

Role of Self-Generated Odor Cues in Contextual Representation

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ABSTRACT: As first demonstrated in the patient H.M., the hippocampus is critically involved in forming episodic memories, the recall of “what” happened “where” and “when.” In rodents, the clearest functional correlate of hippocampal primary neurons is the place field: a cell fires predominantly when the animal is in a specific part of the environment, typically defined relative to the available visuospatial cues. However, rodents have relatively poor visual acuity. Furthermore, they are highly adept at navigating in total darkness. This raises the question of how other sensory modalities might contribute to a hippocampal representation of an environment. Rodents have a highly developed olfactory system, suggesting that cues such as odor trails may be important. To test this, we familiarized mice to a visually cued environment over a number of days while maintaining odor cues. During familiarization, self-generated odor cues unique to each animal were collected by re-using absorbent paperboard flooring from one session to the next. Visual and odor cues were then put in conflict by counter-rotating the recording arena and the flooring. Perhaps surprisingly, place fields seemed to follow the visual cue rotation exclusively, raising the question of whether olfactory cues have any influence at all on a hippocampal spatial representation. However, subsequent removal of the familiar, self-generated odor cues severely disrupted both long-term stability and rotation to visual cues in a novel environment. Our data suggest that odor cues, in the absence of additional rule learning, do not provide a discriminative spatial signal that anchors place fields. Such cues do, however, become integral to the context over time and exert a powerful influence on the stability of its hippocampal representation. © 2014 The Authors. Hippocampus Published by Wiley Periodicals, Inc.

KEY WORDS: hippocampus; spatial; place cells; olfactory; memory

INTRODUCTION

The discovery of “place cells” was a key advance in hippocampal research (O’Keefe and Dostrovsky, 1971; O’Keefe, 1976). Place cells fire primarily as the animal passes through a particular location in space, referred to as the cell’s “place field.” These fields, once developed, can

remain stable indefinitely (Thompson and Best, 1989) and at the ensemble level may be viewed as a spatial representation of the environment (O’Keefe, 1978). Place fields also change as the cues available to the animal changes. They rotate when environmental cues rotate (Muller and Kubie, 1987), and field size and number can change as objects are added to, removed from, or shifted within an environment (Muller and Kubie, 1987; Lever et al., 2002; Renaudineau et al., 2007; Burke et al., 2011). If the relationship between environmental cues [or a task’s contingencies, (Dupret et al., 2010)] is sufficiently altered, the locations of the fields may shift in an unrelated way. Referred to as “remapping” (Muller and Kubie, 1987; Bostock et al., 1991; Muller et al., 1991; Markus et al., 1995; Colgin et al., 2008), this phenomenon results in a unique representation in the hippocampus for each spatial context.

Virtually all of the above studies rely on manipulations of visuospatial cues. This makes experimental sense: visual cues have spatial precision and are easily changed. However, rodents are nocturnal animals, with relatively poor visual acuity (Prusky and Douglas, 2004; Wong and Brown, 2006). One might expect, therefore, that navigation may be guided by other sensory modalities as well, such as olfaction. Olfaction has the potential to be a strong contextual cue for rodents, providing information about food, a mate or predators being present in the surrounding environment. On the other hand, while the strength of an odor cue is a useful guide to the proximity of food or foes, it is relatively less useful to determine direction due to its volatile nature. Nevertheless, previous studies show evidence of odor cues being used both to differentiate context [e.g., Honey and Hall (1989),] as well as for navigation (Lavenex and Schenk, 1998; Save et al., 2000; Wallace et al., 2002; Muzzio et al., 2009). Save et al. (2000) specifically looked into the interaction of visual and self-generated olfactory cues in awake, freely behaving animals. They found that cleaning self-generated olfactory cues from the floor accentuated the loss of directional stability of place fields caused by removal of some or all visual cues. The authors speculate that the reliability of the olfactory cues might depend on their initial association with some visual reference framework [see Lavenex and Schenk (1995)]. Save and colleagues stated two open questions based on their findings: how do visual and olfactory cues interact, and how can a rat discriminate space only on the

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Accepted for publication 11 April 2014.

DOI 10.1002/hipo.22289

Published online 16 May 2014 in Wiley Online Library (wileyonlinelibrary.com).

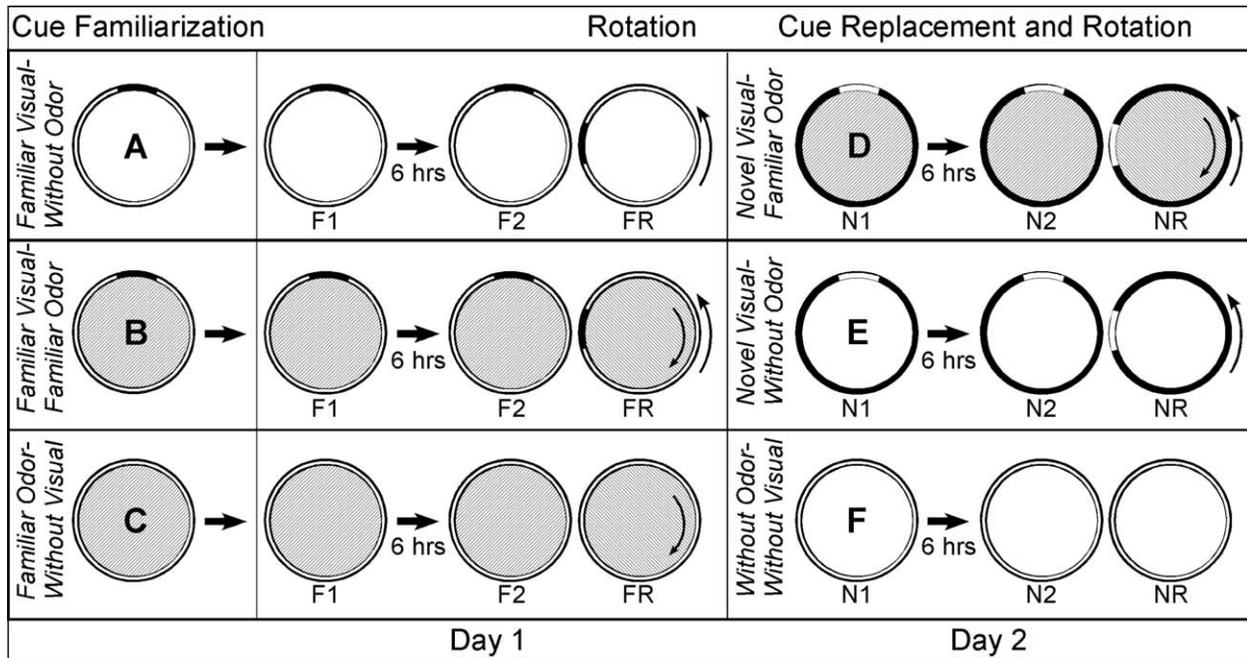


FIGURE 1. The interplay of visual and olfactory cues in spatial representations was examined with six experimental manipulations. Three groups of mice were familiarized: to visual cues (A), visual and self-generated odor cues (B), or odor cues alone (C) for a minimum of 5 days (cue familiarization). In the familiar condition (Day 1), place field stability was assessed across a 6-h delay (F1–F2). Then the ability to follow 90° cue rotations was assessed (FR). Where both visual and odor cues were present (B), these sets of cues were rotated counter to each other. The following day

(Day 2), long-term stability (N1–N2) and rotations (NR) in mice from manipulation (A) were assessed in a novel visual-cued cylinder with preserved odor cues (D). Mice from manipulation (B) were reassessed in the presence of novel visual cues alone (E), and mice from manipulation (C) were reassessed in the absence of both visual and self-generated odor cues (F). Hashes indicate preserved odor cues. Arrows indicate direction of 90° rotations. Cue rotations were counter-balanced across animals.

basis of self-deposited olfactory cues? Our study investigated how much actual spatial information self-generated olfactory cues provide to place cells, both in the absence of asymmetric visual cues and when they are put in conflict with familiar visual cues.

We used a three-stage protocol to characterize how olfactory cues might influence hippocampal place cells: familiarization to the cues, rotation of the familiarized cues, and replacement of familiar cues with novel ones. We familiarized three groups of animals to stable odor cues, stable visual cues, or both. Because rodents are able to navigate in total darkness by using olfactory traces alone (Lavenex and Schenk, 1998), we maintained these cues by re-using the same, animal-specific paperboard in the same configuration relative to available visual cues during familiarization sessions (the Familiar Odor, or FO condition) instead of replacing the floor paper every session with fresh paper, as in the control condition (the Without Odor, or WO condition). While the animals were not in total darkness, we either had clear asymmetric cues (high-contrast geometric shapes) on the cylinder walls (the Familiar Visual, or FV condition) or sought to minimize any asymmetric visual cues by painting the cylinder uniformly (the Without Visual, or WV cue condition). This led to three distinct groups, Familiar Visual/Without Odor (FV-WO), Familiar Visual/Familiar Odor (FV-FO), and Familiar Odor/Without Visual (FO-WV) (Fig. 1).

Having familiarized animals to one or both sets of cues, we recorded hippocampal place cells and assessed long-term field stability and the effects of cue rotation on the spatial representation. Based on previous work (Muller and Kubie, 1987), place fields should follow the rotation of visual cues. We hypothesized that, if odor traces provide spatial information, place fields should also follow the rotation of highly familiar odor cues (i.e., the FO-WV condition). However, where both sets of cues were present, these cues were counter-rotated to each other, placing the two sets of cues into conflict. In this case, we expected to see a dissociation of the spatial representation, with some fields following one set of cues and others following the other set of cues (Renaudineau et al., 2007). Finally, we examined how familiarity influenced the neural response to these manipulations by repeating them in entirely novel conditions (Anderson and Jeffery, 2003).

METHODS

Animals

Twenty male C57Bl6/J mice (Jackson laboratories, Sacramento, CA) were chronically implanted with depth-adjustable four-tetrode microdrives to record the activity of CA1 neurons

during spontaneous exploration of a circular arena. All procedures described were performed in accordance with guidelines approved by University of Oregon's Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 80-23).

Surgical Procedures

Surgeries were performed using aseptic techniques. Ketamine (100 mg/kg) was administered as a pre-anesthetic, and surgical anesthesia was maintained with isoflurane gas (1.25–2.0%, adjusted as necessary for appropriate depth of anesthesia). Eyes were covered with a triple antibiotic ointment to prevent drying. Dexamethasone (0.1 mg/kg) and atropine (0.03 mg/kg) were administered prophylactically to reduce inflammation and respiratory irregularities, respectively. Under stereotactic guidance (David Kopf Instruments, Tujunga, CA), a hole was drilled in the skull 1.8 mm posterior to bregma and 1.4 mm left of the midline for insertion of the recording tetrodes. Four additional holes were drilled; two per side 4 mm lateral from the midline, for the insertion of two stainless steel screws (00–90 × 1/8") and ground wires. The tips of the tetrodes were lowered to a depth of approximately 700 μm from the dura. Grip Cement (Dentsply, Milford, DE) was used to secure the array to the screws and skull. Vaseline was applied to isolate the individual tetrodes from the cement, preserving the ability to adjust tetrode depth. Mice were administered buprenorphine (0.06 mg/kg) postoperatively for analgesia to minimize discomfort. All mice were individually housed following surgery and were allowed 7 days of postoperative recovery.

Behavioral Protocol

The goal of this study was to characterize the contribution of self-generated odor cues in the spatial firing of CA1 place cells, specifically in relation to distal visual cues that are known to strongly affect CA1 place fields. Therefore, we tested the effects of familiarization to visual and/or odor cues, and to their subsequent rotation and removal.

We recorded place cell activity from three independent groups of mice in two separate conditions. The organization of these groups and conditions is illustrated in Figure 1. First, we familiarized each group to visual cues alone (Familiar Visual/Without Odor group or FV-WO), to a combination of visual and odor cues (Familiar Visual/Familiar Odor group or FV-FO), or to odor cues alone (Familiar Odor/Without Visual group or FO-WV). Following this, we rotated the cues by 90° to observe the response of the stabilized place fields; where both sets of cues were present, these were rotated counter to each other. Finally, we placed the animals in a novel visual environment (Novel Visual or NV) while reversing the treatment of the odor traces. Thus, the previously Familiar Visual/Without Odor (FV-WO) group now becomes the Novel Visual/Familiar Odor group (NV-FO) and is exposed to a novel visuospatial environment and the same (familiar) odor cues throughout three sessions; the previously Familiar Visual/

Familiar Odor group (FV-FO) now becomes the Novel Visual/Without Odor group (NV-WO), being exposed to a novel visuospatial environment and no familiar odor cues anymore due to a fresh floor paper being used in every session; and the previously Familiar Odor/Without Visual group (FO-WV), now becomes the Without Odor/Without Visual group (WO-WV), essentially receiving neither any asymmetrical visual cues nor any familiarized odor cues due to fresh floor paper used every session. Details of the three manipulations are explained below.

Cue familiarization

The FV-WO group was familiarized exclusively to visual cues, the FV-FO group was familiarized to both visual and self-generated odor cues, and the FO-WV group was familiarized exclusively to self-generated odor cues. All mice were allowed to explore the cylindrical arena (a plywood cylinder, 60 cm in diameter, 45 cm in height) freely for 20 min daily for at least five days. During these familiarization sessions, neuronal activity was monitored, and the tetrodes were lowered by 45–90 μm daily until place cell activity was obtained. Geometric shapes painted on the inside wall of the cylinder served as the visual cues, while self-generated odors accumulated over successive sessions on the paperboard flooring served as the odor cues. For the absorption and accumulation of the self-generated odor cues, a thick paperboard capable of absorbing urine without changing texture or scaling was used. Each paperboard was stored in its own plastic sleeve between sessions to preserve the odor cues and to prevent cross-contamination between animals. Fecal boli were removed before storage. For the FV-WO group, fresh floor paper was used in each session. The FO-WV sessions were conducted in a cylinder painted uniformly white on the inside. Between all sessions, the cement floor beneath the paper/paperboard and the cylinder wall were wiped with ethanol. A circular, uniformly black curtain surrounded the arena. Illumination came from four equally spaced light sources above the arena.

After at least 5 days of familiarization and having recorded stable CA1 place cell activity, the animals were run in the familiar visual and odor conditions for two 20-min sessions (F1 and F2, chronologically) at an interval of 6 h, while their neuronal activity was recorded. The F1 session served as a baseline for neuronal activity in the familiar environment, and the F2 session under identical conditions was used to establish consistency of neuronal response to the familiar cues, specifically place field stability.

Cue rotation

Following session F2, the mice were returned to their cages and held inside a black box immediately outside the recording room, while the cue conditions were altered. For the mice in both FV-FO and FO-WV groups, the cylinder and the floor were counter-rotated by 90° relative to their original configuration (the blank cylinder was rotated for the FO-WV group animals to control for any unintended visual cues on the

cylinder). For the mice in the FV-WO group, the floor paper was replaced and the cylinder rotated by 90°. Immediately following this manipulation, the animals were returned to the arena for a third and final session (familiar rotation: FR session). Direction of cue rotation was counter-balanced across animals.

Cue replacement

On the second day, the three-session sequence was repeated, but the visual and/or odor cue conditions were replaced by novel cue elements. A different cylinder of identical dimensions but with novel visual cues (NV) was used (white cylinder with black geometric shapes, instead of the black one with black geometric shapes used on the first day) for the previously FV-WO and FV-FO groups. The odor conditions were changed as follows: for the formerly designated FV-WO group, instead of fresh paper in every session, the same paperboard was used in all three sessions, thereby preserving the self-generated odor cues; for the formerly designated FV-FO and FO-WV groups, instead of using the same paperboard, a fresh paper was used in every session, removing the familiar self-generated odor cues. Therefore the former FV-WO group now had novel visual cues and had self-generated odor cues for the first time, becoming the Novel Visual/Familiar Odor or NV-FO group. The previous FV-FO group now had novel visual cues and no preserved odor cues, thereby becoming the Novel Visual/Without Odor or NV-WO group. Finally, the FO-WV group now had neither asymmetric visual cues nor familiarized self-odor cues, thereby becoming the Without Odor/Without Visual group (WO-WV). As with the previous day, baseline activity was recorded, followed 6 h later by assessment of place field stability (sessions N1 and N2, respectively).

Novel cue rotation

Following the N2 session, the visual and odor cues were rotated as previously done in the familiar condition. The cylinder was rotated by 90° for the Novel Visual/Without Odor and Without Odor/Without Visual groups, while the cylinder and the floor paperboard were counter-rotated for the Novel Visual/Familiar Odor group. Immediately after this, a third and final session was recorded (novel rotation: NR session).

Single Neuron Recording

Microdrives used for recording neuronal activity were constructed from methods adapted from Gray et al. (1995). Briefly, four lengths of 18 μm diameter 10% Platinum/Iridium wire (California Fine Wire, Grover Beach, CA) were spun together and fused to form tetrodes. The ends were plated with platinum (Technic, Cranston, RI) to an impedance of 250–750 k Ω . The individual wires of four tetrodes and the two ground wires were connected to an EIB-16 electrode interface board (Neuralynx, Bozemann, MT). This combination of EIB-16, tetrodes, and ground wires was housed on a Teflon stage mounted on three drive screws. The drive screws (0–80 \times 3/

8”) provided adjustability of depth for the array as well as the structure between the array and the skull.

Screening

Screening for place cells took place during the familiarization sessions. A tethered HS-16 operational amplifier (Neuralynx, Bozeman, MT) was plugged into the EIB-16 for monitoring/recording neuronal activity and providing spatial tracking information. If complex spike cells (Fox and Ranck, 1975) were not observed, the array was lowered 45 μm at the end of the familiarization session. Day 1 recordings commenced when complex spikes of sufficient amplitude as to be discriminable from the activity of neighboring neurons were observed and the mouse had completed a minimum of five familiarization sessions.

Data acquisition

Neuronal data were acquired using a 24-channel Cheetah system (Neuralynx, Bozeman, MT). Neuronal signals were buffered through the HS-16 and passed via a 2-meter tether through the ceiling of the recording chamber. In an adjacent room, the signal was amplified and captured using Neuralynx data acquisition software. Thresholds were set such that only waveforms of a specified minimum voltage were stored. Spiking activity was high-pass filtered from 600 to 6,000 Hz and sampled at 32 kHz. A digital camera fixed to the ceiling of the recording chamber linked to the Cheetah system enabled the recording of the animal’s position during the course of each session by tracking two LEDs fixed to the HS-16.

Data Analysis

Spikes were sorted offline using MClust (A.D. Redish, University of Minnesota, Twin cities, MN). Pairs of waveform measures were plotted to form clusters of points corresponding to waveforms of individual neurons. The axes of cluster space used were spike height, valley, and energy. MClust provides the flexibility to apply cluster boundaries across multiple recording sessions. Neurons were judged to be “stable” (the same from one session to the next) if similar cluster boundaries could be applied across consecutive sessions without losing cluster separation on at least one pair of axes. Only cells with clearly separable clusters across all the three sessions on either day were included in the analyses. This process is illustrated in Figure 2.

Maps of all behavioral and neuronal data were generated using custom MATLAB routines. A motion filter of 2 cm/s was used to discard spiking activity during periods of immobility. Data corresponding to the 60-cm-diameter cylinder were parsed into a 2D matrix of 2 cm \times 2 cm pixels. The binned spikes were then divided by the binned occupancy to create an unsmoothed rate map. This was convolved with a 3 bin \times 3 bin Gaussian kernel to create a smoothed rate map. Correlations were based on comparisons of smoothed rate maps between sessions. A Pearson’s correlation (r) was calculated between equivalent bins, discarding unvisited and common-zero bins. Correlations were calculated between sessions 1 and

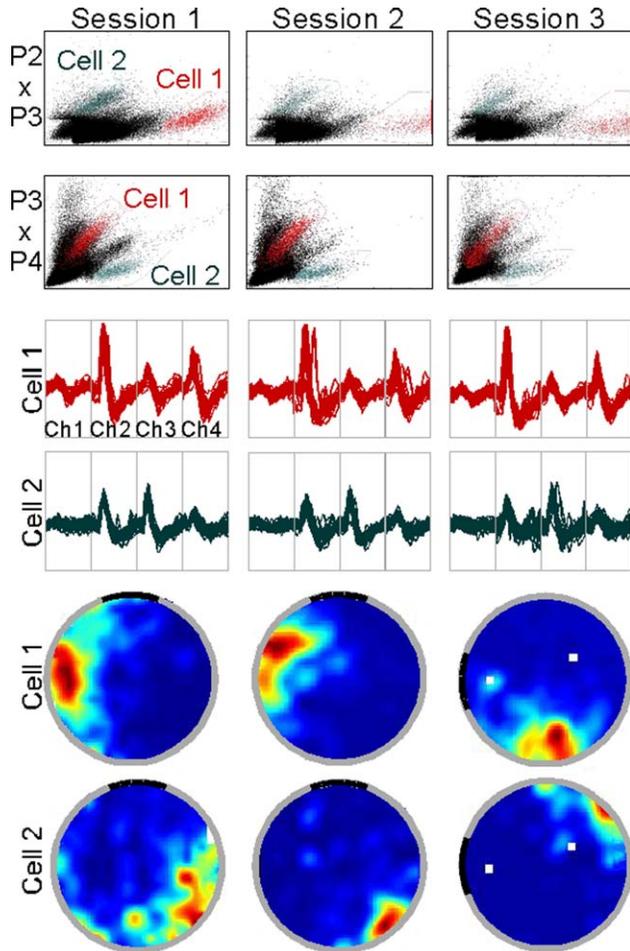


FIGURE 2. Recording stability was verified across sessions. To accurately determine how counter-rotation of odor and visual cues influenced spatial representations in the hippocampus, it was first essential to demonstrate that the same cells were recorded across all three sessions. As described in the Methods section, pairs of waveform measures were plotted to form clusters of points corresponding to waveforms of individual neurons (A). Boundaries set around clusters in one x - y plot, corresponding for example to peak voltage on channels 2 and 3 of the tetrode (P2 \times P3), could be refined on other plots (e.g., P3 \times P4). In this way, we isolated the activity of individual neurons [e.g., Cell 1 (in red) and Cell 2 (in green)]. These boundaries set in one session could then be applied to other sessions. With stable recordings, these boundaries faithfully captured the spiking activity of individual neurons over time (B), and made it possible to assess long-term spatial stability and rotation to cues (C). Ch1–4: channels 1–4 of the tetrode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

2, as well as between sessions 2 and 3 using a best-fit angle of rotation. Only data from cells exhibiting correlations across the first two sessions equal to or greater than 0.3 were included in the subsequent rotation session analyses (see below).

The best-fit angle of rotation was the angle at which the rate map from session 2, rotated in steps of 6° , correlated maximally with the rate map from session 3. It has been shown in mice that under conditions of minimal attentional load, roughly 1/3 of place cells spontaneously remap (Kentros et al.,

2004). We sought to minimize the impact of spontaneously remapping neurons on the rotation analysis and therefore only calculated the best-fit angle of rotation for those neurons that were stable across the 6-h delay from session 1 to session 2. We used a moderate correlation score of $r = 0.3$ as the arbitrary threshold. A Kolmogorov-Smirnov Z -test was performed to compare the distributions of place field rotation.

Comparisons of correlation scores between two groups were performed using unpaired t -tests. Comparisons between multiple groups for each of the spatial measures (correlation score, coherence, mean firing rate, peak firing rate, field size, spatial information) were performed using one-way ANOVA, with Tukey's correction for multiple comparisons.

Mean firing rate was calculated as the total number of spikes divided by the total length of the session (20 min). Neurons exhibiting mean rates > 6 Hz were classified as interneurons and excluded from additional analysis. Coherence was measured by the z -transformed Pearson's correlation score between a pixel (a $2 \text{ cm} \times 2 \text{ cm}$ bin) and its eight nearest neighbors in the unsmoothed rate map (Kubie et al., 1990). The peak firing rate was the highest firing rate bin in the smoothed rate map. A field was defined as a contiguous minimum 80 cm^2 region where the cell fired above 20% of its peak firing rate for the whole arena. Spatial information was calculated as $\sum p_i (\lambda_i/\lambda) \log_2 (\lambda_i/\lambda)$, where i is the bin number, p_i is the probability for occupancy of bin i , λ_i is the mean firing rate for bin i , and λ is the overall mean firing rate (Markus et al., 1994). All statistical analyses were conducted using SPSS 20.0 (IBM).

Histology

Marking lesions for the identification of electrode placement were made by passing D.C. current through a wire from each tetrode from which data were recorded. Mice were then given a lethal dose of pentobarbital sodium (Euthasol 150 mg/kg) and perfused transcardially with 0.9% saline, followed by a 10% formalin solution. Sectioning was performed on a sliding microtome. Coronal sections ($50 \mu\text{m}$) were collected and mounted on gelatin-coated slides, stained with Cresyl violet, and examined under light microscope. The locations of all recording electrodes were confirmed to be in the CA1 cell layer.

RESULTS

In this study, we examined whether and how stable, self-generated odor cues influence the fields of CA1 place fields in relation to distal visual cues. Specifically, do cues from the two modalities combine to determine the firing field of an individual place cell, or is the field of an individual place cell preferentially anchored to one cue modality or the other? We addressed this question by assessing the place cell activity of three groups under the three manipulations—familiarization (to visual and/or odor cues), rotation (of both the familiar and

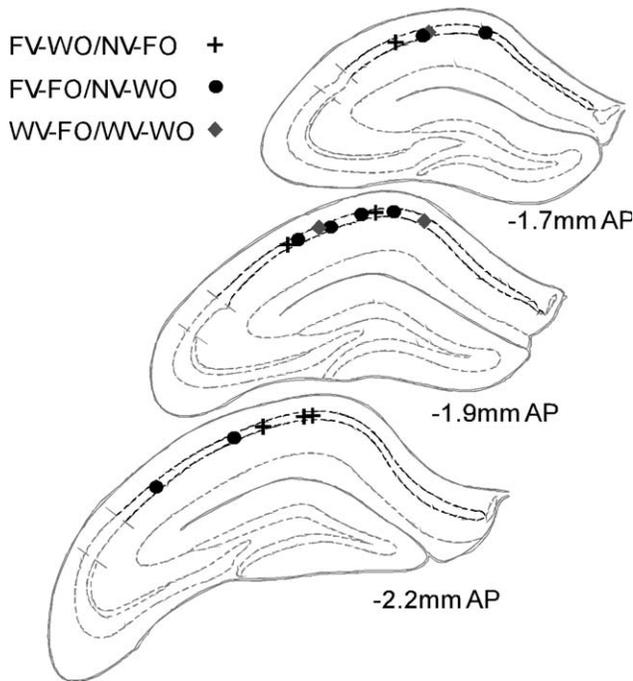


FIGURE 3. Recording sites were localized to the CA1 region of the hippocampus. Marking lesions were made in tetrodes yielding analyzed CA1 place cell data. These sites were distributed evenly along the proximal-distal axis of CA1 across the three groups. Sites corresponding to mice initially exposed to stable visual cues alone (FV-WO/NV-FO) are indicated by the “plus” sign. Filled black circles and gray diamonds represent recording sites in mice initially exposed to familiar visual and odor cues (FV-FO/NV-WO) and familiar odor only (WV-FO/WV-WO), respectively.

novel visual and/or odor cues), and replacement (of the familiarized visual and/or odor cues). The first comprised familiarizing them for several days to distal visual and/or preserved self-generated odor cues to observe if access to these stable, sensory cues can lead to the formation of stable CA1 place fields. The second manipulation comprised rotating the cues relative to their original position (and relative to each other, for the combined visual and odor groups) to observe if the place fields follow the rotation of one cue or the other. The third manipulation comprised replacing the familiarized cues with novel cues and observing the stability and rotation of place fields in a novel environment. The cues to which the mice were exposed were either visual (geometric shapes stenciled on the wall of the enclosing cylinder), olfactory (self-generated odor cues preserved on absorbent paperboard flooring), or a combination of the two. The majority of recording sites for each group of mice were located at the mid-point of the CA1 cell field, equidistant from proximal and distal poles (Fig. 3).

Cue Familiarization

The data collected in the familiar condition from the FV-WO group provided a baseline with which to compare data from the other groups. Place field stability and rotation to cues were assessed in the presence of familiar visual cues only. Data

for a total of 46 place cells were collected from five mice in the FV-WO group (two mice were excluded due to shifts in cluster boundaries). These mice were familiarized (i.e., screened) for an average of 7 ± 1.23 days. Of the 46 cells recorded, 38 cells (38/46 or 83%; all cells: mean $r = 0.54 \pm 0.04$, stable cells: mean $r = 0.64 \pm 0.03$) had stable place fields across the 6-h delay (with “stable” defined as F1/F2 correlations ≥ 0.3). For the FV-FO group, the familiar condition involved both visual cues and preserved, self-generated odor cues. Data for a total of 59 place cells were collected from seven mice in the FV-FO group (an eighth mouse was excluded due to shifts in cluster boundaries). These mice were familiarized for an average of 7.14 ± 0.8 days. Of the 59 cells recorded, 47 cells (47/59 or 80%; all cells: mean $r = 0.52 \pm 0.04$, stable cells: mean $r = 0.64 \pm 0.02$) had stable place fields across the 6-h delay. Thus, in the absence of task contingencies, simply maintaining odor cues does not appear to greatly increase place field stability.

Cue Rotation

For the animals in the FV-WO group, immediately following F2 the visual cues were rotated by 90° and the mice were returned to the arena for the third session (FR). As expected, place fields rotated consistently with the visual cues (for examples, see Fig. 4A, Cells 1–4) and were tightly clustered around the cylinder’s -90° angle of rotation [Fig. 4C; 84.2% (32/38 cells) within $\pm 30^\circ$ of the cylinder rotation angle; mean $\theta = -70.9 \pm 6.6^\circ$, SE].

For the FV-FO animals, immediately following F2 the visual cues and the self-generated odor cues (i.e., the cylinder and the floor) were rotated by 90° in opposite directions, and the mice were returned for the third session (FR). Despite the presence of preserved odor cues, fields continued to rotate with the visual cues (for examples, see Fig. 4B, Cells 1–3). Of the 47 stable cells recorded, 34 had fields rotating with the visual cues [Fig. 4D; 72.3% (34/47 cells) within $\pm 30^\circ$ of cylinder rotation angle]. In the rare instances ($n = 2$) where the cells appeared to follow odor cues according to the rotational analysis, it is clearly visible from the fields that they are actually following the visual cues (Fig. 4B, Cell 4, F1 vs. FR).

The distributions of the two familiar conditions were not significantly different (Kolmogorov-Smirnov $Z = 0.70$, $P = 0.71$). Furthermore, an analysis of best-fit correlations before and after the cue rotation indicates that the fields do not undergo remapping. Correcting for the rotation, fields were well correlated with no significant difference between the FV-FO condition and the FV-WO control condition (FV-FO: mean $r = 0.59 \pm 0.02$ SE, FV-WO: mean $r = 0.63 \pm 0.03$ SE; $t(83) = -1.06$, $P = 0.29$). Of the remaining 12 unstable cells (12/59 or 20%), 4 rotated with the visual cues and 8 rotated to an intermediate angle; none rotated to within $\pm 30^\circ$ of the odor rotation angle. The presence of familiar odor cues did not dramatically alter the spatial properties or stability of FV-FO place fields. No significant differences were found for

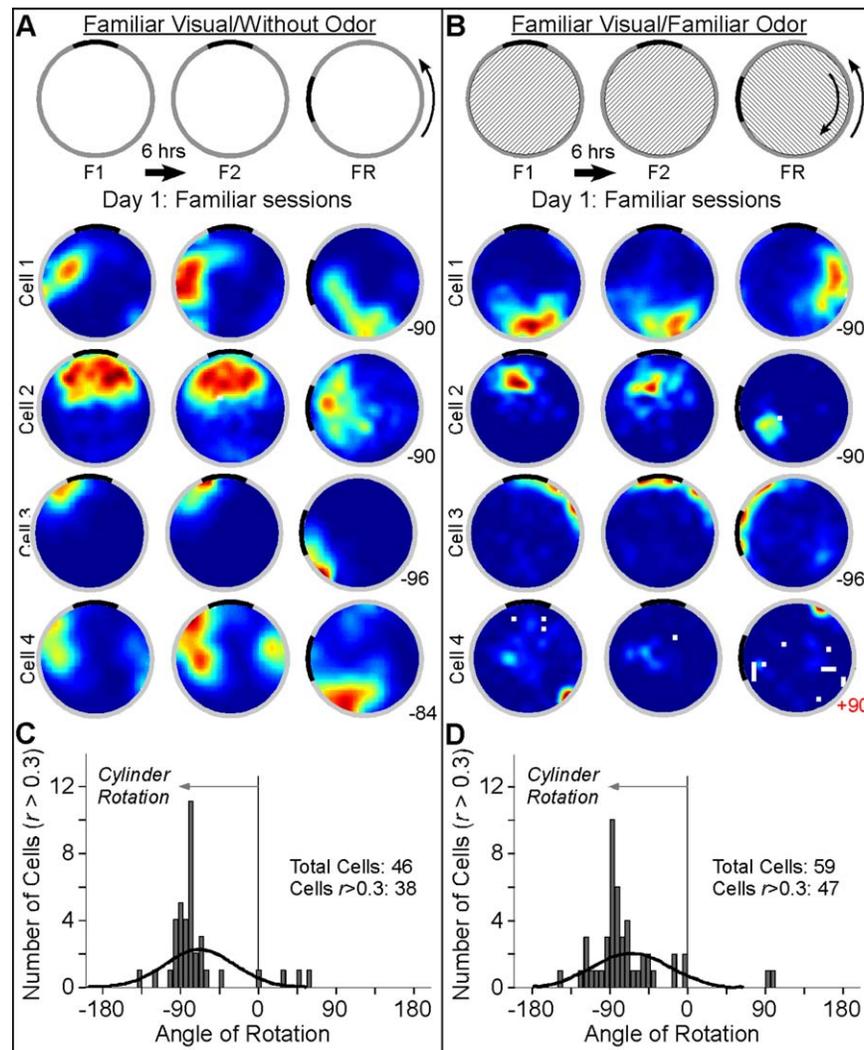


FIGURE 4. Visual cues guide place field rotations, even in the presence of stable odor cues. Place cells in mice familiarized exclusively to visual cues rotated faithfully with those cues, as illustrated by Cells 1–4 (A). The vast majority of the cells rotated to within $\pm 30^\circ$ of the visual rotation (C). Surprisingly, a comparable proportion of cells in mice familiarized to both visual and self-generated odor cues also rotated faithfully with the visual cues (B,

Cells 1–3; D). In rare instances, rotation toward the odor cues was seen (Cell 4, odor cue angle of rotation indicated in red). However, even here, comparison of sessions F1 and FR suggest the cell is still following the visual cues. The distributions of best-fit angles of rotation (C versus D) were similar. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

measures of coherence, spatial information, or field size during the first familiar session between conditions (Table 1).

On the basis of previous studies, we hypothesized that the presence of familiar odor cues would influence the spatial characteristics and anchoring of place fields, which would become particularly evident with counter-rotation of the two sets of cues. Specifically, we expected some place cells to rotate with the odor cues. Surprisingly, this was not the case.

Cue Replacement

One day later, place cell activity of the FV-WO group of mice was assessed in a second, novel condition (NV-FO) where a second cylinder provided a new set of visual cues, and each

animal's self-generated odor cues were preserved across the three sessions. Data for a total of 69 place cells were collected from seven mice. Of these, 53 cells (53/69 or 77%; all cells: mean $r = 0.51 \pm 0.03$, stable cells: mean $r = 0.63 \pm 0.02$) had stable fields across the 6-h delay, exhibiting N1/N2 correlation scores ≥ 0.3 . The proportion of cells/mouse that had stable fields was comparable in the FV-WO and the NV-FO groups (Fig. 5A). Therefore, replacement of visual cues and preservation of self-odor cues did not alter the spatial characteristics of the place cells or the stability of their fields.

The place cell activity of FV-FO mice was similarly assessed in a second, novel condition. In this condition (Novel Visual/Without Odor or NV-WO), a second cylinder provided a new set of visual cues, and fresh floor paper was present for each

TABLE 1.

Firing properties of pyramidal neurons

Condition	<i>n</i>	Spatial information ^a (bits/spike)	Place field size ^a (sq. cm)	Coherence ^a	Session 1–Session 2 correlation scores
Familiar Visual/Without Odor	46	1.65 ± 0.11	54.82 ± 6.63	0.79 ± 0.05	0.54 ± 0.04
Novel Visual/Familiar Odor	69	1.69 ± 0.11	57.69 ± 7.10	0.80 ± 0.04	0.51 ± 0.03
Familiar Visual/Familiar Odor	59	1.82 ± 0.15	41.85 ± 4.62	0.66 ± 0.04	0.52 ± 0.04
Novel Visual/Without Odor	65	1.65 ± 0.13	59.77 ± 9.18	0.57 ± 0.04	0.32 ± 0.04
Familiar Odor/Without Visual	20	0.96 ± 0.14	99.62 ± 16.35	0.47 ± 0.08	0.13 ± 0.06
Without Odor/Without Visual	20	0.93 ± 0.12	52.86 ± 10.11	0.50 ± 0.07	0.18 ± 0.06

All values are mean ± standard error.

^aSession 1 values.

session such that self-generated odor cues were no longer preserved. Data for a total of 65 place cells were collected from the eight mice. Surprisingly, field stability in this condition was markedly reduced, with only half the place fields (33/65 or 50.7%; all cells: mean $r = 0.32 \pm 0.04$, stable cells: mean $r = 0.58 \pm 0.03$) stable across 6 h, where the corresponding number for the other three visually cued manipulations (FV-WO, FV-FO, NV-FO) had been around 80%. Correlations between sessions N1 and N2 were significantly lower compared to the other three visual conditions (FV-WO, NV-FO, FV-FO, $F(3) = 7.24$, $P < 0.001$, Tukey's test; Table 1), with a correspondingly lower proportion of cells/mouse with stable fields (see Fig. 5A).

This difference indicates that the development of stable fields in a novel environment is disrupted when highly familiar self-generated odor cues are no longer present. The spatial properties of NV-WO fields did not differ dramatically from those of the other conditions (see Table 1). The only significant effect was lower spatial coherence, compared with the FV-WO group of animals in the familiar environment (NV-WO: 0.57 ± 0.04 , FV-WO: 0.79 ± 0.05 , NV-FO: 0.80 ± 0.04 , $F(5) = 6.99$, $P < 0.01$, Tukey's HSD). These results indicate that while the removal of familiar self-generated odor cues had a marked effect on the stability of the new NV place fields, it had minimal effect on the spatial characteristics of the fields themselves.

Novel Cue Rotation

Immediately following the N2 session for the NV-FO group, the visual cues (cylinder) and self-generated odor cues (floor) were counter-rotated by 90°, and the mice were returned to the arena for the third session (NR). Place fields in the NV-FO condition rotated with the visual cues (e.g., see Fig. 6A, Cells 1–4; Fig. 6C, mean $\theta = -82.5 \pm 3.6^\circ$ SE). The proportion of visually cued fields/mouse was comparable to that observed with familiarized visual cues alone, in the FV condition (Fig. 5B). Measures of field size, coherence, and spatial information did not differ between the two conditions (Table 1).

The visual cues for the NV-WO group were rotated immediately after the N2 session, and the animals were returned for

the third session (NR). Rotation with the visual cues was far less consistent (e.g., see Fig. 6B, Cells 1 and 2 versus Cells 3 and 4). Although the mean angle of field rotation was in the direction of the visual cues (Fig. 6D; mean $\theta = -29.1 \pm 14.9^\circ$ SE), the proportion of cells/mouse rotating to within $\pm 30^\circ$ of visual cue rotation was significantly lower than that of the preceding three conditions (Fig. 5B). This resulted in a significantly different distribution compared with that of the FV-FO condition (K-S: $Z = 1.41$, $P = 0.037$) as well as FV-WO (K-S: $Z = 1.5$, $P = 0.022$) and NV-FO (K-S: $Z = 1.76$, $P = 0.004$) conditions. Given the fact that these place cells now had only visual cues to follow, and considering their random rotation from the N2 to NR sessions, it can be concluded that the non-rotation is evidence of instability of the fields, even across the few minutes delay between the two sessions. Therefore, the replacement of visual cues along with removal of familiar odor cues resulted in a marked loss of place field stability, both long-term and short-term, in those animals that had previously been acclimated to preserved self-generated odor traces.

FO-WV/WO-WV Group

The data from previous groups indicate that sufficiently familiar self-generated odor cues do influence the stability of the spatial firing properties of CA1 place cells, even if they do not provide a spatial orientation, *per se*. Given these results, it was important to determine whether such cues could guide place cell firing independently. However, the data from the FO-WV/WO-WV group suggest that, even with familiarity, self-generated odor cues alone will not anchor place fields to specific locations within the environment.

Familiar odor/without visual group

For the Familiar-Odor/Without Visual (FO-WV) group, place field stability and rotation were assessed in a featureless cylinder in the presence of familiar odor cues. These mice were familiarized for an average of 9.3 ± 0.7 days. Data for a total of 20 place cells were collected from three FO-WV mice. Firing fields in the FO-WV condition were atypical, lacking the spatial specificity characteristic of place cells (e.g., see Fig. 7A, Cells 1–4),

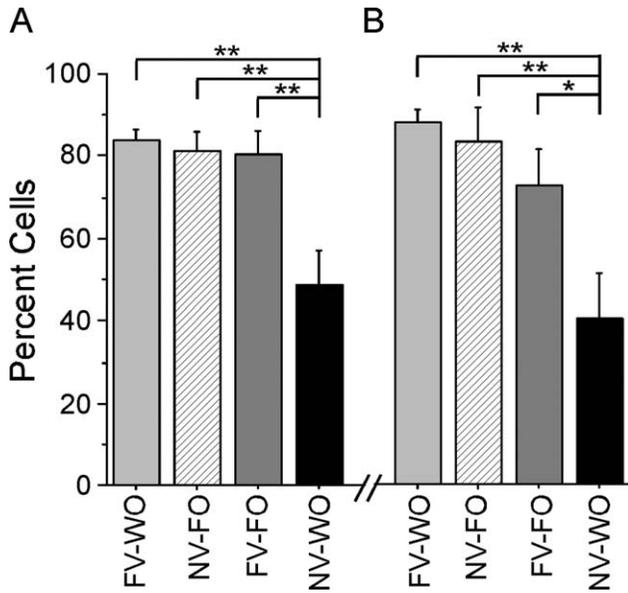


FIGURE 5. Long-term stability and rotation in a novel environment is disrupted by removal of familiar odor cues. The proportion of cells/mouse exhibiting long-term stability and accurate rotation to novel visual cues alone (NV-WO) was significantly reduced to the other three manipulations involving visual cues. Each of the other manipulations exhibited comparable proportions with long-term stability and visually guided rotation. FV-WO: Familiar Visual/Without Odor; NV-FO: Novel Visual/Familiar Odor; FV-FO: Familiar Visual/Familiar Odor. [* $P < 0.05$; ** $P < 0.01$].

and were both significantly larger (FO-WV: 99.6 ± 16.3 sq. cm., FV-WO: 54.82 ± 6.6 sq. cm., $F(5) = 3.025$, $P < 0.05$, Tukey's HSD) and with less spatial coherence (FO-WV: 0.47 ± 0.08 , FV-WO: 0.79 ± 0.05 , $F(5) = 6.99$, $P = 0.005$, Tukey's HSD) compared with fields in the FV-WO control condition. Stability of firing across the 6-h delay was also significantly reduced compared with the control condition (FO-WV: $r = 0.13 \pm 0.06$, FV-WO: $r = 0.53 \pm 0.04$, $P < 0.0001$), with only 5/20 cells achieving the minimum criterion for field stability across the 6-h delay (all cells: mean $r = 0.13 \pm 0.06$, stable cells: mean $r = 0.46 \pm 0.07$). The low incidence of stability precluded a subsequent angle of rotation analyses and comparisons with the FV-WO control condition. These data indicate that typical place fields do not form in the presence of familiar, self-generated odor cues alone.

Without odor/without visual group

One day later, mice were placed in the final condition in which no stable visual or odor cues were available (Without Odor/Without Visual or WO-WV). Data for a total of 20 place cells were collected from the three WO-WV mice. Results in this condition were as expected, based on the literature. As with the FO-WV condition, firing fields in the WO-WV condition were atypical, lacking the spatial specific-

ity characteristic of place cells (e.g., see Fig. 7B, Cells 1–4). Only 6/20 cells exhibited the minimum criterion for field stability across the 6-h delay (all cells: mean $r = 0.17 \pm 0.06$, stable cells: mean $r = 0.50 \pm 0.06$). Consistent with this observation, spatial measures of field size, coherence, and spatial information were similar to those seen during the previous session when familiar, self-generated odor cues were available (Table 1). The low incidence of stability precluded a subsequent angle of rotation analyses and comparisons with the FV-WO control condition.

DISCUSSION

In this study, we investigated the relative roles of self-generated odor cues and visual cues on the activity of hippocampal CA1 place cells. We familiarized mice extensively to a set of visual cues and/or to preserved, self-generated odor cues. We then assessed long-term field stability and the effects of cue rotation. Maintenance of odor traces did not appear to have a discernible effect upon place field stability. Unsurprisingly, fields that had developed in the presence of stable visual cues alone (FV-WO condition) faithfully rotated with those cues. Contrary to our expectations, however, fields in the presence of both highly familiar, self-generated odor cues and visual cues (FV-FO) rotated entirely with the visual cues as well, essentially ignoring the counter-rotated odor cues. Moreover, when visual cues were made ambiguous (FO-WV condition), odor traces alone did not lead to stabilization of the place cell map. Surprisingly, removal of familiar odor cues severely disrupted the stability of newly made fields in a novel, visually cued environment. These results indicate that while self-generated odor cues do not provide intrinsically spatial information, they nonetheless become increasingly important contextual cues over time and can exert a pronounced influence on basic information processing in the hippocampus.

Our study design allowed for several distinct outcomes, each of which would inform on how odor cues are represented in the activity of CA1 place cells. On the first day, when familiar visual and self-generated odor cues were counter-rotated (FV-FO), fields could have (1) collectively followed the visual cues, (2) collectively followed the odor cues, (3) individually followed either one set of cues or the other, or (4) remapped. Outcomes 3 and 4 could conceivably have been interpreted as a degree of inherent field instability in FV-FO mice. Therefore, we added a second day during which stability and rotation were assessed in a novel, exclusively visual-cued environment. Our expectation for this second day was a recapitulation of numerous previous studies: long-term stability and accurate field rotation. This second "control" condition would therefore allow us to confirm that any effects observed on the first day were attributable specifically to the presence of stable, self-generated odor cues. Surprisingly, this second condition revealed an interesting effect. As noted above, familiar odor cues produced no discernible effect on field rotation with the

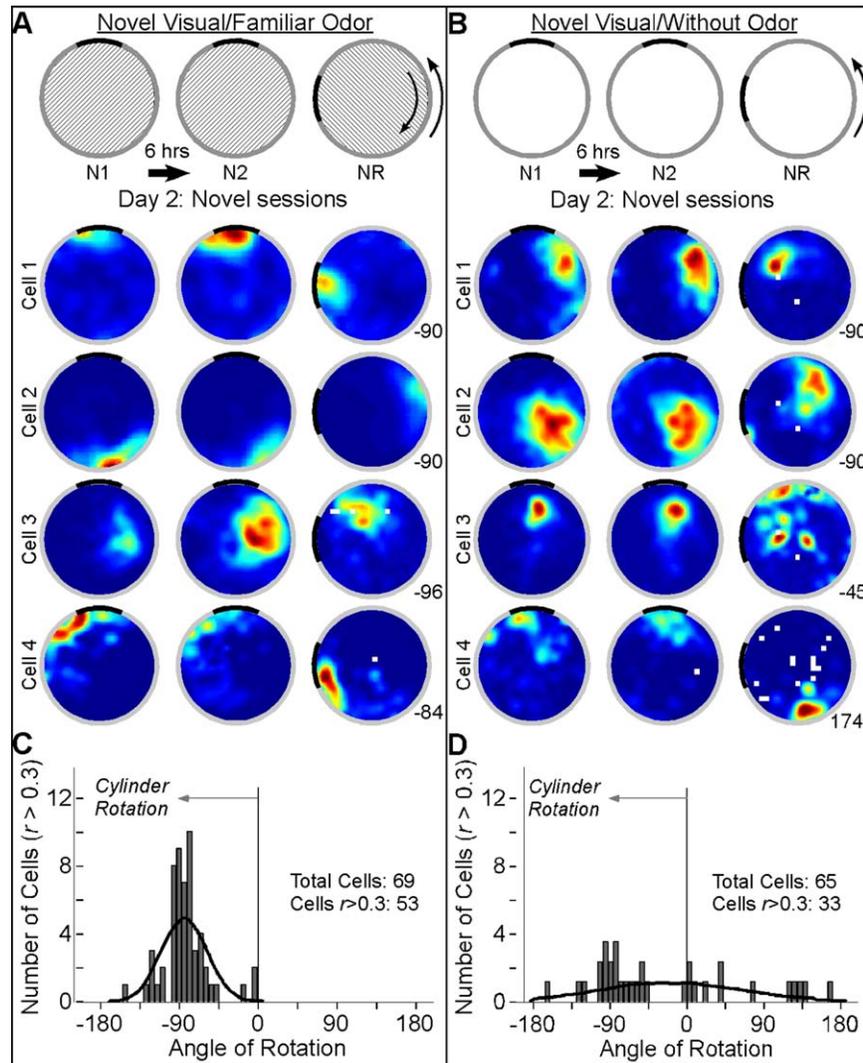


FIGURE 6. The removal of familiar odor cues significantly disrupted visually guided rotations. The long-term stability and the ability to follow novel visual cues, even in the presence of preserved odor cues (A, NV-FO), was comparable to that observed in the visual-alone control condition from Day 1 (Cells 1–4; see Fig. 4A for comparison). In contrast, the limited proportion of cells from NV-WO mice that exhibited long-term stability (B,

Cells 1–4) appeared to rotate largely randomly. The inability to rotate with the novel visual cues is illustrated by the distribution of best-fit angles of rotation (D), which differed significantly from that of cells in the presence of Novel Visual/Familiar Odor cues (C), as well as both sets of cells from Day 1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

visual cues. This result, in conjunction with the familiar, exclusively odor-cued condition, indicates that self-generated odor cues do not effectively anchor fields. However, the field instability and disrupted rotation observed when familiar odor cues were no longer present (NV-WO) indicates that preservation of odor cues nonetheless impacted on hippocampal information processing: while not providing a spatially discriminative stimulus, the odors still impinged upon the stability of a spatial representation.

As noted in the Introduction section, ours is not the first study to investigate the involvement of odor cues in establishing spatial representations. We confirm the finding of Save and colleagues (2000) that odor cues can influence spatial representations. However, there are significant procedural differences between the studies. Save and colleagues removed a salient

familiar visual cue (a cue card) and found that cleaning the floor of self-generated odor cues increased the rotation of place fields relative to their start point. The animal remains in the environment throughout all cue manipulations, so the path integration signal is maintained. This is by no means a trivial difference: Rotenberg and Muller (1997) showed that the exact same cue manipulation has the opposite effect upon place cells depending upon whether the animal remains in the environment for it or whether it happens when the animal is in its home cage. By removing the animal between manipulations, we investigate the relative strength of visual and olfactory cues on the recall of a map from memory, rather than the persistence of an already instantiated map following removal of familiar cues.

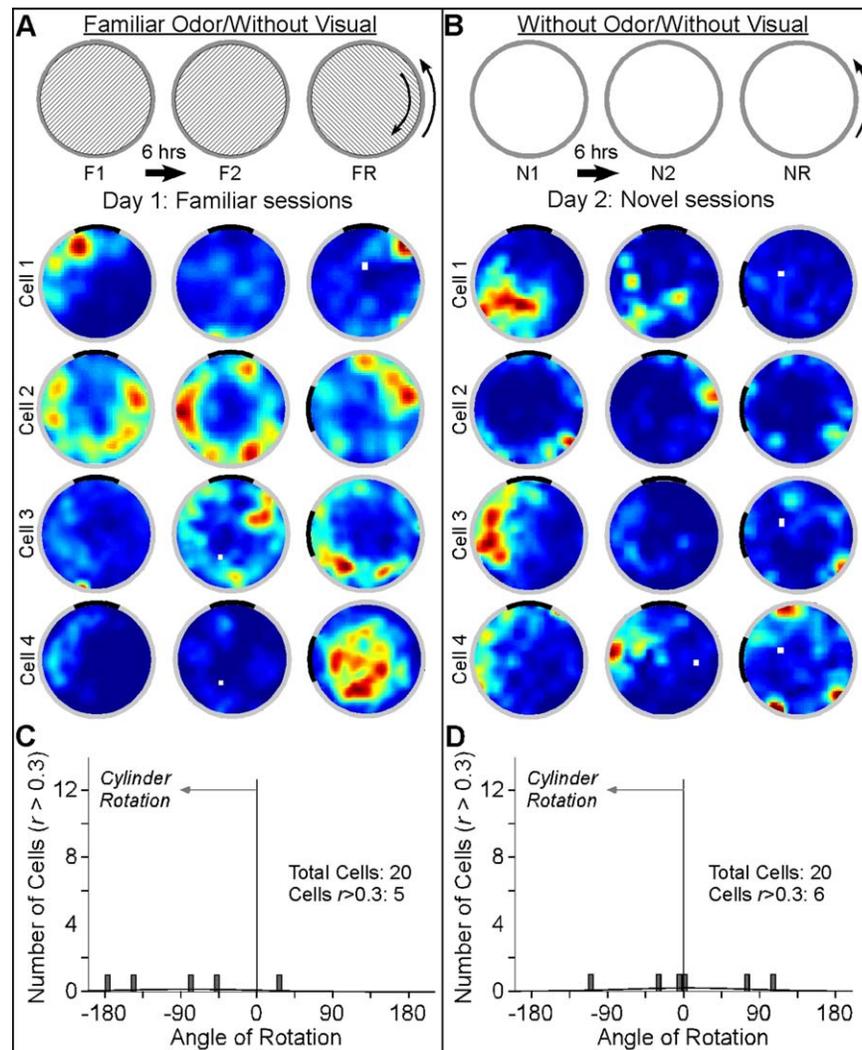


FIGURE 7. Familiar odor cues alone failed to elicit stable fields or effectively guide field rotations. Fields in the absence of visual cues were typically only poorly defined and did not correlate well across the 6-h delay. This was true both in the presence (A) and absence (B) of stable odor cues. The number of cells that did achieve the minimum correlation of $r > .03$ was insufficient for

an analysis of distributions of best-fit angles of rotation. However, visual inspection of the distribution clearly indicates that rotations were largely random both with stable odor cues (C) and without such cues (D). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The two studies taken together suggest that odor cues will anchor place cell activity only when such representations are initially visually cued. We also found that when compared head-to-head, visual cues are much stronger spatial cues compared to olfactory cues. This finding is also corroborated by Save et al. (2000) who found that place fields formed in the presence of visual cues are more enduring than place fields formed in their absence. Building on the finding that olfactory cues stably maintain place fields generated in the presence of a visual cue, we examined if they are capable of generating stable place fields without the aid of visual cues, and found that not to be the case. Finally, our novel condition data on Day 2 lead directly to our interpretation that odors become integral to the context over time. This is the result that allows us to begin addressing the “fully open”

question posed by Save and colleagues (2000): How do visual and olfactory cues interact?

The observed results are best interpreted as evidence of the strong contextual role of odor cues. The “context” of an environment is, of course, not limited to geometric cues providing spatial orientation. In fact, the visual cues are but a subset of the cues available. The shape of the environment, the texture of the floor, the sounds in the room, and the odors that are present also contribute, as do procedural elements, such as being removed from the cage and placed into the environment. We have controlled for each of these factors with the six distinct conditions included in this study. The disruptions caused by the removal of familiar, self-generated odor cues in the novel environment are clear evidence that these cues had become an integral part of the arena context.

How, precisely, the removal of familiar odors disrupted spatial processing is less clear. This could reflect a conflict between expectations and outcomes, and indicate the influence of top-down control on processing of the novel visual cues. With the familiarization sessions, odors became part of the environment: their presence was expected. Their removal set up a conflict between incoming sensory information and the representation of the context stored in memory. One could speculate that this conflict would potentially engage medial prefrontal and anterior cingulate cortical regions involved in error detection and conflict monitoring (Botvinick et al., 2004; Carter and van Veen, 2007; Kim et al., 2010). In mice, the anterior cingulate cortex responds to the absence of familiar stimuli (Weible et al., 2012), and both structures have been shown to influence hippocampus-dependent behaviors (Goshen et al., 2011; Tse et al., 2011). This would set up a competition between the novelty of the new visual cues and the unexpectedly odorless floor. The hippocampus is highly responsive to visual novelty, as evidenced by the rapid and robust reorganization of spiking activity characterizing remapping in novel environments (Breese et al., 1989). Novel odors also have a pronounced influence on hippocampal function (Gold et al., 2011; Irwin and Byers, 2012) and activation of the hippocampus is associated with recall of odor recognition behavior (Lehn et al., 2013). Therefore, the removal of familiar odor cues likely acts either as a distracter from the spatial cues, or convinces the animal that arena cues are not necessarily stable. More experiments are required to distinguish between these possibilities.

Together, these data clearly demonstrate that, over time, self-generated odor cues can become integral to contextual representations in the hippocampus, and that their unexpected absence adversely impacts on hippocampal function. As such, odor cues are likely to substantially influence the development and recall of episodic memories in the hippocampus. Importantly, this work was done without any externally applied task contingencies, which have been shown to exert a strong influence on the firing of hippocampal place fields (Kentros et al., 2004; Muzzio et al., 2009; Dupret et al., 2010). In the presence of competing visual cues, animals may simply not pay attention to the spatial information available in odor traces, especially when they are not cues for goal-directed behaviors. Prior work demonstrating that spatial behavior in total darkness can be guided by odor traces (Lavenex and Schenk, 1998) by definition has externally applied task contingencies: the animals are motivated to attend to olfactory cues. Moreover, place cells have been shown to respond to odor cues when they are task-relevant (Shapiro et al., 1997; Eichenbaum, 1998). In addition, the animals in our study freely explored a circular environment without following any pre-defined path, and it is possible that if the animals had to follow a well-defined trajectory, then a combination of ease of following scent trails as well as idiothetic cues might have enhanced the directional value of the olfactory cues. Therefore, future work exploring whether task contingencies with attentional demand and the availability of idiothetic cues allow animals to generate odor-linked place fields will be of great interest.

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