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Sex differences in memory for landmark arrays in C57BL/J6 mice

Tania J. Bettis · Lucia F. Jacobs

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Abstract The most robust sex differences in cognition across polygynous mammalian species are the sex-specific patterns of the use of spatial cues during encoding and orientation. In laboratory rats, wild rodents, and humans, females orient preferentially to the features and arrangement of local landmarks, while males preferentially attend to distant landmarks. Yet this sex-specific pattern is often absent or reversed in the laboratory mouse, a species representing a major laboratory model of neural mechanisms. We explored sex differences in the C57BL/J6 strain of laboratory mouse by employing tasks that were motivated by the natural patterns of exploration. We predicted that such tasks would unmask the predicted default polygynous patterns of cue use by females and males. We used two standard tasks, a novel object recognition task and a five-stage serial object dishabituation task. On the first task, the results showed a female advantage in detecting the novel object, as predicted by prior results from other polygynous species. In the second task, we found, also as predicted, a male advantage in performance when the polarization of the array was distorted and a female advantage in performance when the local array was re-arranged. The pattern of sex-specific advantages in performance in C57BL/J6 mouse is thus concordant with that found in other polygynous mammals.

Keywords Spatial orientation · Navigation · Geometry · Object recognition · Evolution · Parallel map theory

T. J. Bettis (✉) · L. F. Jacobs
Department of Psychology, MC 1650, University of California, Tolman Hall, Berkeley, CA 94720-1650, USA
e-mail: tjbettis@berkeley.edu

Introduction

Sex differences in the encoding of objects have emerged as a powerful tool for understanding spatial cognition in polygynous mammals. For example, in humans, women consistently outperform men on tests of object location memory (Voyer et al. 2007). On such tasks, women are consistently more accurate than men in identifying changes in an array of objects observed for a short period of time. This sex difference, along with the male advantage in tasks of mental rotation, is consistent across cultures suggesting an evolutionary origin (Silverman et al. 2007); however, see Hoffman et al. (2011) for an effect of culture on this sex difference in another spatial task. Similarly, females may rely on an array of local objects for navigational cues, a well-studied phenomenon in the laboratory rat (*Rattus norvegicus domesticus*), using the radial arm maze. At acquisition, female, but not male rats, show impaired performance if local landmarks are deleted. Moreover, this female advantage in performance is organized by their exposure to gonadal hormones in the postnatal period (Williams and Meck 1991).

The mechanism for this female advantage in performance may be attentional. The evidence comes from tasks developed originally to exploit a natural inclination to explore novel objects in the subject's environment. In this task, a rodent first habituates to an array of objects. Its recognition of these objects, and their associated locations, is then measured in a series of probe tests, where single objects are either rearranged in space or one object is substituted for another (Save et al. 1992; Ennaceur and Delacour 1988). In such tasks in laboratory rats, female rats are more sensitive to the displacement of objects in an array than are male rats (Saucier et al. 2008). In the same task, female laboratory rats show better object recognition than male rats after longer periods of retention (Sutcliffe et al. 2007).

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In male laboratory rats, the attention to objects is quite different, as seen in their performance on the radial arm maze. Here, performance is not impaired with the randomization of small object arrays, as in females, but only when the shape of the room is obscured with a circular curtain around the maze (Williams et al. 1990). While this result is consistent with studies of the sex differences in the laboratory rat, it is inconsistent with a more recent report in the laboratory mouse (*Mus musculus*). Frick and colleagues have shown, in an object recognition and location memory task, that male mice display an increased response to both changes in object location and identity (Frick and Gresack 2003). In another study, no sex difference was reported for a similar task (Benice and Raber 2008). In general, there appears to be a disparity between a clear pattern for predictable sex differences in the laboratory rat but the absence or reversal of the same pattern in the laboratory mouse (see Jonasson 2004 for a meta-analysis).

While the ecology and natural history of the two species would predict similarities in response to spatial cues, there are several potential reasons why the results obtained might indicate a species difference. In many studies, the comparison is made between object and spatial memory, suggesting that the two are unique kinds of memory while categorizing all spatial changes together (Thinus-Blanc et al. 1996; Dere et al. 2007). Not all spatial changes provide or alter the same kinds of information. Spatial cues can be classified as those that primarily support a sense of direction in a space and those that are used to localize precise locations; these classes have been described as directional and positional cues, respectively (Jacobs and Schenk 2003). If the shape of a symmetrical array, such as a square or circle, is distorted into a polarized shape, such as a rectangle or ellipse, then the new array provides more directional information than the prior array. In contrast, an exchange of one unique object for another, where the shape of the array is not changed, may provide new positional but not new directional information. In species where males are more sensitive to directional cues and females more sensitive to positional cues, such as laboratory rats and humans (Benhamou and Poucet 1998; Chai and Jacobs 2010), one would predict that changes that result in the polarization of the array shape would be detected more accurately by males. In contrast, changes that result in a new distribution of object features, either by replacement or by substitution, we would predict to be detected more accurately by females.

The goal of the current study was to test this prediction in the laboratory mouse and thereby also address the question of sex differences in memory for object feature and location in this species (strain C57BL/6J). We predicted that the pattern of sex differences in this mouse when placed in appropriate housing and tested using low-stress tasks would be similar to that of other polygynous mammalian species. Therefore, males would notice and explore changes that polarized the array, whereas females would notice and explore changes in the object features. We adapted two standard object memory tasks to test these predictions. In the first task, we used the object recognition task of Ennaceur and Delacour (1988). In this task, mice explore object conditions on three successive days: an empty arena on day 1, with the addition of two identical objects on day 2, and then on day 3, with one of the now familiar objects replaced by a novel object (Fig. 1). Preferential exploration of the novel object is evidence for recognition of its novelty and hence the mouse's ability to discriminate the two objects. We predicted that this task would reveal a sex difference, with females more likely to explore the novel object than males. In the second task, we adapted the serial dishabituation task of Save et al. (1992). After habituation to an array of five unique objects (Fig. 2), the mice experience a series of probe tests where one object is changed, by being moved to a new location, being swapped with another object, being substituted or being newly added to the array. We predicted that object changes that altered the polarity, and hence, the inherent directionality of the array, should elicit a response from the male mice. In contrast, other changes that resulted in changes in object feature but did not change polarization should, as in Experiment 1, elicit a stronger response from the female mice.

Methods

Study animals

C57BL/6J mice (15 females, 15 males) were obtained at the age of 2 months and were tested at the age of 3 months for the object recognition task. A subset of nine female and

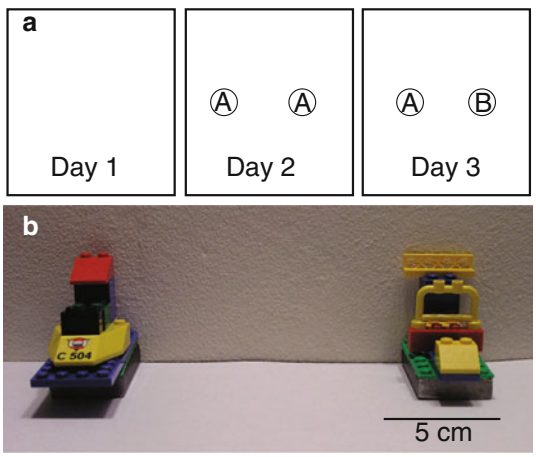

Fig. 1 Object recognition task. **a** A representation of the experimental design and spatial layout. The side of the arena in which the novel object was presented was counterbalanced across subjects. **b** Objects constructed from Lego™ blocks used in the object recognition task

Fig. 2 Serial dishabituation task. **a** Stimuli used in the task. **b** A representation of the experimental design and spatial layout

ten male mice were tested the following month in the serial dishabituation task. This was a random subset as six female and five male mice from the original group had been chosen at random for a pilot of another study.

Husbandry

The females were housed in same-sex groups, three per standard mouse cage (19 cm × 29 cm × 13 cm). The males were housed individually in standard mouse cages, to replicate more closely the natural social environment of this species (Deacon 2006) and thereby reduce anxiety (Palanza et al. 2001). Each cage was supplied with a disposable cardboard igloo and commercial bedding material (Nestlets™), in a 14:10 light cycle (lights on 22:00, off at 08:00). All testing was conducted during the dark phase of the light cycle. Water was available ad lib, and a ration of 4 g standard mouse chow was supplied on alternate days; this was sufficient to ensure that the mice were rarely without food, and never for more than 12 h, so that they maintained ad lib weight.

Object recognition task

Apparatus and procedure

We adapted the object recognition task from Ennaceur and Delacour (1988). The task consisted of three successive days of exposure to three similar environments for a period of 15 min per day. The arena was an opaque guinea pig cage (40.2 cm × 50.8 cm × 20.3 cm). The objects were unique constructions from Lego® blocks. A video camera was mounted to the ceiling and was connected to a recorder, monitor, and computer in an adjacent room. The door between the rooms was kept closed. External cues were available but were not prominent because the walls of the arena were opaque.

On day 1, the mouse was released into the empty arena for 15 min of exploration and habituation. On day 2, for the sample phase, the mouse was again released into the arena, which this time contained two identical objects, placed near the center. On the day 3, for the choice phase, the mouse was again released into the arena, which now contained a duplicate of the object from the sample phase and a novel object (Fig. 1). The location of the novel object was counterbalanced among mice to control for side biases.

The sample and choice phases were recorded during days 2 and 3 for subsequent analysis using Ethovision Pro (Noldus, Inc.). Two measures of exploratory 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 mouse's presence within the 4-cm zone was defined as its body's center of gravity, as defined by Ethovision, being within that zone. Although it is possible for the mouse to be facing away from the object and still be recorded in the zone surrounding the object, this was rare. It was extremely rare that the Ethovision calculated center of gravity crossed into the zone without the head of the animal being oriented toward the object. Significant agreement between this recording and manually recorded behavior has been shown in other studies (Benice and Raber 2008). Sex differences were calculated using ANOVA tests.

Serial dishabituation task

Apparatus

All trials took place on an elevated circular platform (90 cm diameter) constructed from white sheet vinyl plastic. This platform was elevated 31 cm above a white floor (122 cm × 122 cm × 5 cm) and did not have any walls. Trials were carried out during the dark phase of the light cycle under low light conditions. 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 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.

All the objects used in the serial dishabituation task were glazed ceramic objects (mostly commercial salt

shakers), ca. 9 cm in height (Fig. 2a). There are several advantages to using this type of object for an object recognition task in mice. First, they cannot be altered by the mice by chewing; second, they are too tall for mice to climb on which would increase that object's saliency; third, they can be completely cleaned of odor cues between trials; and finally, such objects are easily obtained in different shapes and patterns.

Procedure

The design of this task was adapted from Save et al. (1992). Each mouse experienced 11 consecutive 6-min trials. The first four trials habituated the mouse first to the arena and then to the array of objects. Subsequent trials introduced serial changes to this array, as illustrated in Fig. 2. Between trials, the mouse was placed in a holding cage for a 3-min inter-trial interval. In Trial 1 (Open Arena), the mouse is released into the empty arena for habituation to the environment. In Trials 2, 3, and 4 (Habituation), the mouse is released into the arena, which now contains a square array of five objects. In Trial 5 (New Location), the center object is moved to a position outside of the square. This array is replicated in Trial 6, so that the habituation to the change can be measured by comparing the changes in exploration of the changed array between Trials 5 and 6. In Trials 7 (Spatial Switch), two objects are switched in location and again, the same array is experienced in Trial 8, for a measurement of changes in exploration. In Trial 9 (Substitution), the familiar object is substituted with a novel object; again, the same array is experienced in Trial 10. In Trial 11 (Addition), a new object is added to the array, for a total of six objects.

Analysis

Each session was recorded on videotape for subsequent analysis by the Ethovision Pro software program (Noldus, Inc.). During Trial 1, distance traveled (cm) and velocity (cm/s) were recorded to assess differences in baseline activity levels. In all other trials, 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 mouse's presence within the 4-cm zone was defined in terms of the location of its body's center of gravity, as defined by the Ethovision tracking algorithm. An ANOVA was performed to assess any sex differences in initial activity level (average velocity and distance traveled). Habituation to the objects in Trials 2, 3, and 4 was analyzed using a repeated-measures MANOVA, with trial as the within-subjects variable and sex as the between-subjects variable. To assess sex differences in response to changes in the arrangement of the objects, MANOVAs were used to assess the difference in time spent with each object category (e.g. displaced vs. non-displaced) before and after the change. This number will be positive if the mice demonstrated an increase in attention to the object category and negative if the mouse habituated further to the object category. To assess a significant change in behavior toward the object categories, a one-sample *t* test was done to determine whether the distributions of the discrimination indices differed from zero. During the last trial, a new object was added and this does not allow for the same kind of analysis. To analyze Trial 11, we compared duration and number of visits to each object category using MANOVA tests.

Results

Object recognition task

Figure 3a, b show that female mice made more visits and spent more time exploring the objects than did male mice during the sample phase. However, both male and female mice explored the two objects evenly in this phase. There was a significant effect of sex on time spent exploring the objects ($F_{1,\ 28} = 13.79$, $P < 0.01$). However, there was no significant effect of object ($F_{1,\ 28} = 1.19$, $P = 0.28$) nor was there a significant sex by object interaction ($F_{1,\ 28} = 0.27$, $P = 0.61$). The same pattern was seen when frequency of visits to the object was analyzed. There was a significant effect of sex ($F_{1,\ 28} = 17.5$, $P < 0.01$), but no effect of object ($F_{1,\ 28} = 0.01$, $P = 0.68$) and no interaction ($F_{1,\ 28} = 0.07$, $P = 0.17$). During the sample phase on day 2, female mice made more visits to both of the objects than the male mice did (left object: ($F_{1,\ 28} = 11.27$, $P = 0.02$), right object: ($F_{1,\ 28} = 14.19$, $P < 0.01$)). Females also spent more time exploring the objects than the males did (left object: ($F_{1,\ 28} = 12.60$, $P = 0.01$), right object: ($F_{1,\ 28} = 23.20$, $P < 0.01$; Fig. 3a, b). Both male and female mice divided their exploration evenly between the two objects in the sample phase. A one-sample *t* test revealed no significant difference from 50 % of the exploration time spent on either object for either females ($t_{14} = 1.29$, $P = 0.22$) or males ($t_{14} = 0.62$, $P = 0.54$).

Figure 3c, d show that, during the choice phase, female mice preferentially explored the novel object while the male mice did not. There was a significant effect of sex ($F_{1,\ 28} = 24.47$, $P < 0.01$) and of object ($F_{1,\ 28} = 8.65$, $P < 0.01$) and a significant sex-by-object interaction ($F_{1,\ 28} = 9.29$, $P < 0.01$) on the time spent exploring the objects. This same pattern is seen when the frequency of visits to objects is analyzed. Again, there is a signifi-

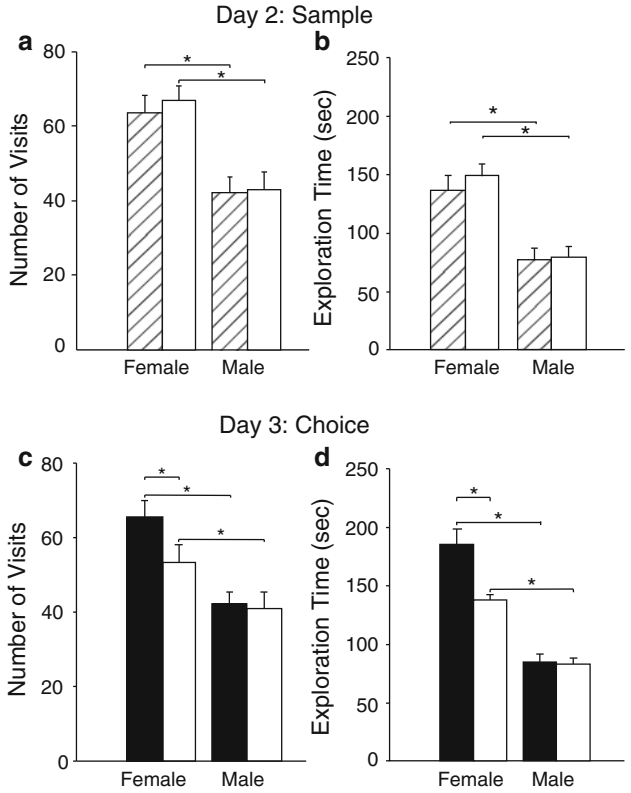

Fig. 3 Object recognition task. **a** Day 2: number of visits made to the objects during the sample phase. *Striped bars* indicate the object on the right, and *open bars* indicate the object on the left. **b** Day 2: duration of time spent exploring the objects during the sample phase. *Striped bars* indicate the object on the right, and *open bars* indicate the object on the left. **c** Day 3: number of visits to the objects during the choice phase. *Solid black bars* indicate novel object, *open bars* indicate familiar object. **d** Day 3: duration of time spent with the objects (*asterisk* indicates a significant difference, $P < 0.05$). *Solid black bars* indicate novel object, *open bars* indicate familiar object

cant effect of sex ($F_{1,\ 28} = 12.24$, $P < 0.01$) and object ($F_{1,\ 28} = 5.82$, $P = 0.02$) as well as a significant interaction between sex and object ($F_{1,\ 28} = 5.07$, $P = 0.03$). Females made more visits to the novel object than did males ($F_{1,\ 28} = 17.36$, $P < 0.01$), but they did not make more visits to the familiar object ($F_{1,\ 28} = 3.54$, $P = 0.07$; Fig. 3c). The female mice also spent more time with both the novel ($F_{1,\ 28} = 38.86$, $P < 0.01$) and the familiar objects than did male mice ($F_{1,\ 28} = 14.13$, $P < 0.01$; Fig. 3d). Paired samples t tests reveal that females made more visits ($t_{14} = 2.35$, $P = 0.03$) and spent more time ($t_{14} = 2.84$, $P = 0.01$) with the novel objects than the familiar objects. The males did not make more visits ($t_{14} = 0.27$, $P = 0.80$) or spend more time ($t_{14} = 0.09$, $P = 0.93$) with the novel than the familiar object. Together, these results show that male mice spent the same amount of time and made approximately the same number of visits to the objects on both day 2 and day 3. The female mice spent more time with the objects overall and, on day 3, selectively explored the novel object more than the familiar one.

Serial dishabituation task

There were no correlations across female or male mice in performance between the novel object recognition task and any of the measures of performance on probe trials in the serial dishabituation task. During Trial 1, in the open arena with no objects, females and males showed similar patterns of spatial exploration: female mice traveled 26.1 ± 1.1 m (mean ± SEM) and male mice traveled 25.2 ± 1.1 m. The average velocity of the female mice was 8.9 ± 0.43 cm/s, and the average velocity for the male mice was 8.4 ± 0.42 cm/s. Neither of these measures of activity were significantly different between the sexes [distance: ($F_{1,\ 17} = 0.81$, $P = 0.38$), velocity: ($F_{1,\ 17} = 0.30$, $P = 0.59$)]. We also examined the duration spent in the center of the arena as a measure of willingness to explore the outer ring of the platform, where, in subsequent trials, the objects would be placed. The center was defined as an area 33.3 cm in diameter. While the mean times spent in the center by males and females differed substantially (males: 15.35 ± 2.67 s; females: 7.39 ± 2.81 s), this difference was not significant ($F_{1,\ 17} = 4.22$, $P = 0.06$).

Figure 4 shows that both male and female mice habituated to the objects similarly across trials as is indicated by the reduction in exploration frequency of visits and time spent with the objects. A repeated-measures MANOVA, with number of visits to objects in each trial as the dependent measure, revealed a significant effect of trial ($F_{2,\ 16} = 56.62$, $P < 0.01$), but no effect of sex ($F_{1,\ 17} = 0.85$, $P = 0.37$) and no significant interaction ($F_{2,\ 16} = 3.27$, $P = 0.06$; Fig. 4a). The same pattern of results was seen using the time spent with objects as the dependent measure; there was significant effect of trial ($F_{2,\ 16} = 18.64$, $P < 0.01$), but no effect of sex ($F_{1,\ 17} = 3.31$, $P = 0.09$), and no interaction ($F_{2,\ 16} = 0.24$, $P = 0.78$; Fig. 4b). When all trials containing objects are included in the analysis a somewhat different pattern emerges. A repeated-measures MANOVA with number of visits to objects in each trial as the dependent measure reveals a significant effect of sex ($F_{1,\ 17} = 20.25$, $P < 0.01$) and trial ($F_{9,\ 9} = 17.45$, $P < 0.01$) but no significant interaction ($F_{9,\ 9} = 0.73$, $P = 0.68$). An assessment of the total time spent exploring objects reveals only a significant trial effect ($F_{9,\ 9} = 10.02$, $P < 0.01$) but no significant effect of sex ($F_{1,\ 17} = 2.63$, $P = 0.12$) and no interaction ($F_{9,\ 9} = 0.91$, $P = 0.56$).

Figure 5 shows that, when a central object was shifted to a new location in an outside position, male mice differentially explored it while female mice did not. A two-way

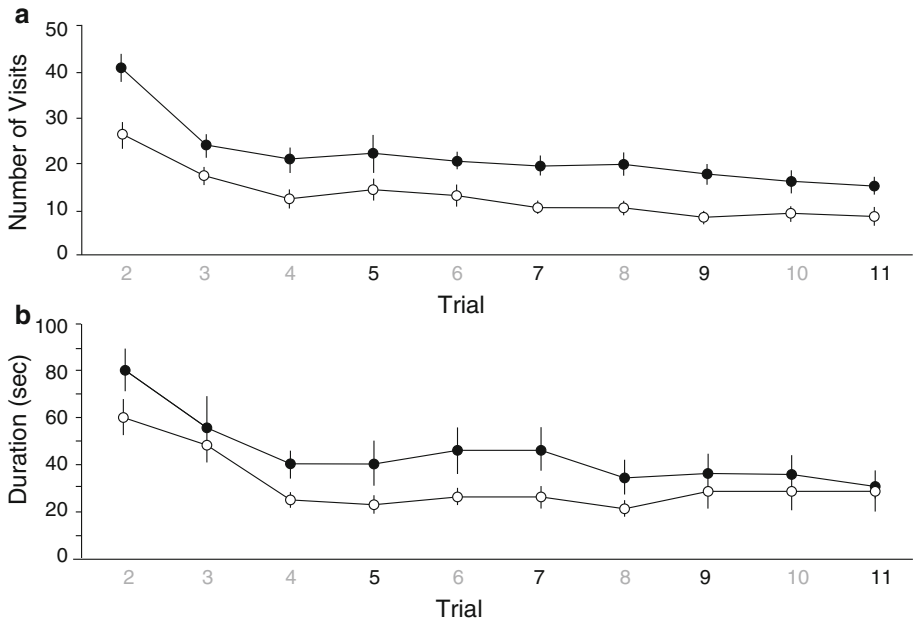

Fig. 4 Serial dishabituation task. **a** The mean number of visits to objects in each trial. Note that number of visits declines across trials for both male and female mice. **b** The average duration spent exploring objects in each trial. *Closed circles* indicate data from females, *open circles* indicate data from males

ANOVA reveals a significant effect of object category (either displaced or fixed) ($F_{1,\ 17} = 11.80$, $P < 0.01$) but no significant effect of sex ($F_{1,\ 17} = 0.00$, $P = 0.96$) and no interaction ($F_{1,\ 17} = 0.18$, $P = 0.68$) on the duration spent exploring the objects. This is the same pattern seen in the number of visits to the objects with a significant effect of object category ($F_{1,\ 17} = 4.5$, $P = 0.05$), but not of sex ($F_{1,\ 17} = 0.35$, $P = 0.56$), and no interaction ($F_{1,\ 17} = 1.67$, $P = 0.21$). This indicates that both the male and female mice changed their behavior differentially to the displaced and fixed objects and that there is no significant sex difference. However, the male mice showed a significant increase (above a zero change level) in number of visits to the displaced object across trials ($t_9 = 2.52$, $P = 0.03$) and the time spent exploring it ($t_9 = 2.54$, $P = 0.03$). Neither the change in number of visits to the displaced object ($t_8 = -0.35$, $P = 0.74$) nor the time spent exploring ($t_8 = 1.04$, $P = 0.33$) differed from zero for females. Hence, the female mice did not show the same level of response and only males appeared to dishabituate to the object after this displacement.

Figure 6 shows that, when two of the objects switched locations, the female mice did not further habituate to them while the male mice did. A two-way ANOVA with the change in time spent exploring objects as the dependent variable reveals a significant interaction between sex and object category ($F_{1,\ 17} = 5.74$, $P = 0.03$) but no main effect of either sex ($F_{1,\ 17} = 0.26$, $P = 0.62$) or object category ($F_{1,\ 17} = 0.56$, $P = 0.47$). This indicates that male and female mice responded differently to the object

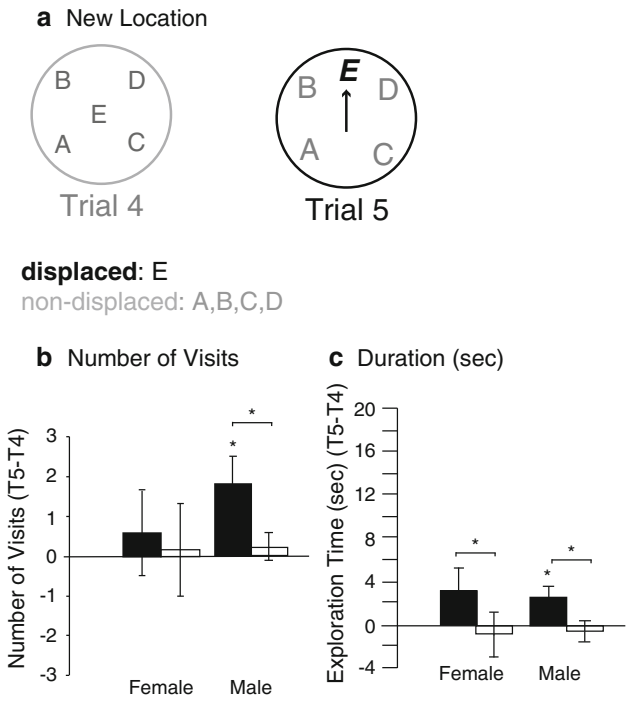

Fig. 5 Serial dishabituation task, new location **a** A familiar object is moved to a new location, which changes the shape of the array. **b** Change in mean number of visits between Trials 4 and 5 for displaced and non-displaced object categories. *Solid bars* represent the displaced object, and *empty bars* represent the average of the fixed objects. **c** Change in mean exploration time spent with objects between Trials 4 and 5 for displaced and non-displaced object categories. *Solid bars* represent the displaced object, and *empty bars* represent the average of the fixed objects. (*asterisk* indicates a significant difference from 0 in a *t* test, $P < 0.05$)

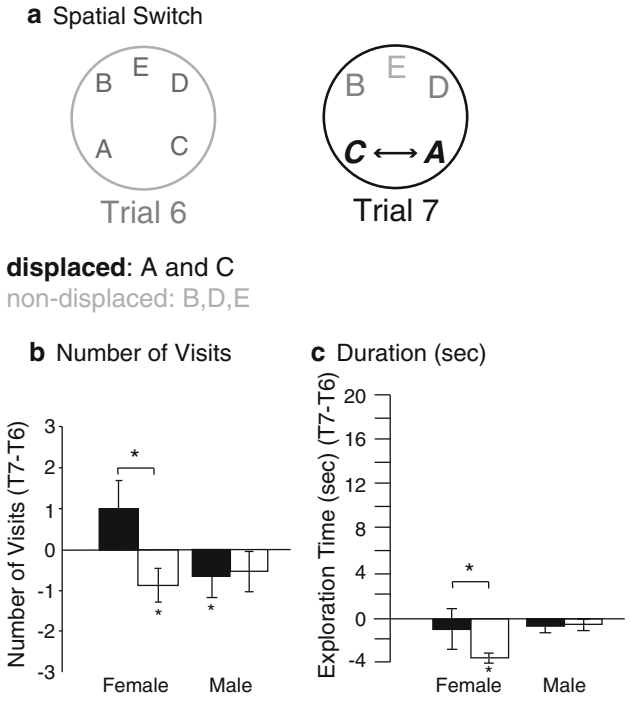

Fig. 6 Serial dishabituation task, spatial switch. **a** The location of two familiar objects is switched. **b** Change in mean number of visits between Trials 6 and 7 for displaced and non-displaced object categories. *Solid bars* represent the average of the switched objects, and *empty bars* represent the average of the fixed objects. **c** Change in mean exploration time of objects between Trials 6 and 7 for displaced and non-displaced object categories. *Solid bars* represent the average of the switched objects, and *empty bars* represent the average of the fixed objects (*asterisk* indicates a significant difference from 0 in a *t* test, $P < 0.05$)

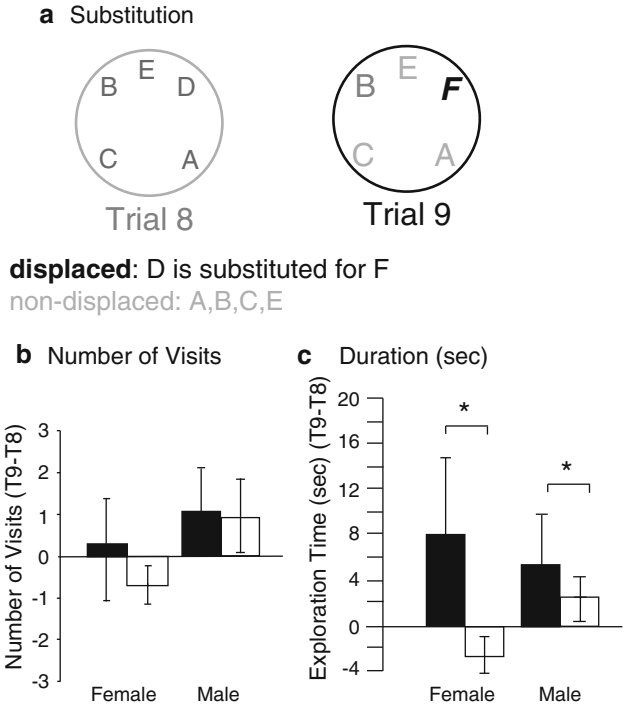

Fig. 7 Serial dishabituation task, Substitution. **a** A substitution of a novel object for a familiar object in a familiar location. **b** Change in mean number of visits between Trials 8 and 9 for substituted and familiar object categories. *Solid bars* represent the substituted object, and *empty bars* represent the average of the fixed objects. **c** Change in mean exploration time of objects between Trials 8 and 9 for substituted and familiar object categories. *Solid bars* represent the substituted object, and *empty bars* represent the average of the fixed objects. Note that none of the measures differed significantly from zero

categories. However, when the change in number of visits to the objects is the dependent variable, then there is no significant interaction ($F_{1,\ 17} = 3.74$, $P = 0.07$), no main effect of sex ($F_{1,\ 17} = 1.38$, $P = 0.26$), and no significant effect of object category ($F_{1,\ 17} = 2.90$, $P = 0.11$). The change in time spent exploring the different object categories across trials is not significantly different from zero in either the fixed ($t_8 = 0.38$, $P = 0.71$) or the switched objects ($t_8 = 1.39$, $P = 0.20$) for females. However, the change in the number of visits to the fixed objects was significantly less than zero ($t_8 = -2.68$, $P = 0.03$), which indicates that females continued to habituate to these unchanging objects. The change in the number of visits to the displaced objects across Trials 6 and 7 was not significantly different from zero ($t_8 = 0.11$, $P = 0.92$) for females. Males displayed a different pattern. The data indicate that they continued to habituate to both sets of objects though not significantly so for the fixed objects. The change in time spent exploring the switched objects differs significantly from zero ($t_9 = -3.04$, $P = 0.01$), indicating an increase in habituation. This was not true for the fixed objects ($t_9 = 0.49$, $P = 0.63$). When the change in number of visits is assessed, males did not differ from zero, either for the fixed objects ($t_9 = -1.61$, $P = 0.14$) or the switched objects ($t_9 = -2.02$, $P = 0.07$).

Figure 7 shows that, in Trial 9, when an old object is substituted with a novel one, both male and female mice preferentially explore the novel object. A two-way ANOVA reveals a significant effect of object category on the change in time spent exploring ($F_{1,\ 17} = 5.53$, $P = 0.03$), but there was no significant effect of sex ($F_{1,\ 17} = 0.11$, $P = 0.75$) and no significant interaction ($F_{1,\ 17} = 0.23$, $P = 0.64$). In females, there was no significant change in their behavior, in the number of visits either to the substituted object ($t_8 = 0.16$, $P = 0.87$) or to the familiar objects ($t_8 = -1.42$, $P = 0.19$). There was also no difference in the time spent with the substituted object ($t_8 = 1.15$, $P = 0.28$) or the familiar objects ($t_8 = -1.50$, $P = 0.17$). The same pattern was seen in males, with no significant change between Trials 8 and 9, in the number of visits either to substituted object ($t_9 = 1.10$, $P = 0.30$) or to the familiar objects ($t_9 = 1.37$, $P = 0.21$), or the time spent

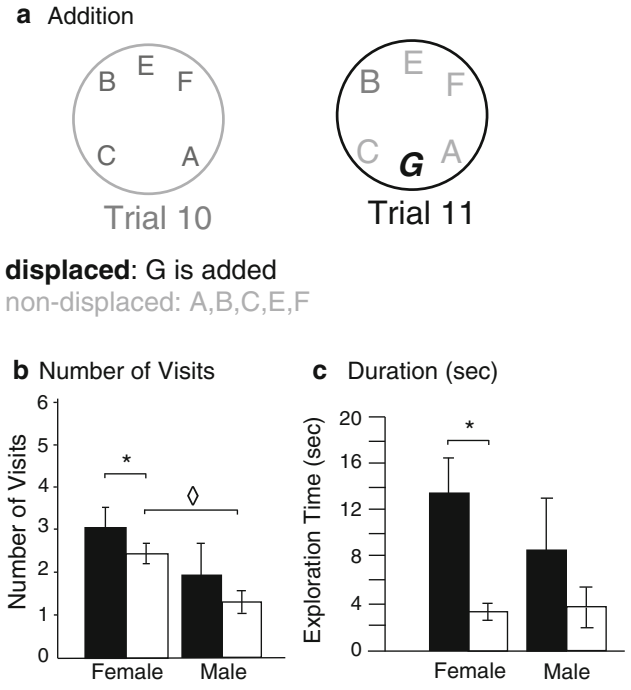

Fig. 8 Serial dishabituation task, addition. **a** A novel object is added to a novel location. **b** Number of visits to the novel object and mean number of visits to the familiar objects. *Solid bars* represent the added object, and *empty bars* represent the average of the fixed objects. **c** Exploration time spent with the novel object and mean exploration time of the familiar objects. *Solid bars* represent the added object, and *empty bars* represent the average of the fixed objects. (*asterisk* indicates significance at $P < 0.05$, paired samples t test; *diamond* indicates significance at $P < 0.05$, ANOVA)

with the substituted object ($t_9 = 1.46$, $P = 0.18$) or the familiar objects ($t_9 = 1.14$, $P = 0.29$).

Figure 8 shows that, in Trial 11, when a new object is added to a new location, both male and female mice explored this object more than the old objects. While it appears that the female mice differentially explored the novel object and that the male mice explored of all objects, there is not a significant interaction between sex and object category. A two-way ANOVA reveals a significant effect of object ($F_{1,\ 17} = 6.20$, $P = 0.02$), but not of sex ($F_{1,\ 17} = 0.55$, $P = 0.47$) and no significant interaction ($F_{1,\ 17} = 0.82$, $P = 0.38$) on the time spent with either the novel or familiar objects. The same test of number of visits shows no significant effect of object ($F_{1,\ 17} = 2.48$, $P = 0.13$) or sex ($F_{1,\ 17} = 4.30$, $P = 0.06$), and no interaction ($F_{1,\ 17} = 0.00$, $P = 0.98$). Female mice visited familiar objects more than did males ($F_{1,\ 17} = 4.86$, $P = 0.04$). The females also made more visits to the novel object than to familiar objects ($t_8 = 2.43$, $P = 0.04$) and spent more time with the novel object than with the familiar object ($t_8 = 2.53$, $P = 0.04$). The male mice did not visit the novel object more than the familiar objects ($t_9 = 1.00$, $P = 0.34$), and they did not spend more time with the novel object than the familiar object ($t_9 = 1.02$, $P = 0.33$).

Discussion

In the present study, we predicted that, with appropriate test procedures, sex differences in the laboratory mouse would parallel those seen in other polygynous species, such as humans and the laboratory rat. We adapted two tasks where mice are motivated only by their natural inclination to explore novel objects, and therefore minimized any stressful effects of training. In these two tasks, females were more sensitive to changes in the association of object and location and changes in object feature than were males as predicted, while, as predicted, males were more sensitive to changes that altered the polarity of an array, and hence the quality of directional information that could be deduced from the array.

The pattern of learning in female laboratory mice observed in these tasks is consistent with that seen in the laboratory rat, where females are more accurate than males in detecting changes in an object (Saucier et al. 2008; Sutcliffe et al. 2007). It is important to note, however, that we have not identified the mechanism of this differential response in females. It would not be surprising whether the females' greater time spent attending to the objects resulted in improved object memory and recall, and hence greater response to changes in one of the learned objects. The goal of our study, however, was not to identify this mechanism but rather to determine whether such a sex difference could be demonstrated consistently in the laboratory mouse, in a manner that is concordant with sex differences found in other polygynous mammalian species.

The serial dishabituation task revealed intricacies of the sex differences in spatial cognition in mice. Four changes were explored: New Location, Spatial Switch, Substitution, and Addition. These four changes can be categorized by the effect on polarization: in only one change, New Location, did the symmetrical array become asymmetrical and hence polarized. In the last change, Addition, the added object made the array symmetrical once more. Thus, based on the hypothesis that female mice attend to object identity and relationships within an array and that male mice attend to geometry and directional information, one would predict a relatively larger response in male mice on the first change, New Location, which is what we found. The increased response of the male mice to the shifting of an object to a new location has been found in prior studies

of male laboratory rats (Sutcliffe et al. 2007) and male laboratory mice (Frick and Gresack 2003). In these studies, the male advantage has been interpreted as the result of superior memory for an object's location, as males explored a familiar object in a novel location more than did females (Frick and Gresack 2003; Sutcliffe et al. 2007). However, in the study by Frick and Gresack (2003), this shift in location also changed the shape and the polarity of the object array, as in the current experiment. Future research is necessary to systematically determine the influence of array geometry on object memory in the male laboratory mouse.

In the Spatial Switch and Substitution conditions, based on work in other polygynous species, we would predict that females would attend more to the displaced objects than would males. Only the first of these predictions was confirmed, in the Spatial Switch change. In the Substitution change, we predicted a relatively greater female response but found that both females and males attended to the change. In a written task for humans, women detect that two objects have switched locations more accurately than men do, but this female advantage is not seen when the location of the object was simply shifted (James and Kimura 1997). Female rats show a similar sensitivity to the locations of objects being switched, where the change is strictly the spatial relationship of the objects with each other, and not the shape of the array (Saucier et al. 2008). Therefore, our finding a lack of sex difference seems anomalous.

In a previous study, we have shown that the relative degree of similarity between the novel and familiar objects can have an effect on novel object recognition and the sex differences therein. When the novel and familiar objects vary dramatically in shape and pattern, then both male and female mice show a clear recognition of the novel object. However, when the novel and familiar object are more similar, then it becomes more difficult to distinguish them. Female mice still recognize the novel object in the more difficult scenario, while male mice did not (Bettis and Jacobs 2012). Thus, we may have observed no sex difference in this trial as a result of the high degree of difference between the old object and the novel object that was substituted into the array. We may also have observed such low exploration of objects just as a result of fatigue. Because Substitution was assayed late in the sequence, that is, Trials 9 and 10, such decreases in motivation could have differentially affected this probe test. These speculations must await further experimentation.

The result of the Addition probe also supports the hypothesis that females attend to object identity and relationships within an array, while males attend to geometry and directional information. We predicted both that females would attend to the novel object and that males would attend to the novel object in the novel location. Instead, only females detected the change, despite the apparently conspicuous addition of a new object to the by-now extremely familiar objects. One possible reason for males not detecting this change could be the location of the novel object, which had the effect of transforming an asymmetrical space enclosed by five objects to a symmetrical space, enclosed by six objects.

Our study demonstrated a female response to novel object substitutions (both tasks), while other studies have found only a male response after such manipulations (Frick and Gresack 2003). A factor that might have led to this discrepancy is the housing of the animals. In this study, the male and female mice have different housing conditions in order to eliminate the confound of sex and stress that is produced in standard housing conditions. This variable—whether a male is housed alone or in a group of males—may affect the outcome of object recognition tasks in the laboratory rat (Sutcliffe et al. 2007). Also, Voikar et al. (2005) conclude that the effects of housing on mice are minimal and some tests suggest that individually housing males are less anxiogenic than group housing with the same sex. Yet these single-housed males, though less stressed overall, performed less accurately than group-housed males on the object recognition task (Voikar et al. 2005). Stress has well documented effects on the structure and function of the hippocampus of rats and mice, the forebrain structure recruited for spatial encoding of novel spaces and objects in mice (Bowman et al. 2003). More specifically, stress leads to structural changes in the hippocampal CA3 field (McEwen 1999). Based on the predictions of the parallel map theory of hippocampal function, damage to this subfield should specifically impair the encoding of directional but not positional cues (Jacobs and Schenk 2003). The result, therefore, of group housing on cognition in male mice should theoretically be their decreased reliance on directional cues and their increased reliance on the use of positional cues, that is, to attend, like females, more closely to the features of objects. Thus, spatial strategy in group-housed males should be more similar to that of females than of males, as observed in Voikar et al. (2005) and in concordance with the effects of housing on spatial strategy in laboratory rats (Hermes et al. 2005).

One explanation for this pattern of results, concordant with the present study, is that solitary housing more closely mimics the natural mating system of the house mouse, the ancestral species of the laboratory mouse. In general, the mating system of this species is that of a solitary male defending a territory, on which several females communally nest and rear their offspring (Bronson 1979). Given the natural species pattern of male solitary defense of the mating territory, it makes sense that in the laboratory, a solitary-housed male (at the least, a male that is not housed

with other male mice) might exhibit a more male-typical pattern of spatial cognition. Such a hypothesis must be tested and validated with future work, but it highlights the potential of using sex differences in spatial memory in laboratory mice as a powerful tool for understanding the cognitive mechanisms underlying spatial orientation.

In summary, sex differences in the serial dishabituation task were generally predicted by the hypothesis that males and females in polygynous mating systems differ in their use of directional and positional cues (Jacobs and Schenk 2003). Females, encoding space according to the position of close objects, attend carefully to object features and are more likely to notice a change, such as a switch or substitution. Males, encoding space at a coarser resolution, based on distal landmarks and other directional cues, attend to objects that polarize the landmark array, because such changes increase the directional information that the mouse can extract from the array.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The experiments carried out and reported herein were done in compliance with all United States laws and were approved by the Animal Care and Use Committee of the University of California at Berkeley.

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