

Working Memory and Reference Memory Tests of Spatial Navigation in Mice (*Mus musculus*)

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Researchers in spatial cognition have debated for decades the specificity of the mechanisms through which spatial information is processed and stored. Interestingly, although rodents are the preferred animal model for studying spatial navigation, the behavioral methods traditionally used to assess spatial memory do not effectively test the predictions of specificity in their representation. To address such issues, the present study tested the ability of mice to use boundary geometry and features to remember a goal location across 2 types of tasks—a working memory task with a changing goal location, and a reference memory task with 1 rewarded goal location. We show for the first time that mice, like other animals, can successfully encode boundary geometry in a working memory spatial mapping task, just as they do in a reference memory task. Their use of a nongeometric featural cue (striped pattern), in contrast, was more limited in the working memory task, although it quickly improved in the reference memory task. We discuss the implications of these findings for future research on the neural and genetic underpinnings of spatial representations.

Keywords: spatial navigation, geometric module, reorientation, boundaries

Spatial navigation encompasses a range of behaviors influenced by a variety of both internally generated and external sources of information. Because the ability to represent, remember, and return to particular locations in one's environment is essential to the daily functioning and survival of any navigating species, it is likely that there are multiple dissociable mechanisms underlying such spatial behaviors. Recent advances in the study of hippocampal representations of spatial information in freely moving rodents have transformed our understanding of the neural representations underlying navigation behavior (see Barry & Burgess, 2014, for a review). Nevertheless, although rodents are the most common animal model for spatial cognition, the widespread traditional methods of training and testing (e.g., Morris water maze) do not provide much

insight into the specificity of the processes underlying navigation (see Lee & Spelke, 2010a; Gallistel & Matzel, 2013). The purpose of the present paper is to address this issue using mice.

One of the main difficulties in observing behavioral manifestations of distinct underlying processes is that behavior is a result of a combination of outputs from several potentially independent computations. We address two such factors that have been found to influence spatial navigation: the environmental cues provided and the task involved in learning them.

Experiments on various species of animals have shown that, in a spatial working memory reorientation task (requiring subjects to return to a target location after a short delay and inertial disorientation), subjects relied consistently on environmental boundaries to compute spatial relationships, while only showing limited use of other nongeometric features (e.g., scent markings, visual contrast patterns, wall color/brightness) as direct goal markers and often failing to use them altogether (e.g., rats: Cheng, 1986; toddlers: Hermer & Spelke, 1994; chicks: Lee, Spelke, & Vallortigara, 2012; fish: Lee, Vallortigara, Ruga, & Sovrano, 2012). Subsequent tests showed that although distance and direction information provided by even subtle 3-dimensional extended surface structures successfully define a goal location, an array of free-standing objects or high-contrast 2-dimensional lines on the ground are not effective (Lee & Spelke, 2010b, 2011; Lee, Spelke, & Vallortigara, 2012; Lee, Sovrano, & Spelke, 2012), supporting the view that boundary-based navigation is highly specific. Nevertheless, when given repeated reinforcement training at an unchanging target location in reference-memory navigation tasks, animals do eventually learn to use spatial relationships with respect to various featural cues, and such results are often cited as evidence against

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the existence of boundary-specific representations (see Cheng & Newcombe, 2005, for a review; Pecchia & Vallortigara, 2010). Features and boundaries have also been shown to be dissociable in controlled-rearing studies in which feature-based but not boundary-based navigation was influenced by rearing conditions (Chianetti & Vallortigara, 2010; Brown, Spetch, & Hurd, 2007). Converging evidence from studies of human spatial navigation (Doeller & Burgess, 2008; Doeller, King, & Burgess, 2008) and scene recognition (Greene & Oliva, 2009; Park, Greene, Brady, & Oliva, 2011) show that boundary processing is behaviorally distinctive from other features (e.g., do not generally follow rules of reinforcement learning) and specifically associated with hippocampal and parahippocampal activation.

In behavioral studies, the differences in the type of training implemented across tasks and studies, and their potential interaction with the type of spatial cues provided, make it especially challenging to disentangle the effects of general learning processes from specific spatial computations. Given the evidence that flexible spatial updating in working memory navigation depends more heavily on the hippocampus, in contrast with fixed response learning that relies more heavily on areas such as the striatum (e.g., McDonald & White, 1994; Packard & McGaugh, 1996), it is crucial to take task specificity into account when studying spatial behavior. Moreover, to form a coherent theory of navigation, such task differences must be understood in light of the dissociation between boundaries and other featural cues.

Like most reorientation studies with nonhuman animals to date (see Cheng & Newcombe, 2005), the existing studies on spatial reorientation in mice have only implemented reference memory tasks (Fellini, Schachner, & Morellini, 2006; Twyman, Newcombe, & Gould, 2009). Because mice are a widely used animal model for testing the neural and genetic underpinnings of spatial abilities, it is especially important to develop tasks that characterize distinctive systems of spatial representation and memory. The present study aimed to provide tests of cue specificity and task specificity in spatial reorientation in mice using two types of environmental cues (i.e., boundaries and features) in two types of tasks (i.e., working memory and reference memory).

The Present Study

The present study implemented four tests of spatial orientation and memory with mice. The first experiment tested spatial working memory (varying target locations across trials) in accord with boundary geometry in a rectangular arena (Experiment 1a) and in accord with one striped, featurally distinctive wall in a square arena (Experiment 1b) devoid of competing or interacting boundary cues (see Pearce, 2009). The second experiment tested the same two environments with boundary geometry (Experiment 2a) and features (Experiment 2b) but through a reference memory task, with an unchanging, reinforced target location.

General Method and Materials

The present protocol provided a goal-finding task for mice by simultaneously harnessing their aversion to bright open spaces and to getting wet. This was achieved without the use of a traditional water maze (Morris, 1984) by filling an arena with only enough water to wet their paws. At the corners of the arena were four small

boxes, only one of which had a small opening on one side; this opening served as the target location, where the mice could be dry and safely hidden. For each experiment, the mouse was first allowed to explore the arena until it took shelter in the target location; the mouse was then removed, disoriented, and then released back into the environment.

For the working memory task, the target hole was varied across trials and blocked off (made inaccessible) for the test phase, when the mouse was returned to the arena immediately after disorientation. We recorded and analyzed the search behavior of the mouse for 60 s after its release. For the reference memory task, the target remained open, accessible, and constant over trials.

Subjects

Subjects were 12 male mice (C57BL/6, 8–10 weeks old), obtained from Charles River Laboratories (Calco, Italy). This common black strain of mice was chosen because it is the most widely studied lab rodent in the world, for both behavioral and genomics research (e.g., Matsuo et al., 2010). Two weeks prior to testing, the mice were housed in groups of 2–3 in standard Technoplast Type II cages (267 × 207 × 140 mm), which were cleaned weekly and filled with fresh bedding, cardboard domes, and strips of paper (for enrichment). Mice were checked daily for their condition, weight, and visible injuries to ensure the absence of aggressive behaviors and general well-being. All home cages, as well as the testing setup, were kept in the same room, which was maintained at 21–23 °C. The mice were provided with a grid (13 × 25 mm) full of standard food pellets (Mucedola 4RF21GLP, Certificate PF1610 for Mice and Rats) and a bottle of water (food and water checked daily), and put on a 12-hr light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). After completing this study, the mice were kept for use in other experiments.

Each subject was tested in Experiment 1 (working memory) first, and then Experiment 2 (reference memory), because Experiment 2 required learning of a single-reward location, which would interfere with Experiment 1 if Experiment 2 were administered after it. In each experiment, six subjects were tested in the geometry condition first, and the other six were tested in the feature condition first.

Apparatus

The experiments took place in a circular testing space formed by uniformly colored, light-proof, black curtains. One bright central light (round; diameter, 10 cm) illuminated the circular testing space from directly above, and all other lights in the room were extinguished. A camera was mounted on the ceiling. At the center of the testing space was either a uniformly colored gray rectangular arena (40 × 80 × 20 cm) or a square arena (40 × 40 × 20 cm) with three gray walls and one striped black/white, featurally distinctive wall (stripe thickness, 4.5 cm; see Figure 1). The arena was filled with 5 mm of water. In each corner was a black box (8 × 8 × 12 cm), one of which had an opening (7.5 × 7.5 × 4 cm) on one side. To minimize the availability of any potential visual cues, the opening was always on the side of the box that faced away from the center of the arena.

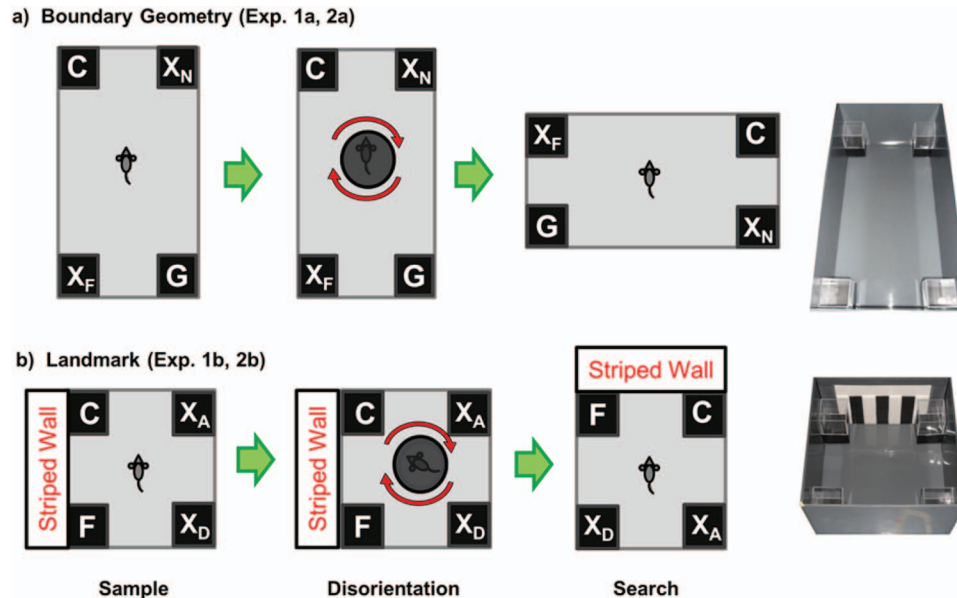


Figure 1. Depictions of the target sampling, disorientation, and search procedures for the two arenas testing rectangular environmental geometry (Experiments 1a and 2a) and a distinctive visual feature (Experiments 1b and 2b). The rotational symmetry of the rectangle provides two geometrically correct responses: the correct location (C) and the rotationally symmetric corner (R) (a). The geometrically incorrect corners are labeled near (X_N) and far (X_F) errors. Target locations were varied across trials (in Experiment 1) or subjects (Experiment 2). For the striped wall to be used as a relative spatial mapping cue, the target corner (C) should be distinguished not only from the corners with completely different featural properties—the diagonal (X_D) and adjacent (X_A) errors—but also from the corner with mirror symmetry with respect to the feature (S) (b). Alternatively, the striped wall can be used partially as a goal marker, allowing mice to distinguish the two corners near the striped wall from the two corners across from it (C + S vs. X_D + X_A). Because target locations were varied, only one of the four possible corner designations is shown. See the online article for the color version of this figure.

Design

Experiment 1a tested spatial working memory using boundary geometry within a rectangular arena with plain, gray walls (see Figure 1a). A total of nine trials were administered on 2 consecutive days—five on the first day of testing and four on the second. After the sampling phase of each trial, in which the mouse found the refuge corner, the mouse was disoriented and placed back into the arena for the test phase with all corners inaccessible, such that its searches were not reinforced again. This was done for several reasons, including the use of time measures for preference and minimization of interference with the next trial. The target locations were varied across trials and counterbalanced, such that each corner was tested equally.

Approaches to the four corners in the test trial were labeled as shown in Figure 1a: The correct target (C) and rotationally symmetric (R) corners were the *geometrically correct* corners, and the near (X_N) and far (X_F) errors were the *geometrically incorrect* corners.

Experiment 1b tested working memory using a distinctive striped feature on one wall of a square arena (see Figure 1b). A total of eight trials were administered on 2 consecutive days—four trials on each day. The target locations were counterbalanced, such that each corner was tested equally, and varied across trials. The approaches to the four corners in the test trial were labeled as follows: the correct target (C) and featurally symmetric (S) corners

shared the presence or absence of the striped wall (if the correct corner was near the striped wall, the featurally symmetric corner was the other corner near the striped wall; if C was across from the striped wall, then S was the other corner across from the striped wall), and the diagonal (X_D) and adjacent (X_A) corners had the opposite relationship to the featural cue. If the featural cue is used to compute relative positions, then C should be distinguishable from S; if the featural cue is used only as a direct goal marker, then C and S should be distinguishable from X_D or X_A (i.e., striped vs. not striped) but not from each other (i.e., left vs. right of striped wall).

Experiment 2a applied reinforcement training to the test of geometry in the same rectangular arena used in Experiment 1a. Experiments were carried out exactly as in Experiment 1, except that the goal location remained constant over trials and that the target escape hole remained open after the disorientation procedure. The trial was completed once the mouse successfully located the target corner. Equal numbers of subjects were assigned to each target corner, and each subject performed 10 trial pairs (total of 20 rewarded searches).

Experiment 2b applied the stable goal location and reinforcement to the test of features with the same square arena and striped wall feature used in Experiment 1b. Procedures were identical to Experiment 2a.

Given the past findings on reorientation behavior and goal-directed spatial memory tasks across a wide range of species, including mice, successful use of geometry and feature were tested

using one-tailed directional comparisons for a goal preference whenever applicable.

Procedures

The mouse was removed individually from its home cage in a covered cylinder (10×15 cm) and transported to the testing arena within the same room. The mouse was released from the center of the arena and allowed to explore the environment until it found the target hole and took refuge inside. After 30 s, it was removed and disoriented for 30 s in the covered cylinder (0.3 rotations/s in one direction and then the other). Meanwhile, the arena was rotated 90° with respect to the rest of the environment and the position of the experimenter, the target box was rinsed with water, and (for the working memory condition) the target hole was closed shut. The mouse was released from the center again and given 1 min to explore the arena. After 1 min, the mouse was removed from the testing arena, again using the covered cylinder, and placed back inside its home cage. Testing was conducted across 2 consecutive days per arena (geometry, feature) per experiment (working memory, reference memory) for a total of 8 days. Daily intertrial interval was about 1 hr.

Behavioral measures were coded offline from the video recordings by two coders: for the spontaneous navigation task, we analyzed the first approaches and the total time spent in each corner during the 60-s test trial; for the reference memory task, we analyzed the first approaches and total time spent at the three incorrect corners before reaching the target hole. A corner choice was defined as any instance in which any part of the head or body

of the mouse was within 3 cm from a corner box. Intercoder reliability was checked for 15% of the trials; the coders agreed 100% on the first approached corner and 92% on total number of seconds spent at each corner. For trials with initial coding discrepancies, an agreement was reached by recoding.

Experiment 1a

Experiment 1a tested spatial working memory using rectangular boundary geometry in mice, in the absence of featural cues. The target location changed on each trial, and the refuge was made inaccessible during the 60-s test phase to measure the amount of time spent at each corner during that period. Successful use of geometry predicts that mice will prefer the geometrically correct corners (C + R) over the other two.

Results and Discussion

Time measure. A repeated-measures analysis of variance (ANOVA) was conducted with geometry (correct vs. incorrect geometry) and trials (see Figure 2a). First, a main effect of geometry showed that the total time spent at the geometrically correct corners (C + R = 23.3 s), 95% confidence interval (CI) [20.5, 26.2], was significantly higher than the total time spent at the incorrect corners ($X_N + X_F = 18.7$ s), 95% CI [16.5, 20.9], $F(1, 11) = 6.92$, $p = .02$, $\eta_p^2 = .39$. Moreover, a main effect of trials revealed a general increase in the time spent at the corners over trials, $F(1, 11) = 3.82$, $p = .01$, corrected for sphericity using Huynh–Feldt and Greenhouse–Geisser adjustments of p value,

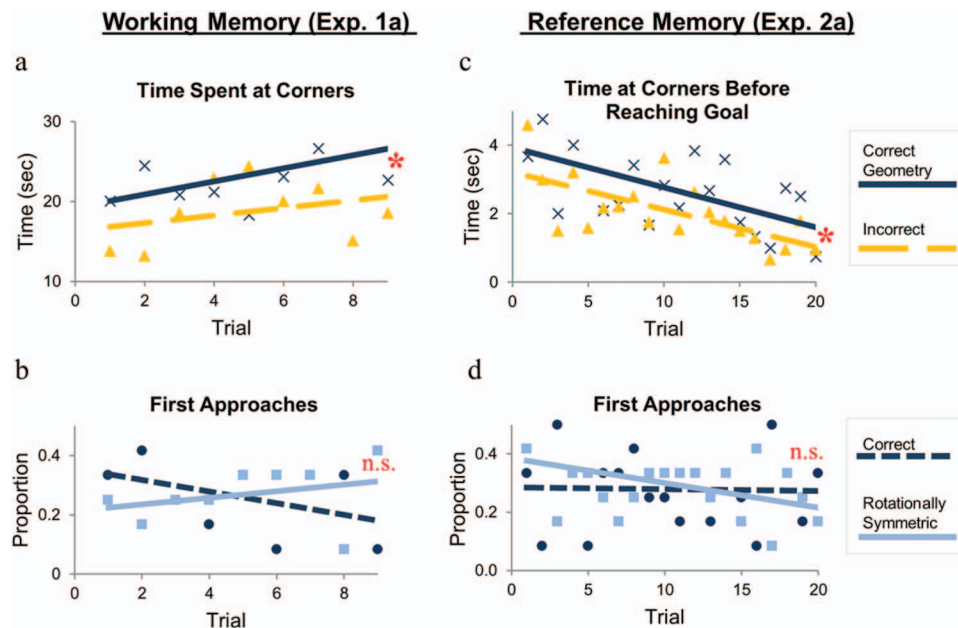


Figure 2. Proportion of total time spent, as well as first choice, at the corners for tests of boundary geometry in the working memory task (a and b, respectively) and in the reference memory task (c and d, respectively). In the first choice measure, there was no distinction between the target corner and the rotationally symmetric corner, showing that the rats were disoriented. The time spent at the geometrically correct corners provides evidence that, in both working memory and reference memory tasks, mice were using boundary geometry to guide their search behavior. * Significant differences between overall means at $p < .05$. See the online article for the color version of this figure.

$\eta_p^2 = .26$, and a significant linear trend, $F(1, 11) = 12.89$, $p = .004$, $\eta_p^2 = .54$. However, because there was no significant Trials \times Use of Geometry interaction, $F(8, 88) = 1.16$, $p = .34$, $\eta_p^2 = .10$, we concluded that although there was no increase in their ability to use geometry over trials, the mice spent more time at the relevant corner locations in general, perhaps “searching” for the goal. To ensure that this success was not attributable to a preference for the one target corner (due to a lack of disorientation or the use of polarizing visual or olfactory cues), the time spent at C (11.5), 95% CI [9.8, 13.1], was compared with R (11.9), 95% CI [8.8, 14.9]; no differences were found ($t < 1$, *ns*).

First choice. In contrast to the time measures, the proportion of first choices to the geometrically correct corners (C + R = 52.8%), 95% CI [44.2, 61.4], did not differ significantly from a chance value of 50%, $t(11) = .71$, $p = .25$, one-tailed directional hypothesis, $d = .430$; there were no significant differences or trends in this measure across trials ($F_s < 1$, *ns*; see Figure 2b). To provide a test for the null hypothesis, as well as a positive effect of geometry, a Bayesian analysis (see Gallistel, 2009) was performed to compare the odds of a geometrically correct first search between 50% and 70% (based on effect sizes of previous studies using a working memory task in animals: e.g., Hermer & Spelke, 1994; Lee, Spelke, & Vallortigara, 2012; Lee, Vallortigara, et al., 2012) with the odds of the null hypothesis (50% chance). The resulting Bayes factor for geometrically correct choice in Experiment 1a was 0.25, with corresponding odds of the null hypothesis at 4.01, providing significant support for the null hypothesis (see Gallistel, 2009). There were no differences in performance between subjects that were tested in the geometry condition first versus feature condition first.

The results of this experiment are the first demonstration of a working memory representation of environmental geometry in mice. Despite the absence of repeated training at a single-reward target location, mice exhibited a preference for the geometrically correct corners of the arena, and updated this location from trial to trial. Furthermore, the rotational symmetry of the rectangular environment provided an internal check for the effectiveness of the disorientation procedure and the absence of other cues that could distinguish the correct corner from its geometrically identical corner. One thing to note is that the first approach measure did not reveal any geometric sensitivity. A possible explanation is that in this novel, wet environment, the mice tended to approach and stay close to the walls of the arena (i.e., thigmotaxis), introducing error and noise to the first choice measures and to the sequence of approaches (as the mice run around the environment along the walls). Nevertheless, the time measure showed a clear successful use of boundaries to compute relative positions (e.g., “corner to the east of the long wall”)—the mice stopped and spent more time at the geometrically correct corners than the geometrically incorrect ones.

Experiment 1b

Experiment 1b tested spatial working memory using one featurally distinctive, striped wall, in a square arena lacking informative environmental geometry. A successful use of the feature as a relative positioning cue would predict a preference for the correct corner over the others (e.g., “corner to the east of the striped wall”). A partial use of the feature as a direct goal marker could

result in a preference for the two striped corners when the target is a striped corner, and a preference for the two plain corners when the target is a plain corner (i.e., a preference for C + S).

Results and Discussion

Time measure. A repeated-measures ANOVA with trials and all four corners as the within-subjects measures was conducted. There was no main effect of corner, $F(3, 33) = .69$, $p = .56$, $\eta_p^2 = .06$, or Corner \times Trial interaction, $F(21, 231) = 1.40$, $p = .12$, $\eta_p^2 = .11$, but there was a significant effect of trial, $F(7, 77) = 3.09$, $p = .006$, $\eta_p^2 = .22$ (see Figure 3a). The linear trend analysis for trial was significant, $F(1, 11) = 8.61$, $p = .014$, $\eta_p^2 = .44$, reflecting the same general increase in time spent at the relevant corner locations found in Experiment 1a. When partial feature use to distinguish between the corners near the stripes from the all-gray ones was tested (C + S vs. $X_D + X_A$), the repeated-measures ANOVA still showed no significant main effect of corner, $F(1, 11) = 2.34$, $p = .15$, $\eta_p^2 = .18$, but did reveal a significant effect of trial, $F(7, 77) = 3.10$, $p = .006$, $\eta_p^2 = .22$, and a significant Feature \times Trial interaction, $F(7, 77) = 2.50$, $p = .023$, $\eta_p^2 = .19$. A linear trend analysis of this interaction was also significant, $F(1, 11) = 9.74$, $p = .01$, $\eta_p^2 = .470$, reflecting an increasing use of the striped wall to distinguish the striped corners from the plain gray ones over trials (despite the failure to use it as a relative positioning cue). Interestingly, the location of the target corner with respect to the striped feature (i.e., whether it was a striped corner or an all-gray corner) did not have a significant effect on performance ($F < 1$, *ns*).

First choice. The proportion of first choices to the correct corner did not differ significantly from chance of 25% (21.9%), 95% CI [11.6, 32.1], $t < 1$, *ns*. The Bayes factor comparing the odds of a correct first search between 25% and 45% to the odds of the null hypothesis was 0.03, with corresponding odds of the null hypothesis at 38.80, providing strong support for the null hypothesis (see Figure 3b). There was no evidence for even a partial use of the feature, when compared with a chance level of 50% (C + S = 52.1%), 95% CI [46.4, 57.8], $t < 1$. The Bayes factor comparing the odds of a feature-based first search between 50% and 70% to the odds of the null hypothesis was 0.01, with corresponding odds of the null hypothesis at 78.91, again providing strong support for the null hypothesis. There was no difference in performance between those trials for which the goal was near the striped wall or at an all-gray corner, $t(11) = 1.23$, $p = .24$, for C + S, $d = .53$; $t(11) = 1.45$, $p = .17$, for C, $d = .56$. No significant changes in accuracy were found across the eight trials ($F_s < 1$, *ns*). There were no differences in performance between subjects that were tested in the geometry condition first versus feature condition first.

Mice failed to use the striped wall as a relative location or directional cue. Yet, despite the fact that there was no repeated training at one corner, the mice improved in their use of the striped versus gray distinction to remember the target location, spending more time in the two striped-wall corners when the goal was near the striped wall and spending more time in two gray corners when the goal was far from the striped wall. As in Experiment 1a, we found that the first approach did not reveal feature sensitivity in this working memory task.

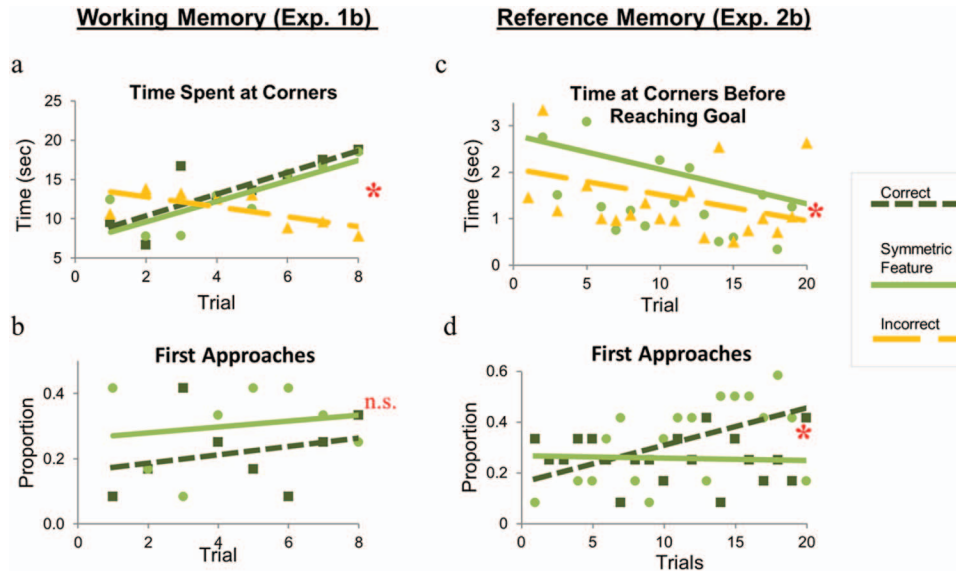


Figure 3. Total time spent and proportion of first approaches at the corners for tests of the striped feature in working memory (a and b, respectively) and in reference memory (c and d, respectively). In the working memory task, mice showed evidence of a partial use of the striped wall, preferring the correct and featurally symmetric corners over the other two; nevertheless, their inability to distinguish between the correct and symmetric corners suggests that they could not compute the location of the goal using its relative position to the feature. In the reference memory task, however, the first choice measures show an increasing preference for the one correct corner, over the symmetric corner, with a significant difference in the second half of the test trials. Mice tended to spend more time in the featurally symmetric, before reaching the target corner. * Significant differences between overall means at $p < .05$. See the online article for the color version of this figure.

Although the use of the feature in the present experiment was limited, the partial success using the feature as a direct marker could be interpreted as potentially contradicting the findings of the original working memory study in rats, in which the features were ignored (Cheng, 1986). One possible explanation is that the sharp contrast edges of the striped feature in this experiment were visibly more salient than those used in previous studies (see Sheynikhovich, Chavarriga, Strösslin, Arleo, & Gerstner, 2009). Further along these lines, the simplicity of the present environment (blocked external cues, no cue competition with geometry, and one polarizing feature) may have made the striped wall effectively more salient. In human children, for example, making the featural information part of the target locations (i.e., containers) within a circular arena, rather than a property of one wall of a rectangular arena, induces children to use them adeptly to distinguish between individual locations, although they continue to be unable to use them as relative positioning cues (Lee, Shusterman, & Spelke, 2006; Lee, Winkler-Rhoades, & Spelke, 2012). Finally, in contrast with many other studies of disoriented behavior, our task used an escape from an aversive environment as the goal; this may have heightened motivation and performance for feature use even in a working memory task (Dudchenko, Goodridge, Seiterle, & Taube, 1997; Golob & Taube, 2002).

Experiment 1 demonstrated that mice reorient by boundary geometry and exhibit a limited use of features in a task that requires spatial updating of the goal from trial to trial. To test whether this pattern of behavior is specific to spatial working memory tasks, Experiment 2 implemented a reference memory task using the same environmental cues.

Experiment 2a

Experiment 2a tested reference memory navigation by boundary geometry in the same rectangular arena used in Experiment 1a. Because the trial ended once the mouse found the target corner, the dependent variables for Experiment 2 were first choices and the time spent at the other corners before reaching the target. Successful use of geometry predicts that first choices to C + R will be higher than a chance value of 50%. For trials in which mice did not make a correct first choice, we predicted that they would spend more time in the geometrically equivalent corner than in the incorrect ones before finding the target corner.

Results and Discussion

Time measure. On analyzing the errors made with the other corners before reaching the target corner, we found that the mice preferred the geometrically equivalent corner (R) over the geometrically incorrect ones (X_N or X_F) based on the amount of time spent at the corners (R vs. mean of X_N and X_F), $t(11) = 2.22$, $p = .024$, one-tailed, $d = .59$ (see Figure 2c).

First choice. Looking at the first approaches, the mice chose the geometrically correct corners 57.5% of the time, 95% CI [43.1, 71.9], but this was not quite significantly different from 50% chance using a one-tailed t test, $t(11) = 1.15$, $p = .068$, $d = .69$ (see Figure 2d). Nevertheless, the Bayes factor comparing the odds of a geometrically correct first search between 50% and 70% was 159.6, with corresponding odds of the null hypothesis at 0.006, providing strong support for the successful use of geometry over the null hypothesis. There was no main effect of trials ($F < 1$, ns).

and no difference in choices between C (27.9%), 95% CI [18.4, 37.4], and R (29.6%), 95% CI [22.1, 37.1], $t < 1$, *ns*. Mean percentages of geometrically correct first choices in the reference memory task (Experiment 2a) were not significantly higher than performance in the working memory task (Experiment 1a, 52.8%; $t < 1$, *ns*). There were no differences in performance between subjects who were tested in the geometry condition first and the feature condition first.

Although the Bayesian analysis indicated strong support for geometrically correct first choices, the t test only showed a marginal effect, which was not statistically significant. Given the substantially large effect size (Cohen's d) of 0.69, this may indicate a lack of power in the dataset that would be addressed by increasing the sample size in future studies. Additionally, it is possible that the fact that there was only a 50% reinforcement rate for correct geometric first choice (because there was only one open target location and two correct solutions) may have slowed down learning in this task.

Experiment 2b

Experiment 2b tested reference memory learning using the same square arena and striped-wall feature used in Experiment 1b. Procedures were identical to Experiment 2a. A successful use of the feature predicts a first choice preference for the correct corner over the others. A partial use of the feature predicts a preference for the featurally symmetric corner (S) over the other two corners before reaching the target. A local feature preference predicts performance for goals near the striped wall to be better than for goals across from the feature.

Results and Discussion

Time measure. Analysis of the time spent in the incorrect corners before reaching the target corner showed that mice had a significant preference for the symmetric corner (S) over the average of the featurally incorrect corners (X_D, X_A), $t(11) = 1.94$, $p = .039$, one-tailed (see Figure 3c).

First choice. The first choices to the correct corner was 31.7%, 95% CI [25.1, 38.2], which differed significantly from a chance value of 25%, $t(11) = 2.24$, $p = .024$, one-tailed, $d = 1.35$ (see Figure 3d). The Bayes factor comparing the odds of a correct first search between 25% and 45% to the odds of the null hypothesis was 31.81, with corresponding odds of the null hypothesis at 0.03, providing strong support for correct performance. A repeated-measures ANOVA with trials as the within-subjects variable and the feature distance as the between-subjects factor revealed a significant linear trend across trials, $F(1, 11) = 6.707$, $p = .027$, $\eta_p^2 = .401$, showing a general improvement in accuracy over trials. There were no significant effects of the distance between the featural cue and the target ($F_s < 1$, *ns*). When collapsed over all trials, proportion of first choices to the correct corner (C) was not significantly higher than the first choices to the featurally symmetric corner (S), $t(11) = 1.27$, $p = .12$, one-tailed, $d = .76$. However, given the significant improvement in accuracy over trials, we found that although C was not higher than S for the first half of the test ($t < 1$, *ns*), C was significantly higher than S in the second half of the test, $t(11) = 1.86$, $p = .045$, one-tailed, $d = .89$.

Comparing first choice performance across experiments, there was a significant improvement in first choices to the correct corner

in the reference memory task (Experiment 2b) over the working memory task (Experiment 1b), as would be expected given the stability of the target, $t(11) = 2.09$, $p = .03$, one-tailed, $d = .73$. There were no differences in performance between subjects that were tested in the geometry condition first and the feature condition first.

In an arena with one distinctive feature, the mice learned in 20 reinforced trials to distinguish the one target corner from the others—this was equally true both when the goal was near the striped feature or more distal from it. Interestingly, the pattern of errors showed that despite the above-chance choices to the correct corner, there was still some evidence of a partial use of the feature to simply distinguish between the striped corners and the all-gray corners.

These findings replicate and extend past findings of Twyman et al. (2009), who demonstrated that mice can learn to distinguish between corners of a square arena with alternating walls of different brightness or patterns, and Fellini et al. (2006), who discovered that even though both young and aged mice can learn a location with respect to a featural cue, the learning of geometry is impaired in aged mice. Although it is clear that mice can learn to use a feature over repeated exposure and reinforcement, the evidence suggests that computing relative position with respect to a feature takes more training and experience to learn than using it as a direct goal marker.

General Discussion

The present study showed that there may be some interesting differences in the ways that boundary geometry and features are processed and learned in working memory and reference memory tasks. In the working memory task in which the target location had to be updated on every trials, mice successfully encoded boundary geometry to compute relative location and direction (e.g., to distinguish between the corners to the left/right of the distal wall of the rectangle). Mice also demonstrated a limited use of a feature to encode locations, but rather than using it as a relative positioning cue, they used it as a direct goal marker to distinguish between the corners near the striped wall from the solid gray corners. In contrast to the failure of Cheng's (1986) rats in using featural cues in a working memory task, disoriented mice successfully used a feature to help guide their spatial navigation in the absence of informative boundary geometry. Because in Cheng's (1986) experiment rats performed a food-finding task, this difference may be partly explained by the higher motivation of the animals in the present escape task using aversive stimuli (see Dudchenko et al., 1997). Another crucial difference is that while Cheng controlled for use of extramaze cues by moving the testing arena to random locations within the lab, the present study was conducted within an otherwise empty, symmetrical curtained testing space, potentially resulting in higher salience of the single striped feature in the absence of other competing environmental cues (including any distinguishing geometry of the test arena itself).

In the reference memory task in which the goal was stable and repeatedly reinforced, the mice, as in past studies, showed use of both geometry and features. However, in contrast with the relatively consistent level of performance in the geometry condition, 20 trials of reference memory training in the feature condition sufficiently improved their ability to remember the location of the

correct corner. Importantly, there was no difference between performance at the goals near the striped wall and the goals on the all-gray side of the arena, suggesting that the mice learned to use the striped wall even when it was not directly at the location of the goal.

Through simple tests of spatial updating in working memory and reinforced training of reference memory, we have reported behavioral evidence that shows qualitative differences in the two types of spatial tasks with interesting distinctions in the representation of boundaries and features. Nevertheless, it is important to recognize the limitations of the present study. First, although we used a rectangular arena for consistency with past reorientation experiments, and one polarizing feature to promote cue salience, this makes it difficult to directly compare performance in the feature and geometry conditions. This may be addressed in future studies by either testing an asymmetric arena (e.g., triangular or kite-shaped) for the geometry test, or a symmetric arena (e.g., striped wall on two opposite walls) for the feature tests. Another possible objection to the present approach is that some cues (like features) are just harder to learn than other (like boundaries). There might be an issue of inherent salience, but this interpretation cannot explain the difference in the use of the feature across the two tasks. Finally, although using the same animals for the various conditions of this study is effective to the extent that the same animal is observed to exhibit different behaviors across experimental manipulations, it may have slowed down learning in general, especially with the presence of two different arenas (for geometry and feature testing). Therefore, further investigation using a between-subjects design could provide cleaner results.

Conclusion

Future research must investigate the extent to which the mechanisms underlying spatial coding of features and boundaries may be dissociable and how they interact with the different types of spatial memory. The cue specificity reported in the present findings are consistent with studies of single-cell recordings in the rodent hippocampal formation identifying neurons that respond to wall-like boundaries in a particular direction in space but not to the featural properties of these surfaces (Solstad, Boccara, Kropff, Moser, & Moser, 2008; Lever, Burton, Jeewajee, O'Keefe, & Burgess, 2009). Moreover, the task specificity found here supports the view that separable mechanisms underlie flexible spatial updating and fixed learning in rodents (i.e., that contributions of the hippocampal and striatal systems of place learning vary depending on the level of experience and learning; McDonald & White, 1994; Packard & McGaugh, 1996). Experiments on humans have shown that striatum-dependent navigation by landmarks and hippocampus-dependent environmental boundaries have distinct neural substrates (Doeller, King, & Burgess, 2008; Bird, Capponi, King, Doeller, & Burgess, 2010) and are differentially impaired by genetic disorders such as Williams syndrome (Lakusta, Dessalegn, & Landau, 2010). Dissociating potential cue specificity (e.g., boundaries vs. features) from task specificity (e.g., working memory vs. reference memory), as we have done here in a mouse model, will offer new insights into the network of representations underlying spatial navigation and memory.

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