

# Mice are not little rats: species differences in a one-day water maze task

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The present study examined species differences in spatial and non-spatial memory in the Morris water maze. Male Wistar rats and C57BL/6 mice were tested in a one-day water maze task in which spatial learning, retention, and non-spatial learning were assessed within 3 h. Rats and mice appeared to use different strategies for locating the hidden escape platform. Whereas rats evinced a clear spatial strategy, mice appeared to

rely less on spatial cues and more on alternative non-spatial strategies. The sensitivity of this behavioral protocol to subtle species differences highlights the potential use of this one-day water maze task as a tool for evaluating rapidly learning and memory in rodents. *NeuroReport* 11:3461–3465 © 2000 Lippincott Williams & Wilkins.

**Key words:** Mice; Rats; Spatial and non-spatial memory; Species differences; Water maze

## INTRODUCTION

Historically, rats have been most commonly utilized in animal studies of learning and memory. However, advances in transgenic and knockout mouse technology, which allow manipulation of putative genes involved in learning and memory (e.g. [1–3]), have thrust mice increasingly into the behavioral arena. In mice, the cognitive effects of a host of genetic alterations have been tested using learning and memory tasks originally designed for rats. However, it is unclear whether these tasks are suited equally well to both species.

One behavioral task widely adapted for use with mice is the Morris water maze [4], originally designed to capitalize on rats' swimming abilities in their natural habitat [5,6]. In this hippocampal-dependent test of spatial reference memory, rodents must use extramaze cues to find an escape platform submerged in a tank of water. Young rats and mice generally learn this task over the course of several days [7,8]. However, it is unclear whether mice perform the task as well as rats or employ similar strategies to find the platform. Surprisingly, few studies have addressed directly these issues. Whishaw and colleagues report superior performance of rats relative to mice in a spatial water maze task, a conclusion based largely on latencies to find the hidden platform [5,9]. However, both species were tested in the same sized tank (~150 cm), seriously confounding interpretation of the data. Rats are larger and stronger than mice, and it is not surprising that mice take longer than rats to find the platform given the same distance to traverse. Nevertheless, Whishaw and Tomie's [5] finding that mice spent less time than rats in a corridor

drawn between the start position and platform suggests differences in the way the species locate the platform.

The present study was designed to compare the performance of Wistar rats and C57BL/6 mice in spatial and non-spatial versions of the Morris water maze. Wistars have been used frequently in learning and memory paradigms [10] and C57BL/6 is a commonly used strain in transgenic studies [11]. Here, rats and mice were tested in swim tanks of a size generally used for each species [12,13]. Rather than the typical multi-day testing protocol [8], the animals were tested using a one-day water maze protocol in which spatial and non-spatial memory were tested within the course of 3 h. This task consisted of three blocks of four spatial learning trials to examine spatial acquisition (each block separated by 30 min), followed 30 min later by one spatial probe trial (to examine retention), and 20 min later by four cued (non-spatial) trials. Because this compressed protocol requires more extensive learning in one day, we consider this task more challenging than the standard multi-day protocol. We postulated that this more challenging protocol would be particularly sensitive to species differences in learning and memory.

## MATERIALS AND METHODS

**Subjects:** Subjects were nine male C57BL/6J mice obtained from the Jackson Laboratories (Bar Harbor, ME) and seven male Wistar rats obtained from Charles River Laboratories (Raleigh, NC). Rats and mice were 6 and 7 months old, respectively, at the beginning of testing and were handled for 5 days prior to testing. Mice were housed up to five/cage and rats were housed singly or in pairs.

All animals were kept in a room with a 12:12 h light:dark cycle (lights on at 06.00 h) and behavioral testing was performed during the light phase of the cycle. Food (Harlan Teklad 2215 Rodent Diet) and water were available *ad lib*.

**Morris water maze apparatus:** A circular tank was filled with water ( $24 \pm 2^\circ\text{C}$ ) and surrounded by various extramaze cues. The apparatus used for both species was identical except for the tank size; 103 cm in diameter for mice and 180 cm for rats. The smaller tank was positioned in the center of the larger tank for mouse testing, ensuring a similar extramaze environment for both species. Because distal extramaze room cues were further from the smaller tank, square geometric patterns were attached to the edge of the large tank ( $\sim 40$  cm from the small tank) for testing mice. The remaining apparatus and four-trial shaping procedure have been described in detail elsewhere [8]. For shaping, animals were tested in smaller rings to decrease total swimming area; 55 cm in diameter for mice and 103 cm for rats. No data were collected during shaping.

**Spatial task acquisition:** Animals in the spatial task (a test of spatial reference memory) were trained to find a submerged platform using extramaze cues. A transparent lucite platform ( $10 \times 10$  cm) was submerged beneath the surface of the water (0.5 cm for mice, 2 cm for rats), in the north-west quadrant of the tank. The sequence of four start positions (N, S, E, W) varied for each trial. Each animal participated in twelve trials, organized into three blocks of four trials (one trial/start position within a block) separated by 30 min. Each animal was given 60 s to reach to the platform, upon which it remained for 10 s. If the platform was not located within 60 s the animal was placed on it by the experimenter. The next trial started immediately after removal from the platform. After completion of the fourth trial of the block, the animal was placed in its home cage for 30 min.

Swim time (s), pathlength (cm), and swim speed (cm/s) were recorded. Because the pathlength measure is biased against rats due to the larger size of the rat tank, and therefore longer distance to traverse to find the platform, we calculated a pathlength ratio for each animal. To compute this ratio, each animal's pathlength was divided by the area of the tank in which it was tested/100. In addition, a corridor measure was recorded to examine whether the animals swam in a straight path from the start location to the platform [8,14]. A corridor (14 cm wide) was drawn between the start location and the platform, the animal's location sampled 10 times/s, and the ratio of time spent in the corridor/total swim time calculated [8].

**Thirty minute probe trial:** One probe trial was conducted 30 min after completion of the spatial task. During this trial, the platform was collapsed, remaining unavailable for escape for 30 s. The platform was then raised and available for escape for an additional 30 s. During the first 30 s of the probe trial, quadrant time (percentage time spent in the training quadrant) and proximity (average distance (cm) to the platform; distances sampled 10 times/s) were recorded. Like pathlength, the proximity measure is distance-based, and is also confounded by differing tank sizes. To reduce this bias, we calculated a proximity ratio similar to the

pathlength ratio. Platform crossings (the number of times the location of the platform was crossed) were also measured during the first 30 s of the probe trial; rats were allowed an area for crossings proportionally larger (1.75 times) than mice to account for differences in tank size.

**Cued task acquisition:** The cued task (a test of non-spatial reference memory) was conducted 20 min after completion of the probe trial. A visible platform (covered with yellow tape) was raised above the surface of the water. For mice, the platform extended just above the water level and had a plastic circle (8 cm diameter, 0.5 cm thick) attached perpendicularly to it [8]. For rats, the platform extended 1 cm above the surface of the water. The platform was moved to a different quadrant for each of the four trials: quadrant 3 for trial 1, quadrant 1 for trial 2, quadrant 2 for trial 3, and quadrant 4 for trial 4. All animals were tested in this sequence. Swim time, pathlength, and swim speed were recorded. A pathlength ratio was calculated as in the spatial task. Intertrial intervals were  $\sim 10$  min.

**Data analysis:** Spatial acquisition and cued task measures were averaged within a group for each block of four trials (spatial task) or single trial (cued task), and analyzed using a one-way repeated-measures ANOVA (SuperANOVA, Abacus Concepts, Berkeley, CA). One-way ANOVAs without repeated-measures were performed on the probe trial measures.

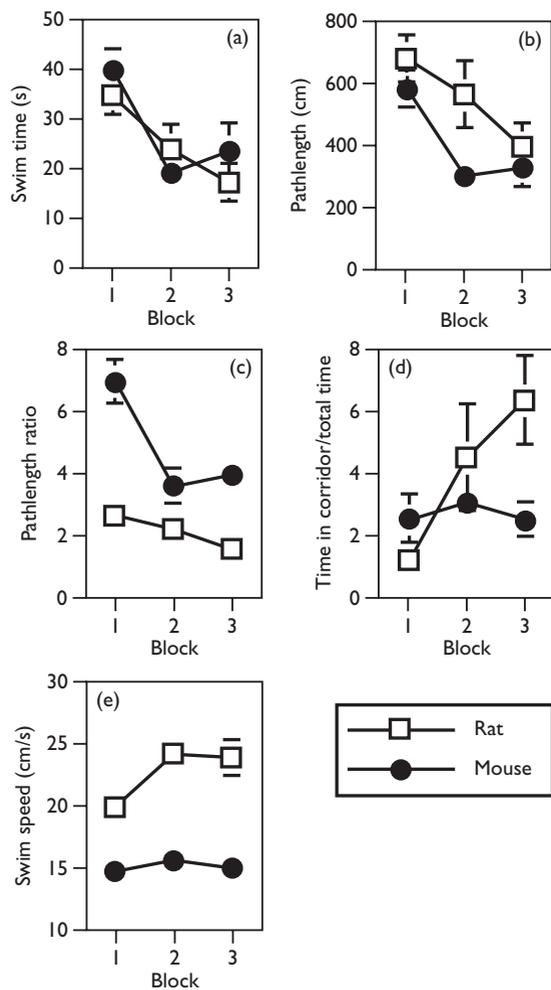
## RESULTS

**Spatial task acquisition:** Rats and mice did not differ significantly in the swim time (Fig. 1a) or pathlength measures (Fig. 1b), suggesting similar abilities to find the platform. However, there was a trend for rats to exhibit longer pathlengths than mice (main effect of species:  $F(1,14) = 3.95$ ,  $p = 0.07$ ), probably due to the longer distances required for rats to traverse relative to mice. When tank size was accounted for, rats outperformed mice. The pathlength ratio, adjusted for the area of the tank, was significantly different between the species ( $F(1,14) = 27.4$ ,  $p < 0.001$ ). Rats exhibited shorter pathlength ratios than mice (Fig. 1c), particularly in the first block of trials (species  $\times$  block interaction:  $F(2,28) = 3.4$ ,  $p < 0.05$ ;  $t$ -test for block 1:  $t(1,14) = 24.9$ ,  $p < 0.001$ ). In addition, rats, but not mice, increased their ratio of time spent in the corridor during testing (Fig. 1d; species  $\times$  block interaction,  $F(2,28) = 4.2$ ,  $p < 0.05$ ; block effect,  $F(2,28) = 4.5$ ,  $p < 0.05$ ). Rats spent significantly more time in the corridor during block 3 than mice ( $t$ -test,  $t(1,14) = 7.4$ ,  $p < 0.05$ ), indicating a more directed search by rats during the last block of trials. Swim times, pathlengths, and pathlength ratios improved throughout testing in both species (block effects:  $F(2,28) = 12.6$ , 10.4, and 8.3, respectively,  $p < 0.05$ ). Rats maintained faster swim speeds (Fig. 1e) than mice throughout testing ( $F(1,14) = 45.6$ ,  $p < 0.001$ ). Swim speeds increased significantly during testing in rats but not mice (species  $\times$  block interaction:  $F(2,28) = 4.7$ ,  $p < 0.05$ ).

**Thirty minute probe trial:** Rats spent a greater percentage of time in the training quadrant during the probe trial than mice (Table 1;  $F(1,14) = 11.6$ ,  $p < 0.01$ ), suggesting greater spatial retention by rats. In contrast, the proximity measure

**Table 1.** Probe trial data.

Task	Measure	Rat	Mouse
Spatial probe	Quadrant time*	47.5 ± 3.2	28.8 ± 4.2
	Proximity†	52.4 ± 1.9	38.2 ± 1.9
	Proximity ratio*	0.2 ± 0.0	0.5 ± 0.02
	Platform crossings	2.6 ± 0.4	1.4 ± 0.4

\*Rats better than mice,  $p < 0.05$ .†Mice better than rats,  $p < 0.05$ .

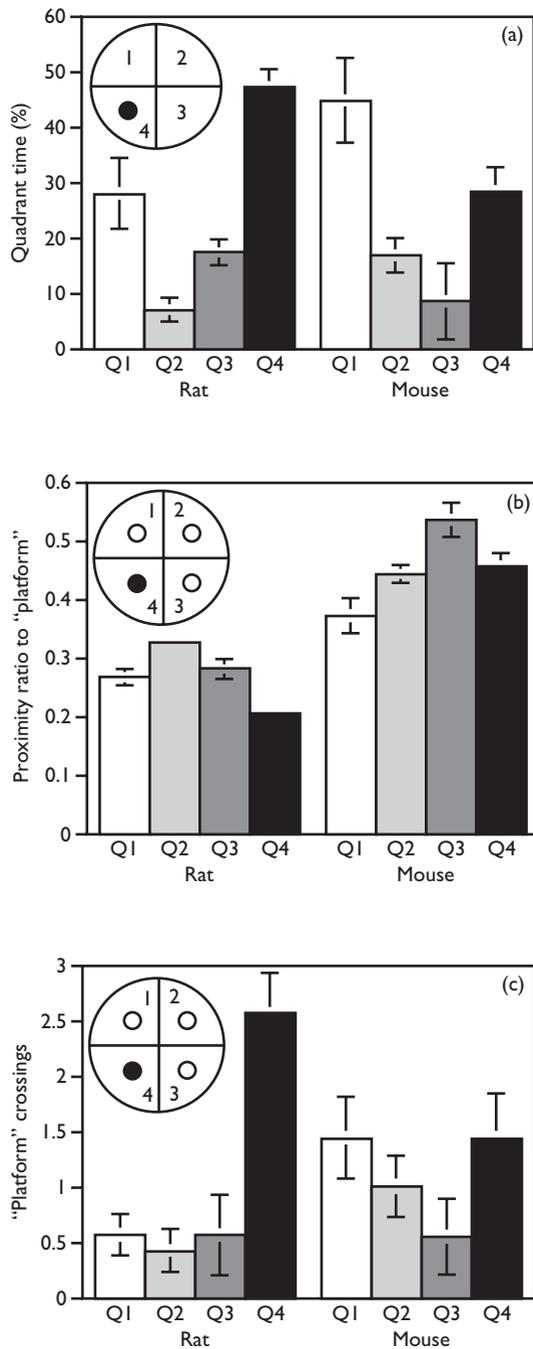
**Fig. 1.** Spatial task acquisition as measured by swim time (a), pathlength (b), pathlength ratio (c), the corridor measure (d), and swim speed (e). Each point represents the mean ( $\pm$  standard error of the mean (s.e.m.)) of each group averaged over four trials. For swim time, pathlength, and pathlength ratio, lower numbers indicate better performance. Higher corridor values represent a more direct swim path to the platform. Both species exhibited similar swim times throughout testing, but rats displayed faster swim speeds, superior pathlength ratios, and increased time in the corridor relative to mice.

indicated that mice swam significantly closer to the former platform position (Table 1;  $F(1,14) = 28.2$ ,  $p < 0.001$ ), suggesting a better knowledge of the precise platform location by mice. However, an analysis of the proximity ratio revealed that, consistent with their superior quadrant

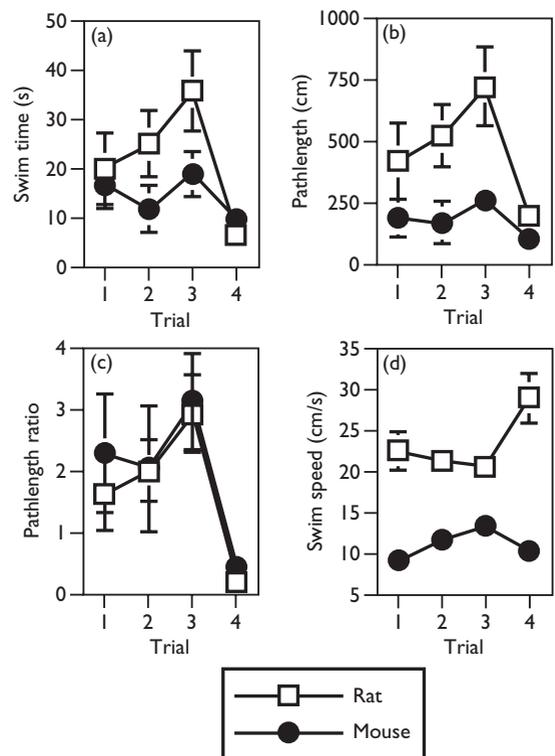
times, rats exhibited closer proximities to the platform than mice (Table 1;  $F(1,14) = 92.9$ ,  $p < 0.01$ ). Rats and mice did not differ significantly in the number of platform crossings, although there was a trend for rats to outperform mice (Table 1;  $F(1,14) = 3.9$ ,  $p = 0.07$ ).

To further investigate species differences in probe trial performance, we examined quadrant time, proximity ratios and platform crossings in all four quadrants. Rats spent the greatest percentage of time (48%) in the training quadrant (Fig. 2a), whereas mice spent the highest percentage (45%) in quadrant 1. A repeated-measures ANOVA conducted on the quadrant time data revealed a significant species  $\times$  quadrant interaction ( $F(3,42) = 4.6$ ,  $p < 0.01$ ). Although only one platform was used during training, virtual platforms were positioned by the computer software in the center of each non-training quadrant. A repeated-measures ANOVA of proximity ratios to each platform position (Fig. 2b) yielded a significant species effect ( $F(1,14) = 256.7$ ,  $p < 0.001$ ), quadrant effect ( $F(3,42) = 6.9$ ,  $p < 0.001$ ) and species  $\times$  quadrant interaction ( $F(3,42) = 6.5$ ,  $p < 0.01$ ). Whereas rats swam closest to the training platform and furthest from the opposite platform, mice swam closest to the quadrant 1 platform, potentially indicating a less spatial search strategy by mice. An examination of platform crossings in each quadrant suggested crossings by mice were more distributed among the four quadrants, whereas rats searched preferentially the center of the training quadrant (Fig. 2c). The effect of species was not significant in a repeated-measures ANOVA, indicating a similar total number of crossings by the species among the combined quadrants. However, a significant quadrant effect ( $F(3,42) = 7.4$ ,  $p < 0.001$ ) and species  $\times$  quadrant interaction ( $F(3,42) = 3.4$ ,  $p < 0.05$ ) demonstrated a differential distribution of crossings by each species.

**Cued task acquisition:** Rats exhibited slower swim times/s (Fig. 3a;  $F(1,14) = 4.7$ ,  $p < 0.05$ ), longer pathlengths (Fig. 3;  $F(1,14) = 21.0$ ,  $p < 0.05$ ) and faster swim speeds (Fig. 3d;  $F(1,14) = 63.1$ ,  $p < 0.001$ ) than mice. Differences in swim time and pathlength were particularly evident when the platform was located in the non-training quadrants (trials 1–3). When the platform was moved to the training quadrant (quadrant 4) during trial 4, both species displayed similarly rapid swim times and short pathlengths. Swim times and pathlengths in both species were the greatest when the platform was located in the quadrant opposite the training quadrant (trial 3). The main effect of Trial was significant for swim time and pathlength ( $F(3,42) = 4.2$ ,  $p < 0.05$ ), however, the species  $\times$  trial interaction was not significant for either measure. Swim speeds remained constant in mice throughout testing, but increased in rats during the last trial, resulting in a significant interaction ( $F(3,42) = 6.0$ ,  $p < 0.01$ ) but not a main effect of trial ( $F(3,42) = 2.7$ ,  $p = 0.06$ ). Although swim time and pathlength suggest that rats were impaired relative to mice, an analysis of pathlength ratio revealed no species differences (Fig. 3c). The fact that this measure adjusts for tank size suggests similar abilities to locate the cued platform. Neither the trial effect nor the species  $\times$  trial interaction were significant for pathlength ratio.



**Fig. 2.** Performance during the spatial probe trial. Each bar represents the mean ( $\pm$  s.e.m.) of each group. In the figure insets, each quadrant is indicated with a number and the former training platform is represented by the filled circle in quadrant 4. Open circles in the (b) and (c) insets represent virtual platforms positioned in the center of each non-training quadrant. (a) Percentage time spent in each of the four quadrants. Rats spent significantly more time in the training quadrant (Q4) than mice. (b) Proximity ratio for each platform position. Lower numbers indicate shorter distances swum from each platform location. Rats swam significantly closer to the former training platform than mice. (c) Number of times each platform was crossed. Although crossings in the training quadrant (Q4) not differ significantly between the species, a significant species  $\times$  quadrant interaction suggested a more focused search for the platform in Q4 by rats. Crossings by mice were distributed more among the four quadrants.



**Fig. 3.** Cued task acquisition as measured by swim time (a), pathlength (b), pathlength ratio (c), and swim speed (d). Each point represents the mean ( $\pm$  s.e.m.) of each group for each trial. For swim time, pathlength, and pathlength ratio, lower numbers indicate better performance. Rats exhibited elevated swim times, pathlengths, and swim speeds relative to mice. However, the absence of species differences in the pathlength ratio measure suggests that the larger tank contributed to rats' apparent difficulty in locating the platform.

**DISCUSSION**

Both species displayed evidence of learning the spatial water maze task, consistent with previous studies of rats [7,15] and mice [8,13]. The data also indicate that rats are superior to mice in learning the hidden platform location, consistent with previous findings by Whishaw and colleagues of rats and mice tested in the same size tank [5,9]. However, the present data suggest that tank size should be taken into account to compare appropriately these species. This point is especially relevant to the interpretation of the cued task, in which several measures suggest a cued impairment in rats relative to mice, whereas the pathlength ratio, which accounts for tank size, reveals no species difference. During trial 3 of the cued task, when quadrant 2 contained the platform, swim times and pathlengths were increased curiously for both rats and mice relative to the other trials. In this, and subsequent experiments in our laboratory, we have found that rats and mice avoid quadrant 2 during the cued task for reasons that we have not been able to determine. Nevertheless, the data suggest that mice and rats perform similarly on cued trials.

Several measures of spatial task performance suggest that rats are more prone than mice to rely on spatial strategies for locating the platform. For example, both species exhibited similar swim times during spatial acqui-

sition, yet only rats increased their time spent in the corridor. The corridor measure provides an index of how direct a path an animal takes to locate the platform [5,14], and suggests that the rats were more accurate in determining the direction of the platform relative to the start position. Furthermore, all of the probe trial measures indicated a strong preference for the training quadrant among rats. The spatial strategy exhibited by rats is consistent with previous findings that young rats typically employ place learning strategies in the water maze [16]. In contrast, mice do not appear to rely on spatial strategies to the same extent as rats, a finding consistent with previous observations in mice [17]. The platform crossings measure is particularly revealing of this difference; whereas rats cross the training platform preferentially, crossings in mice are distributed more among all platforms. These data may suggest that mice search for the platform, not within a specific quadrant, but within a certain radius from the edge of the tank. Other potential strategies include random spiraling, stereotyped motor response strategies, or predatory avoidance strategies, all of which have been observed in mice [17,18]. Furthermore, the current data suggest that mice rapidly switched from the spatial to the non-spatial task, again supporting the notion of alternative non-spatial strategies. Although these non-spatial strategies are effective in helping the mice to locate the platform, they suggest important differences in the way mice and rats solve the task.

The one-day water maze task used here allowed for testing of spatial acquisition, retention, and non-spatial memory within 3 h. The present results illustrate that both species are able to perform all components of this compressed task. Furthermore, the multiple measures of performance recorded in this task (e.g. spatial probe trial measures in addition to acquisition measures) enable more detailed comparisons of between-group differences, such as differing search strategies, than analysis of swim time and pathlength exclusively. Other work from our laboratory indicates that this task is sensitive to mnemonic fluctuations during the estrous cycle in female mice [19]. Thus, this one-day task may be a useful screening tool for

evaluating rapidly the effects of pharmacological treatments or genetic manipulations on memory in rodents.

## CONCLUSION

Both rats and mice are able to locate a hidden and visible platform in the Morris water maze, even when tested in a compressed one-day water maze protocol. However, the species appear to use different strategies for locating the hidden platform; rats evince a robust spatial strategy, whereas mice appear to utilize alternative non-spatial strategies. These species differences highlight the potential of the one-day water maze task as a tool for evaluating rapidly learning and memory in rodents. Furthermore, the strategic differences revealed in this report provide critical information for the design and interpretation of future mnemonic studies in mice.

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