ROLE OF SELF-GENERATED ODOR CUES IN PLACE CELL REPRESENTATION OF SPATIAL CONTEXT

by

DEVDEEP AIKATH

A DISSERTATION

Presented to the Department of Psychology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

March 2012

DISSERTATION APPROVAL PAGE

Student: Devdeep Aikath

Title: Role of Self-generated Odor Cues in Place Cell Representation of Spatial Context

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Psychology by:

Clifford G Kentros	Chairperson
Edward K Vogel	Member
Margaret E Sereno	Member
Philip E Washbourne	Outside Member

and

Kimberly Andrews Espy	Vice President for Research & Innovation/Dean of the Graduate School

Original approval signatures are on file with the University of Oregon Graduate School.

Degree awarded March 2012

DISSERTATION ABSTRACT

Devdeep Aikath

Doctor of Philosophy

Department of Psychology

March 2012

Title: Role of Self-generated Odor Cues in Place Cell Representation of Spatial Context

The importance of the hippocampus in the formation and retrieval of episodic memory has been famously demonstrated in the case of patient H.M. Subsequent studies conducted in animal models have provided considerable insight into the specific functions of the individual components of the hippocampus. In the rodent, the pyramidal neurons of the *CA1* and *CA3* regions of the hippocampus have typically been associated with the encoding of visuo-spatial cues and their utilization in navigation. These 'place cells' fire when the animal is in a specific part of its environment (its place field). However, these cells also encode non-spatial information from other sensory inputs, such as olfaction and audition. This study was conducted to find out how contextual odor cues are represented in the firing of *CA1* place cells and whether these cues could drive stable spatial representations.

One group of mice was first extensively familiarized to a cylinder containing both visual cues and preserved, self-generated odor cues. Then, after assessing place field stability across a six hour delay, the visual and odor cues were rotated in opposite directions by ninety degrees (counter-rotated). Another group of mice was familiarized only to the visual cues that were subsequently rotated. The next day stability and rotation

were re-assessed in a novel cylinder. However, the odor cues of the two groups were switched: the preserved odor cues of the first group were removed, and the odor cues of the second group were now preserved across the three sessions. In a separate experiment, a third group of animals was familiarized only to the odor cues. Firstly, we found that contextual odor cues attenuated rotation with the visual cues, but only following extensive familiarization. Secondly, the removal of familiar odor cues impaired long-term stability of place fields. Third and finally, the self-generated odor cues alone were not sufficient for the generation of stable place fields in a free, open-field exploration paradigm.

We therefore conclude that although they are not as dominant as discrete visual cues, highly familiarized odor cues exert a significant effect on the representation of space of the mouse *CA1* place cell, illustrating the role of contextually relevant information in navigating an ever-changing world.

CURRICULUM VITAE

NAME OF AUTHOR: Devdeep Aikath

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene
University of Calcutta, India

DEGREES AWARDED:

Doctor of Philosophy, Psychology, 2012, University of Oregon
Master of Science, Psychology, 2007, University of Oregon
Bachelor of Medicine & Surgery, 2004, University of Calcutta

AREAS OF SPECIAL INTEREST:

Systems Neuroscience

GRANTS, AWARDS, AND HONORS:

Jhamandas Watamull fellowship, 2006

ACKNOWLEDGMENTS

I wish to express my gratitude to Professor Kentros and Drs. Aldis Weible and David Rowland for their help in collecting and analyzing the data included in this dissertation, and for the preparation of this manuscript. I would also like to thank the members of the Kentros lab, and the members of my advisory committee for their helpful discussions and feedback. Last but not least, I wish to thank my parents and my wife, Preeti who have patiently supported me throughout my doctoral career.

To the memory of my grandmother, Mira Ghosh

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Table Page

CHAPTER I

INTRODUCTION

The hippocampus is one of the most intensely studied parts of the brain. Loss of hippocampal function, as first described in patient H.M., results in the inability to form new autobiographical memories or memories of events in their corresponding spatial and temporal context (Cohen and Eichenbaum, 1994; Krebs et al., 1989; Scoville and Milner, 1957; Tulving, 2002). Much of how contextual information is represented functionally in the hippocampus remains unknown.

The discovery of place cells in rodents was a key advancement in hippocampus research (Kentros, 2006). The pyramidal neurons of *CA1* and *CA3* regions of the hippocampus exhibit spatially localized firing patterns when the animals explore an environment containing discrete visuo-spatial cues (O'Keefe and Dostrovsky, 1971). The location-specific firing of these 'place cells', referred to as the cell's 'place field', follows rotations of such visual cues (Muller and Kubie, 1987; O'Keefe and Speakman, 1987), and are affected by the insertion or removal or barriers within the environment (Lever *et al*., 2002a; Muller and Kubie, 1987), as well as changes in the shape and size of the environment (Lever *et al.*, 2002b; O'Keefe and Burgess, 1996). Generally, the place fields are very stable for a given environment. However, significant changes in the environment may result in changes in the spatial localization of these fields, a phenomenon known as 'remapping' (Muller and Kubie, 1987; O'Keefe and Burgess, 1996). It has since been shown that place cells can also fire in response to non-geometric features such as odor (Anderson and Jeffery, 2003; Hayman *et al.*, 2003), cued (Moita *et al.*, 2003) and contextual (Wang *et al.*, 2010) fear stimuli, and task requirements (Markus

et al., 1995; Smith and Mizumori, 2006b). Nevertheless, the most dominant feature of hippocampal pyramidal neurons in rodents is their firing properties in response to environmental geometry and visual cues. This, along with the findings of spatial learning impairment resulting from hippocampal lesions (Eichenbaum *et al.*, 1990; Morris *et al.*, 1982), establishes spatial navigation as one of the most important functions of the rodent hippocampus.

For rodent place cells, individual environments unique, due to the specific cognitive and behavioral demands associated with each environment. Context-dependent hippocampal activity is responsible for episodic memory, which serves to distinguish one situation from another to retrieve the appropriate response (Ainge et al., 2008). According to Smith and Mizumori (2006a) the high correlation between spatial geometry and context makes spatial information very important to the rodent, and as such significant hippocampal resources are allocated to processing spatial information. Since hippocampal processing plays such an important role in the encoding of spatial context, contextually relevant environmental cue such as odor should affect the hippocampal representation of space, even when it is not as spatially specific as discrete visual cues. It has been found that rats can continue to maintain stable fields formed in the presence of the visual cues, if the visual cues are removed and olfactory and idiothetic cues are preserved (Save *et al.*, 2005). This is consistent with the fact that rats can use odor traces for tracking (Lavenex and Schenk, 1998; Wallace *et al.*, 2002). Muzzio *et al.* (2009) found that when animals were required to pay attention to non-spatial (reward-associated) odor cues and ignore visual cues, their location-specific place fields disintegrated and were replaced by odor-guided fields. However, it remains to be demonstrated definitively

whether place cells utilize odor information in conjunction with visual cues and path integration to create a spatially stable representation of their environment or if there are separate types of cells capable of processing these two types of information independently in a given environment. To address this issue, we familiarized visual and odor cues and then placed them directly in conflict in a single environment, and then assessed the impact of this manipulation on the spatial representation of the *CA1* place cells.

Mice were implanted with chronic recording electrodes, and then familiarized to both visual cues (painted on the walls of an enclosing cylinder) and preserved self-generated odor cues (accumulated on an absorbent paperboard floor) in an open-field environment for a minimum of five days. For the first manipulation, we assessed field stability across a six hour delay and then examined how stable place fields responded to 90 degree counter-rotation of the two sets of cues. We found that a significant proportion of place fields did not follow the rotation of the visual cues and some in fact rotated in the opposite direction (i.e., followed odor cues). This was in contrast to a control group familiarized only to the visual cues which faithfully followed the visual rotation.

The following day we repeated the experiment, but with novel rather than familiar sets of odor and visual cues. The animals were put in a different cylinder with a novel set of visual cues, and the floor conditions were switched. Therefore, the animals previously familiarized to odor cues no longer had those cues, and the control group now had odor cues preserved for a short period (a single familiarization session before the test of rotation). The presence of novel, self-generated odor cues did not disrupt rotation to the

visual cues. However, the absence of familiar odor cues reduced long-term stability across the six hour delay.

Finally, we examined whether odor cues alone could support stable place fields. A third group of animals were familiarized only to the preserved, self-generated odor cues in a blank enclosing cylinder for at least five days. We found that in these cells were unable to form recognizable fields over either the short or the long term.

While these results confirmed the previous findings that visual cues have a dominant role in the orientation and stability of the mouse *CA1* place fields in an open arena, we found clear evidence that self-generated odor cues became an integral part of the spatial context with extensive familiarization. The representation of space by the *CA1* place cells was disrupted when the orientation of familiar odor and visual cues were placed in conflict. Furthermore, the ability to establish stabile place fields to novel visual cues was degraded when these familiar odor cues were no longer present.

CHAPTER II

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 the 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)

Surgery and Implantation

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 the bregma and 1.4 mm left of the midline for insertion of the recording tetrodes. An additional four holes were drilled laterally, two in each hemisphere, for the insertion of two stainless steel anchoring screws (00-90 x 1/8"), one reference wire and one ground wire. The tips of the tetrodes were lowered to a depth of approximately 700 µm from the dura. The ground and reference wires and the microdrive were secured to the skull with Grip

cement (Dentsply, Milford, DE). The tetrodes were coated with paraffin wax to prevent adhesion of Grip cement or debris. Mice were administered buprenorphine (0.06 mg/kg) postoperatively for analgesia. All mice were individually housed, and allowed to recover for at least seven days before familiarization sessions were initiated.

Familiarization and Recording

Familiarization

All mice were allowed to explore the arena (a plywood cylinder, 60 cm in diameter, 45 cm in height) freely for 20 minutes daily for at least five days. During these familiarization sessions, neuronal activity was monitored and the tetrodes were lowered by 15-30 µm daily until place cell activity was obtained. Geometric shapes painted in black and white on the inside wall of the cylinder served as the visual cues, while the animal's self-generated odor accumulated over successive sessions on the floor paper served as the contextual odor cues. Nine animals were familiarized to both visual cues and odor cues (vis-odor group), eight animals were familiarized only to visual cues (vis-only group), and three animals were familiarized only to odor cues (odor-only group). The same visually cued cylinder was used for familiarizing the vis-odor and vis-only groups, while a blank white cylinder was used for the odor-only group. 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 boluses were removed before storage. When preservation of odor cues was not desired, a fresh floor paper was used in each session, as in case of the vis-only animals, and the floor below wiped with ethanol between sessions.

The entire arena was surrounded by a uniform, circular black curtain concentric with the cylinder. Illumination came from four equally spaced light sources above the arena.

Day 1: Familiar Condition

The primary goals of the present study were to examine the effects of counter-rotating visual and odor cues on the *CA1* place fields, and how these effects were influenced by familiarity to the cues. We then addressed whether odor cues alone are capable of guiding spatially specific firing fields. These goals were accomplished with a sequence of three 20-minute sessions carried out on two consecutive days. The sequence on Day 1 began immediately following the minimum five days of familiarization and the verification of place cell activity (Fig. 1A, i-iii). During the first session (F1), the mice explored the familiar environment, and were then returned in their respective cages to the mouse housing room. Six hours later, the mice re-explored the familiar environment (F2). Following this, the mice were returned to their cages and held in a black box immediately outside the recording room while the cue conditions were altered. For the vis-odor and odor-only animals, the cylinder and floor were counter-rotated by 90 degrees with respect to the original configuration. For the vis-only animals, the floor paper was changed and the cylinder rotated as above. Immediately following this manipulation, the animals were returned to the arena and a post-rotation session (FR) was recorded. Direction of rotation of the cues was counter-balanced across animals.

Day 2: Novel Condition

On Day 2, the three session sequence was repeated (Fig. 1B, i, ii), but under novel visual and odor cue conditions. A different cylinder with the same dimensions but a novel set of visual cues was used, and the odor conditions of the vis-odor and vis-only groups

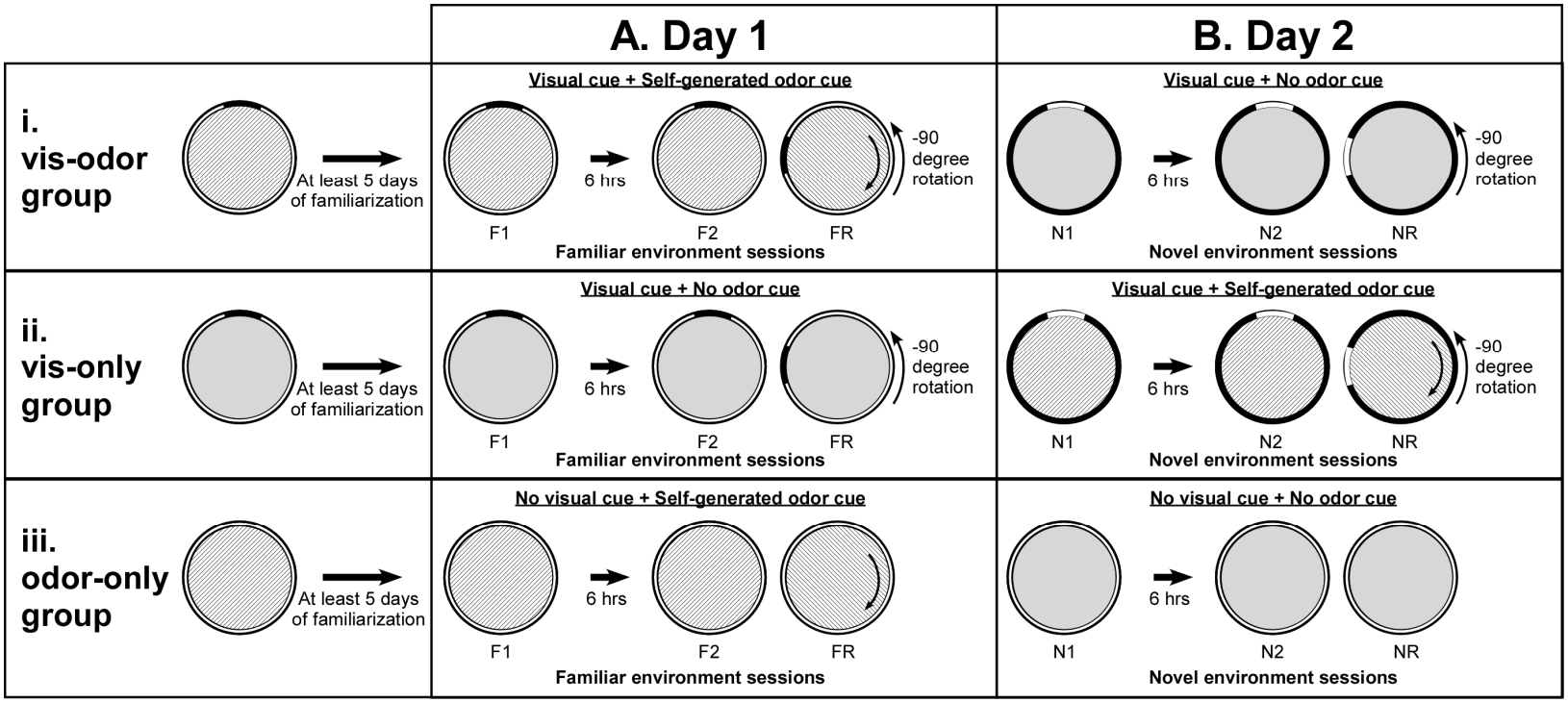

Figure 1. Experimental set up for the different groups and different days of experiment. The vis-odor animals were familiarized to visual cues painted inside the cylinder and to preserved self-generated odor cues (excretion) on the floor paper. The vis-only animals were familiarized to visual cues only and to no odor cues, as a fresh floor paper was used every session. Odor-only animals were familiarized to self-odor cues preserved on the floor paper in a blank cylinder.
1A. Day 1, Familiar environment: All the animals underwent an initial recording session (F1), a second stability check session (F2) and a third session after the rotation of environmental cues (FR). **1B.** Day 2, Novel environment: For the vis-odor and odor-only groups, a novel cylinder was used, and odor conditions were switched. For the odor-only group, fresh floor paper was used in the same blank cylinder. Subsequently, three sessions N1, N2 and NR were recorded in the novel environment.

were switched. Under the novel conditions, the vis-odor animals had fresh floor paper for all three sessions thereby removing the previously familiarized odor cues; On the other hand, the same paperboard was reused for all three sessions for each vis-only animal thereby preserving the self-generated odor cues. For the odor-only animals, only the odor condition was altered as they were given a fresh floor paper every session, but the blank cylinder without cues was retained (Fig. 1B, iii). The first and second sessions were identical in the orientation of the environmental cues, and were recorded six hours apart to examine place field stability (sessions N1 and N2). Immediately following the second session, the cylinder was rotated by 90 degrees for the vis-odor animals, who no longer had the preserved odor cues, and the cylinder and the floor paper were counter-rotated for the vis-only animals, and a post-rotation session was recorded (NR).

Data Acquisition

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 Inc., Cranston, RI) to an impedance of 250-750 kΩ. The individual wires of 4 tetrodes along with the ground and reference wires were connected to an EIB-16 electrode interface board (Neuralynx, Bozemann, MT). This combination of EIB-16, tetrodes and ground wires were housed on a teflon stage mounted on depth-adjustable drive screws.

During exploration of the environment, the EIB-16 electrode affixed to the microdrive was connected to an HS-16 operational amplifier head-stage (Neuralynx)

connected to the ceiling of the environment by a flexible tether. The head direction and position were tracked using two LEDs affixed to the head-stage.

Spiking activity was high-pass filtered between 600-6000 Hz and sampled at 32 kHz online using a 24-channel Cheetah system (Neuralynx, Bozeman, MT). Spikes were sorted offline using MClust (A David Redish, University of Minnesota, Twin cities, MN) and Spikesort 3D (Neuralynx). Waveforms were judged to belong to the same neuron if similar cluster boundaries could be applied across sessions. *CA1* pyramidal neurons were identified on the basis of mean firing rate (<6 Hz), sparse firing activity and complex spiking activity as observed online through an oscilloscope. Only cells with clearly separable clusters across all the three sessions of either day 1 or day 2 were included in the analyses.

Data Analysis

Spiking Activity

The spiking activity of single units was associated with the animal's location during the spike. A motion filter of 2 cm/s was used to discard spiking activity during periods of immobility. The position of the animal and the spikes were then organized into 2 x 2 cm bins. The binned spikes were then divided by the binned occupancy to create an unsmoothed rate map. This was convolved with a 3 x 3 Gaussian kernel to create a smoothed rate map.

Correlation Scores

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 2, as well as

between sessions 2 and 3 using a best-fit angle of rotation (see Rotation Analysis, below). Only data from cells having $r_{session1,session2} \geq 0.3$ were included in the subsequent rotation session analyses.

Rotation Analysis

Rate maps from sessions 2 and 3 were compared for the rotation analysis, the session 2 map being rotated in steps of 6 degrees for to find the angle at which it is maximally correlated with the session 3 map, and this best fit angle was reported. It has been shown in mice that place field stability is necessary for spatial task performance, and that a proportion of cells spontaneously remap (Kentros *et al.*, 2004). We wanted to remove these spontaneously remapping neurons from the rotation analysis, and consequently selected the cells with high stability (across 6 hours, between sessions 1 and 2) for the rotation analysis. Since the correlation score between these sessions was used as the measure of stability, we used a moderate correlation score ($r = 0.3$) as the arbitrary threshold. A Kolmogorov-Smirnov *Z* test was performed to compare the distributions of place field rotation.

Firing Properties

The *mean firing rate* was calculated as the total number of spikes divided by the total length of the session (20 minutes) and allowed distinction between high-firing cells (mostly interneurons) and low-firing cells (mostly pyramidal neurons), the arbitrary cut-off selected being 6 Hz. *Spatial coherence* or simply *coherence* was measured by the *z*-transformed Pearson's correlation score between a pixel (a 2 cm x 2 cm bin) and its eight nearest neighbors in the unsmoothed rate map (Kubie et al., 1990). The *peak firing rate* is 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. *Spatial information content* is a measure of the extent to which the firing of a cell can be used to predict the position of the animal, and calculated as $\Sigma p_i (\lambda_i / \lambda) \log_2 (\lambda_i / \lambda)$, 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 (+5 V) for 3 s 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 trans-cardially with 0.9% saline, followed by a 10% formalin solution. Sectioning was performed on a sliding microtome. Coronal sections (50 µm) 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.

CHAPTER III

RESULTS

Previous research has demonstrated the effect of non-spatial, contextual odor cues on the activity of hippocampal pyramidal neurons (Eichenbaum et al., 1987; Ginther et al., 2009; Wood et al., 1999). The primary aim of the present study was to determine how counter-rotation of highly familiar contextual odor cues and intrinsically spatial visual cues impacted on the representation of space by mouse CA1 place cells.

To address this question, we performed three separate manipulations. First, we extensively familiarized two groups of mice to a visually-cued cylindrical arena by placing them in the arena for twenty minutes per day for a minimum of five days. Self-generated odor cues were collected from mice of one group (vis-odor) by reusing absorbent paper board flooring across familiarization sessions that was unique to each mouse. The goal for this group was to entwine visual and contextual odor cues into the spatial representation. The other group (vis-only) had fresh paper flooring for each familiarization session, ensuring that only visual cues guided the representation. We then screened cells for long-term stability of the place fields from identical sessions recorded six hours apart (sessions 1 to session 2), and then tested the rotation of stable place fields when the visual and odor cues were rotated in opposite directions by ninety degrees (session 2 to session 3).

Second, we examined the role of familiarity in the first manipulation by exposing mice to novel self-generated odor and visual cues only once prior to the test of rotation. This was accomplished on the day following the initial manipulation by repeating the three session sequence, but with the flooring conditions of the two groups reversed: the

self-generated odor cues of vis-only mice were preserved, while fresh paper was provided each session for mice previously assigned to the vis-odor group.

Third, we examined whether self-generated odor cues were sufficient by themselves to maintain stable place fields in the absence of visual cues. A separate group of animals were familiarized to self-generated visual cues in a cylinder lacking discrete visual cues. The activity of place cells from these mice was analyzed through the three session sequence in both the familiar and novel conditions.

Counter-rotation of Familiar Visual and Olfactory Cues

Data were collected from a total of 121 place cells (vis-odor: 64, vis-only: 57) in the Familiar condition. Out of this total, 83 cells had stable place fields [Correlation between F1 and F2 $\geq$ 0.3] which were then selected for the rotation analysis. Only cells that had stable fields across the six hour delay were included in the rotation analysis so as to limit the impact of spontaneous instability on the results. The proportion of cells per mouse that met the correlation criterion for each group were similar (vis-only, day 1: 70.2 $\pm$ 14.5 s.e. % of cells, 6 animals; vis-odor, day 1: 60.4 $\pm$ 9.3 s.e. % of cells, 8 animals; t (12) = 0.6, $p > 0.05$). Immediately following F2, the cues were counter-rotated and the mice were returned to the cylinder. As expected, the place fields of the vis-only animals always followed the visual cues as expected (Fig. 2A). However, many of the place fields of the vis-odor animals did not seem to follow the rotation of the visual cues, rotating instead in the direction of the self-generated odor cues or at an intermediate angle (Fig. 2B). To find out if there was a significant difference between the vis-odor and vis-only groups, the distribution of place field rotation (F2 v/s FR) was compared between the groups using the best-fit angle of rotation θ. Direction of rotation of the cylinder was

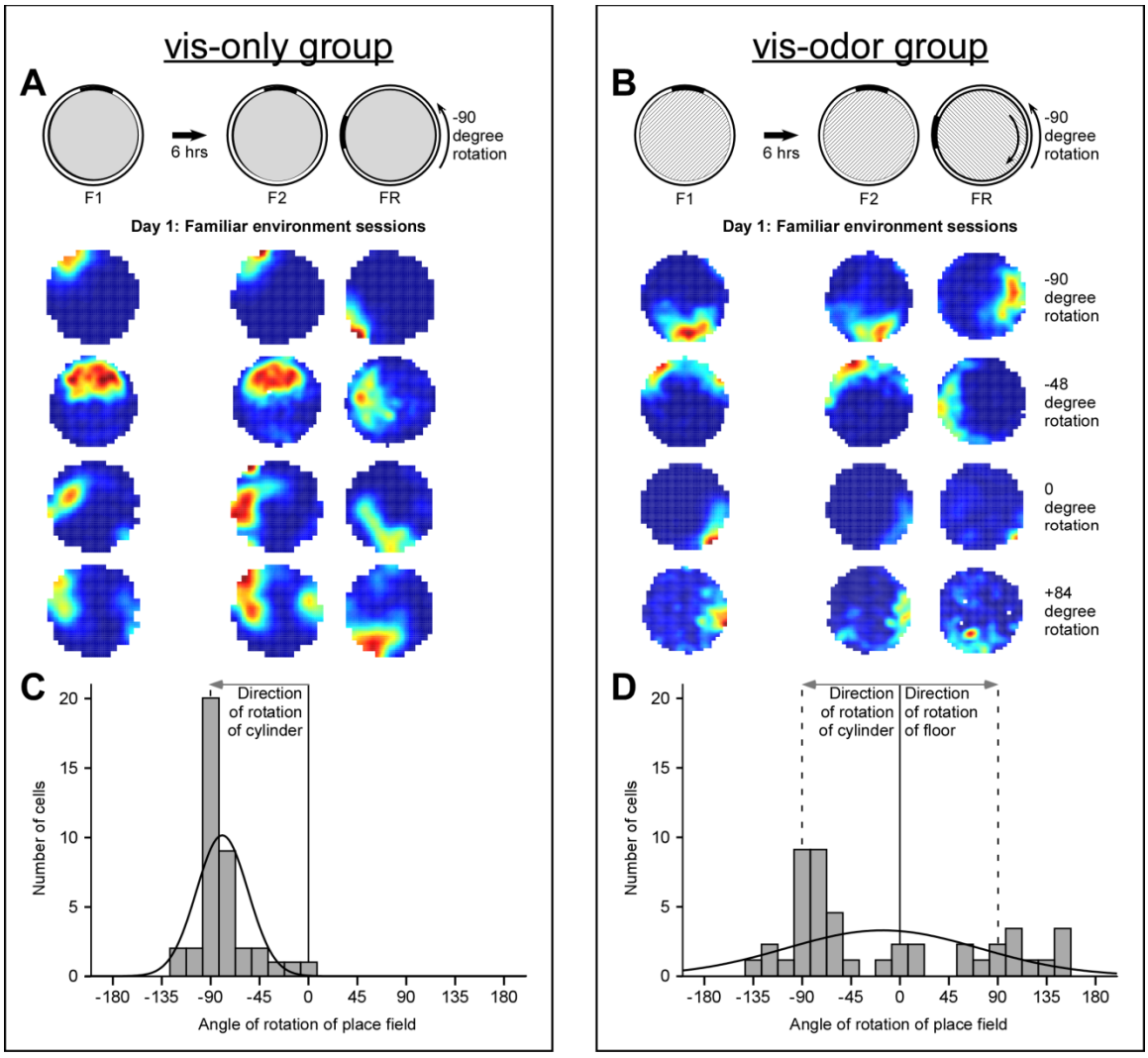

Figure 2. Effect of familiarized visual cues v/s preserved self-generated odor cues on *CA1* pyramidal cells. Sessions F1 and F2 were recorded 6 hours apart, immediately followed by FR, after the visual cues and the odor cues were counter-rotated by 90 degrees. All sessions were 20 minutes long.
Left panel: Vis-only group- only visual cues were familiarized, and rotated.
Right panel: Vis-odor group- animals were familiarized to both visual and odor cues for at least 5 days. Cues counter-rotated.
A. Place fields of the vis-only neurons rotated predominantly with visual cues. **B.** Some place fields of the vis-odor neurons rotated to the visual cue rotation angles, but some also rotated with the odor cues, as well as to intermediate angles. **C & D.** Histograms showing the distribution of angles of *CA1* place field rotation. The distribution was significantly different between the groups (vis-odor group = 43 cells; vis-only group = 40 cells, Kolmogorov-Smirnov $Z = 1.7$, $p < 0.01$).

taken as -90 degrees and the floor rotation angle was taken as +90 degrees. The vis-only group place cells were tightly clustered around the angle of rotation of the cylinder, and none of the fields rotated in the opposite direction (mean θ = -79.5 degrees, S.D. = 23.6 degrees, 87.5% (35 cells) within $\pm$ 30 degrees of cylinder rotation angle) (Fig. 2C). In contrast, a large proportion (37.2%) of the vis-odor place fields rotated away from the direction of cylinder rotation and the general distribution of vis-odor group place cells was broader and centered away from the cylinder rotation angle (mean θ = 15.8 degrees, S.D. = 89.3 degrees, 48.9% (21 cells) within $\pm$ 30 degrees of cylinder rotation angle) (Fig. 2D). The two distributions were significantly different (Kolmogorov-Smirnov Z = 1.7, $p < 0.01$). These analyses indicate that visually-guided field rotations are disrupted by counter-rotation of familiar self-generated odor cues.

Given the group differences for cue rotation, we examined whether these effects could readily be explained by inherent differences in the spatial firing properties of the place cells between groups. No significant differences were found for measures of spatial coherence, spatial information content or field size on comparing the first familiar sessions between the groups (see Table 1). We also looked at the best-fit correlation scores of the neurons before and after the cue rotation for evidence of remapping. Mean best-fit correlation scores were high with no significant difference between the vis-odor and vis-only groups (vis-odor: mean r = 0.55 $\pm$ 0.03 s.e., vis-only: mean r = 0.62 $\pm$ 0.03 s.e., t (81) = -1.43, $p > 0.05$). These data indicate that extensive familiarization to self-generated odor cues did not produce gross differences in *CA1* place fields.

In summary, when familiar visual and odor cues were counter-rotated, rotation to the visual cues was disrupted. The familiarization to odor cues, however, did not have

discernible effects on the long-term stability or the basic firing properties of the place cells.

Table 1. *Mean firing properties during the first familiar session (F1)*

	Group	Cells	Mean	S.D.	S.E.M.
Spatial information content	vis-odor, F1	43	1.45	.81	.12
	vis-only, F1	40	1.33	.66	.10
Spatial coherence	vis-odor, F1	43	.72	.31	.05
	vis-only, F1	40	.84	.29	.05
Field size	vis-odor, F1	43	57.47	48.78	7.44
	vis-only, F1	40	43.20	42.96	6.79

Counter-rotation of Novel Visual and Olfactory Cues

The critical difference in this second manipulation is the reversal of the flooring conditions for the two groups of mice. Data were collected from a total of 103 place cells (vis-odor, with odor cues removed: 54; vis-only, with odor cues preserved: 49) in the novel environment on day 2. The proportion of vis-only cells per mouse that were stable across the six hour delay was comparable to the proportions observed the previous day (vis-only, day 2: 70.7 $\pm$ 8.3 s.e. % of cells, 6 animals). Surprisingly, the proportion of stable vis-odor cells per mouse on day 2 was significantly lower compared with the vis-only group (vis-odor, day 2: 41.2 $\pm$ 8.6 s.e. % of cells, 8 animals; t (12) = 2.41, $p < 0.05$). This result indicates that the absence highly familiar self-generated odor cue impairs the ability to generate stable place fields in a novel environment.

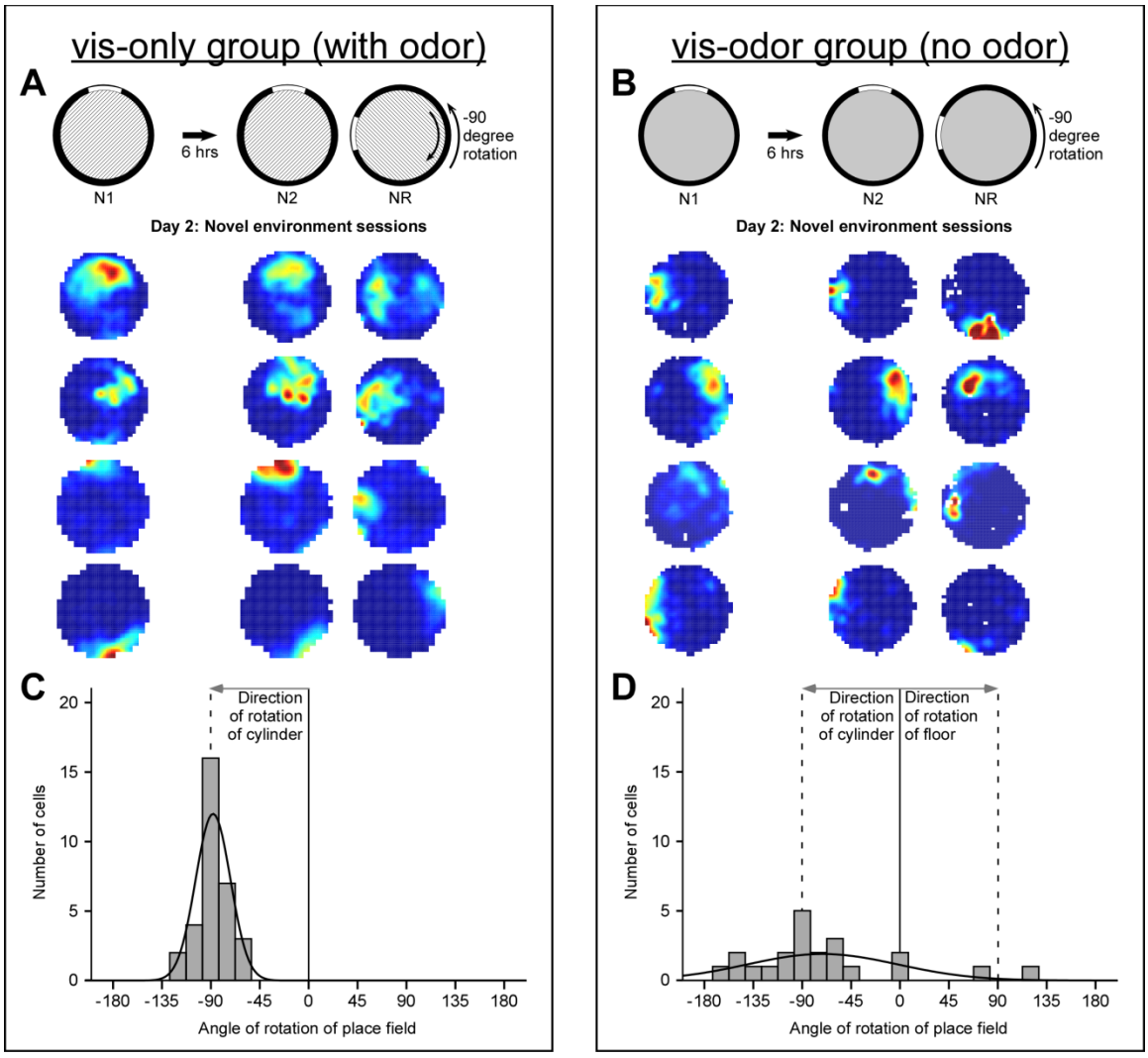

Figure 3. *CA1* place fields under novel visual and olfactory cues (day 2). Sessions N1 and N2 were recorded 6 hours apart, immediately followed by NR, after the visual cues and odor cues were counter-rotated by 90 degrees.
Left panel: Vis-only group- these animals were now provided with preserved odor cues in addition to visual cues, in a novel visual environment. Cues were counter-rotated.
Right panel: Vis-odor group- the animals were placed in a novel visual environment and deprived of familiarized odor cues. Only the visual cues were rotated.
A. Place fields of the vis-only neurons were stable and rotated exclusively with visual cues. **B.** Most place fields of the vis-odor neurons were unstable (59% had correlation scores less than 0.3), but the rotation distribution of the stable cells followed the visual cues. **C & D.** Histograms showing the distribution of angles of *CA1* place field rotation. The rotational distribution for both groups were centered on the angle of rotation of the visual cues and not significantly different (vis-odor group = 22 cells; vis-only group = 32 cells, Kolmogorov-Smirnov $Z = 0.97$, $p > 0.05$).

Place field rotation on the second day of the experiment was compared among a total of 54 stable place cells (vis-odor, with odor cues removed: 22, vis-only, with odor cues preserved: 32). In contrast to the manipulation on day 1, both the vis-odor and vis-only animals rotated predominantly with the visual cues (Fig. 3, A & B). Despite the presence of odor cues, the mean best-fit angle of rotation θ of vis-only fields was centered on the cylinder rotation angle (mean θ = -87.75 degrees, S.D. = 15.9 degrees, 93.8% (30/32 cells) within $\pm$ 30 degrees of cylinder rotation angle). Though the distribution of the vis-odor place cells was more broadly based, it was still centered on the cylinder rotation angle (mean θ = -71.5 degrees, S.D. = 69.3 degrees, 50% (11 cells) within $\pm$ 30 degrees of cylinder rotation angle). A comparison of the two distributions did not differ significantly (Kolmogorov-Smirnov Z = 0.97, $p > 0.05$) (Fig. 3, C & D). Both distributions differed significantly with that of the vis-odor group on day 1 (vis-odor day 1 v/s vis-odor day 2: Kolmogorov-Smirnov Z = 1.38, $p < 0.05$; vis-odor day 1 v/s vis-only day 2: Kolmogorov-Smirnov Z = 1.96, $p < 0.001$). These data indicate that the counter-rotation of familiar, but not novel, self-generated odor cues disrupts field rotation with visual cues.

The decrease in the number of stable fields across the six hour delay following the removal of familiar odor cues was unexpected, as both stability and rotation of these fields was expected to be comparable with fields of the vis-only group on day 1. Investigating this further, we found that the spatial properties of these fields did not differ dramatically from those of the other groups analyzed (see Table 2). The only difference in the properties of cells in the novel cylinder following removal of the familiar odor cues was an increase in field size; this was significantly larger for the vis-odor group on day 2

(N1) compared with the vis-only fields on both days. However, it was not significantly different from the same group on day 1. These results indicate that the removal of familiar self-generated odor cues had minimal effect on the spatial characteristics of place cells.

Table 2. *Mean firing properties during the first familiar and novel sessions for both the vis-odor and vis-only groups*

	Group	Cells	Mean	S.D.	S.E.M.
Spatial information content	vis-odor, F1	43	1.45	.81	.12
	vis-only, F1	40	1.33	.66	.10
	vis-odor, N1	22	1.30	.58	.12
	vis-only, N1	32	1.41	.86	.15
Spatial coherence	vis-odor, F1	43	.72	.31	.05
	vis-only, F1	40	.84	.29	.05
	vis-odor, N1	22	.76	.36	.08
	vis-only, N1	32	.96	.39	.07
Field size	vis-odor, F1	43	57.47	48.78	7.44
	vis-only, F1	40	43.20	42.96	6.79
	vis-odor, N1	22	93.69	83.08	17.71
	vis-only, N1	32	48.52	36.88	6.52

To summarize, novel odor cues did not alter the rotation of fields with the visual cues. Surprisingly, the absence of familiar odor cues significantly reduced the stability of newly formed fields, without dramatically altering the initial development of those fields.

Preservation of Odor Cues in Absence of Directional Visual Cues

Since the previous two manipulations indicated that self-generated odor cues can have a significant influence on visually-guided spatial representations, it was important to determine whether such cues could guide place cell firing independently. We therefore repeated the two manipulations in a featureless cylinder with a separate group of mice.

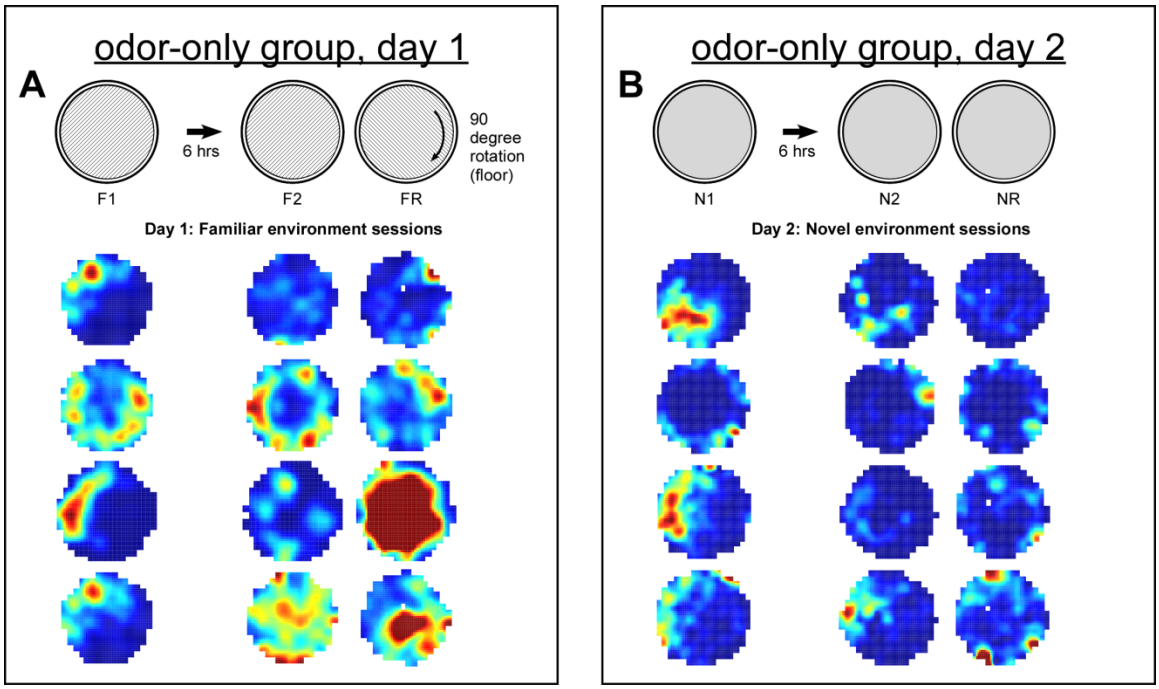

Figure 4. *CA1* place fields in the absence of discrete visual cues: with and without preserved self-generated odor cues.
Left panel: Odor-only group, day 1 (Familiar condition) - animals were placed in a blank cylinder depriving them of discrete visual cues and were familiarized to self-generated odor cues. Sessions F1 and F2 were recorded 6 hours apart, immediately followed by FR after the odor cues had been rotated by 90 degrees.
Right panel: Odor-only group, day 2 (Novel condition)- the odor-only animals were placed in the same blank cylinder, but now the familiar self-generated odor cues had been removed. Subsequently, sessions N1, N2 and NR were recorded.
A & B. Firing rate maps of *CA1* pyramidal neurons showing their place fields. Each row represents firing fields of an individual neuron across the three sessions. Cells of the odor-only animals lacked the characteristic location-specific firing pattern of *CA1* pyramidal neurons seen in the vis-odor and vis-only animals in this study.

Place field data were collected from a total of 20 cells from the odor-only group on day 1 and from a total of 22 cells from the day 2. None of the place fields of the odor-only animals from days 1 and 2 demonstrated the characteristic, location specific firing observed in the vis-only and vis-odor groups from the previous manipulations (Fig. 4, A & B). Stability of across the 6 hour delay for the odor-only group was comparable between day 1 and day 2 and significantly lower on both days than both the vis-odor and

the vis-only groups (Fig. 5A). The means tables and the pairwise comparisons of the correlation scores are given in Table 3 & 4, respectively.

Table 3. *Mean correlation scores of the odor-only, vis-odor & vis-only groups on day 1 and of the odor-only group on day 2*

	Group	Cells	Mean	S.D.	S.E.M.
Session1-Session 2 Correlation scores	odor-only, day 1	20	.13	.26	.06
	odor-only, day 2	22	.20	.27	.06
	vis-odor, day 1	64	.39	.33	.04
	vis-only, day 1	56	.45	.40	.05

Table 4. *Pairwise comparison of correlation scores: the odor-only group on day 1 v/s the vis-odor & vis-only groups on day 1 and the odor-only group on day 2*

Dependent Variable	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.
Correlation scores	odor-only, F1	odor-only, N1	-.07	.10	.981
		vis-odor, F1	-.26*	.09	.032
		vis-only, F1	-.31*	.09	.005

Given the lack of stability in the absence of visual cues with or without odor cues being present, we also compared the spatial firing properties (spatial coherence, spatial information content, and field size) of the odor-only animals during the first familiar session (F1) with those of the vis-odor and vis-only animals to determine whether these cells were capable of developing fields in the presence of odor cues alone. Guided exclusively by self-generated odor cues, place fields exhibited significantly lower spatial information and significantly larger fields compared with F1 visually-guided fields, as well as lower coherence compared with F1 vis-only fields (Fig 5B-D). The means and pairwise comparisons for each of these measures are given in Tables 5 & 6, respectively.

Table 5. *Mean firing properties of the odor-only, vis-odor & vis-only groups on day 1 and of the odor-only group on day 2*

	Group	Cells	Mean	S.D.	S.E.M.
Spatial information content	odor-only, day 1	20	.96	.64	.14
	odor-only, day 2	22	.87	.54	.16
	vis-odor, day 1	64	1.75	1.31	.16
	vis-only, day 1	56	1.43	.78	.10
Spatial coherence	odor-only, day 1	20	.47	.35	.08
	odor-only, day 2	22	.52	.29	.06
	vis-odor, day 1	64	.60	.35	.04
	vis-only, day 1	56	.78	.34	.04
Field size	odor-only, day 1	19	99.62	71.25	16.35
	odor-only, day 2	22	63.56	55.35	11.80
	vis-odor, day 1	64	47.26	44.35	5.54
	vis-only, day 1	56	41.50	39.89	5.33

Table 6. *Pairwise comparison of firing properties: the odor-only group on day 1, v/s the vis-odor & vis-only groups on day 1 and the odor-only group on day 2*

Dependent Variable	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.
Spatial information content	odor-only, F1	odor-only, N1	.10	.31	1.000
		vis-odor, F1	-.78*	.26	.028
		vis-only, F1	-.46	.26	.478
Spatial coherence	odor-only, F1	odor-only, N1	-.04	.11	.999
		vis-odor, F1	-.12	.09	.755
		vis-only, F1	-.31*	.09	.010
Field size	odor-only, F1	odor-only, N1	36.06	18.62	.382
		vis-odor, F1	52.36*	15.53	.011
		vis-only, F1	58.12*	15.78	.004

In summary, stable place fields did not form in the presence of extensively familiarized, self-generated odor cues in an otherwise featureless cylinder. Moreover, in the absence of visual cues, conventional place fields did not develop, with CA1 pyramidal neuron firing evident much more broadly throughout the entire cylinder.

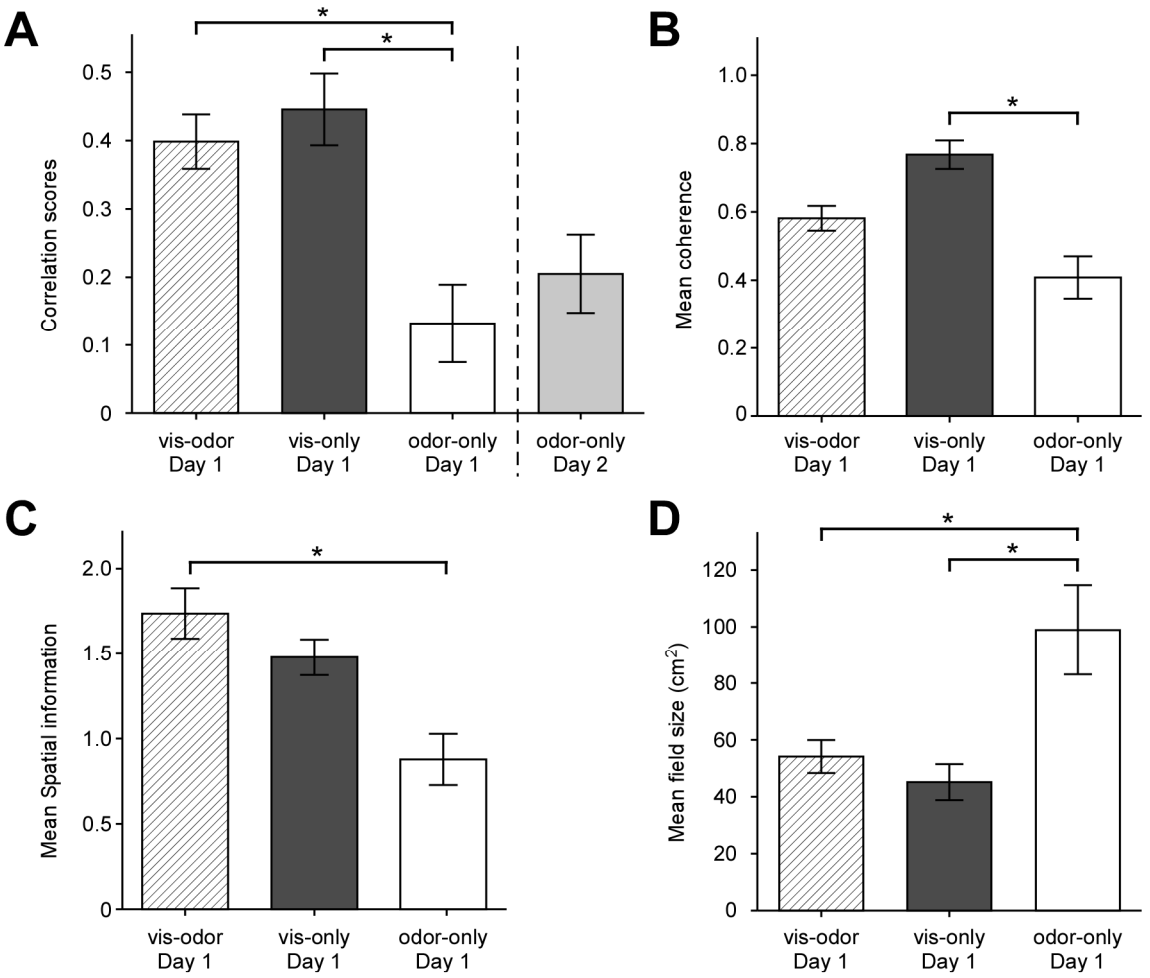

Figure 5. Comparison of place field properties of the odor-only group, with the vis-odor and vis-only groups. **A.** F1-F2 correlation scores of the odor-only group compared to the F1-F2 correlation scores of the vis-odor and vis-only groups, and to the N1-N2 correlation scores of the odor-only group. The place field stability of the odor-only group, even in the familiarized environment, was significantly lower than both the vis-odor and vis-only groups. It was comparable to the stability of the same group under the novel conditions, when neither visual not odor cues were present. **5B.** Mean spatial coherence of the odor-only animals was significantly lower than the vis-only group. **5C.** Mean spatial information content of the odor-only animals was significantly lower than the vis-odor group. **5D.** Mean field size of the odor-only animals was significantly larger than both the vis-odor and vis-only groups.

CHAPTER IV

DISCUSSION

In the present study, we examined the role of highly familiar, self-generated odor cues on the location-specific firing of the *CA1* pyramidal neurons, or 'place cells'. We familiarized mice to a set of visual cues and preserved, self-generated odor cues. Following this familiarization, we examined the stability of the place fields across a six hour delay and then counter-rotated the visual and olfactory cues, putting them into direct conflict with one another. We found that, in the presence of extensively familiarized self-generated odor cues, a significant proportion of the place fields did not rotate with the visual cues, rotating instead either in the direction of the odor cues or to an intermediate angle. In a subsequent manipulation, we found that the absence of the familiar odor cues degraded the ability to develop stable fields to a novel set of visual cues. Importantly, in the absence of discrete visual cues, the ability to form place fields anchored exclusively by self-generated odor cues was virtually non-existent. Taken together, these data provide compelling evidence that contextual, non-spatial odor cues become an integral part of visually-guided spatial representations with familiarity.

After we extensively familiarized animals to visual and self-generated odor cues, we envisioned one of three possible outcomes when the orientation of those cues relative to one another was changed. One possible outcome presumed that fields had been anchored solely by the visual cues, with the result that they would have rotated exclusively with those cues. A second possible outcome was that some or all of the fields would have instead followed the rotation of the highly familiar, self-generated odor cues. The third possible outcome that we identified presumed that familiarization would have

fused the visual and the odor cues to form a representation of the environment, and that counter-rotation of those sets of cues would have represented a different environment, causing the fields to partially or completely remap. In fact, we observed that less than half of the place fields rotated to within 30 degrees of the visual cues, and a significant proportion rotated towards the odor cues or to an intermediate angle instead. On the other hand, place fields in the control animals, familiarized only to the visual cues, rotated exclusively with the visual cues as expected, as shown by previous studies (e.g., Muller and Kubie, 1987). This is a clear demonstration of the fact that familiarized visual cues reliably support the representation of space by the *CA1* place cells, but when there is a conflict of orientation between the visual cues with the self-generated odor cues, the representation of space of a large proportion of the place cell population is disrupted. The effect of the odor cues was not strong enough to drive a definitive rotation of place fields or to cause remapping, as we had predicted, but majority of the place fields lost their orientation even though they did not remap. This implies that long-term familiarization caused the self-generated odor cues to become an integral part of their spatial context, and that the orientation of the self-generated odor cues had formed a component of the place cell's representation of space.

The day following the counter-rotation of extensively familiarized cues, we repeated the three session sequence with the same animals using novel visual and self-generated odor cues. Importantly, the floor odor conditions of the two groups were reversed. Mice that had been familiarized to only the visual cues were instead re-exposed to both visual and self-generated odor cues, and mice previously exposed to both sets of cues were instead guided by visual cues alone. Surprisingly, the animals that had

previously been familiarized to self-generated odor cues demonstrated a significant reduction in the proportion of cells with fields that were stable over the six hour delay when the preserved odor cues were no longer available. Those fields that were stable did rotate with the visual cues. The stability effect was not due to the novelty of the visual cues. The *CA1* place cells of mice re-exposed to both sets of cues developed place fields that were stable over the long-term, and rotated strictly with the visual cues when both sets of cues were counter-rotated. Therefore, we can deduce that the absence of preserved odor cues was the likely reason for the reduction in long-term stability. These observations from day 2 further strengthened the assessment from day 1, that the familiar odor cues had become an integral part of the spatial context. The results on day 2 suggest perhaps that the unexpected absence of the familiar odor cues distracted the mice, disrupting the efficient encoding of the novel visual cues.

Importantly, fields from mice extensively familiarized exclusively to odor cues, in the absence of any discrete visual spatial cues, were not only unstable across the six hour delay, but were in fact lacking in the spatial resolution associated with visually cued place fields even within a single session. Therefore, self-generated odor cues, even in conjunction with idiothetic strategies, were unable to support a stable representation of space.

The above findings allow us define a role for the self-generated odor cues in the representation of space by the *CA1* place cells. The fact that more neurons followed the visual cues during the counter-rotation, and that novel odor cues failed to disrupt this rotation at all confirms the role of the visual cues as the predominant driver of orientation in the environment. However, the fact that some cells did rotate with the odor cues

indicates that the orientation of the odor cues was also learned and remembered. Furthermore, the impact of odor cues was conclusively established by the effect on place field stability when familiar odor cues were no longer available. It is perhaps this latter effect which is in fact most telling. By themselves, odor cues could not stabilize place fields, and relatively novel self-generated odor cues had no influence on rotation to the visual cues. Extensive familiarization to the odor cues in conjunction with visual cues was essential for the main effects we observed. This suggests that the odor cues became an expected part of the context with familiarity, and this familiarity bound the odor cues to the visual spatial cues. Their removal subsequently served as a distractor, limiting the resources that would otherwise normally integrate the new visual cues in the novel environment.

Visual cues are well-localized by nature and the ability to see them from a distance allows them to be stable landmarks. In fact, it has been demonstrated that when the same visual cues were placed in the center of an environment instead of at the periphery, animals could no longer use them as orientation cues (Cressant et al., 1997). This finding points to the fact that distal cues are better than proximal cues for orientation. The odor cues have to be proximal given the paradigm we used and the effective distance for detecting self-generated odors. In addition, a potential problem with olfactory cues is their volatile nature, which negatively affects their permanence and their spatial specificity. All these factors make visual cues better for spatial orientation in relation to odor cues. Nevertheless, odor for a rodent is important both for the purposes of foraging since it helps in find food sources and avoid predator risk. Therefore, it is important for a rodent to recognize the contextual value of odor in conjunction with the

environment associated with it. In corroboration with these principles, we found odor to be a weak orientation cue but a reasonably strong contextual cue when familiarized.

We did find that stable place fields were not formed at all in the absence of visual cues even when self-generated odor cues were present, which is surprising having observed the effects of familiarized odor on the place cells. However, we need to keep in mind that this was a free exploration of an open field, and the studies that have shown evidence of use of odor cues for navigation have involved extensive training and reward-seeking paradigms (Lavenex and Schenk, 1998; Muzzio *et al.*, 2009; Wallace *et al.*, 2002). Several days of familiarization was required to integrate odor into the spatial context, unlike vision that was integrated in a single session. Similarly, we may conjecture that this familiarization might not have been sufficient for the use of odor as an orientation cue, which may have a higher threshold for learning compared to the use of visual cues.

In conclusion, we found that odor cues have a definite spatial effect on the *CA1* place cells in addition to the various non-spatial effects documented before. However, additional study may help to outline the boundaries of such spatial effects and the flexibility of those boundaries.

REFERENCES CITED

Ainge JA, Dudchenko PA, Wood ER. 2008. Context-dependent firing of hippocampal place cells: does it underlie memory? In: Mizumori SJ, editor. Hippocampal place fields: Relevance to learning and memory. New York, NY: Oxford University Press.

Anderson MI, Jeffery KJ. 2003. Heterogeneous modulation of place cell firing by changes in context. J Neurosci 23(26):8827-35.

Cohen NJ, Eichenbaum H. 1994. Memory, Amnesia, and the Hippocampal System. Cambridge, MA: MIT Press.

Cressant A, Muller RU, Poucet B. 1997. Failure of centrally placed objects to control the firing fields of hippocampal place cells. J Neurosci 17(7): 2531–2542.

Eichenbaum H, Kuperstein M, Fagan A, Nagode J. 1987. Cue-sampling and goal-approach correlates of hippocampal unit-activity in rats performing an odor-discrimination task. J Neurosci 7(3):716-732.

Eichenbaum H, Stewart C, Morris RG. 1990. Hippocampal representation in place learning. J Neurosci 10(11):3531-42.

Ginther MR, Walsh DF, Ramus SJ. 2009. The representation of sequential events in the hippocampus and neocortex. Society for Neuroscience Abstract Viewer and Itinerary Planner 39.

Gray CM, Maldonado PE, Wilson M, McNaughton B. 1995. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. J Neurosci Methods 63(1-2):43-54.

Hayman RM, Chakraborty S, Anderson MI, Jeffery KJ. 2003. Context-specific acquisition of location discrimination by hippocampal place cells. Eur J Neurosci 18(10):2825-34.

Kentros CG, Agnihotri NT, Streater S, Hawkins RD, Kandel ER. 2004. Increased attention to spatial context increases both place field stability and spatial memory. Neuron 42(2):283-295

Kentros C. 2006. Hippocampal place cells: the "where" of episodic memory? Hippocampus 16(9):743-54.

Krebs JR, Sherry DF, Healy SD, Perry VH, Vaccarino AL. 1989. Hippocampal specialization of food-storing birds. Proc Natl Acad Sci U S A 86(4):1388-92.

Kubie JL, Muller RU, Bostock E. 1990. Spatial firing properties of hippocampal theta cells. J Neurosci 10(4):1110-23.

Lavenex P, Schenk F. 1998. Olfactory traces and spatial learning in rats. Animal Behaviour 56:1129-1136.

Lever C, Burgess N, Cacucci F, Hartley T, O'Keefe J. 2002a. What can the hippocampal representation of environmental geometry tell us about Hebbian learning? Biol Cybern 87(5-6):356-72.

Lever C, Wills T, Cacucci F, Burgess N, O'Keefe J. 2002b. Long-term plasticity in hippocampal place-cell representation of environmental geometry. Nature 416(6876):90-4.

Markus EJ, Barnes CA, McNaughton BL, Gladden VL, Skaggs WE. 1994. Spatial information-content and reliability of hippocampal ca1 neurons - effects of visual input. Hippocampus 4(4):410-421.

Markus EJ, Qin YL, Leonard B, Skaggs WE, McNaughton BL, Barnes CA. 1995. Interactions between location and task affect the spatial and directional firing of hippocampal-neurons. J Neurosci 15(11):7079-7094.

Moita MA, Rosis S, Zhou Y, LeDoux JE, Blair HT. 2003. Hippocampal place cells acquire location-specific responses to the conditioned stimulus during auditory fear conditioning. Neuron 37(3):485-97.

Morris RG, Garrud P, Rawlins JN, O'Keefe J. 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297(5868):681-3.

Muller RU, Kubie JL. 1987. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J Neurosci 7(7):1951-68.

Muzzio IA, Levita L, Kulkarni J, Monaco J, Kentros C, Stead M, Abbott LF, Kandel ER. 2009. Attention Enhances the Retrieval and Stability of Visuospatial and Olfactory Representations in the Dorsal Hippocampus. PloS Biology 7(6).

O'Keefe J, Burgess N. 1996. Geometric determinants of the place fields of hippocampal neurons. Nature 381(6581):425-8.

O'Keefe J, Dostrovsky J. 1971. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34(1):171-5.

O'Keefe J, Speakman A. 1987. Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res 68(1):1-27.

Save E, Paz-Villagran V, Alexinsky T, Poucet B. 2005. Functional interaction between the associative parietal cortex and hippocampal place cell firing in the rat. Eur J Neurosci 21(2):522-530.

Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20(1):11-21.

Smith DM, Mizumori SJ. 2006a. Hippocampal place cells, context, and episodic memory. Hippocampus 16(9):716-29.

Smith DM, Mizumori SJ. 2006b. Learning-related development of context-specific neuronal responses to places and events: the hippocampal role in context processing. J Neurosci 26(12):3154-63.

Tulving E. 2002. Episodic memory: from mind to brain. Annu Rev Psychol 53:1-25.

Wallace DG, Gorny B, Whishaw IQ. 2002. Rats can track odors, other rats, and themselves: implications for the study of spatial behavior. Behavioural Brain Research 131(1-2):185-192.

Wang M, Wann EG, Yin L, Florian C, Abel T, Muzzio IA. 2010. Remembering the smell of fear: The role of the hippocampus in predator odor fear conditioning. Society for Neuroscience Abstract Viewer and Itinerary Planner 40.

Wood ER, Dudchenko PA, Eichenbaum H. 1999. The global record of memory in hippocampal neuronal activity. Nature 397(6720):613-616.