



Short communication

## Sex differences in object recognition are modulated by object similarity

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## ARTICLE INFO

## Article history:

Received 15 July 2011

Received in revised form 16 April 2012

Accepted 20 April 2012

Available online 8 May 2012

## Keywords:

Mouse

C57BL/J6

Spatial memory

Evolution

## ABSTRACT

How females and males differ in performance in object recognition tasks appears to vary among mammalian species, with female superiority found in the laboratory rat and humans but not in the laboratory mouse. Here we assessed sex differences in object recognition in C57BL/J6 mice by varying the similarity between objects to be learned. Females outperformed males in object recognition when the novel object was similar to a previously learned object.

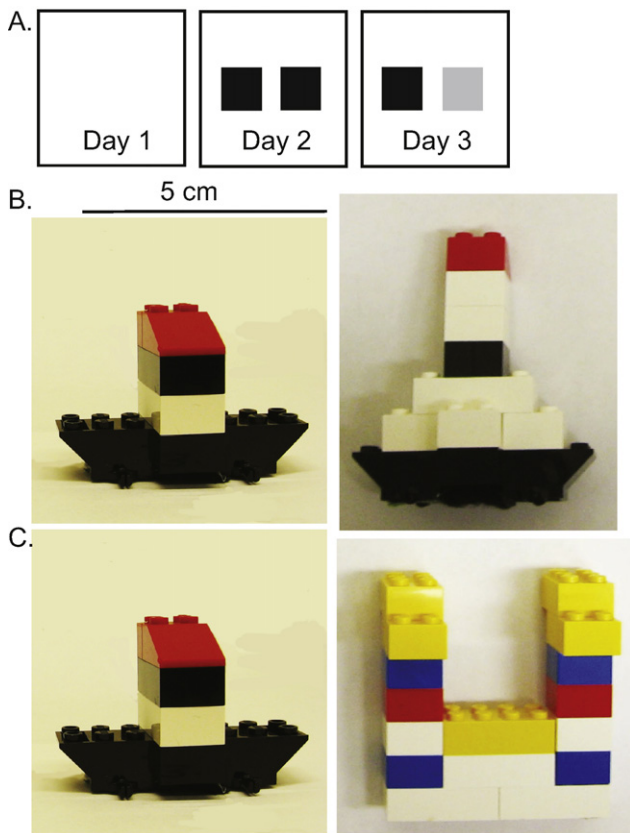
Published by Elsevier B.V.

An important task for the study of sex differences in memory is object recognition. In laboratory rats, object recognition tasks produce a female advantage, with female rats discriminating between the novel and familiar object, indicating memory for the familiar object [1]. The pattern of sex differences in this task in the laboratory mouse is controversial, however, with reports of no sex differences or superior performance of males in some studies and of female mice in others [2–4]. In our previous study of C57BL/6J mice, females differentially explored a novel object more than a familiar object and this discrimination was not seen in males [5]. Despite the controversy on the relative performance of females and males in mice, nonetheless it is clear from several studies that male mice can discriminate the novelty of some test objects [2,6,7]. Thus, it is unclear why male mice are not able to discriminate the quality of novelty in some studies but not others. There are several variables that differ among studies, which may offer some insight, including the duration of habituation to the arena, exposure to the objects during the sample phase, differences in the length of the inter-trial interval, the shape of the arena and the perceptual features of the objects used as training stimuli. As discussed by Ennaceur, the question of object perception by rodents is important and understudied, yet it is clearly critical to the pattern of performance in object recognition studies [8]. For example, it is important to avoid objects with special affordances that might induce preferences, thereby masking the detection and exploration of an object as a result of its novelty. The affordances of objects have been defined as “the relation between the abilities of animals and the properties of objects” [9]. This can include aspects of the object that allow a mouse to climb

on top of it or elicit other types of exploratory behaviors for reasons other than novelty discrimination. Affordances of objects may also refer to characteristics that distinguish them from one another and allow for the recognition of differences between them. Objects should therefore be maximally discriminable yet similar enough to not incite preferential exploration irrespective of novelty, a difficult balance to attain. Because many studies supply only brief descriptions, and not color photographs or detailed descriptions that would allow for a comparison of the objects used in different studies, it is difficult to reconcile conflicting results across such studies. Other researchers have raised similar issues, such as the need to control difference in odor absorption and texture [9], and to control object height, which can cause an exploratory bias independent of the relative novelty [10].

In the present study, we hypothesized that sex differences in object recognition in mice may emerge from sex differences in the ability to discriminate objects. In several mammalian species, including humans as well as the laboratory rat (*Rattus norvegicus*), and two species of kangaroo rats (*Dipodomys merriami*, *Dipodomys microps*), females have demonstrated a greater attention to the identity of objects [11–14]. Therefore we predicted that, when the novel object was similar to the familiar object, females would outperform males, but if the novel object was highly dissimilar, we predicted that there would be no sex difference. A high degree of difference will afford both the male and female mice the ability to discriminate, while a low degree of difference will only afford the female mice the ability to discriminate objects. To test this hypothesis, we constructed objects where features (color, shape, size, texture) could be carefully controlled and varied. Using the classic method of Ennaceur and Delacour [15], we constructed sets of objects from commercial plastic blocks (Lego®), using a readily available product to facilitate standardization across laboratories.

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**Fig. 1.** Experimental design and stimuli. (a) A schematic representation of the experimental design. (b) Experiment 1 objects. (c) Experiment 2 objects.

The subjects were C57BL/6J mice ( $N=22$ , 11 female, 11 male), which were 12 weeks old at the time of testing. Mice were housed in groups of three. Each cage was supplied with a disposable cardboard igloo and bedding material (Nestlets®). Mice were kept on a 12:12 light cycle (lights on 20:00, off at 08:00). Cages were all kept below the top tier of the racks in order to reduce the stress of being directly under a light source. Water and food (standard mouse chow) were available ad lib.

Two object recognition experiments were carried out as previously described [5]. Each experiment was carried out over three consecutive days, during which the mice were exposed to the apparatus for a period of 5 min per day (Fig. 1). 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. 1). The retention delay was thus 24 h. The location of the novel object was counterbalanced among mice to control for side biases. Different objects were constructed for each replication of the task such that all objects presented were completely novel to the mouse.

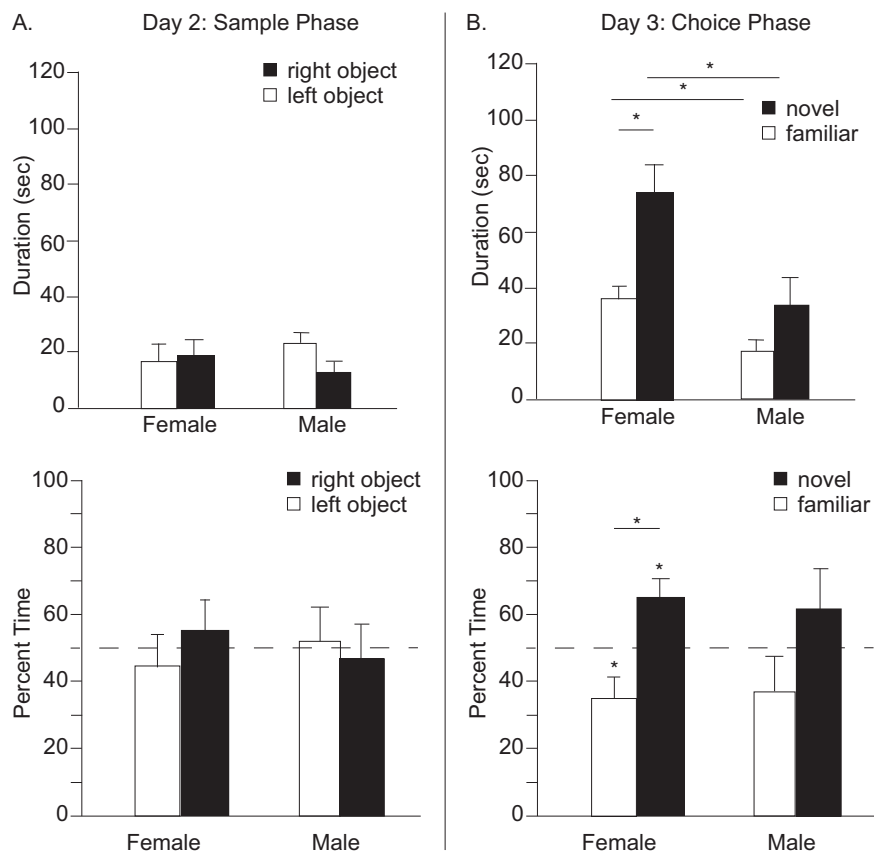
The only differences between Experiments 1 and 2 were the stimuli used and the relative degree of similarity between the novel

and familiar objects. In Experiment 1, two objects of relative similarity were used. Both objects were constructed with identical blocks on the bottom, thus the objects occupied identical footprints. The objects were also designed such that they would not allow for climbing. This was done in order to prevent the mice from sitting on the objects and observing the room beyond the apparatus. In addition, these objects were constructed using the same color scheme and a similar pattern of alternating white/dark blocks, which makes the contrast striking in both objects. The most salient difference between the two objects appeared to be the difference in height. Experiment 2 was carried out one week later. Here one of the objects was replaced with an object that differed dramatically in many features: height, color, contrast and shape, as one object had only one 'tower' and the other had two (Fig. 1B and C). While color is probably not a feature that mice will attend to, contrast is [16,17]. And the contrast apparent in the black, white and red object is greater than the contrast between colors in the yellow, blue, white, and red object. While this is a qualitative description of the level of similarity between stimuli paired for these studies, it provides a starting point from which a more detailed understanding of the nature of object recognition in mice can be built.

All behavior was recorded on videotape for subsequent analysis using Ethovision Pro (Noldus, Inc.). Two measures of behavior were made: the time spent within 4 cm of 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 the Ethovision Pro tracking algorithm. The Ethovision tracking system results in high correlations between manual recording and automatic scoring in object recognition tasks in mice [18,19]. Using the center of gravity as the criterion for entering the zone of interest has the potential to record instances where the mouse enters the zone but faces away from the objects, as the program does not distinguish between nose and tail. However, in prior studies and in pilot studies we found no differences between manually recorded instances of attention to the objects (being within 4 cm and facing the object) and automated recorded instances as defined above [5]. In addition a discrimination index was calculated for each of the choice phases. The discrimination index was calculated by subtracting the duration spent exploring the familiar object from the duration spent exploring the novel object and dividing by the total duration spent exploring both objects. All statistical tests reported are two-tailed except where indicated.

During the sample phase, on Day 2 of Experiment 1, no significant differences in total duration were observed between objects for either female mice (paired samples  $t$ -test:  $t_{10}=0.54$ ,  $p>0.10$ ) or male mice (paired samples  $t$ -test:  $t_{10}=1.58$ ,  $p>0.10$ ). Nor were there any differences between the sexes in either measure of total duration (ANOVAs of right object:  $F_{1,20}=0.33$ ,  $p>0.10$ , and left object:  $F_{1,20}=1.13$ ,  $p>0.10$ ) or percentage of time spent exploring the objects (ANOVA of right object:  $F_{1,20}=0.12$ ,  $p>0.10$ , and left object:  $F_{1,20}=0.16$ ,  $p>0.10$ ; Fig. 2A). In addition, none of the percentages differed significantly from 50% (one-sample  $t$ -tests for female mice:  $t_{10}=0.09$ ,  $p>0.10$  and for male mice:  $t_{10}=0.5$ ,  $p>0.10$ ).

On Day 3 of Experiment 1, in which the novel and familiar objects were similar, significant differences were observed between the sexes (Fig. 2B). Analysis using one-way ANOVA revealed a significant difference between the sexes in total duration spent with both the novel ( $F_{1,20}=4.63$ ,  $p=0.04$ ) and the familiar objects ( $F_{1,20}=5.88$ ,  $p=0.03$ ). A two-tailed, matched-pairs  $t$ -test resulted in a significant difference in the total duration spent exploring the novel vs. the familiar objects by the female mice ( $t_{10}=3.28$ ,  $p=0.01$ ) but not for the male mice ( $t_{10}=1.74$ ,  $p>0.10$ ). When percentage of exploratory time was analyzed, the female mice spent a significantly greater percentage of time exploring the



**Fig. 2.** Experiment 1: Similar Objects. (a) Duration and percent time spent exploring identical objects during the training phase. (b) Duration and percent time spent exploring familiar and novel objects during the testing phase. \* indicates  $p < 0.05$ .

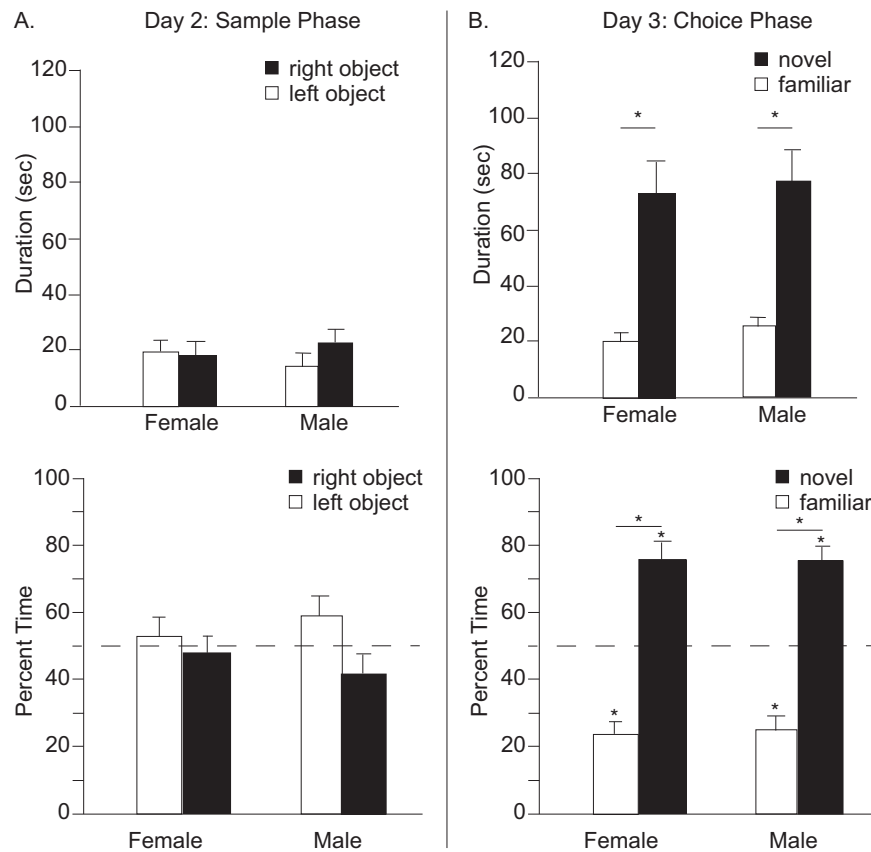
novel object than the familiar object (one-tailed  $t$ -test:  $t_{10} = 1.95$ ,  $p = 0.04$ ). There was no significant difference in the percent of time devoted to each of the objects by the males (one-tailed, paired-samples  $t$ -test:  $t_{10} = 1.48$ ,  $p = 0.06$ ). Lastly, a discrimination index was calculated by subtracting the time spent with the familiar from the novel object and dividing by the total time spent exploring objects. An ANOVA of this discrimination index revealed no significant sex difference ( $F_{1,20} = 0.00$ ,  $p > 0.10$ ). However, one-sample  $t$ -tests of the distributions revealed that the discrimination index calculated for the female mice was significantly greater than zero (one-tailed  $t$ -test:  $t_{10} = 1.94$ ,  $p = 0.04$ ). In contrast, the same test of the distribution from the male mice did not reveal a discrimination index significantly different from zero ( $t_{10} = 1.47$ ,  $p = 0.09$ ).

The sample size was reduced by one male mouse in Experiment 2, as this subject did not participate in the experiment due to health concerns. On Day 2, the sample phase of Experiment 2, in which the objects to be discriminated differ greatly, no significant differences were observed between sexes or objects and a repeated measures ANOVA with duration as the dependent measure reveals no significant effect of Sex ( $F_{1,19} = 0.22$ ,  $p > 0.10$ ) or Object ( $F_{1,19} = 1.59$ ,  $p > 0.10$ ) and no significant interaction ( $F_{1,19} = 3.16$ ,  $p = 0.09$ ). In addition, the percent of time devoted to each object was not significantly different than 50% for either the female ( $t_{10} = 0.31$ ,  $p > 0.10$ ) or the male ( $t_9 = 1.98$ ,  $p = 0.08$ ) mice as analyzed with a one-sample  $t$ -test (Fig. 3A).

On Day 3, the choice phase, only a significant effect of Object type ( $F_{1,19} = 80.66$ ,  $p < 0.01$ ) was observed in a repeated measures ANOVA with duration as the dependent variable (Fig. 3B). There was no significant effect of Sex ( $F_{1,19} = 0.70$ ,  $p > 0.10$ ) and no significant Sex by Object interaction ( $F_{1,19} = 0.00$ ,  $p > 0.10$ ). One-sample  $t$ -tests revealed that the female ( $t_{10} = 10.42$ ,  $p < 0.01$ ) and male

( $t_9 = 8.04$ ,  $p < 0.01$ ) mice spent significantly more than 50% of their exploratory time with the novel object, and therefore significantly less than 50% of their time with the familiar object. Lastly, a one-way ANOVA of the discrimination index reveals no significant difference between the sexes ( $F_{1,19} = 0.02$ ,  $p > 0.10$ ). One-sample  $t$ -tests demonstrate that the discrimination index calculated for both the female ( $t_{10} = 10.40$ ,  $p < 0.01$ ) and the male mice ( $t_9 = 8.06$ ,  $p < 0.01$ ) were significantly different from zero (Fig. 4).

The results from Experiment 1 replicated our earlier findings that female mice are better able to discriminate the identity of similar objects than male mice [5]. This is consistent with the literature on sex differences in object recognition in other mammalian species. It is also consistent with a model of spatial orientation in mammals where sex differences in navigational strategies emerge from the preferential reliance of females on local cues and males on distal cues. For example, both female laboratory rats and women show a sex-specific advantage in object location memory [1,20,21]. In addition, female rats demonstrate impaired performance in a radial arm maze only when local cues were rearranged, while male rats become disoriented if distal cues were obscured, even when local cues are available [22]. In the present study, female and male mice both attended to the objects to the same degree during the sample phase, in the presence of identical objects. During the choice phase, female mice differentiated between the novel and familiar objects by exploring the novel object for a longer duration. There was a trend for male mice to show this pattern of exploration, but there was also high variance in responses between individuals, and the result was not significant. While Experiment 1 demonstrated a female advantage, Experiment 2, utilizing objects that differed in several features, demonstrated that male mice can discriminate remembered objects, even after delays of 24 h. This is consistent



**Fig. 3.** Experiment 2: Different Objects. (a) Duration and percent time spent exploring identical objects during the training phase. (b) Duration and percent time spent exploring familiar and novel objects during the testing phase. \* indicates  $p < 0.05$ .

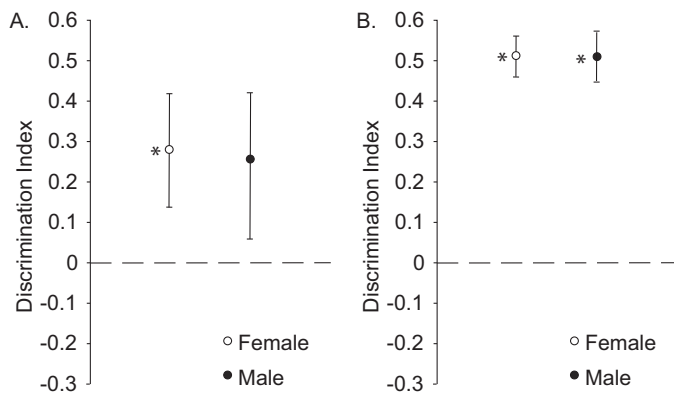
with previous findings from studies studying object recognition using only male mice, which is the standard design in rodent studies of learning and memory [7]. Studies of both sexes, and their differences in perception and memory, are therefore important to document the complete range of cognitive abilities expressed by a species, particularly such an important laboratory model species as the C57J/B6 mouse. A further implication of our results is the importance of controlling the features of the object in discrimination tasks. The use of commercially available building blocks, such as Lego®, in these experiments will greatly increase our ability to

compare results across diverse mouse strains and laboratories. Finally, there are no published studies reporting a retention of object memory for delays as great as one week. We therefore assumed that there would be no order effects between the two experiments. While we are fairly confident the order of testing did not influence our results, further studies must address this question experimentally.

In conclusion, the female advantage for memory of object feature has been reported in mammalian species as diverse as the laboratory rat [20] and the human [11]. Thus our finding of the same female advantage in mice, both in the present study and in a prior study [5], suggests that this pattern of sex difference in spatial memory may be found generally in mammalian species where the sexes rely differentially on different types of cues in spatial navigation [23].

**Acknowledgments**

We would like to acknowledge Sam Evans, Amy Cook, Mikel Delgado, Jennifer Arter, Anna Waisman, and Paul Elsen both for technical assistance and discussion of this research. This research was supported by a grant from the JD French Alzheimer’s Association, a Hillblom Foundation Network Grant to Stanford University and sabbatical support to L.J. from the Santa Fe Institute. The authors declare that they have no conflict of interest. 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 affiliated institution.



**Fig. 4.** Discrimination indices for each of the sexes during the test phase on Day 3 of each experiment. (a) Experiment 1 (similar objects) (b). Experiment 2 (different objects) \* indicates a significant difference from 0 ( $p < 0.05$  in a one-sample  $t$ -test).

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