

# Place recognition and heading retrieval are mediated by dissociable cognitive systems in mice

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**A lost navigator must identify its current location and recover its facing direction to restore its bearings. We tested the idea that these two tasks—place recognition and heading retrieval—might be mediated by distinct cognitive systems in mice. Previous work has shown that numerous species, including young children and rodents, use the geometric shape of local space to regain their sense of direction after disorientation, often ignoring nongeometric cues even when they are informative. Notably, these experiments have almost always been performed in single-chamber environments in which there is no ambiguity about place identity. We examined the navigational behavior of mice in a two-chamber paradigm in which animals had to both recognize the chamber in which they were located (place recognition) and recover their facing direction within that chamber (heading retrieval). In two experiments, we found that mice used nongeometric features for place recognition, but simultaneously failed to use these same features for heading retrieval, instead relying exclusively on spatial geometry. These results suggest the existence of separate systems for place recognition and heading retrieval in mice that are differentially sensitive to geometric and nongeometric cues. We speculate that a similar cognitive architecture may underlie human navigational behavior.**

navigation | scene perception | spatial representation | geometry processing | neural specialization

A navigator who becomes lost must solve two tasks to regain her bearings. First, she must identify her current location, a process we term place recognition. Second, she must identify her current facing direction, a process we term heading retrieval. These two tasks are logically dissociable from each other: A “you are here” map identifies location without revealing heading, whereas a compass reveals heading without identifying location. Neurophysiological work on rodents suggests that the outputs of these two processes are represented by distinct neural populations: Location is coded in the hippocampus, in both general terms (different environments elicit different hippocampal maps) and specific terms (place cells fire at specific coordinates within an environment), whereas heading is encoded by head direction (HD) cells in several structures including the postsubiculum, thalamus, and retrosplenial cortex (1–3). However, little is known about the systems that determine these quantities from perceptual inputs. In particular, it is not known whether place recognition and heading retrieval are mediated by the same or different processing streams.

Here, we use a novel behavioral paradigm to test the hypothesis that the mechanisms that mediate place recognition at the coarse level (i.e., identification of the current environment) in mice are dissociable from the mechanisms that mediate heading retrieval. We use a variant of a spatial reorientation task that has been used extensively to study navigation behavior in a variety of species, including rodents and human children (4–7). In the standard version of the task, the animal (or human) navigator is first familiarized with a rectangular chamber with a hidden reward in one of the corners. Once it learns the location of the reward, the navigator is then removed from the chamber, disoriented, and placed back into the center of the chamber by facing a randomly

chosen direction. By observing which corner the navigator chooses when searching for the reward, it is possible to determine which cues it uses to orient itself in space. Many studies using this task have demonstrated that geometric cues (i.e., the shape of the chamber) exert strong control over behavior, often to the exclusion of other cues. For example, rats trained in this task will search equally often in the correct corner and in the corner that is diagonally opposite. This pattern of behavior indicates that the animal is using geometry as a cue, because these two corners have the same spatial relationship to the chamber geometry. The animals will often ignore other orienting cues such as odors, visual patterns, and wall color, even when these cues provide polarizing information that could potentially resolve the geometric ambiguity (6, 8). Although the exclusive reliance on geometric cues is not found under all circumstances (9, 10), it has been observed in a large number of studies.

An important aspect of this classical paradigm, which to our knowledge has not been previously commented on, is the fact that there is no ambiguity about the identity of the environment, because the experiment is typically performed within a single chamber (although, see refs. 11 and 12). Thus, in the standard version of the task, the navigator needs only to reestablish his or her heading direction to find the reward. Therefore, to examine place recognition and heading retrieval simultaneously, we used a novel version of the task in which there were two chambers, each with unique identifiable features and a different reward location. We first taught the mice the locations of the reward in each chamber, and then tested them while alternating between the two chambers on different test trials. To find the reward in this case, the mouse must both identify the chamber and determine which direction it is facing within the chamber—in other words, it must perform both place recognition and heading retrieval.

## Significance

The ability to recover one's bearings when lost is critical for successful navigation. To accomplish this feat, a navigator must identify its current location (place recognition), and it must also recover its facing direction (heading retrieval). Using a novel behavioral paradigm, we demonstrate that mice use one set of cues to determine their location and then ignore these same cues when determining their heading, although the cues are informative in both cases. These results suggest that place recognition and heading retrieval are mediated by different processing systems that operate in partial independence of each other. This finding has important implications for understanding the cognitive architecture underlying spatial navigation.

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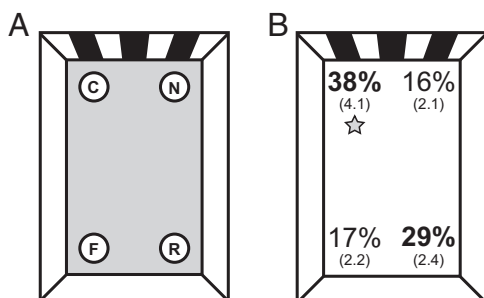
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We hypothesized that these two processes—place recognition and heading retrieval—would be differentially controlled by geometric and nongeometric cues. To test this hypothesis, we used two geometrically identical rectangular chambers that contained unique features that allowed them to be discriminated. In Experiment 1, each chamber contained a striped feature attached to the short wall, which was vertically aligned in one chamber but horizontally aligned in the other. In Experiment 2, each chamber contained a vertically striped feature, which was attached to the short wall in one chamber but attached to the long wall in the other. Critically, in both cases, the feature was potentially informative about both the identity of the chamber and heading within the chamber. To anticipate, we found that mice used the features to disambiguate the chambers but not to disambiguate headings within the chambers. In other words, they used features for place recognition but not heading retrieval, thus demonstrating a dissociation between these two processes.

## Results

We first set out to show that mice trained in a classical single-chamber reorientation paradigm use geometric cues to reorient themselves while ignoring nongeometric cues—a pattern often found in other species. Previous work has demonstrated that mice use geometry for reorientation, but the effect of polarizing nongeometric cues in the presence of orienting geometry has not been tested (13). We trained 16 disoriented mice to locate a reward in the corner of single rectangular ( $20 \times 30 \times 25$  cm) chamber with a polarizing cue along one short wall (Fig. 1A). Fig. 1B presents the average proportion of trials that mice searched in each of the four chamber corners over 16 total test trials. Mice searched for the reward more often in the two corners that were geometrically appropriate (C and R in Fig. 1A) than in the two corners that were geometrically inappropriate (F and N), replicating the previous finding of sensitivity to geometry [Cohen's  $d = 1.30$ ,  $t(15) = 5.20$ ,  $P < 0.001$ ]. Moreover, they failed to use the orienting feature to distinguish the correct corner (C) from the geometrically equivalent corner that was diagonally opposite (R), thus showing the same insensitivity to nongeometric cues when determining facing direction often found in other species [Cohen's  $d = 0.39$ ,  $t(15) = 1.57$ ,  $P = 0.14$ ].



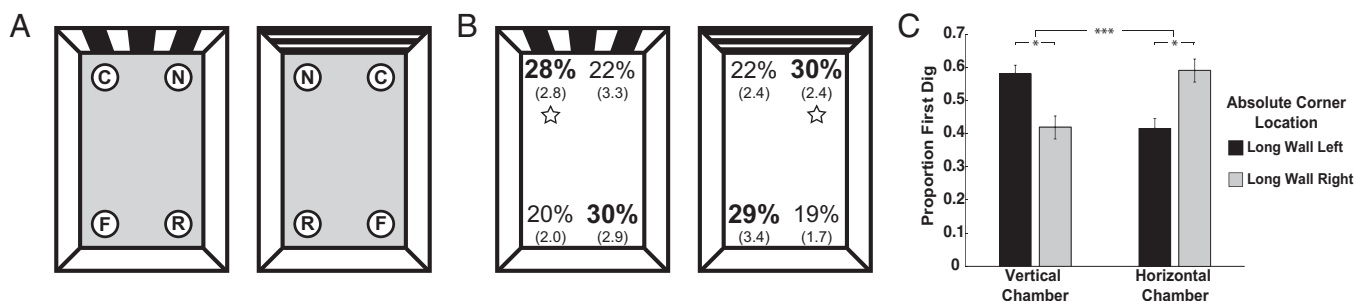
**Fig. 1.** Design and results for the preliminary experiment, which used the classical one-chamber reorientation paradigm. (A) Disoriented mice were trained to locate a reward in a single rectangular chamber with a visual feature along one short wall. C, R, N, and F denote the four cups in the corners of the chamber, where C denotes the correct corner (i.e., the corner with the hidden reward), R the rotationally equivalent corner (i.e., the corner geometrically equivalent to C), N the near corner (i.e., the corner that is closest to C), and F the far corner (i.e., the nonrotationally equivalent corner farthest from C). (B) Percentage of first digs in each of the four corners of the chamber (and SEMs). The star denotes the rewarded location. Mice searched significantly more often at C and R (bolded) than N and F, but there was no significant difference between the percentage of digs at C and R. This pattern reprises the classical results.

We then examined the navigational behavior of 16 disoriented mice in a two-chamber paradigm. The animals were presented alternately with two rectangular chambers that were geometrically identical ( $20 \times 30 \times 25$  cm) but distinguishable by stripes along one short wall. The stripes were vertical in one chamber and horizontal in the other (Fig. 2A). Because this feature both differentiated between the chambers and acted as a polarizing cue, it could be used for both place recognition and heading retrieval. In one chamber, mice were rewarded when they searched in the left corner nearest the striped wall, and in the other, when they searched in the right corner nearest the striped wall. We predicted that mice would use the stripes to identify the chamber in which they were located, but would not use the stripes to disambiguate between geometrically equivalent headings.

The results upheld our predictions. Fig. 2B presents the average proportion of trials that mice searched in each of the four corners in each of the two chambers (16 total test trials per chamber). Typical animal behavior in this task can be viewed in [Movie S1](#). In neither chamber did the distribution of search frequencies across all corners (C, R, N, and F in Fig. 2A) differ significantly from those of the control animals trained in the classical single-chamber paradigm [both  $X^2(3) < 6.05$ ,  $P > 0.11$ ]. In both chambers, the animals searched more often in the geometrically appropriate corners (C and R in Fig. 2A; bolded in Fig. 2B) than the geometrically inappropriate corners (N and F in Fig. 2A) [horizontally striped chamber: Cohen's  $d = 0.65$ ,  $t(15) = 2.59$ ,  $P = 0.02$ ; vertically striped chamber: Cohen's  $d = 0.68$ ,  $t(15) = 2.73$ ,  $P = 0.02$ ; Fig. 3A]. This observation was confirmed by a 2 (absolute corner location: long wall left or right)  $\times$  2 (chamber: vertically striped or horizontally striped) repeated-measures ANOVA, which revealed a significant interaction between absolute corner location and chamber [ $F(1,15) = 22.72$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.60$ ; Fig. 2C]. Because the geometrically appropriate corners differed between the two chambers, this pattern of performance indicates that the mice must have distinguished between the chambers. Given that the identity of the feature (horizontal vs. vertical) was the only thing that differed between the two chambers, these findings strongly suggest that the animals used the feature for chamber discrimination (i.e., place recognition).

We then performed an additional statistical test to see whether the animals used this feature to distinguish between geometrically equivalent headings within each chamber. The classic finding with rectangular chambers is that animals do not distinguish the rewarded location from the diagonally opposite location, even in the presence of a nongeometric polarizing cue. We replicate the classic finding here: In neither context did animals search more at the correct location than the diagonally opposite corner [both Cohen's  $d < 0.14$ ,  $t(15) < 0.57$ ,  $P > 0.58$ ]. Thus, the mice used the striped feature to distinguish between the chambers, but simultaneously failed to use this potentially informative feature to disambiguate between headings. That is, for heading retrieval, the mice solely relied on geometry.

In Experiment 2, we further explored the range of features that are used for place recognition. In particular, we asked whether the mice could discriminate between the chambers based on the spatial location of a feature relative to chamber geometry. The paradigm was similar to Experiment 1. A new group of disoriented mice ( $n = 16$ ) were trained to locate rewards in the corners of two rectangular chambers, with different reward locations in each chamber. In this case, the same vertical striped feature was present in both environments, but in different locations: In one chamber, the feature was on a short wall, whereas in the other chamber, it was in the center of a long wall (Fig. 3A). Thus, to disambiguate the chambers, the animals had to process the location of the feature relative to the chamber geometry. They could not distinguish the chambers on the basis of feature identity alone.



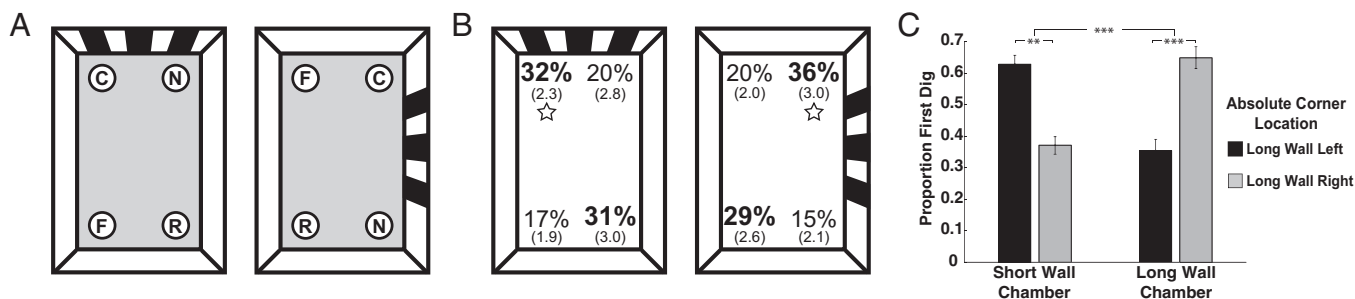
**Fig. 2.** Design and results for Experiment 1. (A) Mice were trained to locate a hidden reward in two rectangular chambers that had identical geometry but were distinguishable by the orientation of stripes (vertical vs. horizontal) along a single short wall. C, R, N, and F denote the four cups in the corners of the chambers, where C denotes the correct corner, R the geometrically equivalent corner, N the near corner, and F the far corner. Note that the location of the rewarded cup differed between the two chambers. (B) Shows the average percentage of first digs (and SEMs) in each corner of the two chambers. Stars denote the rewarded locations; bolded numbers indicate digs in geometrically appropriate corners. (C) The bar chart shows the same data as in B, but averaged over geometrically equivalent corners. Error bars denote  $\pm 1$  SEM. Mice dug more often in the corners that were geometrically appropriate for each chamber, thus indicating that they distinguished between the chambers. Moreover, they did not distinguish between geometrically appropriate corners.  $*P < 0.05$ ,  $***P < 0.001$ .

Fig. 3B shows the average proportion that mice searched in each of the four corners, separately for each context. In neither chamber did the distribution of search frequencies across all corners differ significantly from those of the control animals trained to locate a reward in the classical single-chamber paradigm [both  $X^2(3) < 2.61$ ,  $P > 0.46$ ]. We again found that in both chambers, mice searched more often in the geometrically appropriate corners than in the geometrically inappropriate corners [long-wall chamber: Cohen's  $d = 1.31$ ,  $t(15) = 5.58$ ,  $P < 0.0001$ ; short-wall chamber: Cohen's  $d = 0.92$ ,  $t(15) = 3.93$ ,  $P = 0.001$ ; Fig. 3C]. Confirming this finding, a 2 (absolute corner location: long wall left or right)  $\times$  2 (chamber: long wall feature or short wall feature) repeated-measures ANOVA revealed a significant interaction between absolute corner location and chamber [ $F(1,15) = 54.578$ ,  $P < 0.001$ ,  $\eta_p^2 = 0.78$ ; Fig. 3C]. Moreover, we once again observed that animals searched in the correct corner and the geometrically equivalent corner with equal frequency [both Cohen's  $d < 0.39$ ,  $t(15) < 1.59$ ,  $P > 0.14$ ].

These results replicate the pattern of findings from Experiment 1. Once again, mice used a cue to distinguish between the chambers and then ignored the same cue when determining their facing direction. In this case, the cue in question was the location of the striped feature relative to the geometry. These results suggest that the place recognition system can use a variety of cues, including both spatial and nonspatial features, and that information about the location of a cue relative to chamber geometry can be incorporated into its calculations. The heading retrieval system, however, seems to rely solely on geometry (at least in our experiments; see Discussion).

A possible alternative account of the results in Experiment 2 is that the animals did not, in fact, distinguish between the chambers, but rather treated the two contexts as identical and used the feature to specify a principal orientational axis for the environment (14). We think such an account is unlikely, because it would require the animals to ignore the geometry of the room when determining heading; moreover, it would require them to use the feature as an axis-defining cue (North–South vs. East–West) but not as a polarizing cue (North vs. South). Nevertheless, to test this possibility, we ran 15 of the 16 animals in Experiment 2 in two square chambers (one large and one small) following the last day of testing. Each of these chambers had the vertical striped feature along one wall (Fig. S1). We reasoned that if mice were using the feature to define the principal axis while ignoring geometry, then they should continue to use this strategy in the square chamber. In this case, they should search in the location on the left side of the feature and also in the diagonally opposite corner. However, this is not what we observed. Instead, the mice searched no more often at left-of-feature corner and the corner diagonally opposite than they did at right-of-feature corner and the corner diagonally opposite [small-square chamber: Cohen's  $d = 0$ ,  $t(14) = 0.00$ ,  $P = 1.0$ ; large-square chamber: Cohen's  $d = 0.23$ ,  $t(14) = 0.90$ ,  $P = 0.38$ ; Fig. S1]. Thus, the mice did not use strategy of going to the corners on the left-of-feature diagonal during the main part of Experiment 2, but rather used the location of the feature to distinguish the chambers and the geometry of the chamber to determine their heading.

Taken together, the results from Experiments 1 and 2 indicate that when disoriented mice were faced with a situation in which



**Fig. 3.** Design and results for Experiment 2. (A) The design was the same as Experiment 1, but in this case, the two chambers were distinguished by the location of vertical stripes either on the short wall or the long wall. (B) Shows the average percentage of first digs (and SEMs) in each corner of the two chambers. Stars denote the rewarded locations; bolded numbers indicated digs in geometrically appropriate corners. (C) The bar chart shows the same data as in B, but averaged over geometrically equivalent corners. Error bars denote  $\pm 1$  SEM. Mice dug more often in the corners that were geometrically appropriate for each chamber, thus indicating that they distinguished between the chambers. Moreover, they did not distinguish between geometrically appropriate corners.  $**P < 0.01$ ,  $***P < 0.001$ .



they had to both identify their environment and also reestablish a sense of direction within that environment, they used both nongeometric (Exp. 1) and geometric (Exp. 2) information to identify their environment, but only geometric information to reestablish their sense of direction. To further test this account, we calculated the Bayes factor (15, 16) comparing the alternative hypothesis that the geometrically appropriate corners were chosen more often than the inappropriate corners to the null hypothesis that the geometrically appropriate and inappropriate corners were chosen equally often. Combining data from both experiments, this analysis revealed an average Bayes factor of 3.81 in favor of the alternative hypothesis that animals used the feature to discriminate chambers, a magnitude that is considered to provide “substantial” evidence (17). To verify that the same cues that were used for place recognition were ignored for heading retrieval, we computed the Bayes factor comparing the alternative hypothesis that animals searched more at the correct corner than the diagonally opposite corner to the null hypothesis that the proportion of searches at both geometrically appropriate corners was equal. In this case, the average Bayes factor was  $1.79 \times 10^{-6}$ , which provides evidence in favor of the null hypothesis.

## Discussion

Using a two-chamber spatial reorientation paradigm, we found a dissociation between two fundamental components of spatial navigation: place recognition and heading retrieval. When disoriented mice were faced with a situation in which they had to both identify their environment and also reestablish a sense of direction within that environment, they used both geometric and nongeometric information to identify their environment, but relied solely on spatial geometry to retrieve their heading. Critically, the same cue that was used for place recognition was ignored for heading retrieval, although it was highly informative in both cases. Thus, our results cannot be explained by unequal salience of cues.

We demonstrated this dissociation between place recognition and heading retrieval in two experiments. In Experiment 1, the animals searched for hidden rewards in two geometrically identical rectangular chambers, each of which had a distinguishing feature (horizontal vs. vertical stripes) along one of the short walls. In Experiment 2, the chambers were also geometrically identical rectangles, but in this case, the distinguishing feature was the location of a vertically striped feature relative to the chamber geometry (along short wall vs. along long wall). In both experiments, we reprised the classic results from the literature by showing that the animals searched for the reward more often in the two corners that were geometrically appropriate for each chamber than in the corners that were geometrically inappropriate; furthermore, they did not distinguish between the two geometrically appropriate corners (i.e., the correct corner and its rotational opposite). The fact that the animals chose the corners that were geometrically appropriate for each chamber indicates that they must have used the identity (Exp. 1) or location (Exp. 2) of the striped feature to distinguish between the chambers, because these were the only disambiguating cues. However, the fact that they did not distinguish between the two geometrically appropriate corners indicates that they did not use the striped features to distinguish between headings, although these features clearly polarized the environment. These results demonstrate a functional dissociation between place recognition and heading retrieval: The striped feature acts as a treatment that selectively affects one process (place recognition) but does not affect the other (heading retrieval). (See [SI Discussion 1](#) for further consideration of this point.)

To our knowledge, this is the first demonstration of this dissociation. A previous reorientation study by Horne et al. reported that rats could discriminate between a rectangular chamber with all black walls and a rectangular chamber with all white walls (12). This result is consistent with ours insofar as it indicates that the

animals can use nongeometric cues for place recognition. However, because the wall colors in the Horne study did not specify a unique heading within the chambers, their design did not allow them to dissociate between place recognition and heading retrieval as we do here.

Why might heading retrieval and place recognition rely on distinct cognitive systems? One possibility is that solving these two tasks requires different computations. Place recognition likely involves identification of scenes or landmarks that a navigator can use to determine her general environmental context. Identification might be achieved by matching the contents of the current view with the contents of a previously stored view consisting of a combination of geometric and nongeometric information (18). In this account, place recognition would be akin to object recognition, but performed on navigationally relevant stimuli. By contrast, heading retrieval might involve interpreting the environment in terms of a spatial reference system from which orientational axes can be recovered (14). Although the precise computations underlying heading retrieval are unknown, previous work suggests that at least for humans, heading retrieval is not performed by view matching (4, 19, 20). (See [SI Discussion 2](#) for further consideration of the implications of the present experiments for view-matching theories of reorientation.)

Notably, previous work has identified a possible neuroanatomical basis for this behavioral dissociation. In humans, neuroimaging and neuropsychological work suggests that place recognition is primarily mediated by the parahippocampal place area (PPA), a region of medial occipitotemporal cortex that responds strongly when subjects view environmental scenes or landmark objects (21–23), whereas heading retrieval is primarily mediated by a system centered around the retrosplenial complex (RSC) in the medial parietal lobe (24–28). Analogous to the current findings, the PPA appears to be sensitive to both geometric and nongeometric information (22, 29–33), whereas RSC appears to be especially sensitive to geometry when people retrieve spatial information from memory (28). In rodents, the homologous regions are postrhinal cortex (34), which has been shown to be important for place recognition (35), and retrosplenial cortex, which has been shown to be important for deriving directional information from environmental cues (36). Retrosplenial cortex contains head direction (HD) cells, which discharge selectively when the head of an animal is oriented in a particular facing direction (37), and a previous report demonstrated that these cells are primarily sensitive to environmental geometry rather than nongeometric features after disorientation (38). In addition, neurons that code allocentric locations relative to geometric boundaries have been identified in the entorhinal cortex (39) and subiculum (40) of the rodent, and these cells might be important for retrieving the location of the reward within the chamber after chamber identity and heading have been reestablished.

A possible caveat concerning our interpretation of the present experiments in terms of separable systems for place recognition and heading retrieval is that, as with any behavioral dissociation, we cannot know for certain the identity of the processes that we have dissociated. Although we think that place recognition and heading retrieval provide the most parsimonious descriptions of these processes, other accounts may also explain the data. For example, rather than distinguishing between the chambers as distinct environments, the animals might be distinguishing between two different situations that occur in the same environment, just as a person might distinguish between a wedding and a funeral that both occur in the same building. Although the spatial environment of both events is the same in this example, the contextual features surrounding each situation and the appropriate behaviors are different. Relatedly, we cannot know for certain that the second system supports retrieval of heading. An alternative possibility is that it codes egocentric locations relative to geometric boundaries, and that the animals choose their dig

locations based on a strategy of approaching a corner with a particular local geometric configuration (e.g., short-wall left), without recovering heading at all. In addition, we emphasize once again that our results pertain to the mechanisms that allow the animal to recover its bearings after disorientation and do not necessarily provide insight into the mechanisms that allow the animal to maintain its bearings when oriented.

Finally, it is worth considering the implications of our findings for the ongoing debate about the nature of the cognitive mechanisms underlying spatial reorientation. Two theories are most prominent. The first theory builds on the classic results by arguing that reorientation is mediated by an encapsulated cognitive module (41) that specifies a navigator's position and orientation relative to the geometric structure of the environment but is insensitive to nongeometric features (5–7). The second theory argues that a range of environmental cues, including both geometry and nongeometric features, can guide spatial reorientation (9, 10, 42) and that the combination of cues used in any given situation can vary depending on their salience and reliability. Although our results might seem at first glance to fit more closely with the first view insofar as we postulate the operation of independent mechanisms, one of which is especially sensitive to geometry, it is important to note that our argument does not require that these two mechanisms be modular. More specifically, the dissociability of the place recognition and heading retrieval systems that we demonstrate here does not require heading retrieval to be impervious to nongeometric information under all circumstances. The key point is that we have found one set of circumstances in which nongeometric information is used for one function but not the other, thus establishing the independent operation of the two mechanisms. That said, if our conclusion that there are separate cognitive systems for place recognition and heading retrieval is correct, it may affect the interpretation of cue competition effects that have been taken as evidence in favor of nonmodular theories (43–48). In particular, some cue competition studies have observed that when animals learn to find a goal in a chamber containing both featural and geometric cues, and the featural cues are then altered or removed, then the animals are impaired at finding the goal. These findings have been interpreted as indicating that the learning of locations relative to featural cues can overshadow the learning of locations relative to geometric cues, in contradiction to the predictions of the modular theory. However, our results suggest an alternative account: When the featural cues are changed, animals may believe that they are in a different place for which they do not know the location of the reward. Thus, some cue competition effects may be explained by the existence of a place recognition system that is sensitive to nongeometric features. Conversely, under this interpretation, the failure of a feature to interfere with learning based on environmental geometry (49–52) may indicate that the feature did not form an integral part of the representation of that place.

In sum, our experiments demonstrate a dissociation between place recognition and heading retrieval in mice. Whereas place recognition is sensitive to both featural and geometric information, heading retrieval is primarily guided by spatial geometry. These findings indicate that place recognition and heading retrieval are mediated by different cognitive systems that operate with some degree of independence from each other. For a lost navigator to regain her bearings, she must solve not one but two problems, and both systems must work in concert to get her on her way.

## Methods

**Subjects.** Distinct groups of 16 male C57BL/6 mice, 2–5 mo old (Jackson Laboratory), participated in the classical single-chamber paradigm, Experiment 1, and Experiment 2 (48 animals total). Mice were housed individually and kept on a 12-h light/dark cycle for at least 2 wk before the beginning of the experiments. They had access to water ad libitum, but to increase motivation to participate in the task, they were maintained at 85–90% of their

free-feed weight. Starting 4 d before the experiment, animals were shaped to dig in a medicine cup for a food reward (Kellogg's Cocoa Krispies) in their home cage by providing them once daily with the reward gradually buried deeper under scented bedding. Animal living conditions were consistent with the standards set forth by the Association for Assessment and Accreditation of Laboratory Animal Care. All experiments were approved by the Institution of Animal Care and Use Committee of the University of Pennsylvania and were conducted in accordance with NIH guidelines.

**Apparatus.** The classical single-chamber experiment was conducted in a rectangular ( $20 \times 30 \times 25$  cm) chamber. Exp. 1 and 2 were both conducted in two geometrically identical rectangular ( $20 \times 30 \times 25$  cm) chambers. The walls and floor of all chambers were covered in white laminate. In the single-chamber experiment, there were three black stripes (either vertical or horizontal, balanced across animals) along one short wall (Fig. 1A). In Exp. 1, the two chambers were distinguished by three black stripes along the short wall, which were vertical in one chamber and horizontal in the other (Fig. 2A). In Exp. 2, chambers were distinguished by the location of three vertical black stripes, which were placed along the short wall in one chamber and in the center of the long wall in the other (Fig. 2B). In all experiments, stripes were 4 cm in width. Testing in all chambers occurred in the same location in the experimental room. The chambers were surrounded by a square black curtain with rounded corners, were uniformly lit from overhead, and a white noise generator was hung centrally above the chamber to ensure that animals could not use extraneous sounds as beacons. Cups were embedded in each of the four corners of the chamber floors. The cups contained odor-masked bedding, consisting of 1 g of odor mask (either ground cumin or ginger) for every 100 g of bedding. Mouse behavior was recorded by using LimeLight video tracking system (Coulbourn Instruments) via an overhead, centrally located camera.

**Design and Procedure.** A pilot experiment showed that mice could discriminate the horizontal and vertical stripes to a performance criterion of 75% correct after eight training trials. Thus, all experiments began with a training phase consisting of four training trials per chamber per day for 2 d, with successive trials alternated across chambers (8 trials total in the one chamber experiment; 16 trials total in the two chamber experiments). During this training, mice were taught to search for a reward, which was visible for the first two training trials per chamber and buried in the remaining training trials. In the single-chamber experiment, the reward was always located in one of the two corners nearest the striped feature. In Exp. 1 and 2, the reward was always located in one of these two corners in one chamber, and in the other feature-adjointing corner in the other chamber. These locations were counterbalanced across animals; however, for all analyses and figures, the percentage of searches at each corner are reflected such that correct corner is the same for all animals.

Animals were disoriented before the start of every trial. To disorient an animal, it was placed in a PVC cylinder with a detachable base and lid. The experimenter slowly rotated the cylinder on a turntable roughly four full clockwise then four full counterclockwise revolutions. The cylinder was then carried to the chamber, and the base was slid out from underneath the animal. The cylinder was lifted to start a trial. To ensure that the animals could not use any room cues that were not completely eliminated by use of a surrounding curtain and a white-noise generator, chambers were rotated  $90^\circ$  or  $180^\circ$  before each trial, counterbalanced so that all orientations relative to the room were experienced equally often. The chambers were cleaned with ethanol at the end of each trial to remove odor trails. The intertrial interval was 3–5 min.

Following training, animals were tested in one session per day for 4 d. In the single chamber experiment, testing sessions consisted of two rewarded and two unrewarded trials (interleaved). In Exp. 1 and 2, testing sessions consisted of two rewarded and two unrewarded trials per chamber. Thus, in all cases, there were a total of 16 test trials per chamber. In Exp. 1 and 2, chambers were tested in an interleaved fashion, as were the rewarded and unrewarded trials. So, a sequence for one session might be: Chamber 1 (rewarded), Chamber 2 (rewarded), Chamber 1 (unrewarded), Chamber 2 (unrewarded), etc. The order in which chambers were tested was counterbalanced across sessions. During reward trials, mice were removed from the apparatus after they had found the reward. During unrewarded trials, they were removed after their first dig, or after 45 s (whichever came later). Digs were counted whenever an animal removed bedding from a cup by using one or both paws. Unrewarded trials were included to train the mice to concentrate their first dig at the reward location and as a control for the possibility that mice could smell the reward during rewarded trials. However, there was no difference in the distribution of first digs across corners between rewarded and unrewarded trials in the classical single-chamber experiment [ $\chi^2(3) = 4.67$ ,  $P = 0.20$ ], Exp. 1

[ $\chi^2(3) = 0.70$ ,  $P = 0.87$ ], or Exp. 2 [ $\chi^2(3) = 5.44$ ,  $P = 0.14$ ]. Therefore, we collapsed across rewarded and unrewarded trials for all analyses.

Dig locations were coded following testing by an experimenter blind to condition. The dependent measure was the first corner in which the animal dug. Paired sample *t* tests were used to assess whether the proportion of digs were distributed in the chambers according to the geometry. For Exp. 1 and 2, repeated measures ANOVA with absolute corner location (long wall left or right) and chambers as within-subjects factors were used to compare the search behavior across chambers. All reported statistics are based on two-tailed significance tests.

The day following the final Exp. 2 testing session, 15 of the 16 animals that participated in Exp. 2 were then run in a control experiment in which the animal's search behavior was observed in two square chambers, one large

(30 × 30 × 25 cm) and one small (20 × 20 × 25 cm). Cups were embedded in each of the four corners of the chamber floors. Both chambers had the same vertical stripe feature along one wall that used in Exp. 2. There were four interleaved probe trials per square. Every trial per chamber, the chambers were rotated 90° or 180°. Animals were disoriented before the start of each trial.

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