Behavioral Neuroscience
2003, Vol. 117, No. 6, 1283–1291

0735-7044/03/$12.00 DOI: 10.1037/0735-7044.117.6.1283

Sex Differences in the Behavioral Response to Spatial and Object Novelty in Adult C57BL/6 Mice

Karyn M. Frick and Jodi E. Gresack
Yale University

The present studies examined sex differences in object localization and recognition in C57BL/6 mice. Experiment 1 measured responses to spatial novelty (object displacement) and object novelty (object substitution). Males strongly preferred displaced and substituted objects over unchanged objects, whereas females showed a preference in only 1 measure of object novelty. Experiment 2 further examined object recognition by presenting mice with 2 identical objects, followed 24 hr or 7 days later by testing with a familiar and a novel object. After 24 hr, males preferentially explored the novel object, whereas females exhibited no such preference. Neither sex displayed a preference for the novel object after 7 days. The data suggest that male mice are superior to females at localizing and recognizing objects.

Considerable evidence suggests that men outperform women in mental rotation and spatial navigation tasks (see Kimura, 1999; Linn & Peterson, 1985; Maccoby & Jacklin, 1974, for reviews). The male advantage in spatial navigation has been documented in humans using various methods, including pencil-and-paper route learning tasks (Galea & Kimura, 1993), computer virtual navigation tasks (Astur, Ortiz, & Sutherland, 1998; Moffat, Hampson, & Hatzipantelis, 1998; Sandstrom, Kaufman, & Huettel, 1998), and real-world navigation tasks (Saucier et al., 2002). A similar male superiority in spatial navigation has been observed in several rodent species. For example, males of polygamous vole species range over wider territories, exhibit better spatial navigation abilities, and have larger hippocampi than females (Gaulin & FitzGerald, 1986; Jacobs, Gaulin, Sherry, & Hoffman, 1990). In contrast, sex differences in spatial abilities and hippocampal size are not observed in monogamous vole species, in which males and females have similar range sizes (Gaulin & FitzGerald, 1986; Jacobs et al., 1990). Among rats and mice, several studies have reported that males are superior to females in spatial tasks such as the radial arm maze and Morris water maze (Berger-Sweeney, Arnold, Gabeau, & Mills, 1995; Mishima, Higashitani, Kazuhiko, & Yoshioka, 1986; Roof, 1993; Williams, Barnett, & Meck, 1990; Williams & Meck, 1991; although see Bimonte, Hyde, Hoplight, & Denenberg, 2000; Bucci, Chiba, & Gallagher, 1995; Healy, Braham, & Braithwaite, 1999; Lamberty & Gower, 1988; van Haaren, Wouters, & van de Poll, 1987; Voikar, Koks, Vasar, & Rauvala, 2001). The sex difference in rats stems, at least in part, from organizational effects of sex steroid hormones (Roof, 1993; Williams et al., 1990; Williams & Meck, 1991).

In contrast to spatial navigation, sex differences in nonspatial memory are not well characterized. Studies in humans and rats have illustrated that females navigate using both landmarks and Euclidean geometry, whereas males rely primarily on Euclidean information (Galea & Kimura, 1993; McGuiness & Sparks, 1983; Miller & Santoni, 1986; Sandstrom et al., 1998; Saucier et al., 2002; Williams et al., 1990; Williams & Meck, 1991). This may suggest that females have a greater tendency than males to encode information about objects and object locations. Indeed, some work in humans has shown that women are better than men at noticing object displacements in both two and three dimensions (Eals & Silverman, 1994; McBurney, Gaulin, Devineni, & Adams, 1997; Silverman & Eals, 1992). However, sex differences in the ability of rodents to identify objects and recall object locations have rarely been examined (Ghi, Orsetti, Gamalero, & Ferretti, 1999; Ricceri, Colozza, & Calamandrei, 2000). Memory for object location and identity can be examined in rodents using a spatial–object novelty task that takes advantage of the natural tendency of rodents to investigate novelty (Ammassari-Teule, Tozzi, Rossi-Arnaud, Save, & Thinus-Blanc, 1995; Poucet, 1989; Ricceri et al., 1999, 2000; Thinus-Blanc, Save, Rossi-Arnaud, Tozzi, & Ammassari-Teule, 1996). In this task, rats or mice are allowed to explore several different objects in an open field. Once they have habituated to the objects, one or two objects are moved to new locations in the arena and reaction to the spatial displacement is recorded. Following habituation to the displaced object, one of the nondisplaced objects is replaced by a new object. Animals typically respond to the changes by preferentially exploring the displaced and substituted objects over the nondisplaced and nonsubstituted objects. These preferences are generally thought to indicate the formation of memories for the locations and identities of the objects.

The present studies were designed to examine sex differences in spatial and object memory in C57BL/6 mice. In Experiment 1, male and female mice were tested in the novelty task described

Karyn M. Frick, Department of Psychology and Interdepartmental Neuroscience Program, Yale University; Jodi E. Gresack, Department of Psychology, Yale University.

Funding for this research was provided by Yale University and an American Foundation for Aging Research/Pfizer Research Grant in Hormones and Aging to Karyn M. Frick. We thank Paulette MacRae, Tovia Martirosian, Anwar Parker, Julie Rosenthal, Tony Taglienti, and Susan Vanderhill for assistance with behavioral testing and Mark G. Baxter for his critical reading of the manuscript.

Correspondence concerning this article should be addressed to Karyn M. Frick, Department of Psychology, Yale University, 2 Hillhouse Avenue, PO Box 208205, New Haven, Connecticut 06520. E-mail: karyn.frick@yale.edu

above. One previous study using this task has reported that 3-month-old male and female CD-1 mice both show robust preferences for displaced and substituted objects (Ricceri et al., 2000). However, the lack of a sex difference in response of the outbred CD-1 strain to spatial and object novelty may reflect the fact that the gonads of these mice are approximately 16 times less responsive to estrogen than those of inbred strains, such as the C57BL/6 (Spearow & Barkley, 2001; Spearow, Doemeny, Sera, Leffler, & Barkley, 1999). This estrogen insensitivity could interfere with the normal organizational and activational effects of estrogen on the brain (Diamond, 1987; Juraska, 1991; Loy & Sheldon, 1987) and thus preclude the development of sex differences in memory abilities in the CD-1 strain. Therefore, the current study used C57BL/6 mice, a strain that is not only responsive to estrogen (Frick & Berger-Sweeney, 2001; Frick, Fernandez, & Bulinski, 2002) but also exhibits excellent spatial and contextual learning and memory abilities (Hyde, Hoplight, & Denenberg, 1998; Nguyen, Abel, Kandel, & Bourtchouladze, 2000; Upchurch & Wehner, 1988). Although C57BL/6 mice are inbred rather than outbred like CD-1 mice, they are commonly used as a background for many genetically altered strains (Banbury Conference on Genetic Background in Mice, 1997), and, thus, these data would be of critical importance in interpreting the effects of genetic alterations on object memory. Based on our findings from Experiment 1, we were interested in further examining sex differences in object recognition. Thus, in Experiment 2, male and female C57BL/6 mice were tested in a second object recognition task (Clark, Zola, & Squire, 2000; Ennaceur & Delacour, 1988). The results of both experiments indicate that male C57BL/6 mice exhibit better memory for object locations and identities than female C57BL/6 mice.

Experiment 1

Mice in Experiment 1 were tested in the spatial–object novelty task described above. This task involves four objects, three of which are presented at a time. After habituation to three objects, one object is moved to a new location, followed two trials later by the replacement of one familiar object with a novel object. Previous studies in humans suggest that the sexes do not differ in noticing object displacements when objects are moved to novel locations (James & Kimura, 1997) but that women are better than men in identifying novel objects added to an object array (Eals & Silverman, 1994; Silverman & Eals, 1992). Thus, we expected to find no sex difference in reaction to spatial novelty but an increased reaction in females to object novelty.

Method

Subjects. Male ($n = 25$) and female ($n = 23$) C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were handled 5 min per day for 5 days prior to testing. Five mice were housed per cage in a room with a 12-hr light–dark cycle (lights on at 0700). Behavioral testing started at approximately 3 months of age and occurred during the light phase of the cycle. Food and water were available ad libitum for the duration of testing. 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.

Apparatus. The apparatus was an open field box (60 cm wide × 60 cm long × 47 cm high) constructed of wood and painted white. The open field box was located in a quiet room under fluorescent lighting. Four objects were used for testing: a clear plastic rodent toy shaped like a cube with a pyramid attached to one side, a large clear plastic clothespin, a silver metal box, and a brown ceramic cup. The objects were similar in size, approximately 10 cm × 6 cm × 6 cm. During testing, the objects were placed approximately 5 cm from the walls of the box to allow for investigation of all sides of the objects. A video camera was mounted on the ceiling above the box and was connected to a video recorder, monitor, and computer in an adjoining room.

Procedure. The object novelty procedure was based on previously published methods (Ammassari-Teule et al., 1995; Poucet, 1989; Ricceri et al., 1999, 2000; Thinus-Blanc et al., 1996). Each mouse completed one test session consisting of seven successive trials. Trials were 5 min in duration and were separated by a 3-min intertrial interval (ITI). After completing each trial, the mouse was removed from the box and placed in a holding cage next to the testing box for the duration of the ITI. During this period, the box and objects were wiped with 70% alcohol. During each trial, the door to the testing room was closed and movements were observed on the monitor in the adjoining room.

In Trial 1, each mouse was placed in the empty open field and allowed to freely explore the testing arena (see Figure 1A). For approximately half the mice ($n = 14$ for females, $n = 13$ for males), locomotor activity in the open field was assessed by recording the number of crossings of a 5 × 3 grid laid over the field on the computer monitor. The number of total grid crossings, outer grid crossings, and inner grid crossings were recorded. During Trial 2, three objects were placed near the corners of the box (see Figure 1B). The mouse was then placed in the unoccupied corner facing the wall and allowed to freely investigate the objects. The configuration of the objects remained unchanged during Trials 3 and 4 to allow the mice to habituate to the objects. Response to spatial novelty was examined in Trial 5 by displacing one object to a new location in the box (see Figure 1C). The object configuration remained the same in Trial 6 to permit habituation to the new configuration. Response to object novelty was examined during Trial 7 by replacing one of the familiar objects that was not moved in Trial 5 with a novel object (see Figure 1D). At the completion of Trial 7, the mouse was returned to its home cage. Using the video tracking system and a custom-written computer program, we recorded the duration (time) and frequency (number of visits) of object exploration during each trial. Object exploration was scored only when the mouse's nose or front paws were in contact with the object.

Data analysis. Each locomotor activity measure recorded during Trial 1 was analyzed using separate one-way analyses of variance (ANOVAs).

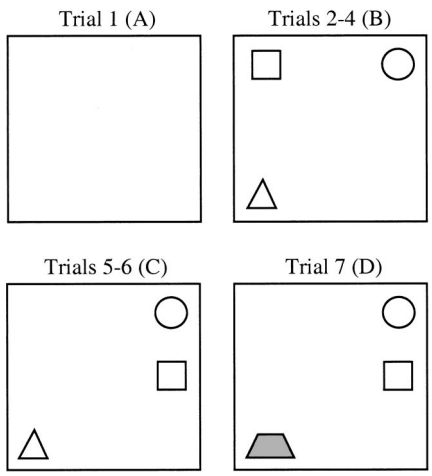

Figure 1. Schematic diagram illustrating the object configurations in the spatial–object novelty task (A–D). Each symbol (square, circle, triangle, and parallelogram) represents a different object.

Habituation to the objects was examined using 2 (sex) × 3 (session) repeated-measures ANOVAs conducted for time and number of visits during Trials 2–4. To assess response to spatial novelty, we compared exploration of the displaced object (DO) with exploration of the nondisplaced objects (NDOs) in Trial 4 (last trial with the original configuration) and Trial 5 (first trial with the new configuration). Specifically, the time spent in Trial 4 with the object that was displaced in Trial 5 was subtracted from the time spent with that object in Trial 5 (positive values indicate an increase in the amount of time spent with the DO in Trial 5 relative to Trial 4). Similarly, the time spent in Trial 4 with the NDOs was subtracted from the time spent with the NDOs in Trial 5 (negative values indicate a reduction in the time spent with the NDOs in Trial 5 relative to Trial 4). Number of visits to the DO and NDOs were compared in the same manner. Values for time and number of visits to the DO and NDOs were compared using 2 (sex) × 2 (DO, NDO) repeated-measures ANOVAs. Paired sample *t* tests comparing the time and number of visits with the DO versus the NDOs were conducted within a sex to determine whether each sex showed a preference for the DO over the NDOs.

To assess response to object novelty, we compared exploration of the substituted (i.e., novel) object (SO) in Trial 7 with exploration of the nonsubstituted (i.e., familiar) objects (NSOs) in Trial 6. Time spent in Trial 6 with the object that was replaced in Trial 7 was subtracted from the time spent with the new object in Trial 7 (positive values indicate an increase in the amount of time spent with the SO in Trial 7 relative to Trial 6). Similarly, the time spent with the NSOs in Trial 6 was subtracted from the time spent with the NSOs in Trial 7 (negative values indicate a reduction in the time spent with the NSOs in Trial 7 relative to Trial 6). Frequency of visits to the SO and the NSOs were compared in the same manner. Values for duration and number of visits to the SO and NSOs were compared using 2 (sex) × 2 (SO, NSOs) repeated-measures ANOVAs. Paired sample *t* tests comparing the duration and number of visits with the SO versus the NSOs were conducted within a sex to determine whether each sex showed a preference for the SO over the NSOs.

Results

Subjects. One female was not included in the analyses because of missing data during Trial 7. One male was not included because he did not explore any object during Trials 4–7.

Locomotor activity. Total grid crossings did not differ significantly between males (105.7 ± 9.1) and females (134.8 ± 13.7), although females tended to be more active than males, $F(1, 23) = 3.1$, $p = .09$. A similar trend was found for outer grid crossings (males = 88.8 ± 6.8, females = 114.9 ± 11.2), $F(1, 23) = 3.8$, $p = .06$. Neither males nor females made many inner grid crossings, so this measure did not differ between the sexes (males = 16.9 ± 2.4, females = 19.9 ± 2.9), $F(1, 23) = 0.6$, $p > .05$.

Habituation. Object exploration decreased during Trials 2–4, as illustrated by significant main effects of trial in both duration, $F(2, 88) = 9.9$, $p < .01$, and number of visits, $F(2, 88) = 9.4$, $p < .01$. However, nonsignificant sex and Sex × Trial effects for both measures indicated that the sexes did not differ in habituating to the objects (see Figures 2A and 2B).

Spatial novelty. Overall, mice spent more time with the DO than the NDOs during Trial 5 relative to Trial 4, $F(1, 44) = 9.5$, $p < .01$. Similarly, mice visited the DO significantly more than the NDOs in Trial 5 relative to Trial 4, $F(1, 44) = 12.9$, $p < .01$. Although the main effects of sex and the Sex × DO–NDO interactions were not significant for either duration or number of visits, *t* tests revealed that males, but not females, explored the DO more than the NDOs. This effect was observed for both duration of visits: males, $t(23) = 3.29$, $p < .01$; females, $t(21) = 1.06$, $p = .30$ (see Figure 3A), and number of visits: males, $t(23) = 3.49$, $p < .01$; females, $t(21) = 1.74$, $p = .10$ (see Figure 3B). In contrast, females seemed to increase their exploration of all of the objects in response to the displacement.

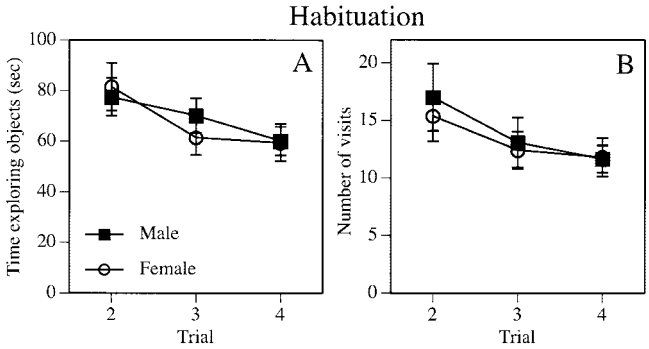

Figure 2. Males and females habituated similarly to the objects during Trials 2–4 of the novelty task, as illustrated by the time (in seconds) spent exploring the three objects (A) and the number of visits paid to the objects (B). Each symbol represents the mean (± *SEM*) of each group for one trial.

Object novelty. Mice spent significantly more time with the SO than the NSO during Trial 7 relative to Trial 6, $F(1, 44) = 19.7$, $p < .01$. Furthermore, a significant Sex × SO–NSO interaction for time, $F(1, 44) = 8.4$, $p < .01$, indicated that males, but not females, showed a significant preference for the SO over the NSOs (see Figure 3C). This sex difference was confirmed by *t* tests, which illustrated a significant difference in the amount of time spent with the SO and NSOs in males, $t(23) = 5.19$, $p < .01$, but not in females. Overall, mice visited the SO more often than the NSOs, $F(1, 44) = 17.9$, $p < .01$. The main effect of sex and the Sex × Session interaction were not significant because the sexes showed a similar preference for the novel object (see Figure 3D); *t* tests indicated that both males and females visited the SO significantly more than the NSOs: males, $t(23) = 3.38$, $p < .01$; females, $t(21) = 2.45$, $p < .03$.

Experiment 2

The data from Experiment 1 indicate that males respond more selectively than females to both spatial and object novelty. That is, males responded to both the object displacement and object substitution by increasing exploration of the moved or new object and decreasing exploration of the nondisplaced and nonsubstituted objects. In contrast, females did not generally direct their exploratory behavior toward the moved or new objects. Rather, they appeared to respond to the changes by increasing exploration of all objects. The exception to this pattern in females was in the number of visits paid to the novel object; in this measure, females exhibited preferential exploratory behavior toward the novel object.

Given the fairly ambiguous result for object novelty among females, we tested a subgroup of males and females from Experiment 1 in a task focusing on object recognition. This task is a modified form of the visual paired comparison task used in humans (Fagan, 1970) and monkeys (Bachevalier, 1990; Gunderson & Sackett, 1984). In this task, two identical objects are presented simultaneously, and then after a delay, one of the familiar objects is presented simultaneously with a novel object

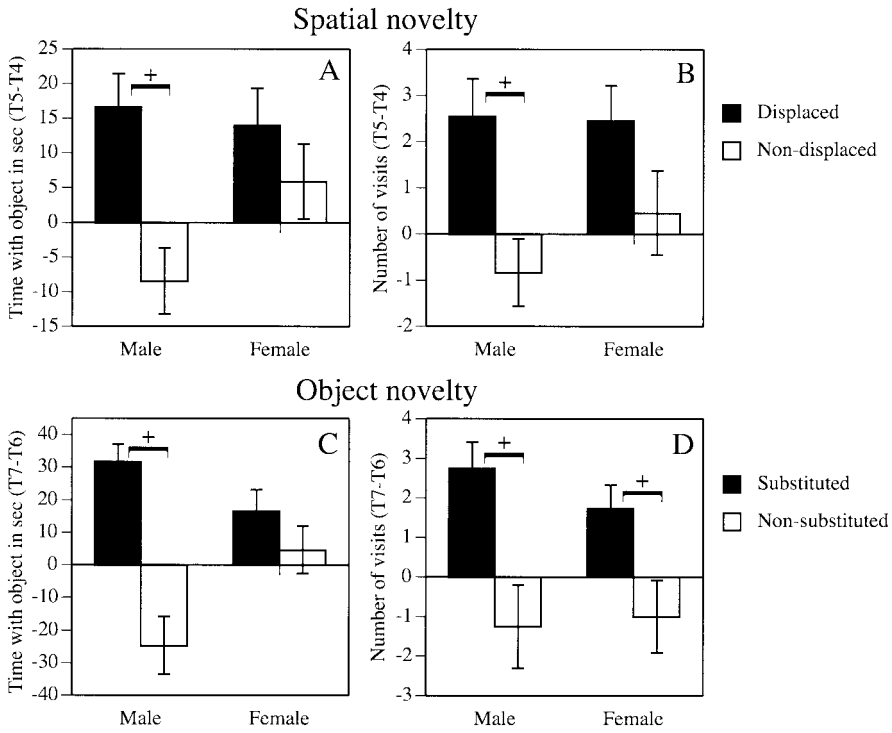

Figure 3. Responses to object displacement (A and B) and object substitution (C and D). In A and B, each bar represents the mean (± *SEM*) time (in seconds) spent with and number of visits to the displaced and nondisplaced objects in Trial 5 (T5) minus that in Trial 4 (T4; positive numbers indicate more exploration, whereas negative numbers indicate less exploration), respectively. Similarly, C and D depict the mean (± *SEM*) time (in seconds) spent with and number of visits to the substituted and nonsubstituted objects in T7 minus that in T6, respectively. Males explored the displaced and substituted objects significantly more than the nondisplaced and nonsubstituted objects (+$p < .05$, between the displaced and nondisplaced objects or the substituted and nonsubstituted objects). In contrast, females did not preferentially explore the displaced object and exhibited a preference for the substituted object only in number of visits.

(Clark et al., 2000; Ennaceur & Aggleton, 1997; Ennaceur & Delacour, 1988). Because rodents have an affinity for novelty, most rodents preferentially explore the novel object over the familiar object. Expression of this preference requires a memory of the familiar (now less interesting) object to recognize that the new object is novel.

One element of the task used in Experiment 1, and in many object recognition tasks, is that the duration of each trial is fixed and independent of the time spent with the objects. With this procedure, measurements of exploration may be influenced by group differences in locomotor activity or motivation. This confound is particularly germane to the assessment of sex differences in object recognition because females in Experiment 1 tended to be more active in the arena than males and because females responded to the object displacement by increasing their overall exploration activity. The object recognition task used in Experiment 2 was modified (Clark et al., 2000; Ennaceur & Aggleton, 1997) to address this limitation by fixing the amount of time spent exploring the objects. Trials did not end until the animal had accumulated a certain amount of time (typically 20–40 s) exploring the two objects, thus minimizing effects of activity and motivation and ensuring similar exposure to the objects.

Method

Subjects. The subjects were 15 male and 15 female C57BL/6 mice previously tested in Experiment 1. Mice were 5 months old at the beginning of behavioral testing and were randomly assigned to either the 24-hr (males $n = 8$, females $n = 7$) or 7-day (males $n = 7$, females $n = 8$) delay condition.

Apparatus. Testing was carried out using the same open field box, testing room, and lighting conditions as in Experiment 1. New objects included a mouse toy shaped like a diamond and a brown plastic doorstop. These objects were similar in size to those used in Experiment 1. The computer tracking system in Experiment 1 was used to record duration and number of visits to the objects.

Object recognition. The object recognition procedure used was based on that reported previously (Baker & Kim, 2002; Clark et al., 2000). The task consisted of three phases—habituation, sample, and choice—which were each completed on separate test days. During habituation, each mouse was placed in the empty testing arena for 5 min (no data were collected during this trial). The next day, all subjects completed the sample phase. Mice were first given 1 min to rehabituate to the empty arena. They were then placed in a holding cage next to the arena while two identical objects were placed in the northeast and northwest corners of the box (5 cm from the walls). The mouse was then immediately placed in the box facing the middle of the south wall and allowed to explore the objects until it accrued

a total of 30 s exploring the objects (after which it was removed to the home cage). The box and objects were wiped with 70% alcohol before the next mouse was tested.

Mice were tested in the choice phase either 24 hr or 7 days after completing the sample phase. The 7-day delay was used because preliminary testing in ovariectomized female C57BL/6 mice showed that mice continued to exhibit a preference for the novel object when tested with the same familiar object 24, 72, and 168 hr after the sample phase. Thus, we were curious to determine whether mice could indeed retain this information for 7 days or whether the persistence of this memory in our preliminary study was the result of repeated exposure to the familiar object. During the choice phase, two objects were placed in the same corners of the open field box occupied during the sample phase. One of the objects was the toy used during the sample phase (familiar object) and the second object was novel. Mice were placed in the box facing the middle of the south wall and allowed to explore until they accrued 30 s exploring the objects. The location of the novel object (northeast or northwest corner) was counterbalanced across mice, such that approximately half of the mice in each sex saw the novel object on the left and half saw it on the right.

Data analysis. For time spent with the objects, a preference for the novel object over the familiar object was assessed using one-sample *t* tests to determine whether the time spent with each object differed significantly from the chance value of 15 s (Baker & Kim, 2002). This type of *t* test was used because the times spent with each object are not independent; the total time exploring must equal 30 s, so time spent with one object reduces time spent with the other. We conducted *t* tests for each sex separately. For number of visits, a 2 (sex) × 2 (object) repeated-measures ANOVA was conducted, followed by paired sample *t* tests within each sex to assess the difference between number of visits to the two objects.

Results

Sample phase. Males did not spend more time with one of the identical objects than the other, suggesting that they were not biased toward either corner of the testing box (see Figure 4A). In contrast, females spent significantly more time with the object on the right side of the box, $t(14) = 2.34$, $p < .04$, compared with the object on the left, $t(14) = -2.34$, $p < .04$ (see Figure 4A). Although this indicates the presence of a side bias, the preference was very modest (13 s vs. 16 s) and thus likely did not bias performance in the subsequent choice phases (in which the location of the novel object was counterbalanced). Furthermore, neither males nor females visited one object more than the other, as indicated by nonsignificant main effects of sex and side, a Sex × Side interaction, and *t* tests (see Figure 4B).

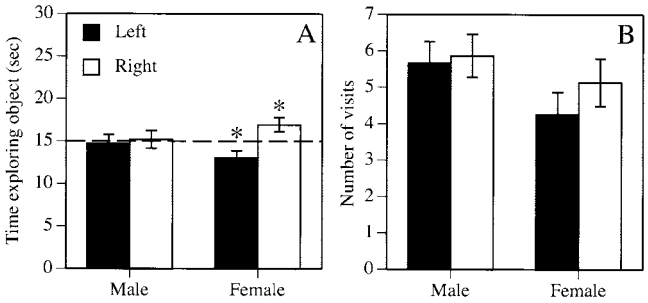

Figure 4. Performance in the sample phase of the object-recognition task, as illustrated by time (in seconds) spent exploring the objects (A) and number of visits (B). Each bar represents the mean (± *SEM*) for each object. The dashed line in A represents chance performance (15 s). Males did not prefer one identical object over the other. Females exhibited an increase in time spent with the object on the right side of the open field box (*$p < .05$ relative to chance), although the number of visits to the two objects was not significantly different.

Choice phase: 24-hr delay. Males exhibited a significant preference for the novel object, as illustrated by the fact that they spent significantly more time than chance with the novel object and significantly less time than chance with the familiar object, $ts(7) = 5.29$ and -5.29, $ps < .001$ (see Figure 5A). Males spent a similar amount of time exploring the novel object, regardless of whether it appeared on the left (16.6 s) or right (18.6 s). In contrast, the time that females spent with each of the objects was not significantly different from chance. Despite the fact that females spent more time exploring the object on the left during the sample phase, females spent a similar amount of time exploring the novel object regardless of whether it appeared on the left (12.7 s) or right (13.1 s). Males also visited the novel object significantly more than females, as illustrated by a significant Sex × Object interaction, $F(1, 13) = 7.8$, $p < .02$ (see Figure 5B). This preference was supported by *t* tests, which indicated a significant difference in the number of visits to the novel and familiar objects in males, $t(7) = 6.00$, $p < .001$, but not in females. The sex and object main effects were not significant for number of visits.

Choice phase: 7-day delay. Neither sex exhibited a preference for the novel object after 7 days, as illustrated by both time and number of visits (see Figures 5C and 5D). Again, males and females spent similar amounts of time exploring the novel object, regardless of whether it was placed on the left (males = 12.9 s, females = 13.5 s) or the right (males = 14.8 s, females = 13.6 s). Although the sex effect and Sex × Object interaction were not significant for number of visits, the object effect was significant, $F(1, 13) = 7.98$, $p < .02$. This effect was driven solely by males, whose *t* test illustrated that they visited the familiar object more than the novel object, $t(6) = -3.46$, $p < .02$ (see Figure 5D). No difference was observed in females.

General Discussion

The results of the present studies demonstrate that male, but not female, C57BL/6 mice respond preferentially to spatial and object novelty. This finding suggests that males are superior to females in remembering the location and identity of an object in an open field. This sex difference was evinced in two tasks that measure response to novelty. In the spatial–object novelty task, both sexes exhibited similar locomotor activity and habituated similarly to objects in the open field. However, a sex difference in response to spatial novelty was indicated by the preferential exploration in males of the displaced object (DO) in the absence of analogous preferential exploration by females. Similarly, in the object novelty phase of the task, males displayed a robust preference for the substituted object (SO), whereas females exhibited a preference in only one measure of performance. Because females showed a mixed response to object novelty, we decided to further explore object memory by testing mice of both sexes in a task that assessed solely object recognition. Twenty-four hours after interacting with two identical objects, males spent significantly more time than chance exploring a novel object. In contrast, females showed no such preference for the novel object. Neither sex preferentially explored

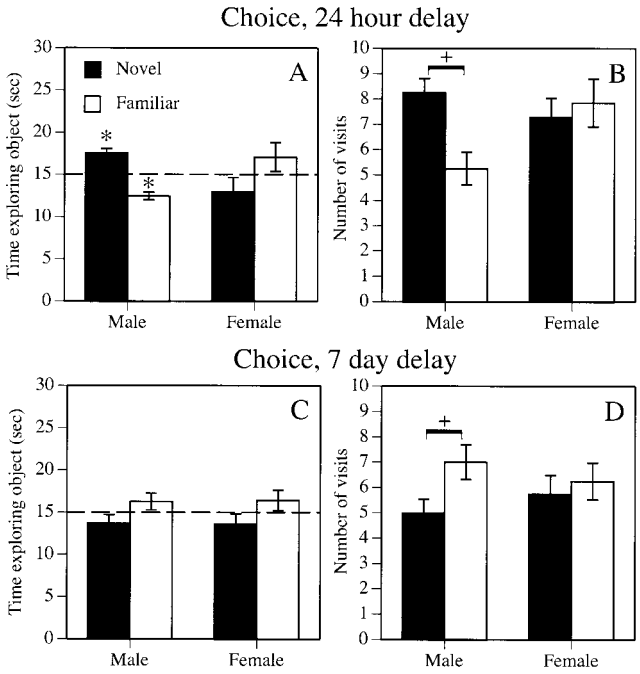

Figure 5. Response to the novel object 24 hr (A and B) and 7 days (C and D) after the sample phase. Each bar represents the mean (± *SEM*) for each object. The dashed lines in A and C represent chance performance (15 s). At the 24-hr delay, males spent significantly more time than chance (A) exploring the novel object and less time than chance exploring the familiar object (*$p < .05$ relative to chance). Males also visited the novel object (B) more than the familiar object (+$p < .05$ between the novel and familiar objects). Females did not exhibit a preference for the novel object in either measure. After 7 days, neither males nor females exhibited a preference for the novel object, as illustrated by time (C) and number of visits (D). Males actually visited the familiar object more after 7 days (+$p < .05$ between the novel and familiar objects).

the novel object after a 7-day delay between the sample and choice phases.

Very few studies (Ghi et al., 1999; Ricceri et al., 2000) have examined sex differences in spatial and object recognition in rodents. Of these, none report that males are superior to females in remembering the location and identities of objects, as was the case in the present study. For example, no sex differences were reported in the ability of 3-month-old male and female CD-1 mice to detect spatial and object novelty in a task similar to that used here (Ricceri et al., 2000). However, as mentioned previously, CD-1 mice are estrogen insensitive relative to other strains of mice, which may contribute to the absence of sex differences in this mouse strain (Spearow & Barkley, 2001; Spearow et al., 1999). Another study in Wistar rats (approximately 6 weeks of age) using an object recognition task in a two-armed maze found that males and females showed similar preferences for a novel object at delays of 30 and 60 min, but only females continued to exhibit a preference after 90 min (Ghi et al., 1999). The inconsistency between this and the present study could be due to multiple factors, including age at testing, procedural differences, or species differences in motor activity or motivation.

Other than exhibiting a preference for the novel object, are there ways in which females may have demonstrated recognition of spatial or object novelty? The spatial–object novelty data in Figures 3A–3C suggest that females do respond to the changes but in a more general manner than males. That is, they appear to increase their exploration of all of the objects rather than exploring the DOs or SOs preferentially. The fact that exploration in females increased from Trial 4 to Trial 5 suggests that females recognize that something has changed. Their failure to concentrate on the DOs or SOs could reflect a memory deficit (i.e., not remembering specific object locations or identities, thus spending more time with all objects) or simply a difference in their propensity to explore the DOs or SOs (i.e., they can recognize which object has been moved or substituted but are not as interested in the object as males). The results of Experiment 2 help to clarify this issue, particularly for object recognition. In Experiment 2, the amount of time spent exploring the objects was fixed, which minimized the differences in novelty-induced activity shown by females in Experiment 1. In this experiment, females spent a similar amount of time exploring the novel and familiar objects after 24 hr and 7 days. The fact that females, but not males, failed to respond differentially to either object in Experiment 2 supports the conclusion that the observed sex differences were due to disparate memory abilities. Nevertheless, we cannot exclude entirely the possibility of a sex difference in the proclivity to explore the novel object.

It could be argued that females did not show a preference for the novel object in Experiment 2 because the delays were too long. It is possible that females can remember the familiar object but only for periods shorter than 24 hr. Although the present data cannot directly refute this argument, the results of the novelty task in Experiment 1 indicate that females do not express a preference for the novel object even with delays as short as 3 min. However, even if systematic testing of females in the object recognition task with shorter delays revealed memory for the familiar object, the present data suggest that this memory lasts longer in males than in females.

Sex differences in object recognition may result from sexual dimorphisms in a number of brain regions. The available data suggest that the neocortex, hippocampus, and septum play a role in spatial and object novelty as tested in Experiment 1 (Poucet, 1989; Save, Poucet, Foreman, & Buhot, 1992; Thinus-Blanc et al., 1996) and object recognition as tested in Experiment 2 (Baker & Kim, 2002; Clark et al., 2000; Ennaceur & Aggleton, 1997; for reviews, see Mumby, 2001; Steckler, Drinkenburg, Sahgal, & Aggleton, 1998). Numerous studies have reported sex differences in these brain regions. In the neocortex, marked differences in the laterality of cortical thickness and estrogen receptor distribution have been noted (Diamond, 1987; Sandhu, Cook, & Diamond, 1986). In the hippocampus, sex differences (typically favoring males) have been demonstrated in many variables, including dendritic and synaptic morphology (Juraska, Fitch, Henderson, & Rivers, 1985; Juraska, Fitch, & Washburne, 1989; Madeira, Sousa, & Paula-Barbosa, 1991; Parducz & Garcia-Segura, 1993), granule cell number (Wimer & Wimer, 1985), and slice excitability (Smith, Jones, & Wilson, 2002). However, because we did not examine the brains of the animals in these studies, it is difficult to speculate about specific neural mechanisms underlying the behavioral sex differences observed here.

Activational effects of sex-steroid hormones may also contribute to sex differences in reaction to spatial and object novelty. The ovarian hormones estrogen and progesterone modulate hippocampal and neocortical morphology and function in adult rodents (for

reviews, see Gibbs & Aggarwal, 1998; McEwen, Alves, Bulloch, & Weiland, 1997; Woolley, 1998). Both brain regions exhibit sexually dimorphic responses to estrogen administration (Miranda, Williams, & Einstein, 1999; Perez, Zucchi, & Maggi, 1986), and some evidence suggests that estrogen given to ovariectomized female rats and mice can improve spatial and nonspatial learning and memory (Bimonte & Denenberg, 1999; Farr et al., 1995; Gibbs, 1999; Gibbs, Burke, & Johnson, 1998; O'Neal, Means, Poole, & Hamm, 1996; Packard & Teather, 1997; Rissanen, Puoliväli, van Groen, & Riekkinen, 1999; Sandstrom & Williams, 2001; although, see Chesler & Juraska, 2000; Fugger, Cunningham, Rissman, & Foster, 1998). Several studies have also reported variations in spatial learning and memory associated with hormonal fluctuations in the estrous cycle, although the effects are subtle and conflicting (Berry, McMahan, & Gallagher, 1997; Frick & Berger-Sweeney, 2001; Frye, 1995; Stackman, Blasberg, Langan, & Clark, 1997; Warren & Juraska, 1997). Because we did not monitor the estrous cycle in this study, our females were likely tested in a variety of hormonal conditions. We have previously found that ovariectomized female C57BL/6 mice receiving an injection of either vehicle or estrogen after the sample phase of the object recognition task show a preference for the novel object after 24 hr (although only the estrogen-treated mice exhibit the preference after 48 hr; Gresack & Frick, 2003). Thus, low estrogen levels (like in males) or high estrogen levels (e.g., after exogenous estrogen) may benefit object recognition in a way not seen in normally cycling females. Examining females in different stages of the estrous cycle will be necessary to adequately address this issue.

In conclusion, the results of the present studies suggest that male C57BL/6 mice are superior to female mice in remembering the location and identity of objects in an open field. The male advantage in object recognition was demonstrated in two tasks, suggesting that this sex difference is fairly robust. These data provide valuable information regarding sex differences in object recognition in an inbred mouse strain.

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Received March 24, 2003
Revision received May 20, 2003
Accepted June 2, 2003 ■