Physiology & Behavior 122 (2013) 216–221

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Effects of pretraining and water temperature on female rats' performance in the Morris water maze

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HIGHLIGHTS

- Spatial pretraining improved female rats' performance in the water maze.
- Water temperature did not alter performance in female rats.
- The relationship between stress and motivation may differ between males and females.
- Alternative paradigms are needed to more accurately assess motivation in female rats.

ARTICLE INFO

Article history:
Received 20 November 2012
Received in revised form 20 March 2013
Accepted 16 April 2013

Keywords:
Pretraining
Spatial learning
Female
Motivation
Memory

ABSTRACT

The water maze is a complex spatial task that requires the coordination of multiple systems to perform efficiently. Various factors have been shown to influence performance in this task, including motivational state and prior experience. Although a consistent sex difference has been observed in acquiring the water maze in rats, the contribution of the various factors in female rat performance has not been fully assessed. Therefore, the current study tested the effects of motivation as manipulated by water temperature of the maze and prior experience in the maze on the performance of female rats. It was hypothesized that females pretrained in the maze would perform better than those without exposure to the water maze, regardless of water temperature, but in naïve rats, colder water would improve performance as shown previously in male rats. For pretraining, female rats were taught to find a visible platform in cold (19 °C, 4 trials on one day) and warm (25 °C, 4 trials on one day) water before acquisition trials, with the order of the water temperature randomly assigned. Control rats were not given any training and were naïve to the water maze procedure. Pretrained and control rats were then tested to locate a hidden platform in either cold or warm water for 5 consecutive days. Overall, pretraining had a significant effect on distance, latency, and directness of path to the platform. Water temperature did not show a significant effect on any measure or a significant interaction with pretraining. Thus, while our hypothesis that pretraining would improve performance was supported, the results did not support the prediction that water temperature would also significantly influence performance. These results show that non-spatial pretraining can critically improve the performance of females in acquiring a place strategy for the hidden platform, irrespective of water temperature.

1. Introduction

Performance on spatial memory tasks has been found to vary between sexes in both humans and rodents [1–5]. In most studies, males show improved performance compared to females on tasks that require spatial memory, such as the water maze, radial arm maze and novel object location [6–11]. In the water maze, our and other laboratories have reported that male rats exhibit shorter latencies and more direct swim paths to a hidden platform during acquisition trials compared to female rats [12,13]. The authors have usually concluded that the observed sex differences were not due to swimming ability or motivation because swim performance to a visible platform has not been found to differ between male and female rats [9,14]. However, few studies have systematically altered the motivational component of the water maze or other spatial tasks when testing female rats.

Motivation in the water maze is assumed to be constant in most studies because the characteristics associated with the water and having to swim to the platform are consistent across trials. One approach that has been taken to manipulate motivation in the water maze is to change the temperature of the water [2,15]. Male rats tested in cold water (19 °C) showed faster rates of acquisition and better retention of the platform location 7 days later than rats tested in warm water (25 °C) [15]. In the radial arm version of the water maze, Salehi and colleagues [2] found that male rats had the fewest errors when tested in 19 °C water compared to two other temperatures (16 °C and 25 °C).

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http://dx.doi.org/10.1016/j.physbeh.2013.04.001

Their findings suggest that water temperatures can be manipulated to alter performance in the water maze, although this relationship may be curvilinear. However, few studies have investigated the effects of water temperature on female rats' performance in the water maze. One study compared water temperature (19 or 33 °C) in a single day of testing in the water maze by comparing females in either estrus or proestrus [16]. The authors reported no differences in spatial memory performance between the water temperatures, but the stage of estrous cycle was found to interact with water temperature. Thus, it is still unclear if water temperature moderates female rats' performance in the water maze across acquisition training days in a similar manner to male rats.

Pretraining in the water maze has also been shown to improve the performance of rats. Twelve trials of training without distal visual cues reduced sex differences observed in naïve female rats [17]. Likewise, pretraining or swimming of male rats prior to a spatial learning task has been shown to improve performance in the water maze following a variety of challenges, such as lesions or pharmacological agents [18–22]. Exposure to the water maze through pretraining may moderate the valence of water temperature and subsequently, performance in the maze. Although prior studies have looked at the effects of water temperature or pretraining on water maze performace, no studies have investigated the interaction between these two variables. Therefore, the current study tested whether spatial pretraining would interact with water temperature to influence the performance of female rats in the water maze. It was hypothesized that pretraining in the water maze would reduce the effect of water temperature compared to female rats naïve to the task, but that water temperature would alter the performance of non-pretrained naïve rats.

2. Materials and methods

2.1. Animals and housing

A total of 42 female Sprague–Dawley rats from Northern Illinois University's Psychology Department animal colony were used for the experiment. Rats (190–290 g) were maintained on a 12/12 h light/dark cycle (lights on at 6:00 and off at 18:00) in a temperature-controlled room (22 ± 2 °C). Rats were pair-housed in a standard Plexiglas cage (27 cm × 48 cm × 20 cm). All procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and approved by Northern Illinois University's Institutional Animal Care and Use Committee.

2.2. Morris water maze

2.2.1. Apparatus

The water maze consisted of a circular galvanized steel tub (170 cm wide, 60 cm high) filled with either 25.0 ± 1.0 °C or 19.0 ± 1.0 °C water for the warm or cold water conditions, respectively. The maze water was made black by adding one full 16 oz jar of black, nontoxic, water-based tempera paint (RichArt, Northvale, NJ, USA). A removable platform (11 cm diameter) was placed into one quadrant of the tub and covered with a sock to increase traction for the rat when climbing out of the water. The platform was located 41 cm from the edge of the pool.

The pool was located in a room with various cues (e.g. cabinets, a poster, geometric shapes on the wall, a door, a chair) outside of the maze. The placement of the pool was marked to ensure that it remained in the same location each day to provide consistent cue locations. Indirect lighting was used for better video tracking of the rat in the pool. An overhead camera was used to record the testing sessions for later analysis with EthoVision 3.0 tracking software.

2.2.2. Procedure

2.2.2.1. Visible platform pretraining. Half of the rats were given 2 days of pretraining in the water maze with a visible platform. For this pretraining, the temperature of the water was counter-balanced with half of the rats swam first in warm water (25.0 ± 1.0 °C) on day 1 and then cold water (19.0 ± 1.0 °C) on day 2 and the other half in the reverse order. The height of the platform was 1 cm above the water level and a white sock was placed on the platform to clearly distinguish the platform from the water. The platform was moved to a novel quadrant in the pool for each of the four trials conducted on each day. In the pretraining, all other procedures were identical to the acquisition training as described below.

2.2.2.2. Acquisition. All rats, pretrained and non-pretrained, were tested in the water maze to locate a submerged platform in a constant location [14]. The platform was lowered 2.5 cm below the water level and disguised by a dark sock. Rats were randomly assigned to either a warm or cold water temperature condition for acquisition and matching-to-place (see below) swimming trials. Each acquisition day consisted of four trials with the rat placed in the water facing the perimeter of the pool at one of four quasi-randomly assigned cardinal directions. The rat was allowed to swim until it located the platform or until 60 s had elapsed. If the animal did not reach the platform within 60 s, the experimenter gently guided her to the platform. She remained on the platform for 30 s for all trials, then was placed in a dry holding cage in the maze room for approximately 120 s and finally returned to her home cage until the next trial. The home cages remained in an adjacent holding area throughout testing. Between trials, the water was strained of feces and gently swirled to diffuse odor cues. Approximately 8 rats were trained per day and all 8 received their 1st training trial before the 2nd training trial occurred for that day (intertrial interval = 20 min).

2.2.2.3. Matching-to-place. Subsequent to acquisition training, all rats were trained for 3 days in a matching-to-place procedure to assess working memory. The submerged platform was moved to a new quadrant each day and never located in the same quadrant for two consecutive days. Two trials were conducted per day and a 30 s intertrial interval was enforced between the trials. Similar to acquisition training, the rats were placed in the water at different starting points for the two trials. Each rat was given 60 s to locate the platform in the novel location for trial one, allowed to remain on the platform for 30 s, placed into a holding cage within the room for 30 s and then placed back into the pool for the 2nd trial. Each rat completed both trials before the next rat was brought into the room to be tested.

2.3. Adrenal gland weights

One week following the matching-to-place procedure, all rats were euthanized using carbon dioxide and their adrenal glands were removed and weighed immediately. To control for body size, the adrenal weight was reported as a ratio of the animal's total body weight.

2.4. Statistical analyses

The recorded swim sessions were entered into EthoVision 3.0 tracking system (Noldus Information Technology, Wageningen, Netherlands). Five samples per second were taken to track the path of the rat. Using the EthoVision program, the total distance traveled, latency to platform, and swim speed were calculated for all trials. Circuity to platform was calculated by taking the shortest possible distance from the start location to the platform and dividing by the distance that the rat swam to the platform for each trial. For the measures taken on the visible platform pretraining task and acquisition training days, means were calculated from all four trials per day. The swim paths for the matching-to-place