Behavioural Processes 82 (2009) 249–255

Contents lists available at ScienceDirect

Behavioural Processes

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

Sex-specific strategies in spatial orientation in C57BL/6J mice

Tania J. Bettis*, Lucia F. Jacobs

Department of Psychology, University of California at Berkeley, 3210 Tolman Hall, Berkeley, CA 94720, United States

ARTICLE INFO

Article history:
Received 24 September 2008
Received in revised form 2 July 2009
Accepted 10 July 2009

Keywords:
Cognition
Cue use
Exploration
Gender
Rodent
Sex difference

ABSTRACT

Sex differences in spatial learning are found in many species of mammals and even in invertebrates. Results from laboratory mouse studies, however, have been inconsistent in comparison to studies of humans, laboratory rats and wild rodent species. Here we re-examined this question in C57BL/6J mice that were exposed to enriched environments using two tasks, an object recognition task and a place learning task where mice were motivated by exploratory drive, not aversive conditioning or food restriction. Using these methods, we found a female advantage for object recognition, similar to the female advantage found in humans and laboratory rats. In the place learning task, male performance was unimpaired by intra-maze cue deletion but impaired by extra-maze cue masking. Female mice, in contrast, were able to navigate accurately under both cue conditions. In summary, by utilizing testing and housing methods that were more species appropriate, we found sex-specific patterns of cue encoding and place learning in better accordance with prior results from other mammalian species. The implication of these results is that the C57BL/6J mouse is an appropriate model for the study of cognitive sex differences in mammals.
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1. Introduction

Sex differences in spatial orientation and encoding of landmarks have been documented widely in mammals (see recent metareviews: human; Voyer et al., 2007, rodent; Jonasson, 2005). Sex differences have also been reported in birds (Range et al., 2006; Vallortigara, 1996) and most recently in an invertebrate, the molluscan cuttlefish (Jozet-Alves et al., 2008). Because of this wide distribution, it is critical to identify the common denominator of sex differences, at least within mammals, so that we can determine if the patterns are similar among taxa. Further, because the genome of the laboratory mouse (strain C57BL/6J) is now known (Kawai et al., 2001), we need to understand its behavior at a comparable level of detail. Understanding cognitive sex differences in the C57BL/6J mouse is therefore an important step towards dissecting the genetic basis of behavior.

Cognitive sex differences in laboratory mice, however, have been less consistent than those seen in the laboratory rat. For example, different patterns of sex differences were found in laboratory rats and laboratory mice on the radial arm and water maze tasks (Jonasson, 2005). Studies of laboratory rats have shown a consistent male advantage (i.e., male performance higher than female performance) on both water and radial arm mazes while laboratory mice demonstrated inconsistent sex differences on these tasks. The pattern seen in laboratory rats is consistent with that seen in other rodent species (meadow vole, Gaulin and Fitzgerald, 1989; deer mouse, Galea et al., 1994; Merriam and Great Basin kangaroo rats, Barkley and Jacobs, 2007; laboratory rat, Williams et al., 1990). Specifically, males rely more heavily on cues that provide directional information such as the geometry of a room, distributed cues or polarized arrays of objects. On the other hand, females have demonstrated sensitivity to positional information such as the relationships and unique features of discrete objects. In the classic study of the hormonal basis of this sex difference in the laboratory rat by Williams et al. (1990), female performance on a radial arm maze declined when the locations of extra-maze objects were randomized, while male performance was unaffected. When the geometry of the room was altered, performance dropped in males but not in females. While laboratory rats demonstrate this pattern of sex differences consistently in radial arm mazes and water mazes, laboratory mice do not display such a consistent pattern of sex differences and have been tested less frequently.

As in the studies of spatial learning in complex mazes, studies of sex differences in laboratory mice on simpler spatial tasks show the same lack of consistency. One such task is the ability to recognize that a new object has appeared in a known location. In this task, two objects are presented for exploration. After a delay, a familiar object is replaced with a novel object. Evidence for object recognition is assayed by the bias in search time directed to the novel object (Ennaceur and Delacour, 1988). This task is related to a paper-and-pencil task for humans where a pair of familiar objects are switched in location after a delay, a task that shows a female advantage (Silverman and Eals, 1992). Similarly, in laboratory rats, there is a female advantage in the object recognition task (Saucier

* Corresponding author.
E-mail address: taniabettis@gmail.com (T.J. Bettis).

0376-6357/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.beproc.2009.07.004

et al., 2007; Sutcliffe et al., 2007). Yet, again inconsistent with the pattern from other species, Frick and Gresack (2003) found a male, not a female, advantage in this task in C57BL/6J laboratory mice. In addition, other laboratories have found either no sex difference (Benice et al., 2006) or a female advantage (Podhorna and Brown, 2002) in this type of task.

The lack of consistency between laboratory mice and laboratory rats could be a genuine species difference, but could also reflect the disadvantage of laboratory mice being tested on tasks designed for laboratory rats. As Frick et al. (2000) have written, "the mouse is not a little rat". Differing by an order of magnitude in average body mass (300 g vs. 30 g), the laboratory rat is a domesticated strain of the semi-aquatic Norway rat while the laboratory mouse is derived from the house mouse, an adept climber. This is reflected in their tail morphology: the laboratory rat's tail is stiff and can be used as a rudder while swimming, while the laboratory mouse's tail is prehensile and is used as a fifth limb while climbing. Tasks that involve swimming should therefore give the laboratory rat an inherent advantage in addition to the better insulation a large body affords while swimming in cold water. In fact, in a direct comparison of different versions of the same maze (i.e., dry vs. water versions), Whishaw and Tomie (1996) showed that laboratory rats outperformed laboratory mice only on the aquatic version of the task.

The goal of our study was therefore to examine sex differences in C57BL/6J laboratory mice using tasks designed specifically for this species. In addition, our study was designed to minimize the stress of handling and testing. Sex differences in spatial learning are affected by stress and therefore it is important to reduce its influence. For example, sex differences in deer mice in spatial learning are modulated by environmental stressors, such as predator odor and the presence of biting flies (Kavaliers et al., 1998; Perrot-Sinal et al., 2000). In our study we therefore used and/or developed tasks that did not depend on swimming or on food restriction but instead shared the single, positive reinforcement of spatial exploration. In addition, mice were tested during their natural active phase and were housed in cages equipped with nest boxes and bedding. To reduce their anxiety in response to novel testing environments, mice were also allowed to explore complex mouse 'playgrounds' on a regular schedule. Because our goal was to measure sex differences in the adult, we also used mice that were fully mature (13 months old) and who had experienced similar tasks as young adults (3 months). We predicted that, under these conditions, C57BL/6J mice would show the pattern typical of the laboratory rat. Specifically, we predicted a female advantage on the object recognition task and sex-specific strategies in place learning, with males and females using different cues for orientation. We expected that males should preferentially orient using cues external to the apparatus, such as the shape of the room or objects on the walls. In contrast, females should preferentially orient to cues closer to the maze such as nearby objects. Such results would be consistent with results found in the laboratory rat (Williams et al., 1990) and in humans (Sandstrom et al., 1998). These sex differences in spatial strategies are also predicted by new theories regarding the cognitive map (Jacobs and Schenk, 2003).

2. Materials and methods

2.1. Study animals

C57BL/6J mice ($N = 22$, 11 females) were obtained at the age of 2 months and were tested at the age of 3 months on a series of object recognition tasks. They were tested again, in the current study, at the age of 13 months. The data for this study refer only to the test at 13 months. They were housed in same-sex groups, three per standard mouse cage (19 cm × 29 cm × 13 cm). Each cage

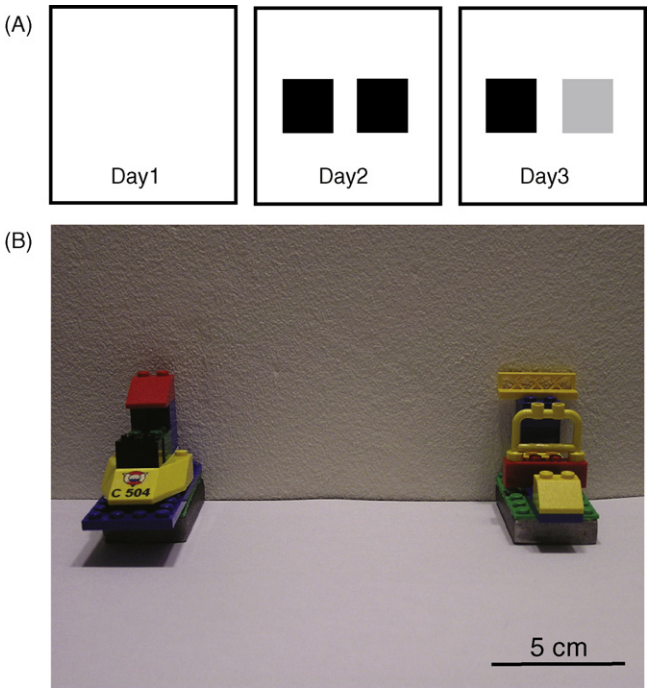

Fig. 1. The apparatus and design of the object recognition task. (A) Spatial layout and experimental design. The squares represent the objects present in the arena. As shown, Day 1 is habituation to the open arena, Day 2 is the sample phase with two identical objects in the arena, and Day 3 is the choice phase with one novel and one familiar object in the arena. (B) Constructed stimuli used in this task.

was supplied with a disposable cardboard igloo and bedding material (Nestlets®). Mice were kept on a 12:12 light cycle (lights on at 20:00, off at 08:00). Water was available ad lib. A ration of 8 g standard mouse chow was provided on alternate days to simulate natural cycles of food availability while maintaining a weight within 1–2 g of ad lib weight. This amount of food meets the daily energetic requirements of the mice (Subcommittee on Laboratory Animal et al., 1995) and reduces the negative effects of ad lib feeding (Keenan et al., 1999). The mice in this study rarely finished the entire 48 h ration, however, and were rarely without food for more than 4 h.

From the age of 6 months, mice were given access to larger environments with novel objects to explore. A cohort of three cagemates was released for 1 h per week into a covered, translucent plastic box (26 cm × 46 cm × 26 cm) with novel objects that could be chewed or climbed. The objects were changed weekly and included a running wheel, plastic tubes, wooden sticks, pine cones, nylon dog bones (Nylabones®), glass jars, pieces of Styrofoam®, tunnels made from wire mesh (i.e. chicken wire) and long upright barriers constructed from white acrylic plastic.

2.2. Object recognition task

2.2.1. Apparatus and procedure

The task was adapted from Ennaceur and Delacour (1988). It consisted of three successive days of exposure to three similar environments (Fig. 1A), for a period of 5 min per day. The arena was an opaque rodent cage (40 cm × 51 cm × 20 cm). Unique objects were constructed using Lego® blocks (Fig. 1B). A video camera was mounted to the ceiling and was connected to a recorder, monitor, and computer in the adjacent room. The two rooms were connected through a partially open door. All experiments took place during the dark phase of the light cycle and were conducted in test rooms with low lighting. Extra-apparatus cues were not masked but were not prominent, as the sides of the arena were opaque.

On Day 1 (habituation phase), the mouse was released into the empty arena for 5 min of exploration and habituation. On Day 2 (sample phase), the mouse was again released into the arena, which now contained two identical objects centered in the arena. On Day 3 (choice phase), the arena now contained a duplicate of the object from the sample phase and a novel object (Fig. 1B). The location of the novel object was counterbalanced among mice to control for side biases. The object that was novel was also counterbalanced between the two object types shown in Fig. 1B. The objects presented to the mice were both completely novel to the mouse, i.e., different objects were constructed for each replication of the task. In addition, 10 months had elapsed between the first and second replication of this task, minimizing carryover effects between replications.

All behavior was recorded on videotape for subsequent analysis using Ethovision Pro (Noldus, Inc.). Two measures of behavior were made: the time spent within a zone of 4 cm distant from an object and the frequency of visits to this zone per object. The criterion for the mouse's presence within the 4 cm zone was the location of the body's center of gravity, as defined by Ethovision. The Ethovision tracking system results in high correlations between manual recording and automatic scoring in object recognition tasks in mice (Roach et al., 2003). We also conducted a pilot study to examine this question and found significant agreement between manual scoring of mouse attention and automatic scoring of this behavior. Though it is possible, it was extremely rare that the Ethovision calculated center of gravity of the mouse crossed into the zone without the head of the animal being oriented towards the object. For this reason we felt confident relying on the tracking system rather than manually recorded behavior.

2.3. Place learning task: ladder-rewarded plus maze

The ladder-rewarded plus maze is a new mouse place learning task that eliminates the need for food restriction or aversive conditioning. Versions of the elevated plus maze are commonly used to measure response to stress in unhabituated mice (Rodgers et al., 1999). In this ladder maze, we used a standard plus maze geometry to assess place learning but used a positive reinforcer, i.e., access to an unexplored area. Mice solved a trial by returning to the learned location of a hidden ladder that led to a novel space that contained tunnels to be explored. Thus, both tasks in our study – object recognition and place learning – exploited the natural tendency of mice to explore novel objects and novel environments.

2.3.1. Apparatus

The ladder maze is an elevated plus maze that is designed to reward performance with access to exploratory space that contains climbable objects (four tunnels). The maze was constructed from white sheet vinyl plastic. It was comprised of four perpendicular arms (45 cm × 4 cm). Each arm was also fitted with a 'speed bump'. This was a U-shaped wire mesh structure (8 cm high) fixed to the maze arm (see Fig. 2C). The function of the speed bump was to increase energetic cost of choosing an arm. In pilot studies, without the speed bumps, the mice would run quickly across the maze and would not attend to their surroundings. Occasionally a mouse would run off the end of an arm, where they may have expected the escape ladder to be. In these pilot studies, the mice did not show evidence of learning the correct location. Once the speed bumps were added the mice slowed down and began to show evidence of learning.

The maze arms were elevated 31 cm above a white floor (122 cm × 122 cm × 5 cm) that contained four plastic tunnels that differed in color and texture (ca. 5 cm diameter × 14 cm length). The plastic ladder (4 cm × 34 cm) was constructed from commercially available white, latticed plastic (1 cm × 1 cm cubes). The layout of the test room is shown in Fig. 2A. The maze and the exploration space were enclosed in a round barrier of transparent acrylic (70 cm high, 122 cm diameter) to prevent escape. Intra-maze cues were attached to this barrier at each of four directions (Northeast, Southeast, Southwest and Northwest), which were positions offset from the directions of the maze arms (North, South, East and West). The directions were absolute cardinal directions. The cues included artificial flowers, colored foam and hard plastic shapes (Fig. 2B).

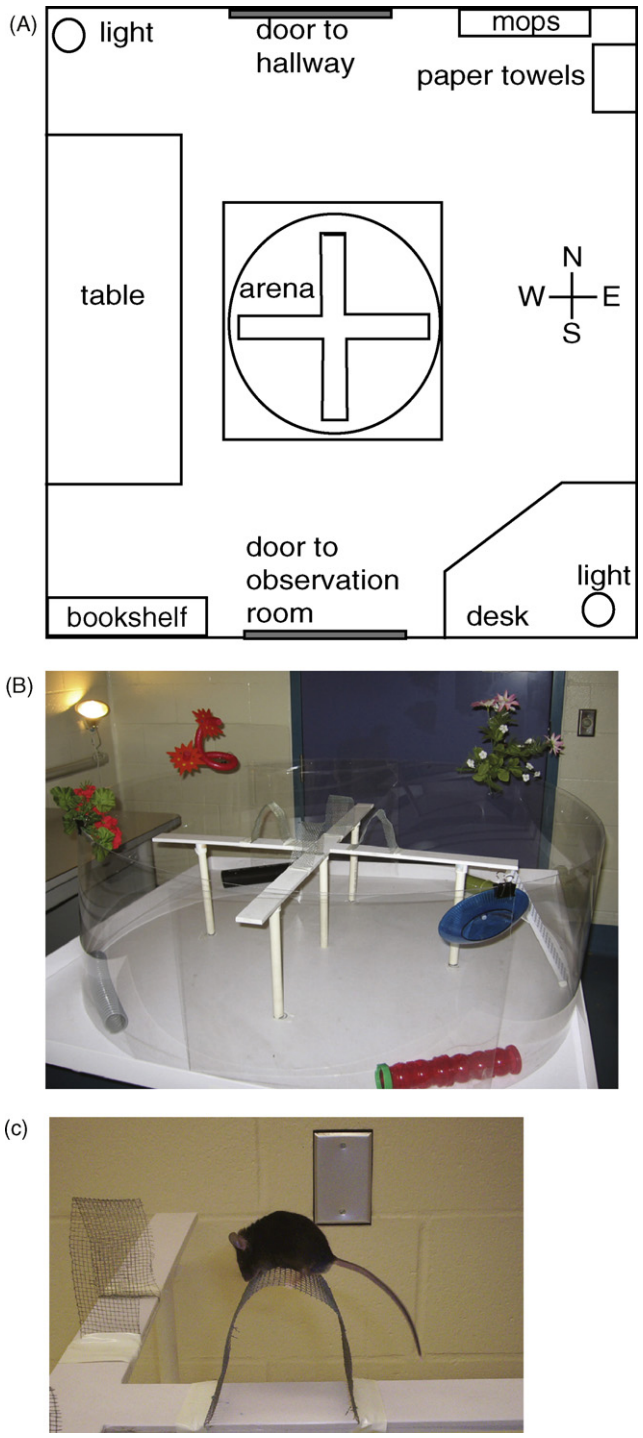

Fig. 2. The apparatus and testing environment of the ladder maze task. (A) Schematic drawing of test room. (B) Photograph of maze, showing intra- and extra-maze objects. (C) A trained mouse traversing the speed bump on an arm of the maze. Reaching the apex of the wire speed bump was the criterion for the choice of a maze arm.

Extra-maze cues in the room included two blue room doors, two spotlights on opposite corners of the test room, a rope of evenly distributed small white lights (decorator lights) that outlined the ceiling of the room and cast an even light, colored foam shapes and posters attached to the walls, a paper towel rack, a mop rack containing a mop and a metal bookshelf (for the room layout, see Fig. 2A). For the extra-maze cue probe test, a white vinyl curtain was hung from a circular hoop (Hula Hoop®) that was suspended from the ceiling, encircling the maze. This curtain masked all visual cues outside of the maze.

2.3.2. Procedure

2.3.2.1. Pre-training. Each mouse was given four pre-training trials in an adjacent room. This procedure habituated the mice to descending on ladders (Trials 1 and 2) and climbing the speed bump (Trials 3 and 4). The sample arm, speed bump and ladder led to the mouse's home cage; after each trial, the mouse spent a 20 s inter-trial interval (ITI) in its cage.

2.3.2.2. Training. Each mouse was assigned either the East (E) or West (W) arm as its goal (ladder) location. The start arm of each trial was pseudo-randomly assigned from the three non-goal arms. The mouse was released on the top surface of the maze at the end of one arm. The mouse was rewarded with exploration when it chose the correct arm. A choice was defined as the mouse climbing to the top of the speed bump. Once the mouse made the correct choice, it descended the ladder and was allowed to explore the tunnels for 20 s. Each mouse was trained in sessions of three trials per session. Thus each non-goal arm served as a start arm once per session. The ITI lasted 60–90 s. During the ITI, the mouse was additionally rewarded with exploration of novel objects in a large arena with novel objects (described above under Section 2.1). Also, during the ITI, the maze arms were wiped and rotated to prevent mice from associating patterns of odor cues or imperfections in the symmetry of the maze with the goal arm location. All training and testing was conducted on a single day; testing lasted between 3 and 5 h. The criterion for learning the location was a first choice of correct arm for all trials in two consecutive sessions, ensuring the mouse oriented correctly twice from each start position.

2.3.2.3. Probe test. Probe sessions were carried out immediately after the training criterion was met. A probe session consisted of three training trials and one probe trial. The probe trial occurred either between training trials 1 and 2 or between training trials 2 and 3; the placement of the probe within the session was counterbalanced among subjects. No ladder was present on the probe trial. We recorded all choices made for 60 s. However, the measure of performance was limited to the first choice. Three types of probe tests, in a fixed order, were used: Probe 1, removal of intra-maze cues; Probe 2, masking of extra-maze cues and Probe 3, removal and masking of both cue sets simultaneously. In Probe 1, we removed all intra-maze cues, including the four colored tunnels and the cues that had been placed close to the maze. In Probe 2, we masked the extra-maze cues with the curtain. In Probe 3, both of the above manipulations were employed simultaneously. We counterbalanced the location of the goal arm across mice. We also counterbalanced the start arm across probe tests and subjects so that the subjects had a new start arm for each probe test. For example, half of the subjects with a West arm goal started from the North arm and the other half with the West arm goal were released onto the South arm in Probe 1. This was balanced so that subjects that started from the North arm in Probe 1 would start from the South arm in Probe 2. This design should have eliminated the effect of side-turning bias.

3. Results

3.1. Object recognition

Two of the female mice were removed from the analysis because they did not attend to the objects during the sample phase, in the presence of two identical objects, and instead remained near a wall for the duration of the experiment.

Using a 2×2 repeated measures ANOVA of time spent near object, with sex as the between-subjects factor and side as the repeated within-subject factor, we found a significant main effect of sex ($F_{1,18} = 23.96$, $p < .01$). There was, however, no effect of side ($F_{1,18} = 0.41$, $p = .53$) nor was there a Sex $\times$ Side interaction ($F_{1,18} = 0.10$, $p = .76$). Further analyses using one-way ANOVA showed that females spent more time than males exploring both the left ($F_{1,18} = 12.12$, $p < .01$) and right ($F_{1,18} = 22.19$, $p < .01$) objects. Using the same analyses, the results for the frequency of visits showed a similar pattern. There was a significant main effect of sex ($F_{1,18} = 9.33$, $p < .01$) but no effect of side ($F_{1,18} = 0.49$, $p = .49$) and no significant interaction between sex and side ($F_{1,18} = 0.83$, $p = .37$). Further analysis using one-way ANOVA showed that female mice made more visits than did male mice to both left ($F_{1,18} = 9.03$, $p < .01$) and right objects ($F_{1,18} = 7.17$, $p = .02$).

In the choice phase on Day 3, a 2×2 repeated measures ANOVA with sex as the between-subjects factor and object type (novel vs. familiar) as the within-subjects repeated factor revealed significant main effects of both sex ($F_{1,18} = 22.96$, $p < .01$) and object type ($F_{1,18} = 8.33$, $p = .01$). This analysis also showed a significant Sex $\times$ Object type interaction ($F_{1,18} = 17.80$, $p < .01$). Further one-way ANOVA revealed that females spent more time with the novel object ($F_{1,18} = 50.45$, $p < .01$) than did males. The data on the frequency of visits reveal the same pattern of behavior. The 2×2 repeated measures ANOVA shows significant effects of sex ($F_{1,18} = 14.73$, $p < .01$) and object type ($F_{1,18} = 4.69$, $p = .04$) with a significant Sex $\times$ Object interaction ($F_{1,18} = 10.86$, $p < .01$). Subsequent one-way ANOVA analyses showed that females visited the novel object significantly more than males did ($F_{1,18} = 47.10$, $p < .01$) but they did not visit the familiar objects more than males did ($F_{1,18} = 3.16$, $p = .09$). As revealed by paired-samples t-tests, females also spent more time exploring novel objects ($t_8 = 8.9$, $p < .01$) as well as making more visits to the novel object ($t_8 = 3.1$, $p = .02$) than to the familiar object. These differences were not significant for male mice (duration: $t_{10} = -.79$, $p = .45$, number of visits: $t_{10} = -1.04$, $p = .32$); see Fig. 3. Finally, the discrimination indices calculated were significantly greater for the female mice than the male mice as determined with a one-way ANOVA ($F_{1,18} = 7.36$, $p = 0.01$). As revealed by one-sample t-tests, the discrimination index was significantly greater than zero for the female mice ($p < 0.01$) but not for the male mice ($p = 0.63$); see Fig. 4.

3.1.1. Place learning

Performance on a training trial was assessed by the number of errors made, i.e., the number of non-goal arms entered before the goal arm was entered. To complete training, mice were required to learn a rewarded location and escape from the maze on the first choice. Therefore, the first arm chosen is the best indictor of the mouse's memory for the correct location in probe tests. Beyond this choice the behavior becomes increasingly difficult to interpret. As seen in Fig. 5, females and males did not differ in their rate of acquisition of this task. The mean number ($\pm$SE) of trials to criterion was 35.4 ± 3.93 for females and 41.14 ± 4.69 for males, which was not significantly different as determined by a t-test ($t_{15} = .94$, $p = .36$). The first female mouse to reach criterion did so after 18 trials (6 sessions) and the last two female mice to reach criterion did so in 54 trials (18 sessions). The first male mice to reach criterion did so in 30 trials (10 sessions) and the last male mouse to reach

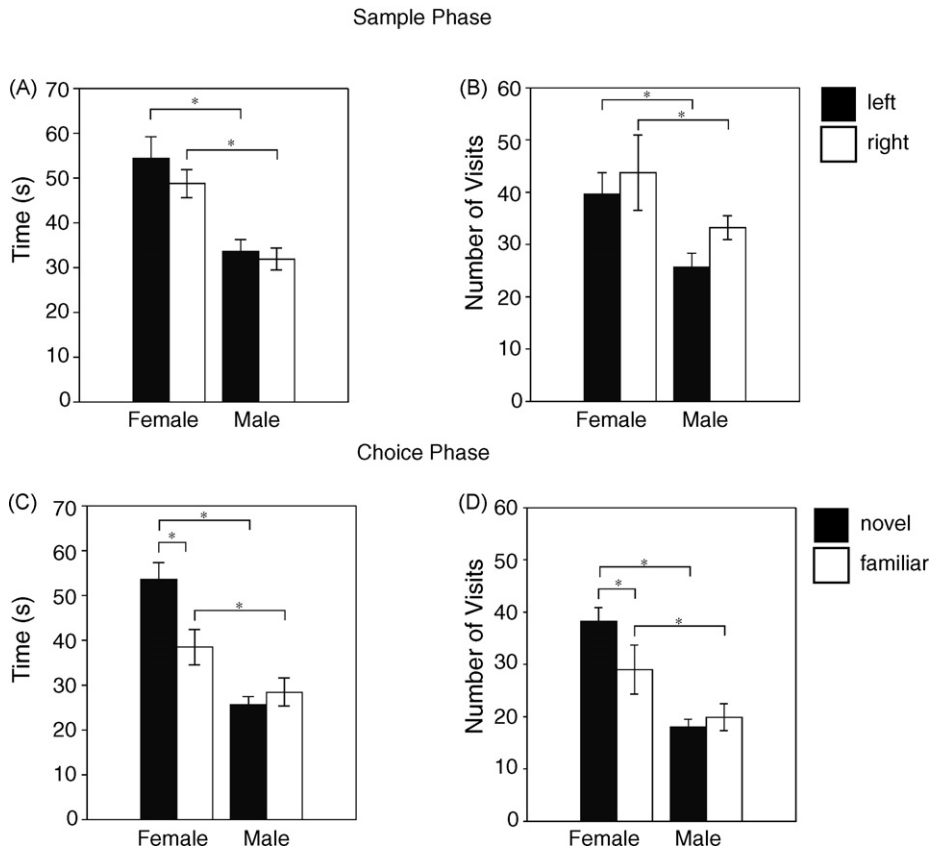

Fig. 3. Results from object recognition task. (A) Duration spent exploring objects during the sample phase. (B) Number of visits to objects on sample phase. (C) Duration spent exploring objects during the choice phase. (D) Number of visits to objects in the choice phase. * indicates $p < .05$.

criterion did so in 54 trials (18 sessions). Overall, after 20 sessions only one female failed to reach criterion while four males failed to reach criterion. These mice were removed from any further analysis. An analysis of errors per session using a 2 × 2 repeated measures ANOVA with sex as the between-subjects factor and session as the repeated within-subjects factor was carried out for the first 6 sessions with the data from the remaining 17 mice (10 females and 7 males). This revealed a main effect of session ($F_{5,11} = 9.37$, $p < .01$), no effect of sex ($F_{1,15} = 3.04$, $p = .10$), and no Sex × Session interaction ($F_{5,11} = 3.04$, $p = .06$).

The results from the probe tests are summarized in Table 1. A single male mouse refused to participate in Probes 2 and 3 by sitting in the start arm for 5 min, thus the sample size is reduced for these two probes. We used the binomial test to determine if more mice chose the correct location than expected by chance, within each

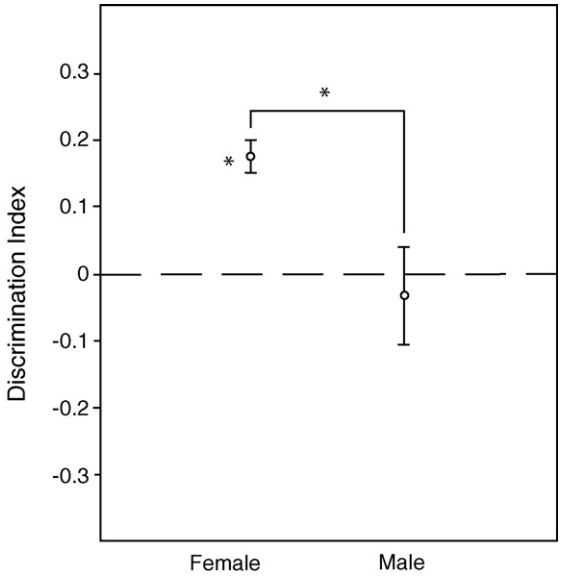

Fig. 4. Discrimination indices for the novel object recognition task. Discrimination index is calculated as the difference in exploration time between the novel and familiar objects, divided by the total time spent exploring both objects. * indicates $p < .05$.

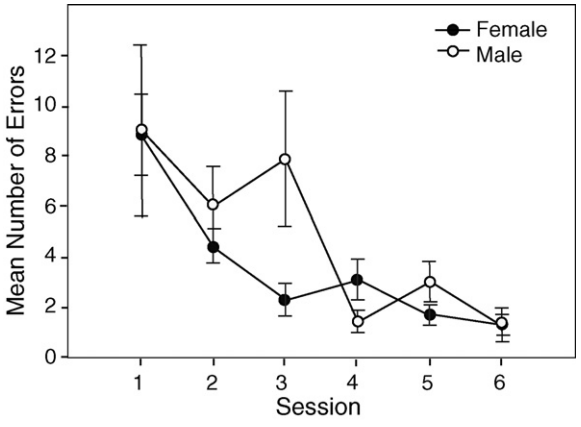

Fig. 5. Task acquisition by female and male C57B/6J mice in the ladder maze assessing mean (±SE) number of errors per session ($n = 10$ females and 7 male mice). Female mice began reaching criterion in the 6th session and male mice began reaching criterion in the 10th session. The last male and female mice to reach criterion did so in the 18th session. The average trials to criterion was 35.4 ± 3.93 for females and 41.14 ± 4.69 for males, which was not significantly different as determined by a t-test ($t_{15} = .94$, $p = .36$).

Table 1
Results for spatial probe tests on the ladder maze.

Type of probe	Group	Mice at criterion			
		Number correct	*n*	4 arms ($p = .25$)	3 arms ($p = .33$)
Intra-maze cues deleted (Probe 1)	Females	9	10	<.01*	<.01*
	Males	6	7	<.01*	<.01*
Extra-maze cues masked (Probe 2)	Females	6	10	.02*	.05
	Males	2	7	.31	.31
All cues deleted/masked (Probe 3)	Females	7	10	<.01*	.01*
	Males	2	7	.31	.31

Note. This table only includes mice reaching training criterion ($N = 17$). The binomial probabilities were calculated twice: first for chance levels with four maze arm choices (i.e., 3 choice arms + 1 start arm) and second with three maze arm choices, disqualifying the start arm as a possible choice (see Section 4).
* $p < .05$.

group, on each probe. Mice could choose any of the four arms once they reached the center. Mice indeed on occasion (albeit rarely) reversed direction and reentered their start arm. For this reason, all four arms were potential choices and the probability of choosing one arm was set at 0.25. However, as the start arm was used less frequently, we repeated the binomial analysis more conservatively by calculating chance as one of three, non-start, arms, i.e., 0.33. When intra-maze cues were removed (Probe Test 1) both female and male mice were significantly above chance in choosing the correct arm (binomial test, $p < .01$). This was true both when the start arm was included as a choice possibility and when it was excluded (Table 1). When extra-maze cues were masked (Probe Test 2), male choice behavior did not differ from chance (binomial test, $p = 0.31$) either when the start arm was included as a choice or not. However, in this probe, females were significantly more likely to choose the goal arm on the first choice (binomial test, $p = .02$). Their choice was not significantly different from chance when the start arm is excluded as an available choice (binomial test, $p = 0.05$). It is important to note that one of the female mice did return to the start arm after entering the center of the maze and this was counted as the first choice. When all cues were removed or masked (Probe Test 3), the first arm choice of males was again at chance levels and again females showed accurate performance, a greater proportion of females choosing the correct goal arm on the first choice than predicted by chance (binomial test, $p < .01$).

4. Discussion

The goal of this study was to determine if sex differences in cue use during spatial orientation in the C57BL/6J mouse strain are consistent with patterns observed in other species. Changes in husbandry and testing methods, that may have reduced stress during testing, led to sex differences in object recognition and place learning largely concordant with those reported for other mammalian species.

In the object recognition task, females were significantly more likely than males to discriminate the novel object from the familiar object by differential exploration. This result could not have been due to either side preference or odor cues. The side on which the novel object was placed was counterbalanced. In addition, during the test phase both objects were new to the mouse, as one was a duplicate of the object used in the training phase. There was also a sex difference in baseline exploration. It is possible that females collected more information during the sample phase than did males and for this reason this study cannot pinpoint the source of the female advantage. The goal of the study, however, was to determine not how but if male and female C57BL/J6 mice differ in their spatial encoding of a novel environment. The present results suggest that females and males pay attention differently to their surroundings, specifically that females pay more attention than males to novel objects and perhaps to discrete objects in general. Regardless of baseline differences in time spent with objects, males nonetheless spent the same amount of time with objects as had been reported in prior studies (Frick and Gresack, 2003). Nonetheless, in the choice phase, they did not discriminate between novel and familiar objects.

The finding that male mice were unable to discriminate between novel and familiar objects may seem contrary to many other novel object recognition experiments in which male mice of this strain are capable of recognizing the novel object (Frick and Gresack, 2003; Rampon et al., 2000; Tang et al., 1999). This may be due to the degree of similarity between the novel and familiar objects. The constructed objects in the present study, composed of Lego® blocks, were perhaps more similar to one another than in other studies, making the task more difficult. Further studies with standardized objects would clearly address this point. In addition, in other object recognition experiments we have found that increasing the difference between objects indeed results in an increase in males' ability to distinguish familiar from novel objects (Bettis and Jacobs, 2009).

The results of the current study confirm the female advantage found in a related object recognition task in C57BL/6J mice (Podhorna and Brown, 2002). This is significant because of several important methodological differences between the studies, most notably that the delay between the sample and choice phases in this study was only 15 min, as compared to 24 h in the current study. On the other hand, in both studies mice were tested during their natural active phase (dark phase of the cycle). The scheduling of testing may be critical. In a similar study that showed a male advantage in this task, after a delay of 24 h, C57BL/6J mice were tested during the light phase of the cycle, i.e. their natural period of inactivity (Frick and Gresack, 2003). Although it is possible that the sexes vary in their sensitivity to activity phase, we can draw no real conclusions until all the factors (cycle, delay duration, object similarity) can be controlled. Such future research will determine the contribution of these factors to the size and magnitude of the sex differences in C57BL/6J mice on this task. Given the similarities in sex differences among other mammalian species, however, it is reasonable to expect that future research will confirm that laboratory mice show a similar pattern to that seen in laboratory rats and in humans, albeit with greater sensitivity to testing conditions.

The hypothesis that females attend more to small objects in their environment than do males was also supported by results from the place learning task. Here males and females experienced the same duration of exploration and exposure to spatial cues and also showed the same pattern of task acquisition. Yet the sexes differed in their response to the removal or masking of cues. The masking of extra-maze cues impaired only male performance. Male performance was also impaired in the last probe test, when both extra-maze and intra-maze cues were masked or removed, but not in the probe test where only intra-maze cues were removed. This clearly points to a male reliance on using extra-maze cues for orientation, in agreement with studies of male vs. female humans

(Sandstrom et al., 1998), desert kangaroo rats (Langley, 1994) and laboratory rats (Williams and Meck, 1991).

Female performance on the place learning task, however, was less affected by changes in visible cues, even the intra-maze cues. This is an unexpected result given their sensitivity to object features in the first task. In fact, female performance remained robust throughout all probe tests. This suggests that females had encoded not simply the visible landmarks but other information as well; some cue that was not controlled with the current experimental design. This interpretation would be consistent with a study of sex differences in laboratory rats, orienting to food rewards on a plus maze (Tropp and Markus, 1999). In this study, females utilized both intra- and extra-maze cues initially and then after training, relied preferentially on extra-maze cues. Males, in contrast, rely preferentially on extra-maze cues from the beginning (Tropp and Markus, 1999). Some potential extra-maze cues that we could not control or mask were the auditory and olfactory cues surrounding the room, such as the noise from the observation room. It is possible that female C57Bl/6J mice show a similar pattern to the laboratory rats in this experiment as a result of the extended training that was necessary to confirm place learning from all release points in the current study.

In conclusion, future work must dissect the contributions of different parameters that influence sex differences in this species. Yet, the concordance of the present results with new models of spatial encoding and mapping (Jacobs and Schenk, 2003), as well as with prior studies of sex differences in other mammalian species suggest that the pattern of cognitive sex differences in the C57BL/6J laboratory mouse will be similar to that found in other mammals. This opens the door for sophisticated work on the genetic and molecular basis of sex differences in cognition in mammals.

Acknowledgements

We thank Paul Elsen, Kristina Coale and Sam Evans for their valuable technical assistance and discussion of the research described here. This study was supported by a grant from the J.D. French Alzheimer's Association, a Hillblom Foundation Network Grant to Stanford University and sabbatical support to L.J. from the Santa Fe Institute. All procedures were approved by the University of California, Berkeley Animal Care and Use Committee and were conducted in facilities that were approved by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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