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Research report

Validation of a 2-day water maze protocol in mice

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ABSTRACT

We present a 2-day water maze protocol that addresses some of potential confounds present in the water maze when using the aged subjects typical of studies of neurodegenerative disorders, such as Alzheimer's disease. This protocol is based on an initial series of training trials with a visible platform, followed by a memory test with a hidden platform 24 h later. We validated this procedure using aged (15–18 m) mice expressing three Alzheimer's disease-related transgenes, PS1(M146 V), APP(Swe), and tau(P301L). We also tested these triple transgenic mice (3xTG) and age and sex-matched wild-type (WT) in a behavioral battery consisting of tests of motor coordination (balance beam), spatial memory (object displacement task) visual acuity (novel object recognition task) and locomotor activity (open field). 3xTG mice had significantly longer escape latencies in the memory trial of the 2-day water maze test than WT and than their own baseline performance in the last visible platform trial. In addition, this protocol had improved sensitivity compared to a typical probe trial, since no significant differences between genotypes were evident in a probe trial conducted 24 h after the final training trial. The 2-day procedure also resulted in good reliability between cohorts, and controlled for non-cognitive factors that can confound water maze assessments of memory, such as the significantly lower locomotor activity evident in the 3xTG mice. A further benefit of this method is that large numbers of animals can be tested in a short time.

1. Introduction

Despite the fact that the water maze is one of the most widely used cognitive assays, there yet remain several logistical and theoretical issues that limit accurate interpretation in some instances. These issues include problems distinguishing performance from memory/learning, lack of sensitivity between treatment groups when controls perform at low levels, sex differences in apparent performance, and confounds such as anxiety and behavioral despair [1–9]. These and other issues are discussed in detail in the following sections. A particular case regards the multiple confounds likely to occur when testing aged subjects, as is essential in many rodent models of neurodegenerative diseases [6,8,10–12]. Here we present a 2-day water maze training protocol that addresses some of these issues and is suitable for assessing long-term memory in aged mice of both sexes.

Typical water maze protocols consist of a visible platform trial, hidden platform trials and a probe trial, the order of which varies [2,9,11]. The hidden platform is concealed beneath water made opaque, generally by non-toxic paint. Acquisition is typically assessed with hidden platform trials as a decreased latency to mount the platform (escape latency) and shorter swim distance across trials, although multiple other measures are also used [2,9,13]. Long-term memory is typically assessed in a probe trial in which the platform has been removed. It is widely assumed 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%). However, in many cases, neither hidden platform nor probe trial procedures control for or assess the many factors governing escape latency, including stress and anxiety, behavioral despair and the robust natural tendency of rodents to explore novel environments, to name a few. In fact, it has also been suggested that probe trials may be accurately described as extinction procedures [12,14–16].

The alternative protocol presented here addresses some of these aforementioned issues. In this paradigm, subjects are trained using

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a visible platform for four trials on the first day. 24 h later they are tested in a hidden platform task. The potential advantages of initial training in a visible platform are many, including reducing variability and providing a baseline for individual performance, permitting habituation and reducing confounds of activity levels, anxiety, stress and behavioral despair. Firstly, this paradigm fosters habituation to the novel environment. Many water maze protocols include habituation trials without a platform because a large number of animals do not initially actively seek escape but continuously explore the pool. However, the absence of a platform (and the inability to control escape) may discourage further attempts to find the platform and is likely to induce a confounding stress response not conducive to habituation and subsequent performance, especially in animals sensitive to the induction of behavioral despair (indicated by floating), or anxiety (indicated by thigmotaxis) [15,17–21]. Secondly, exposing the animals to a situation in which there is no escape, followed by one in which there is, could be considered to be a reversal learning task, which is not a spatial type of learning [14]. This would obviously not be an issue when including initial visible platform trials as a means of habituation.

A further issue with some water maze protocols is that there is no way to distinguish changes in escape latency due to learning from those due to changes in motivation to escape, anxiety, better search strategies, activity levels, etc. when the initial training trials are conducted with a hidden platform [6,12,22–26]. Changing the illumination of the room, the temperature of the water or manipulation of stress hormones is sufficient to change the apparent ability of subjects to learn and/or remember in the water maze [18–20,27–30]. The addition of a single visible platform at the end of all the trials is also unlikely to accurately assess or control for any of these variables. In fact, the data we present below suggest that a single visible platform trial initially is also not sufficient. Although many investigators assume that the visible platform trials monitor the visual acuity of the animals [31], visible platform trials are also potentially useful for many other reasons, not the least of which is to include control assessments within the existing task. Given these considerations, we designed a protocol based on initial training with a visible platform followed by long-term memory assessment 24 h later in a hidden platform trial.

We asked if the escape latency would decrease over time in the initial visible platform trials, resulting in stable, reproducible behavior from cohort to cohort, between individuals, between genotypes and between sexes. We further asked if analyzing the increased escape latency using a hidden platform trial 24 h after the final visible platform trial could sensitively assess long-term memory. We also assessed short-term memory (in mice with long-term memory deficits) by assessing the escape latencies in subsequent hidden platform trials performed 30 min apart. In order to validate this protocol for use in aged animals we used a triple transgenic murine model of Alzheimer's disease (AD). These 15–18-month-old mice express the PS1(M146 V), APP(Swe), and tau(P301L) transgenes and are referred to as 3xTG mice [32]. 3xTG mice and age- and sex-matched WT mice were tested in the novel 2-day water maze procedure presented here, in a typical water maze probe trial, and in a behavioral battery consisting of tests of motor coordination (balance beam), spatial memory (object displacement task) visual acuity (novel object recognition task) and locomotor activity (open field).

2. Methods

2.1. Subjects

Wild-type (WT, $n = 19$) and triple transgenic (3xTG, $n = 22$) mice of both sexes were 15–18-months old at the time of testing [32]. Sample sizes when divided by sex were as follows: WT female $n = 8$, male WT $n = 11$; 3xTG mice males: $n = 13$; females: $n = 9$. They were housed in groups of 3–5 with *ad lib* food and water in a 12–12 light–dark cycle. The rationale for this choice of expression, typical pathology and details of the creation and breeding of these mice are published elsewhere [32]. Briefly, 3xTG mice and controls were created as detailed in [32]. Essentially, two independent transgenes (encoding human APPSwe and human tauP301L, both under control of the mouse Thy1.2 regulatory element) were co-microinjected into single-cell embryos harvested from homozygous mutant PS1M146 V knock-in (PS1-KI) mice. The PS1 knock-in mice were originally generated as a hybrid 129/C57BL6.

Mice were tested in two cohorts with each cohort age and sex matched. All studies were approved by the Animal Institute Ethical Committee of the Albert Einstein College of Medicine and were conducted according to NIH and IUACAAC guidelines.

2.2. Water maze

Animals were trained to criterion (90% escaping in under 60 s) in a series of visible platform trials on day 1 (D1) in a pool measuring 1.2 m in diameter and 50.8 cm deep using Viewer tracking software (Bonn, Germany). The water temperature was $24 \pm 2\,^{\circ}C$. The diameter of the platform (104 cm) was the same for both hidden and visible trials. This required four visible platform trials (V1–V4). The last visible platform trial of any animal is considered to be its post-habituation baseline, and is designated V4 (visible platform trial 4) or D1V4 (day 1, visible platform trial 4). Training of animals was staggered in time so that each mouse had the same inter-trial interval—i.e. 30 ± 10 min between each trial (for both the visible and the hidden trials).

The visible platform was outlined by a dark ring with high contrast to the white background made by the addition of white, non-toxic tempera paint. Animals failing to escape within 3 min were manually guided to the platform. All animals stayed on the platform for 5–10 s before being removed, dried and then placed in a holding cage with a heating pad to prevent hypothermia.

24 h after the last visible platform trial (D2), the animals were tested in a series of three hidden platform trials (T1–T3). As before, the trials were staggered in time to ensure a stable inter-trial interval of about 30 min and each trial was a maximum of 3 min long.

In order to compare the sensitivity and reliability of this protocol to standard training and probe trials, a subset of animals received an additional day of hidden platform trials followed 24 h later by a probe trial.

The platform remained in the same place for all of the trials. Although some protocols vary the position of the platform from trial to trial ([20,33,58]), given the age of the animals we did not utilize this variation. Animals were delivered to either the west or east quadrant (the target quadrant being designated as north) in a random manner for each trial as these are equidistant from the target. Some groups use all quadrants for entry points [34,35], but given that north quadrant entry is substantially closer and the south quadrant entry farther from the target than the other entry points, we chose not to introduce that potential source of variability into the study. High contrast visual cues were placed on the wall of the pool in each quadrant. External cues were not intentionally placed in the room, but the non-symmetrical nature of the testing room (door, sink, computer placement, etc.) possibly provided these.

All trials were recorded and analyzed using Viewer software (Biobserve, Bonn, Germany) and JMP statistical program (SAS, Cary, NC, USA).

2.3. Object placement and novel object recognition tasks

All adjunct tests began 2 weeks after completion of the water maze. The additional behavioral tests were conducted in the following order: open field, object placement, balance beam and object recognition. Comparison to a previous pilot experiment conducted on naïve mice did not indicate altered performance resulting from prior water maze experience (data not shown). The object placement test of spatial working memory [36,37] is based on the natural and robust tendency of rodents to preferentially explore novel objects. The assay was performed essentially as described previously [36,37]. Briefly, in Trial 1, mice were placed in an opaque plastic arena (16 in.^{2}) and allowed to freely explore two identical, non-toxic objects (such as plastic, glass or ceramic items) for 6 min. High contrast visual cues were placed on the walls of the open field. The time spent exploring the objects was recorded manually with timers, after which the animal was returned to the home cage. Following a retention interval of 3 min, the animal was returned to the arena in which one of the objects was displaced in space (Trial 2). Care was taken to ensure that the intrinsic relationship between the objects was changed, and not just the position relative to a visual cue. The mouse was again allowed to explore for 3 min, during which time the exploration of both the displaced (novel) and familiar objects was scored.

In order to assess visual acuity, we employed the novel object recognition task with no retention interval between Trial 1 and Trial 2. In Trial 1, the animals were placed in the open field with two identical objects and allowed to explore for 3 min. The animals were then removed from the arena only long enough to replace one of the objects with a new object (between 30 and 60 s). In Trial 2, mice were allowed to explore again for 3 min and the amount of time exploring both the novel and familiar objects was recorded.