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Male mice exhibit better spatial working and reference memory than females in a water-escape radial arm maze task

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Abstract

The present study examined sex differences in spatial working and reference memory in C57BL/6 mice. Males and females were tested in a version of the spatial 8-arm radial arm maze in which the motivating stimulus was escape from water. To test spatial working memory, four arms were baited with submerged escape platforms, each of which was removed after it was found. Four arms that never contained platforms assessed spatial reference memory. In addition to determining the number of working memory and reference memory errors made in each session, working memory errors made in each trial were analyzed to examine performance as the number of arms to be remembered (i.e. the working memory load) increased. Males committed significantly fewer working memory and reference memory errors than females throughout testing. Within a session, males committed fewer working memory errors than females as the working memory load increased. These sex differences were particularly evident during task acquisition. The data indicate that male C57BL/6 mice learn both the working and reference memory components of a water-escape motivated radial arm maze task better than female mice.

Theme: Neural basis of behavior

Topic: Learning and memory: systems and functions

Keywords: Sex difference; Working memory; Reference memory; Spatial memory; Mouse

1. Introduction

In humans, considerable evidence suggests that men outperform women in a variety of spatial navigation tasks [1,25,41,45,51,57,77,78]. In rodents, the findings are more equivocal. For example, although some studies using a spatial Morris water maze task report superior performance by male rats and mice relative to females [5,68,72,73,83], others report no sex differences in this task [11,87]. Recent work suggests that the observation of sex differences in mice [5,43] and rats [22,68,74] in the Morris water maze depends in large part on task parameters such as the location of start positions or use of non-spatial pre-training.

Similarly inconsistent findings have been reported in studies using another common test of spatial memory, the radial arm maze (RAM). Unlike the Morris water maze, which typically tests spatial reference memory (memory for spatial information that does not change over time, i.e. task-specific information), the RAM tests spatial working memory (memory for spatial information that changes over time, i.e. trial-specific information). As originally described, the RAM is constructed of eight arms radiating out from a round central arena and is surrounded by numerous extramaze cues [61]. At the ends of the arms are placed a food or water reward. Because rewards are not replaced after the animal obtains them, the optimal strategy is to visit each arm only once. As the trials progress, the working memory load increases because more working memory information must be stored. Thus, animals typical-

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ly make more errors in the later trials of a session [61]. However, this task is confounded by the fact that animals can use non-mnemonic strategies to locate rewards. For example, animals may locate rewards by simply entering adjacent arms, a strategy which does not require memory. Sex differences in the use of an adjacent arm strategy have been reported [89,90], which contribute to the fact that reliable sex differences have not been observed using this standard version of the RAM [16,36,72,84,89,95].

An alternative version of the RAM minimizes the effectiveness of non-spatial strategies and thus encourages the use of a spatial memory strategy. In this version, only a subset of arms is baited in order to simultaneously test spatial working memory and spatial reference memory [62]; the addition of arms that are never baited allows for the assessment of spatial reference memory (mice should never enter these arms and, thus, this spatial information remains constant). Using this version of the task, several laboratories have reported that male rats and mice make fewer working memory and reference memory errors than females [3,42,56,89,90]. This difference was particularly evident during the acquisition phase (typically the first half) of training; males and females generally performed similarly during the last half of training [89,90]. Organizational effects of sex steroid hormones have been implicated in these sex differences. For example, female rats treated neonatally with either estrogen or testosterone performed like males in adulthood, whereas male rats that were castrated or treated with testosterone neonatally performed like females in adulthood [72,89,90].

Curiously, two recent studies suggest that female rats and mice are superior to males in learning some aspects of a working/reference memory version of the RAM [7,32]. Animals in these studies were tested using a version of the RAM that uses escape from water, rather than food, as a motivating stimulus [31,32]. In this version, the RAM is placed in a tank of water, and reinforcement is provided by means of escape platforms submerged under the surface of the water. As in dry-land versions of this task, only half of the arms are baited with escape platforms and each platform is removed from the maze once found (similar to food rewards being eaten). In a non-spatial version of the water-escape motivated RAM (with intramaze visual cues provided), male BXSB mice exhibited impaired working memory relative to females [32]. Similarly, in a spatial version of the task, male Wistar rats and BXSB mice made more working memory errors than females during acquisition of the task, whereas males of both species made fewer reference memory errors than females during the last half of training [7]. It is unclear why sex differences in working memory were opposite to those reported in the dry-land RAM. Motivational (aversive versus appetitive) or procedural differences may contribute to the disparate results [7]. However, interpretation of the sex difference in BXSB mice [7] is complicated by the fact that this strain develops a spontaneous inherited lupus-like autoimmune syndrome which is accelerated in males [79,85]. Furthermore, BXSB mice exhibit ectopic neuron clusters in layer I of the neocortex (particularly prefrontal and motor cortex) that are similar to those found in the brains of dyslexic patients [24,33], and these cortical ectopias occur in a greater proportion of males (up to 80%) than in females (up to 50%) [32,79]. Ectopic mice perform better than non-ectopic mice in a Morris water maze test of spatial reference memory [8], whereas ectopic mice are impaired relative to non-ectopic mice in learning a working memory version of the spatial Morris water maze [34,88]. Given that males harbor more cortical ectopias than females, it is possible that the enhanced reference memory and impaired working memory exhibited by male BXSB mice in the RAM [7] was influenced by the predominance of cortical ectopias in this sex.

The present study examined sex differences in performance of the water-escape motivated RAM (WRAM) using C57BL/6 mice. The C57BL/6 mouse was chosen because mice of both sexes exhibit excellent learning and memory abilities in water-escape motivated tasks [20,31,58,86], and because this strain is commonly used as a background for many genetically-altered strains [2]. Thus, this is an excellent strain in which to examine sex differences in the WRAM. Male and female mice were tested in the WRAM using a protocol similar to that of Bimonte et al. [7]. Based on findings from previous dry-land RAM studies in which a subset of arms was baited, it was hypothesized that male C57BL/6 mice tested in the WRAM would exhibit better spatial working memory and spatial reference memory than female mice. This hypothesis was supported by the data.

2. Methods

2.1. Subjects

Female ($n=10$) and male ($n=10$) C57BL/6J mice were obtained at 8 weeks of age from Taconic (Germantown, NY). Upon arrival, mice were handled for 5 days (5 min/day) in order to acclimate them to being picked up by the experimenter. A total of five mice/cage were housed in a room with a 12:12 h light/dark cycle (lights on at 07:00 h). Food and water were available ad libitum for the duration of testing. All behavioral testing occurred during the light phase of the cycle. Behavioral testing began at 12 weeks of age. The estrous cycle of female mice was not monitored by vaginal lavage during testing so that the stress of undergoing this procedure did not influence WRAM performance. Furthermore, working memory in the dry-land RAM is not influenced by stage of the estrous cycle [82], so there is no evidence to suggest that cyclic hormonal fluctuations modulate performance of the WRAM. All procedures followed the guidelines of the National Institutes of Health Guide for the Care and Use of

Laboratory Animals, and were approved by the Animal Care and Use Committee of Yale University.

2.2. Water-escape motivated radial arm maze

The protocol for this task was adapted from Bimonte et al. [7] and was designed to assess spatial working and reference memory simultaneously. The center of the maze (diameter 44 cm) was opaque and the eight arms radiating from the center (38×12 cm) were constructed of clear Plexiglas (Fig. 1). The maze was placed in a large pool of water (24±2 °C) made opaque with white non-toxic tempera paint. Hidden escape platforms (submerged just below the water surface and ~9 cm below the top of the maze) were placed at the ends of four of the arms (Fig. 1). One of the arms was designated as a start arm and it never contained a platform. The sequence of arms with platforms was randomized between mice but remained unchanged within a mouse for all sessions. Platforms were never located in more than two consecutive adjacent arms. Salient extra maze cues in the testing room included pictures on the walls, a sink, a window, and a small table.

Prior to the first test session, each mouse completed a five trial shaping procedure which served to acquaint the mice with the escape platforms. During shaping, only one arm contained a platform, which was made visible by covering it with red tape and lowering the water level to ~0.3 cm below the platform surface. For the first four trials, all eight arms were blocked off and the mouse was confined to the shaping arm. In the first shaping trial, the mouse was placed directly on the platform for 15 s. With each successive trial, the mouse was placed at further distances from the platform such that for the fourth trial, the mouse was placed at the entrance to the shaping arm. During the final trial, the shaping arm was opened to allow access to the center of the maze (the other seven arms remained blocked). The mouse was then placed in the center and allowed to climb on the platform in the shaping arm. If, on any trial, the platform was not found in 30 s, the mouse was gently guided to it. No data were collected during shaping.

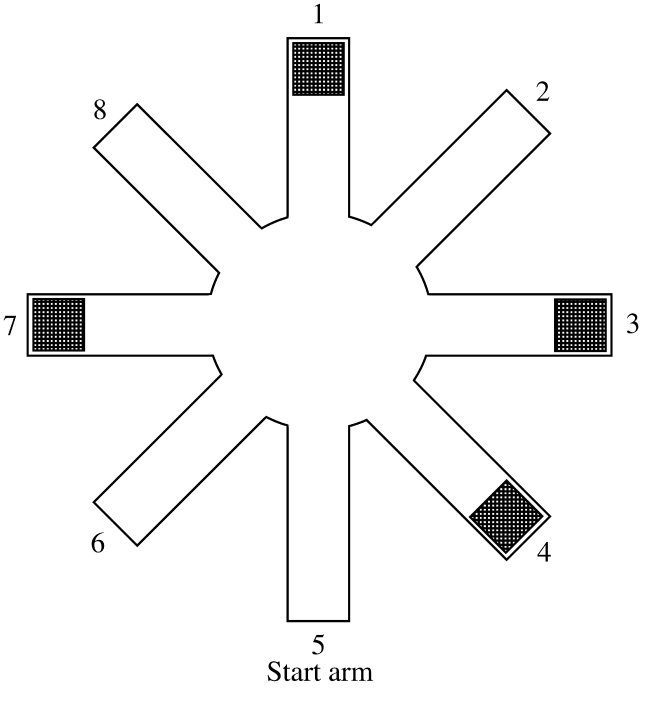

Fig. 1. Schematic diagram illustrating the WRAM apparatus. A sample sequence of arms containing platforms is depicted by the filled squares at the ends of arms 1, 3, 4, and 7. Mice were always placed in the maze in arm 5 (the start arm).

The first day of testing began the day after shaping. Each mouse completed four trials per day (comprising one session) for 15 consecutive days as follows. At the start of trial 1, the mouse was released from the start arm and given 120 s to locate and climb onto a submerged platform. If the mouse did not find a platform within this time, it was gently guided to the nearest one. Once on the platform, the mouse remained there for 15 s, after which time it was removed from the platform, dried off with a towel, and placed in a holding cage for a 30-s inter-trial interval (ITI). During the ITI, the platform that had been found was removed from the maze, leaving three platforms in the pool. The mouse was then returned to the start arm for trial 2. This procedure was repeated until all four platforms were located (one platform per trial). At the end of the fourth trial, the mouse was removed from the maze, dried off with a towel, and returned to its home cage.

Three types of errors were recorded during each trial of the daily sessions as in Refs. [7,32,34]. Mice were considered to have entered an arm when the entire body (excluding the tail) crossed into the arm. Entries into arms from which a platform had been removed during a daily session were considered working memory errors. In each trial, first entries into arms that never contained a platform were considered initial reference memory errors. Finally, repeated entries into arms that never contained a platform were termed repeated reference memory errors. Although an error of this type involves a lapse of both reference and working memory, in that the mouse fails to remember that an arm does not contain a platform (reference) and that it has previously entered this arm during the session (working), we will refer to this error as a reference memory error because it involves entries into arms that never contain platforms. In addition to determining the total number of working memory errors committed in each session, the number of working memory errors committed in trials 2–4 of each session was determined (it was impossible to make a working memory error in trial 1). This allowed working memory errors to be assessed as the trials progressed and the working memory information to be remembered (i.e. working memory load) increased [31]. Values analyzed for trials 2–4 were the average working memory errors made in each individual trial in the sessions of interest (see below).

2.3. Data analysis

Working memory errors, initial reference memory errors, and repeated reference memory errors were each analyzed separately using one between (Sex: female or male) and one within (Sessions) repeated measures analyses of variance (ANOVA). Working memory errors made in each trial were analyzed using a one between (Sex) and one within (Trials) repeated-measures ANOVA. Independent sample *t*-tests measured sex differences in individual trials.

The first day of testing was considered a training day and thus, data from session 1 were excluded from all analyses [7]. Because mice during session 1 are introduced for the first time to the entire maze, the platform locations, hidden platforms, and the concept that platforms disappear once found, this session does not accurately measure any aspect of working or reference memory. Therefore, working and reference memory are analyzed starting in session 2, at which point the mice have been fully exposed to the apparatus and rules of the task. Data for males and females, respectively, during session 1 were: working memory errors, 6.8±1.2 and 4±1.2; initial reference memory errors, 4.8±0.9 and 4.9±0.8; repeated reference memory errors, 1.7±0.7 and 1.9±0.5. In addition, previous studies have demonstrated that the greatest amount of learning in the WRAM task occurs during the first half of testing, specifically during sessions 2–8 [7,32,34]. In order to more closely examine effects on task acquisition, separate ANOVAs were conducted in these studies for the first and last halves of testing [7,32,34]. To facilitate comparison of our data with those of previous studies, we have also conducted separate analyses on the first half (sessions 2–8) and last half (sessions 9–15) of testing.

3. Results

3.1. Working memory errors

Males committed fewer working memory errors than females, particularly during the first half of testing (Fig. 2). During sessions 2–15, the number of working memory errors decreased significantly ($F_{13,234}=5.51$, $P<0.0001$). The main effect of Sex in sessions 2–15 was significant ($F_{1,18}=6.83$, $P<0.02$), with males committing fewer errors than females. The Sex×Session interaction was not significant ($F_{13,234}=1.22$, $P>0.05$). Separate analyses of sessions 2–8 and 9–15 indicated that the significant main effects of Sex and Session in the sessions 2–15 analysis were driven by performance of the groups during task acquisition. Specifically, the number of working memory errors decreased significantly during the first half (sessions 2–8: $F_{6,108}=3.17$, $P<0.007$), but not the second half of testing (sessions 9–15: $F_{6,108}=1.68$, $P>0.05$). Furthermore, males committed significantly fewer errors than females during acquisition (sessions 2–8: $F_{1,18}=8.74$, $P<0.009$), but not during sessions 9–15 ($F_{1,18}=0.25$, $P>0.05$). The Sex×Session interaction was not significant during either half of testing (sessions 2–8: $F_{6,108}=1.05$, $P>0.05$; sessions 9–15: $F_{6,108}=0.64$, $P>0.05$).

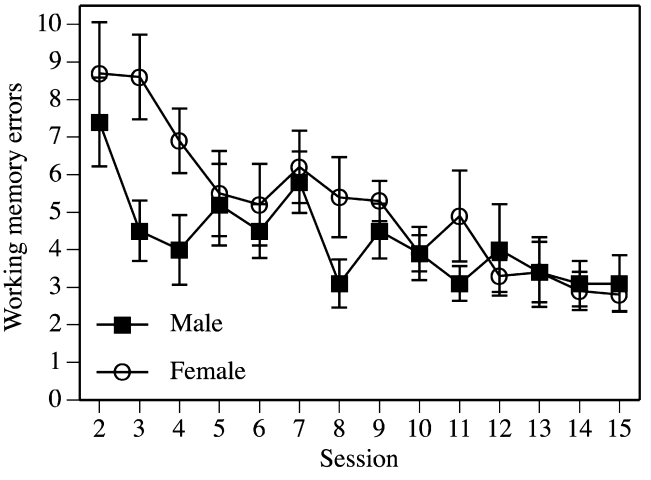

Fig. 2. Males committed fewer working memory errors than females during sessions 2–15. Sex differences were particularly evident during sessions 2–8 (see text). Each point represents the mean±S.E.M. of each group for a single session.

Males also made fewer working memory errors than females within a session as the working memory load increased. Overall, the number of working memory errors increased in each trial of sessions 2–15, as suggested by a significant Trial effect ($F_{2,36}=188.36$, $P<0.0001$). During sessions 2–15, males committed fewer errors than females (main effect of Sex: $F_{1,18}=6.84$, $P<0.02$; Fig. 3A). Although *t*-tests suggested that this sex difference was largely due to performance during trial 3 ($t_{18}=-3.7$, $P<0.003$), the Sex×Trial interaction was not significant ($F_{2,136}=1.67$, $P>0.05$). When performance during sessions 2–8 and sessions 9–15 was analyzed separately, the main effect of Trial was significant for both halves of testing (sessions 2–8: $F_{2,36}=120.67$, $P<0.0001$; sessions 9–15: $F_{2,36}=65.93$, $P<0.0001$). Males made fewer working memory errors than females during trials 3 and 4 of sessions 2–8 (t's$_{18}=-3.03$ and -2.26, respectively, P's<0.04; Fig. 3B), but not in any trial during sessions 9–15 (t's$_{18}=0.23$ to -1.22, P's>0.05; Fig. 3C). Accordingly, the Sex×Trial interaction was significant for sessions 2–8 ($F_{2,36}=3.18$, $P=0.05$), but not sessions 9–15 ($F_{2,36}=0.32$, $P>0.05$).

3.2. Initial reference memory errors

Similar to working memory errors, males made fewer initial reference memory errors than females, particularly during the first half of testing (Fig. 4A). During sessions 2–15, the number of initial reference memory errors

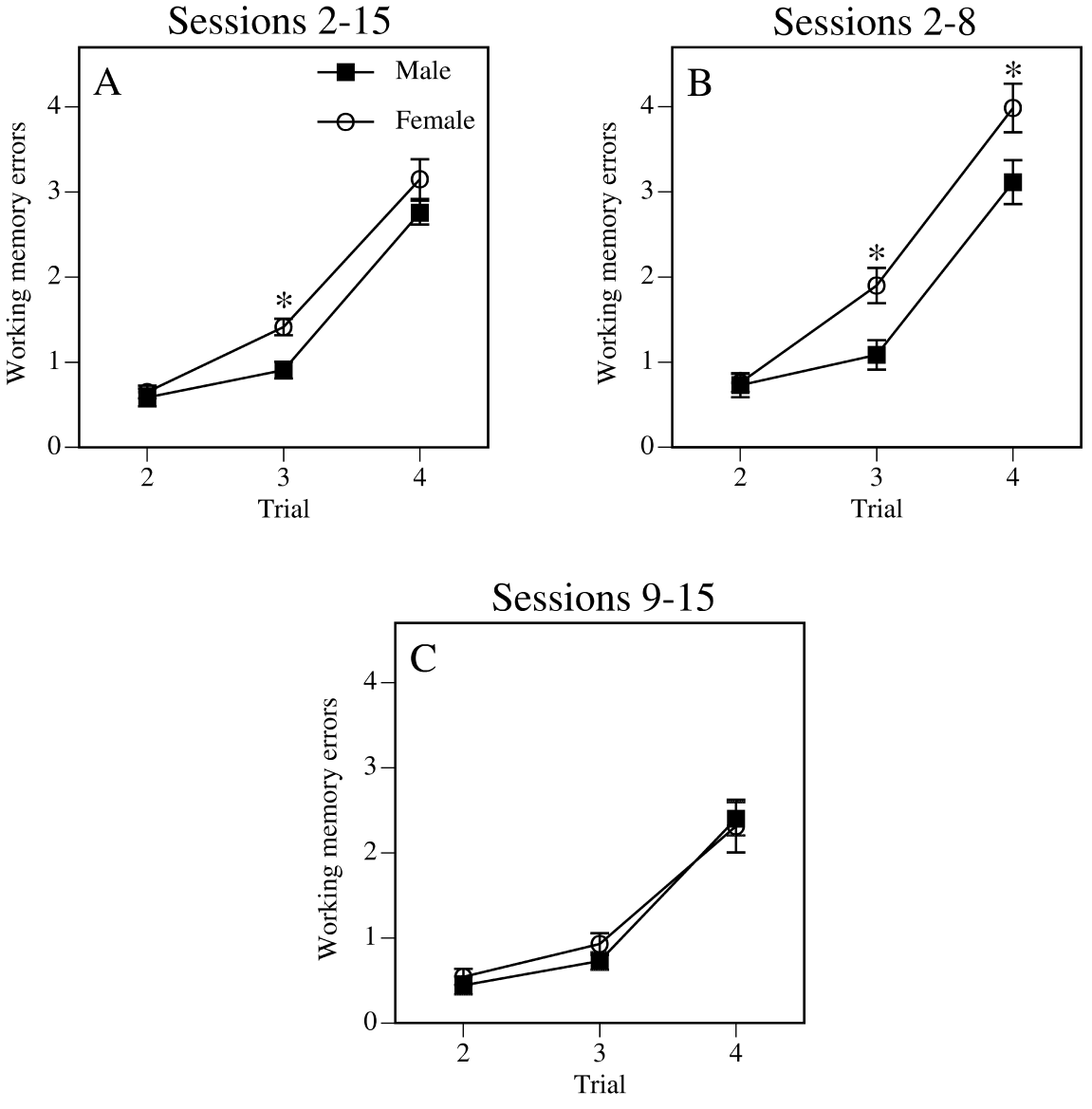

Fig. 3. Working memory errors committed by males and females during trials 2–4 of each session. Overall, males made fewer errors than females as the working memory load increased. During sessions 2–15 (A), males made fewer errors relative to females, particularly during trial 3 (*t*-test, **P*<0.05). During sessions 2–8 (B), males performed better than females during trials 3 and 4 (*t*-tests, **P*'s<0.05). No sex differences were observed during sessions 9–15 (C). Each point represents the mean±S.E.M. of each group for a single trial.

decreased significantly ($F_{13,234}$=15.29, P<0.0001), and males committed fewer initial reference memory errors than females ($F_{1,18}$=4.52, P<0.05). However, the Sex×Session interaction was not significant ($F_{13,234}$=0.92, P>0.05). Similarly, during sessions 2–8, initial reference memory errors decreased ($F_{6,108}$=8.39, P<0.0001), and males committed fewer errors than females ($F_{1,18}$=7.03, P<0.02). Again, the Sex×Session interaction was not significant ($F_{6,108}$=0.97, P>0.05). During sessions 9–15, initial reference memory errors continued to decrease ($F_{6,108}$=2.88, P<0.02), however neither the main effect of Sex ($F_{1,18}$=0.63, P>0.05) nor the Sex×Session interaction ($F_{6,108}$=0.58, P>0.05) was significant.

3.3. Repeated reference memory errors

Males also made fewer repeated reference memory errors than females during acquisition (Fig. 4B). During sessions 2–15, the number of repeated reference memory errors decreased significantly ($F_{13,234}$=7.22, P<0.001). This effect was primarily due to improvements made during the first half of testing (sessions 2–8: $F_{6,108}$=5.17, P<0.0001; sessions 9–15: $F_{6,108}$=1.19, P>0.05). The main effect of Sex during sessions 2–15 ($F_{1,18}$=6.60, P<0.02) was also driven by performance during task acquisition, as males committed fewer errors than females during sessions 2–8 ($F_{1,18}$=11.55, P<0.004) but not

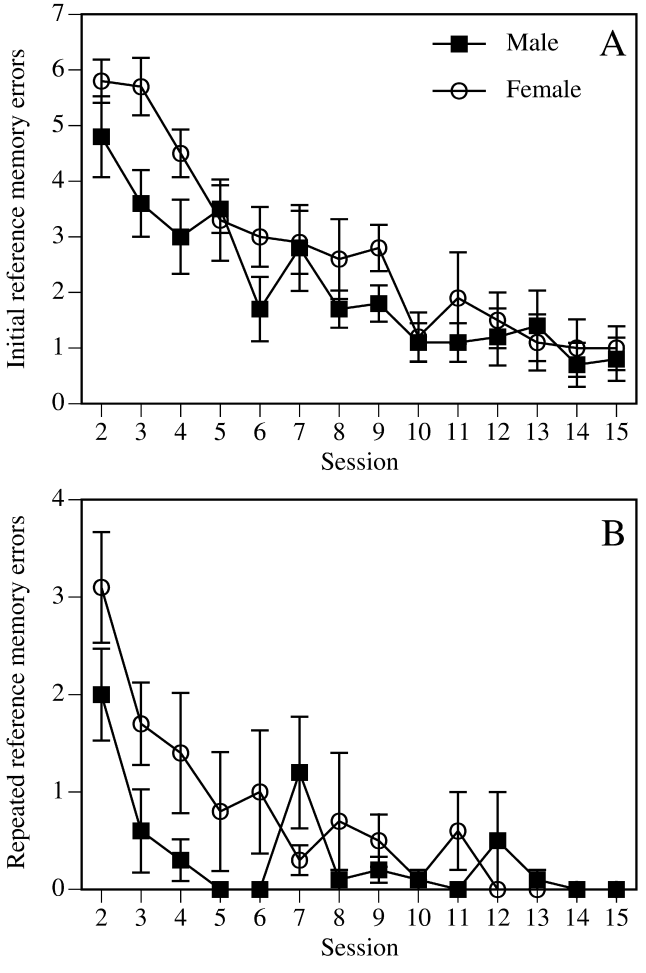

Fig. 4. Males committed fewer initial reference memory (A) and repeated reference memory (B) errors than females during sessions 2–15. Sex differences were particularly evident during sessions 2–8 (see text). Each point represents the mean±S.E.M. of each group for a single session.

during sessions 9–15 ($F_{1,18}$=0.14, P>0.05). The Sex× Session interaction was not significant at any point during testing (sessions 2–15: $F_{13,234}$=1.65; sessions 2–8: $F_{6,108}$=1.18; sessions 9–15: $F_{6,108}$=1.60, P's>0.05).

4. Discussion

The results of the present study indicate that male C57BL/6 mice are superior to female mice in learning both the working and reference memory components of a water-escape radial arm maze task. Males committed significantly fewer working memory, initial reference memory, and repeated reference memory errors than females throughout testing, although the sex difference was particularly evident during task acquisition (i.e. sessions 2–8). Males also committed significantly fewer working memory errors at medium (trial 3) and high (trial 4) working memory loads. As such, these data confirm our hypothesis of a male advantage in both working and reference memory in the WRAM.

The current data are consistent with several studies that have used dry-land versions of the working/reference memory RAM with a subset of arms baited. Similar to the present study, male rats and mice in previous dry-land studies made fewer working memory and reference memory errors than females, particularly during acquisition [3,42,56,89,90]. The current data are also consistent with findings in rats that neonatal manipulation of sex-steroid hormones can masculinize dry-land RAM performance in adult females and feminize RAM performance in adult males [72,89,90]. In contrast, our data are discordant with a previous WRAM study which found that female BXSB mice and Wistar rats made fewer working memory and repeated reference memory errors than males during the first half of testing [7]. Using a similar WRAM task, we found the exact opposite sex difference. This study also found that males committed fewer initial reference memory errors during the last half of testing [7], whereas we found no sex differences during this phase of training. Although our data cannot directly address discrepancies among studies of rats tested in the two versions of the RAM, the consistencies between the current mouse data and those of mice and rats tested in dry-land radial arm mazes indicate that methodological differences may not be the primary contributors to discrepancies between the current and previous WRAM data [7]. The neocortical ectopias that develop prenatally in BSXB mice disrupt the laminar organization of the cortex and alter connections both within the neocortex and to brain regions such as the thalamus [35,80]. A disruption of thalamo-cortical circuitry, which has been shown to play a role in modulating spatial working memory in the dry-land RAM [19], may significantly impair performance in the RAM. The greater incidence of ectopias in male mice [32,79], combined with the fact that ectopic mice exhibit better spatial reference memory and worse spatial working memory relative to non-ectopic mice [8,34,88], suggests that sex differences in BXSB mice [7] may be a consequence of sex differences in ectopia incidence. Nevertheless, the congruence between the present WRAM data and findings from dry-land RAM studies indicates that the two versions of the RAM similarly test spatial working and reference memory. As such, the WRAM provides an alternative method of assessing these types of memory, a conclusion that could prove particularly useful for studies, such as those using cycling females or aged subjects, in which the nutrient restriction required for the dry-land RAM is not desirable.

Multiple factors may contribute to the sex differences in WRAM performance observed in the present study. Among these possibilities are disparate swimming ability or reaction to the stress of swimming. In the standard Morris water maze task, female C57BL/6 mice swim faster than males [20], and thus, it is possible that females made more errors because their faster swim speed allowed

them to make more arm choices. This explanation is unlikely because the sexes made a similar number of arm entries (including all three error types) during session 1 before they knew the rules of the task (males=12.1±1.9 total arm entries, females=9.5±1.8 total arm entries; t_{18}= 0.99, $P>0.05$). If the rapid swim speeds of females had led them to make more arm entries, then a sex difference would be expected in the first session of testing. It is possible that the more complicated and restrictive apparatus of the RAM compared to the open pool of the Morris water maze renders swim speeds less of a factor in RAM choice accuracy. This possibility is supported by data from dry-land RAM tasks which suggest that this task is less influenced by sex differences in activity than other spatial maze tasks [89]. Swim stress may also have contributed to the sex differences observed in the present study. A single day of training in a Morris water maze task has been associated with levels of corticosterone similar to those seen after acute restraint stress [40] (J.J. Kim, personal communication). In the hippocampus, stress severely reduces synaptic plasticity, alters dendritic morphology, and inhibits neurogenesis (see Ref. [39] for a recent review). However, sex differences in the morphological and neurochemical response to stress have been demonstrated in the hippocampus, neocortex, and amygdala [18] (see Refs. [10,47] for recent reviews). Sex differences in the effects of stress on various types of memory have also been reported. For example, classical conditioning in rats is facilitated in stressed males, but impaired in stressed females [92,93]. Also, stress impairs object recognition in group housed males, but not females [4]. Interestingly, in dry-land RAM tasks, 21 days of restraint stress impairs working memory in males [49] and enhances working memory in females [9]. Given that females in the present study performed worse than males, these dry-land RAM findings would seem to argue against stress as a factor in the sex differences observed in this study. However, it is unknown whether chronic restraint stress and chronic escapable swim stress result in comparable levels of stress, and therefore, it remains possible that disparate responses to the stress of swimming influenced the direction of the sex differences in this study.

Interestingly, genetic sex in C57BL/6 mice has also been shown to influence performance of a spatial task, the standard Morris water maze task. One particular study found that XX-females of the C57BL/6JEi-Y^{POS} strain performed significantly worse in the spatial water maze task than XY-females of the same strain or than XY-C57BL/6 males [83]. In contrast, XX- and XY-females did not differ on several other non-spatial tasks [83], suggesting that the Y chromosome confers a specific advantage for spatial navigation or spatial memory. Given these data, it is likely that genetic sex played some role in the sex difference observed in this study. Although XY-female mice have never been tested in a radial arm maze task, the Morris water maze data lead to the prediction that XY-females would exhibit better reference memory (and perhaps better working memory) than XX-females in the WRAM.

The male advantage in the WRAM may also stem from sexual dimorphisms in several brain regions. Considerable evidence suggests that the septohippocampal system and various regions of the neocortex are critical for successful RAM performance [14,44,53,60,69]. Numerous studies report sex differences in these cognitive brain regions. For example, in the neocortex, marked differences in the laterality of cortical thickness and estrogen receptor distribution have been noted [15,75]. In the hippocampus, males exhibit a greater number of mossy fiber synapses [52,66] and dentate granule cells [91], greater slice excitability [81], and higher glutamic acid decarboxylase activity [21]. Dendritic branching of dentate granule and CA3 pyramidal cells is also sexually dimorphic, the direction of which varies in different parts of the dendritic tree [37,38]. Administration of testosterone to neonatal females can induce the development of hippocampal morphology similar to males [73]. Furthermore, sex differences in neocortical choline acetyltransferase activity and hippocampal axonal sprouting have been observed in response to anti-nerve growth factor treatment and lesions, respectively [46,70]. Although a description of all reported sexual dimorphisms in these brain regions is beyond the scope of this discussion, those noted above illustrate the diversity of sexual dimorphisms in cognitive regions of the brain. Attributing the behavioral sex differences observed in the current study to any particular dimorphism would be speculation at the present time.

Activational effects of sex-steroid hormones also likely contribute to the observed sex differences in WRAM performance. For example, estrogen has been shown to modulate hippocampal and neocortical morphology and function in adult rodents of both sexes (for reviews, see Refs. [27,54,94]), and both the neocortex and hippocampus exhibit sexually dimorphic responses to estrogen administration [55,67]. Several studies suggest that exogenous estrogen can improve the spatial working memory of adult females in dry-land and water-escape RAM tasks [6,13,17,29,30,50] (although see Ref. [48]), and in other spatial working memory tasks [26,28,59]. Exogenous estrogen also improves spatial reference memory in the Morris water maze in ovariectomized females [63,64,71,76] (but see Refs. [12,23]) and in males [65], although the spatial reference memory of females in the dry-land RAM is reportedly not affected by estrogen [17,30]. The present study suggests that the low levels of endogenous estrogen to which females are exposed during most of the estrous cycle may impair working and reference memory in intact females. Because RAM testing lasted over 2 weeks, and our intact females were tested over the course of several estrous cycles, the elevated hormone levels present during 2 or 3 days over the course of 15 days of testing are unlikely to have had a major

impact on performance. Furthermore, previous work has demonstrated that working memory in a dry-land RAM task is stable across the estrous cycle [82], and therefore, it is doubtful that cyclic hormonal fluctuations contributed to differences between males and females.

5. Conclusion

The present study demonstrates that male C57BL/6 mice exhibit superior spatial working and reference memory relative to female mice in a water-escape version of the RAM. Multiple factors, including genetic sex, sexual dimorphisms in the brain, and swim stress may have contributed to the male advantage in the WRAM. This sex difference is consistent with those found in dry-land versions of the RAM, suggesting that the WRAM is an effective method of assessing sex differences in rodents (particularly mice) and could be used in future studies to investigate neural correlates of sex differences in spatial working and reference memory.

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