the rapid forgetting observed in aged animals is not enhanced by psychological stressors (13). Environmental enrichment may improve memory to a greater extent in aged mice (14, 15) and we have observed that social isolation impairs the rapid acquisition of hippocampal-dependent memory to a greater extent in older animals. Finally, since training on the water maze involves extensive handling, animals should be habituated to handling by handling them once a day for at least a week prior to training.

2. Materials

1. SWM.

Despite the numerous studies that exist in the scientific literature using the SWM, there is no set guideline as to what materials and pool size to implement. Depending on the color of the animal, the use of paint or dye may be added to the water to provide the visual contrast needed for computer tracking programs. Also, contingent on the animal model (rat or mouse), pool size and escape platform size must be taken into account. Too large a pool results in slower learning and possible exhaustion due to extensive swimming. The size of the apparatus is undoubtedly different for mice and rats and may differ between the various rat and mice strains that are available for research (16, 17). Through experience, as well as comparisons to other water maze protocols, an appropriate diameter for the rat water maze is approximately 1.7–1.8 m and 1–1.5 m for the mice. The size of the platform in relation to the pool size must also be considered. For the measurements listed above, appropriate platform diameters include 12–15 cm for rats and 10–11 cm for mice. The platform can be covered with a fiber or wire net in order to facilitate escape. Furthermore, the platform should be located away from the wall to prevent the animal from finding the platform by circling next to the wall (i.e., thigmotaxis) and discouraging animals on the platform from leaping out of the pool. Finally, the depth of the pool should be at least 20 in. Keep in mind, however, that deeper pools require more time for filling and emptying.

2. Spatial cues.

The pool should be placed in a well-lit, temperature-controlled room that has ample space for the pool itself as well as enough space to move about the perimeter (see Note 3). It is important to control the spatial cues. The use of a solid-color curtain may also be hung around the pool to increase researcher control over the cue variables. The spatial cues should be physically large and provide differences in contrast (lights, adjoining black and white walls), rather than differences in colors or shapes (see Note 4). Finally, the spatial cues should not be localized on the pool wall or directly adjacent to the pool such that the animal can use a single cue as a beacon.

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³The animals should be monitored through the camera. If the researcher is in view or enters the pool area from the same place, the animal may focus on the researcher as the route of escape.

⁴Make sure that there are no visible markings on the pool wall that can be potentially used as a visual cue. All visual cues should be outside of the pool (spatial version of the task).

3. Methods

The procedure by which rodents are tested in the SWM varies greatly and consists of multiple versions. In order to insure that differences are due to cognitive function, the tests should minimize stress. In general, we provide the animals with a pool habituation phase, followed by cue discrimination training to provide the procedural aspects of the task, and finally spatial discrimination training and testing. Animals are first habituated to the pool by allotting 30-s free swim and four trials to climb onto a platform from four different directions. For cue and spatial discrimination, trials are arranged in training blocks containing several training trials per block, where the animals are allowed to explore the pool and learn the strategy to escape from the water. The number of trials/blocks is at the discretion of the investigator but usually does not exceed 5–6 blocks a day with three trials in each block. With each trial, the rodent is gently placed in the pool at the designated starting position. Do not drop the animal into the water or lower them by the tail. Again, the point is to reduce the stress of the task. The release positions are randomly varied across the trials. The time spent searching for the platform should not exceed 60–90 s as swimming for an extended period of time can be stressful on the animal (especially for the older subjects). If the animal fails to find the platform, gently guide it to the platform and allow it to climb out of the water. The animal is left on the platform for 10–20 s. During this time, the animals generally explore their surroundings, sniffing and rearing (see Notes 5–⁸).

Several behavioral measures can be used to examine performance during training. A decrease in the escape latency or path length to find the escape platform is a common indication in the acquisition of the tasks, at least within a group. However, latency is a poor measure of cognition when examining performance across groups. Aging and drug treatments can produce motor disturbances and fatigue and influence swim speed (18). While a decrease in latency is indicative of learning, it is unclear from this measure alone whether a spatial or nonspatial strategy is employed to navigate to the platform. To assist with that determination, a measure of the animal's path length during the trial is employed. Unlike escape latency, path length is not impacted by the animal's swim speed, only the distance traveled to reach the platform. However, this measure is also subject to confounds when measuring learning. For example, an animal may initially adopt a thigmotaxic strategy, swimming along the wall. With continued training, the animal may switch to a strategy of simply floating until rescued as the trial times out. In this case, there would be a decrease in the path length over training that would not reflect the use of a cue or spatial discrimination strategy. Thus, the researcher should record whether the animals actually escaped onto the platform.

3.1. Water Maze Procedure

1. One week of handling
2. Habituation phase training
3. Cue or procedural training
4. Spatial training
5. Probe trials

⁵Remove any animal feces floating in the water in between each trial. A fish tank net works best.

⁸Clean the pool regularly, especially when there are a large number of animals in the study. After the water is removed from the pool, a 70% ethanol or 10% bleach solution can be used to disinfect the walls and bottom of the pool. (If paint is used in the water, there could be discoloration on the pool's walls over time. Thus, be sure to scrub thoroughly).

3.2. Cue Discrimination and Procedural Learning

Habituation training is followed by cue discrimination training. The cue discrimination task is used in order to identify any sensory motor or motivational deficits that would impede acquisition of a spatial search strategy. For cue discrimination training, the spatial cues are minimized usually by putting a curtain or solid colored wall around the pool. In addition, the platform is fitted with a flag or marker that is visible to the animal. The starting location and the platform location are varied on each trial to prevent the use of a spatial strategy or simple motor programs. Animals that fail to reach the escape platform during the last block of cue training are considered to have a sensory motor or motivational deficit and are removed from further training.

In addition to identifying animals with sensory motor deficits, prior training on the cue task can insure that animals have learned procedural aspects of the task, including how to swim, and the fact that the pool wall is not a route of escape. Typically, a cue discrimination task is employed several days prior to training on the spatial version of the task. We find that when animals are trained on the cue task prior to spatial training, they perform better on the spatial version of the task. Similarly, when animals are initially trained on the spatial version, they perform relatively poorly, but exhibit superior performance on subsequent cue training. Thus, when the spatial task is employed prior to cue training, an animal may exhibit poor performance on the spatial task due to impairment in acquiring the procedural aspects of the task. This deficit in procedural learning may be overcome by the end of spatial training such that the animal appears normal on subsequent cue training.

3.3. Spatial Discrimination: Reference Versus Working Memory

For the spatial discrimination task, the platform is hidden beneath the surface of the water and the animal must learn the relationship between spatial cues in order to find the platform. Depending on the protocol, the SWM task can be employed to examine spatial reference memory or spatial working memory (see Note 9). A reference memory can be viewed as a stable archive of information that can be accessed and recollected repeatedly on a daily basis. In this case, the platform remains hidden in the same location across days of training.

In the case of spatial working memory or short-term memory, the usefulness of the spatial information is temporally limited. In this case, the location of the hidden platform is moved prior to the start of the first trial of each day such that it is located in a different quadrant and at a different distance from the edge of the pool relative to the previous day's training. On the subsequent trials within a day, the submerged platform is to be located in the same position as the first trial. A decrease in the latency or path length indicates learning/memory for the spatial location. Furthermore, the inter-trial interval or the interval between training and testing can be varied in order to determine the persistence of the memory. Previous studies have shown that older animals can retain information on the location of the hidden platform over minutes; however, an age-related increase in forgetting is observed as the interval extends to hours (8, 19, 20). Similar effects are observed under conditions of NMDA receptor blockade (21, 22).

3.4. Probe Trials

Probe trials are employed to assess the acquisition and retention of a spatial search strategy. For the probe trial, the platform is removed from the pool. The rodent is placed in the pool

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⁹In many cases, researchers want to determine an animal's cognitive function before treating and then retest the animal following treatment. The water maze may be used to identify animals with memory impairments. Following treatments, spatial memory can be examined in the same pool as long as the platform position is moved. We find that untreated animals with memory impairments consistently exhibit memory impairments.

and allowed to freely explore the maze for a predetermined amount of time (e.g., 60 s). The time the animal spends searching each quadrant of the pool and the number of times they cross the previous location of the platform (platform crossings) is recorded (see Note 10).

Platform crossings can be used when comparing within a group, across different probe trials (e.g., acquisition and retention). However, this measure may be flawed for comparing across groups. An age-related decrease in the number of platform crossings can result from differences in motor ability (23). Young animals make quick sharp turns while aged animals make more sweeping turns resulting in a reduction in the number of crossings.

The use of a spatial search strategy is evident when a significant portion of time is spent searching in the goal quadrant that originally held the escape platform. A probe trial delivered shortly after training can be used to determine whether an animal has acquired information on the location of the escape platform. Once it is clear that the animal has acquired a spatial search strategy, a subsequent probe trial may be delivered in order to access retention (e.g., 24 h after the acquisition probe trial). Animals have acquired or retained the spatial information if they spend greater than 25% (i.e., chance) of the time searching the goal quadrant. Alternatively, a discrimination index (DI score) can be calculated for probe trials using the time spent searching the goal and opposite quadrants according to the formula $DI = (\text{time in goal} - \text{time in opposite}) / (\text{time in goal} + \text{time in opposite})$. Since the DI score is dependent on discriminating two quadrants, it is less susceptible to influence of motor function. A larger DI score indicates better performance and a score near zero indicates chance performance. In this case, the researcher may want to release the animal in the quadrant opposite the goal to insure that some time is spent in this quadrant. Finally, it is possible that the animal changes its search strategy within a probe trial. For example, after initial search of where the platform should be, the animals may then search other quadrants. Alternatively, it may take other animals some time searching the pool before they get their bearings and begin to search the correct quadrant. In this case, the probe trial can be broken down into smaller time segments. Thus, one may want to separately examine the first and second 30 s of a 60-s probe trial.

It has become good practice to employ a probe trial at the end of training to access acquisition in conjunction with the retention probe trial delivered later. The acquisition probe trial is generally followed by a refresher-training block. The implementation of the refresher-training block is used to insure that the animal has not learned that there is no longer a platform to use as a means of escape and performance for the refresher block is generally similar to the training block prior to the probe trial. Alternatively, a specialized escape platform, the *Atlantis platform*, can be remotely lowered and elevated back to position during the probe trial (24). Thus, the rodent is probed for a specific amount of time (i.e., 30 s) and then the platform can be raised to allow for a means of escape, all within the same trial.

3.5. Statistics

Repeated measures analyses of variance (ANOVAs) on latency and path length should be employed to examine learning and subsequent ANOVAs or post hoc tests can be used to localize differences. The ANOVA can be repeated over blocks of trials and a significant repeated measures effect is an indication of learning. In some case, there is an interaction of the repeated measure and one factor. For example, we consistently observe an interaction of age and latency due to the fact that older animals swim more slowly. In this case, separate

¹⁰For animals with learning deficits, the water maze procedure may be used again following treatments. However, unless learning impairments are serious, there tend to be considerable savings across multiple acquisition probes. Therefore, it is advisable that a different room is employed in order that all animals are required to learn a novel spatial configuration.

ANOVAs within each group can be used to determine whether learning, observed as a significant decrease in latency over trials, has occurred. For probe trials, multifactor ANOVAs can be employed or the tests can be repeated (acquisition and retention probes). One-tailed *t*-tests can be used to determine whether a group is using a spatial escape strategy. In this case, the percent time in goal or the DI scores should be greater than chance (time in goal = 25%; DI score = 0).

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