Cued and spatial learning in the water maze: Equivalent learning in male and female mice

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ABSTRACT

Mammals navigate a complex environment using a variety of strategies, which can operate in parallel and even compete with one another. We have recently described a variant water maze task in which two of these strategies, hippocampus-dependent spatial learning and striatum-dependent cued learning, can be dissociated. Male rodents perform better at some spatial learning tasks, while female rodents more readily learn certain striatum-dependent behavioral strategies. We therefore predicted that sex would differentially influence spatial and cued learning in the water maze. We trained adult male and female C57Bl/6 mice for 7 days in the two-cue variant of the water maze, with probe trials on days 5 and 7. In two independent experiments, males and females performed similarly, with both groups showing good spatial learning after 5 and 7 days of training, and both groups showing trend-level cued learning after 5 days and robust learning after 7. Therefore, contrary to our hypothesis, sex does not significantly affect cued or spatial learning in this task.

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Navigating a complex environment requires flexible use of learning strategies that can accommodate both reliable regularities and unpredictable deviations from the expected [4]. The multiple memory systems hypothesis proposes that different brain circuits, which can be dissociated by experimental manipulations, employ different logics of learning and are invoked under different circumstances [26]. We have recently described a water maze learning task in mice that permits assessment of cued or spatial learning in two otherwise identical task variants [13]. We found spatial learning to depend on the hippocampus, while cued learning depended on the striatum, consistent with earlier literature [16,17,15]. We found that the two systems compete with one another during learning [21], such that disruptions of striatal function can actually enhance spatial learning, while hippocampal disruptions enhance cued learning [13].

Male and female rodents have been shown to learn differentially under some conditions. Male rats show consistently superior performance in hippocampus-dependent spatial reference and working memory tasks, in both the Morris water maze and the radial maze [11]. Male mice have also been reported to show better spatial memory in a radial maze [9,12], though findings in the water maze have been more equivocal [11,8,6]. Interestingly, 'sex-reversed' female mice (which carry the male Y chromosome) perform better in the Morris water maze than normal females, suggesting an important role for male genotype (as opposed to hormonal complement) on hippocampus-dependent spatial learning [25]. Several explanations for this sexual dimorphism have been proposed, including evolved, adaptive differences in brain wiring between the two sexes (e.g. [24]), differential glucocorticoid responses to new tasks [1], differential responsivity to appetitive motivation [14], and differential response to non-spatial pretraining [19].

In contrast, limited data suggest that female mice more rapidly acquire certain striatum-dependent learning tasks. In an instrumental habit task, which is sensitive to striatal disruptions in both rats [27] and mice (J.J. Quinn, C. Pittenger, et al, unpublished observations), genotypically female mice show more rapid acquisition of habitual responding than males or 'sex-reversed' females [22]. In navigation tasks, it has been suggested that females rely more on landmark cues rather than on spatial cognition [11,23]. Earlier studies in rats [17,18], our results in a cued water maze task in mice [13], and neuroimaging studies in humans (e.g. [10]) suggest that such cue-based navigation depends on the striatum.

We therefore speculated that our variant water maze task, which tests both spatial and cued learning and can detect competition between them [13], would reveal predictable sexual dimorphisms in behavior. Specifically, we predicted that male mice would perform better than females in the spatial task, while female mice would show superior performance in the cued task. We tested this hypothesis in adult C57Bl/6 mice.

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All experiments were conducted under the supervision of the Yale University Institutional Animal Care and Use Committee (Animal Welfare Assurance Number A3230-1). Food (standard laboratory chow) and water were available *ad libitum*. All experiments used adult male and female C57Bl/6J mice, aged 2.5–4 months; male and female mice were of the same age in each experiment.

Animals were trained on either the cued or spatial variant of the water maze, using a slight variation on the protocol described in detail by Lee et al. [13]. The apparatus consisted of a large circular pool (172 cm diameter) filled with opaque water, which is maintained at 25 °C. A 12 cm circular Atlantis platform (Med Associates, Vermont), 1.5 cm below the surface of the water, permits escape from the water. The platform is marked with a visible cue, consisting of a plastic cylinder (2.5 cm diameter × 11 cm high), painted either a uniform gray or with high-contrast 1-cm horizontal or vertical stripes, as detailed below. During two-cue training (see below), a second cue was held on a stand elsewhere in the pool, such that it was not possible to discern from above the water which cue was associated with the platform and thus the possibility of escape.

All training days consisted of four trials with a 20-min inter-trial interval. The first 5 days consisted of shaping to the task. On day 1, the gray cue was used and the animal was placed on the platform for 30 s for each trial. On days 2–5 the gray cue was again used; animals were placed in the water at the edge of the pool and allowed to search for the escape platform for up to 120 s. Any animal unable to find the platform in this time period was guided to it by the experimenter. After 15 s on the platform, animals were returned to the home cage. Following shaping, animals were returned to the vivarium and left undisturbed for 1–3 days.

Shaping was followed by 7 days of cued or spatial 2-cue training. Vertically and horizontally striped cues were used for this phase; in each trial, one of these cues marked the location of the platform, while the other was placed elsewhere in the pool and did not permit escape. Each animal was assigned to either cued or spatial learning. In the cued task, the platform was consistently associated with one of the two cues (vertical or horizontal stripes, counterbalanced across animals), but varied in location among the four quadrants of the pool. In the spatial task, the platform was consistently in the same place (counterbalanced across animals), but the cue marking it varied pseudo-randomly. In both tasks, the other cue (termed the lure cue) was placed on the stand, not permitting escape, in an adjacent quadrant. On each training trial, the animal was placed in the pool against the edge, opposite both cues, and permitted to search for 120 s for the escape platform. After finding the escape platform, the animal was permitted to rest there for 15 s before being removed to its home cage.

Probe trials were performed on the fourth trial of days 5 and 7 of 2-cue training. On a probe trial, the Atlantis platform was lowered so that it did not permit escape. Search was monitored for 60 s using an overhead digital camera and automated tracking software (Any-Maze: Stoelting), after which the platform was raised and the animal permitted to escape to it. Any animal failing to escape after 60 s (or a total of 120 s of search) was guided to the platform. Systematic bias towards the goal cue in the probe trial was interpreted as evidence of learning, in either the cued or the spatial task. Probe trial performance was quantified as either quadrant occupancy or occupancy in a circular zone, 25 cm in diameter, centered on the goal or lure cue. For probe trials, the time of each animal's first touch of the goal cue was used as a latency measure in the latency analysis (Fig. 1).

Analysis of latency and probe trial data was by RM-ANOVA; effects within individual tasks were probed using lower-order ANOVAs. Analysis was performed using SPSS.

61 adult C57Bl/6J mice were trained in the cued and spatial water maze tasks [13] in two independent balanced experiments ($n = 16$ male/spatial, 16 male/cued, 15 female/spatial, 14
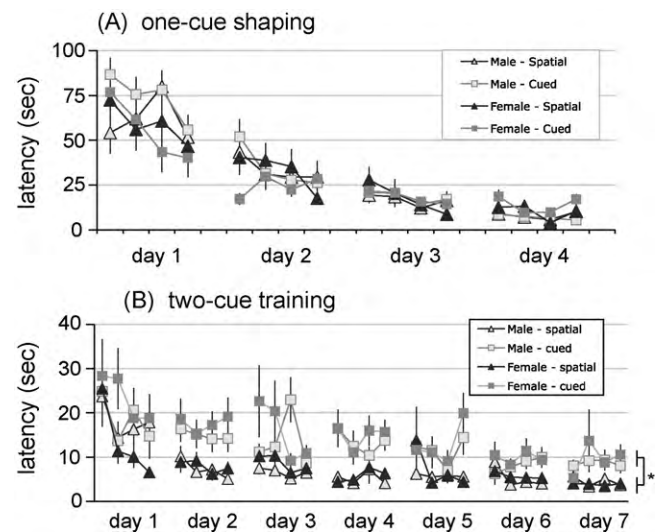

Fig. 1. Escape latencies during shaping and training. (A) Male and female mice showed similar escape latencies during 1-cue training during the shaping phase. 'Cued' and 'Spatial' mice received identical training during this phase but are separated out for illustrative purposes. All values are mean ± SEM. (B) In the 2-cue task, animals learning the spatial task showed faster escape than those learning the cued task, but there was no difference between male and female mice. See text for statistical analysis.

female/cued). Similar results were found in the initial experiment and the replication, and data were pooled for analysis, with experiment number as an independent variable in all primary analyses.

Latency to find the escape platform improved, as expected, across training trials. There was no difference between sexes in escape latencies in the one-cue shaping task (Fig. 1A). In the two-cue task, latencies improved with training for both the cued and spatial task (Fig. 1B RM-ANOVA: day, $F[6,318] = 22.6$, $p < 0.0001$; trial, $F[3,159] = 3.53$, $p < 0.02$; day × trial interaction, $F[18,954] = 2.17$, $p < 0.005$). Latencies were shorter in the spatial task than in the cued task ($F[1,53] = 34.7$; $p < 0.0001$), consistent with what we have observed previously [13]. There was no effect of sex on latency in either task, and no interactions (all $p > 0.05$). Similarly, there was no effect of experiment number and no interactions (all $p > 0.05$).

Probe trials were substituted for normal training trials on the fourth trial of days 5 and 7. Analysis of all probe trial data (RM-ANOVA of quadrant occupancy data with sex, task, and experiment as between-subjects factors and with probe trial day and quadrant as within-subject factors) showed a clear bias towards the goal quadrant (main effect of quadrant: $F[1,53] = 68.36$, $p < 0.0001$) that differed between spatial and cued tasks (quadrant × task interaction: $F[1,53] = 33.8$, $p < 0.0001$) but was not influenced by sex (quadrant × sex: $F[1,53] = 0.036$, $p > 0.1$; interactions of sex with task and trial were likewise non-significant).

In the spatial task (Fig. 2A), significant bias towards the goal quadrant was apparent across both probe trials (RM-ANOVA restricted to the spatial task: main effect of quadrant, $F[1,27] = 84.1$, $p < 0.0001$) that increased from days 5 to 7 (quadrant × trial interaction: $F[1,27] = 9.013$, $p = 0.006$). However, there was no effect of sex on quadrant bias (quadrant × sex: $F[1,27] = 0.03$, $p > 0.5$), or any interactions involving sex. Similarly, significant goal-quadrant bias but no effects of sex were found in ANOVA analysis restricted to day 5 or to day 7.

Less dramatic learning was apparent in the cued task (Fig. 2B), consistent with our previous findings [13]. Bias towards the goal quadrant was at trend level across the experiment (RM-ANOVA