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The Influence of Objects on Place Field Expression and Size in Distal Hippocampal CA1

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Abstract

The perirhinal and lateral entorhinal cortices send prominent projections to the portion of the hippocampal CA1 subfield closest to the subiculum, but relatively little is known regarding the contributions of these cortical areas to hippocampal activity patterns. The anatomical connections of the lateral entorhinal and perirhinal cortices, as well as lesion data, suggest that these brain regions may contribute to the perception of complex stimuli such as objects. The current experiments investigated the degree to which 3-dimensional objects affect place field size and activity within the distal region (closest to the subiculum) of CA1. The activity of CA1 pyramidal cells was monitored as rats traversed a circular track that contained no objects in some conditions and 3-dimensional objects in other conditions. In the area of CA1 that receives direct lateral entorhinal input, three factors differentiated the objects-on-track conditions from the no-object conditions: more pyramidal cells expressed place fields when objects were present, adding or removing objects from the environment led to partial remapping in CA1, and the size of place fields decreased when objects were present. Additionally, a proportion of place fields remapped under conditions in which the object locations were shuffled, which suggests that at least some of the CA1 neurons' firing patterns were sensitive to a particular object in a particular location. Together, these data suggest that the activity characteristics of neurons in the areas of CA1 receiving direct input from the perirhinal and lateral entorhinal cortices are modulated by non-spatial sensory input such as 3-dimensional objects.

Keywords

aging; entorhinal cortex; perirhinal cortex; place fields; plasticity; rat

Introduction

The hippocampus is hypothesized to play a role in episodic memory by associating salient sensory information with the environment in which it was experienced (e.g., Eichenbaum et al., 2007; Nadel et al., 1985). Moreover, an intact hippocampus is required for the normal ability to use the context in which a stimulus was perceived to later facilitate recognition (i.e., the capacity for stimulus recollection; Fortin et al., 2004). Importantly, both episodic memory (e.g., Mishkin et al., 1998) and recollection (e.g., Fortin et al., 2004) involve the

association of stimulus features with a spatial context. Principal cells within the hippocampus increase their firing rates at discrete locations within an environment (i.e., they express place fields; O'Keefe and Dostrovsky, 1971), and an animal's preparedness for movement is required for this activity to be expressed (Foster et al., 1989). Therefore a large body of empirical research describes the effect of movement-related information (e.g., Foster et al., 1989; Maurer et al., 2005; McNaughton et al., 1983a; Terrazas et al., 2005) and spatial input (e.g., Brun et al., 2008; Fyhn et al., 2007; McNaughton et al., 1983a; Muller and Kubie, 1987; O'Keefe, 1976; O'Keefe and Conway, 1978; O'Keefe and Dostrovsky, 1971) on hippocampal neuron firing characteristics. Recently, it has been shown that a portion of hippocampal neurons in rodents respond to individual stimuli (e.g., Manns and Eichenbaum, 2009; Muzzio et al., 2009; Shapiro et al., 1997). Although there is evidence that hippocampal neurons respond to objects or to the conjunction of a stimulus in a certain spatial location (Komorowski et al., 2009; Manns and Eichenbaum, 2009), it is not known whether this information influences the probability that a CA1 pyramidal cell will express a place field, or whether there is an effect of objects on place field size.

Importantly, the information content of the afferent input to CA1 is segregated. Unlike CA3 pyramidal cells and granule cells of the dentate gyrus, in which inputs from the medial and lateral entorhinal cortices converge onto the same cells, the medial and lateral entorhinal cortical inputs to CA1 are segregated. The CA1 pyramidal cells that reside closest to the CA2 border (i.e., proximal CA1) receive afferent input from the medial entorhinal cortex, while the cells closer to the subiculum (i.e., distal CA1) receive projections from the lateral entorhinal cortex as well as a direct projection from the perirhinal cortex (Amaral and Witter, 1995). These anatomical differences between distal and proximal CA1 are notable because the lateral and medial entorhinal cortices show anatomical, functional and physiological differences. The medial entorhinal cortex is heavily interconnected with the postrhinal cortex and other cortical areas that are involved in the processing of information related to space and movement. In contrast, the lateral entorhinal cortex shares reciprocal connections with the perirhinal cortex and cortical regions that are necessary for stimulus perception (e.g., Burwell, 2000; Burwell and Amaral, 1998; Furtak et al., 2007; Hargreaves et al., 2005). Therefore, within CA1, information related to space and movement is segregated from information related to sensory perception.

Because of differences in anatomical connectivity, lesions of the perirhinal and lateral entorhinal cortices produce behavioral deficits that are dissociable from lesions of the postrhinal and medial entorhinal cortices. Damage to the perirhinal cortex leads to object recognition impairments (Norman and Eacott, 2005; Winters and Bussey, 2005), and lateral entorhinal cortical lesions produce deficits in visual discrimination (Myhrer and Andersen, 2001). Conversely, lesions of the postrhinal (Norman and Eacott, 2005) and medial entorhinal (Steffenach et al., 2005) cortices result in spatial memory impairments.

Given the anatomical and functional distinctions between the medial and lateral entorhinal cortices, it is not surprising that the neurons in these areas also show different physiological correlates with behavior. Neurons in layers II and III of the medial entorhinal cortex show strong spatial selectivity (Fyhn et al., 2004; Hafting et al., 2005; Hargreaves et al., 2005). These cells are activated when the animal's position coincides with any vertex of a regular grid of equilateral triangles spanning the surface of the environment, earning these neurons the name 'grid cells' (Hafting et al., 2005). In contrast to the strong correlation of activity in the medial entorhinal cortex with an animal's position in space, the lateral entorhinal cortex shows very little spatial selectivity (Hargreaves et al., 2005).

Thus, it appears that the medial entorhinal cortex exerts a strong influence on the place-specific firing characteristics of CA1 pyramidal neurons (e.g., Brun et al., 2008;

McNaughton et al., 2006; Solstad et al., 2006). For example, the scale at which space is represented (that is, the size of place fields and grid cell firing field size and spacing) increases systematically along the dorsoventral axis in both the hippocampus (Jung et al., 1994; Maurer et al., 2006a; Maurer et al., 2005) and the medial entorhinal cortex (Hafting et al., 2005). This suggests that the spatial metric of medial entorhinal cortical cell output modulates the spatial gain of the hippocampus (McNaughton et al., 2006). Moreover, place field remapping in CA1 is associated with a realignment of the orientation of the grid patterns of medial entorhinal cortical cells (Fyhn et al., 2007). In contrast to medial entorhinal cortical input, little is known regarding how perirhinal and lateral entorhinal cortical projections modulate CA1 place field size, expression and map stability.

Although it is known that CA1 neuron activity can carry some information about object identity, this effect appears to be relatively weak compared to information about spatial location (Cressant et al., 1997; Manns and Eichenbaum, 2009). There are two possible reasons for the small effect sizes of objects on CA1 activity patterns that have been reported. First, previous studies have recorded from CA1 pyramidal cells that are further from the subiculum and closer to CA2, which may not receive direct lateral entorhinal (Amaral and Witter, 1995) and perirhinal cortical input (Naber et al., 1999). Second, only dorsal areas of CA1 have been examined (Battaglia et al., 2004; Cressant et al., 1997; Cressant et al., 1999; Manns and Eichenbaum, 2009). The probability of place field expression is higher in dorsal relative to intermediate CA1 (Jung et al., 1994; Maurer et al., 2006a). Additionally, the average size of place fields is smaller in dorsal compared to intermediate CA1 (Jung et al., 1994; Maurer et al., 2005). Thus, in dorsal CA1 it may be difficult to detect an effect of increasing sensory input on the number of place fields, because the probability of expression is closer to ceiling levels under baseline conditions. Likewise, it may also be difficult to measure a significant decrease in place field size when the fields are already relatively small.

The current experiment recorded CA1 neurons from the distal hippocampus (area nearest the subiculum) in order to investigate how adding 3-dimensional objects of different sizes, shapes and textures might alter place field characteristics in areas that receive direct lateral entorhinal (Amaral and Witter, 1995) and perirhinal (Naber et al., 1999) input. Neurons at levels intermediate between dorsal and ventral CA1 were recorded from, as opposed to the standard dorsal coordinates, in order to increase the possibility of detecting an effect of objects on place field expression and size as young rats circumnavigated a track for food reward.

Methods

Subjects and behavioral training

Electrophysiological studies were conducted on four Fischer-344 male rats between 8 and 12 months old. The rats were housed individually and maintained on a 12:12 light–dark cycle, and all training and electrophysiological recordings took place during the dark phase of the rats' light–dark cycle.

During electrophysiological recordings, the animals were food deprived to about 85% of their *ad libitum* weight and trained to circumnavigate a circular track (~335 cm in circumference) in both the counterclockwise and clockwise directions for food reinforcement. The food reward was a mixture of rat food pellets made soft by soaking them in water, applesauce, and the diet supplement *Ensure*. Food rewards were given in a small plastic food dish (4 cm × 4 cm) at two positions on the track. Both food dishes were located at the position on the track that marked the completion of one lap on opposite sides of a barrier, that is, where the rat was required to turn around and run in the opposite direction (Figure 1A). During all electrophysiological recording sessions, rats were required to run 20

laps (10 in the counterclockwise direction and 10 in the clockwise direction) during two distinct episodes of behavior. Each track running epoch was flanked by a rest period in which the rat was placed in a towel-lined pot located in a position that was central to the circumference of the track. Thus, the activity of CA1 neurons was monitored during an initial 20 min rest session (before behavior), during the first epoch of track running, during a second 20 min rest session after the first behavioral epoch, during a second epoch of track running, and then finally during a third 20 min rest period. Data from the rest periods were used to assess firing stability across the entire recording session.

After learning to circumnavigate the circular track in both directions for a food reward, all four rats participated in three different behavioral procedures. Thus, the track and the environment were familiar to the rats before the electrophysiological data were collected. During the first procedure (Day 1) rats ran on an empty track (Figure 1A) for both epochs of behavior (**no objects, both epochs condition**; Table 1). For the second procedure (Day 2), during epoch 1, eight novel objects that varied in size (~5–13 cm in width, and 4–15 cm in height), color and texture were placed at eight different locations along the track (Figure 1B). During the second epoch of Day 2, six of the same objects used in epoch 1 remained in the same location on the track while two objects (pseudo-randomly selected from different locations) were replaced with novel objects (**objects, both epochs condition**; Figure 1B; Table 1). These eight objects from the second epoch of Day 2 were then placed on the track again for the third procedure (Day 3). The rats ran 20 laps around these familiar objects that were located in familiar locations. During epoch 2 of Day 3, the positions of the eight familiar objects were pseudo-randomly shuffled such that no object was located in the same position between epochs 1 and 2 (**configuration change condition**; Figure 1B; Table 8.1). Each rat completed all of these behavioral procedures on three consecutive days, and this process was repeated four times for each rat for a total of 12 days of electrophysiological recording. Eight unique objects were used during epoch 1 of every object condition, except the configuration change condition.

In two of the four rats, electrophysiological recordings were conducted during two additional behavioral procedures. These included a “**no object-objects condition**” (Table 1), and an “**objects-no objects condition**” (Table 1). For the no objects-objects condition, the rats ran 20 laps during epoch 1 with nothing on the track except the two food dishes and the divider. During epoch 2, however, eight novel objects were placed on the track. Conversely, in the objects-no objects condition, eight novel objects were placed on the track for epoch 1, but these objects were then removed during epoch 2. The rats that were exposed to these conditions participated in both the objects-no objects and no objects-objects conditions four times (eight additional days). For these additional conditions, 8 unique objects were used for every recording session.

For all conditions in which objects were placed on the track, the objects were fixed in place using *Velcro*, thus, rats could actively explore, rear, and climb onto the objects without displacing them. Additionally, during all rest periods the objects were removed from the track so that the rat could not see them during the intervening 20 minute delay.

Surgical Procedures

Surgery was conducted according to National Institutes of Health guidelines for rodents and protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Prior to surgery, the rats were administered penicillin G (30,000 units intramuscularly in each hind limb) to combat infection. The rats were implanted with a “hyperdrive” manipulator device that held an array of 14 separately moveable “microdrives”. The hyperdrive recording device, implantation methods, and the parallel recording methods have been described in detail elsewhere (Gothard et al., 1996). Briefly,

each hyperdrive consisted of 14 drive screws coupled by a nut to a guide cannula. Twelve of these cannulas contained tetrodes (McNaughton et al., 1983b; Recce and O'Keefe, 1989), four-channel electrodes constructed by twisting together four strands of insulated 13 μm nichrome wire (H. P. Reid, Inc., Neptune, NJ). Two additional tetrodes with their individual wires shorted together served as an indifferent reference and an electroencephalographic recording probe. A full turn of the screw advanced the tetrode 318 μm . The hyperdrive was grounded through the animal using a ground wire connected to a dental screw that was placed over the left parietal cortex that touched the surface of the brain. For all four rats, recordings were made from the distal (closest to the subiculum) intermediate CA1 region, which receives a direct projection from neurons in the perirhinal cortex and from neurons in layer III of the lateral entorhinal cortex. In two rats the implant coordinates were 6.0 posterior, 3.0 lateral to bregma, and angled 14° towards the midline. The implant coordinates for the other two rats were more lateral (6.0 posterior, 5.5 lateral to bregma, and angled 14° towards the midline), but in each of these rats several tetrodes were medial enough to reach the portion of the CA1 subregion closer to the subicular border. Tetrode placement was verified histologically. Figure 2A shows a representative example of tetrode recording tracks in this region of CA1. Figure 2B shows the areas of CA1 in which pyramidal neuron recordings were obtained from the four rats.

During surgical implantation the rats were maintained under anesthesia with isoflurane administered at doses ranging from 0.5% to 2.5%. The implant was cemented in place with dental acrylic anchored by small screws. Immediately after surgery, all tetrodes were lowered approximately 1 mm into the cortex, and rats were orally administered 26 mg of acetaminophen (Children's Tylenol Elixir, McNeil, PA) for analgesia. Oral administration of acetaminophen was continued for 3–5 days after surgery. Additionally, all rats were given either 25 mg of ampicillin (Bicillin, Wyeth Laboratories, Madison, NJ) or a combination of 20 mg of sulfamethoxazole and 0.4 mg trimethoprim (Hi-Tech Pharmacal Co., Inc, Amityville, NY) on a 10 days on/10 days off regimen for the duration of the experiment.

Neurophysiology

The tetrodes were lowered after surgery towards the hippocampus, allowed to stabilize for several days just above the intermediate CA1 hippocampal subregion, and then gradually advanced into the CA1 stratum pyramidale. The neutral reference electrode was placed in or near the corpus callosum. During recording sessions, the four channels of each tetrode were attached to a 50-channel unity-gain head stage (Neuralynx, Inc., Tucson, AZ). A multi-wire cable connected the head stage to digitally programmable amplifiers (Neuralynx, Inc.). The head stage also contained an array of LEDs that could be detected by an overhead CCD camera, enabling the tracking of the position of the rat on the track at 60 frames per second. The sampling resolution was such that a pixel was approximately 0.3 cm.

All data were recorded using a Cheetah recording system (Neuralynx). Spike signals from each tetrode channel were amplified by a factor of 1,000–5,000 (depending on the signal amplitude), filtered between 0.6 and 6 kHz (Assembly Hunter amplifiers; Neuralynx), and digitized at 32 kHz. Single units were recorded with respect to a reference electrode placed in or near the corpus callosum, and spike events that reached a predetermined threshold were recorded for 1 msec.

Electroencephalogram (EEG) data were collected from each tetrode with respect to the corpus callosum reference. The EEG from the tetrode with the most pyramidal cell activity was analyzed and filtered for the hippocampal theta rhythm. This is a prominent 6–10 Hz oscillation that occurs in the hippocampus during active exploration (Vanderwolf, 1969). The theta signal was band pass-filtered between 1 and 300 Hz and sampled at a frequency of 2.4 kHz.

Putative single neurons were isolated using an interactive procedure based on the relative action potential amplitudes on the different tetrode channels and other wave-form parameters (McNaughton et al., 1983b; Wilson and McNaughton, 1994). Initially spikes were sorted by means of a semi-automated clustering algorithm (KlustaKwik, author: K. D. Harris, Rutgers–Newark). The resulting classification was corrected and refined manually with custom-written software (MClust, author: A. D. Redish, University of Minnesota; updated by S. L. Cowen and D. R. Euston, University of Arizona), resulting in a spike-train time series for each of the well-isolated cells. Pyramidal cells were identified on the basis of wave shape, a bimodal interspike interval distribution reflecting complex bursting, and a mean firing rate below 5 Hz across the recording session (Muller et al., 1987; Ranck, 1973). Position during each video frame was extracted by finding the center of mass of the image of the head stage LEDs.

Analyses and Statistics

Place field occupancy normalized firing rate histograms and spike raster diagrams were constructed by plotting the circular trajectories of the animals on a linearized, one-dimensional scale, using a linear interpolation (Maurer et al., 2005). Place field location and size was identified by capitalizing on the observation that a unitary place field does not extend beyond a single cycle of theta phase precession (Maurer et al., 2006a). It is well documented that as a rat passes through a CA1 neuron's place field, the spike timing shows a systematic shift such that the spikes occur at successively earlier phases of the theta cycle until there is a complete cycle of phase advancement (O'Keefe and Recce, 1993; Skaggs et al., 1996). By using the phase precession criterion for place field size, the start location of a field was defined as the location at which spikes fired late in theta phase (approximately 360°) and began a cycle of precession. The end location of the place field was defined as the location of spikes with the largest phase angle. This was accomplished by creating a two-dimensional plot of occupancy normalized position (*x*-axis) versus phase of spikes (*y*-axis), as well as occupancy normalized position versus phase density plots. To construct the theta phase of firing versus position plots, each spike was assigned a nominal theta phase. Precisely, the phase assigned to a spike at time *t* was $360^\circ (t - t_0)/(t_1 - t_0)$, where *t*₀ and *t*₁ are the times of the preceding and following peaks of the filtered reference EEG signal (Skaggs et al., 1996). The phase is always a number between 0 and 360. Place field boundaries were manually drawn using the theta phase precession information by an experimenter who was blind to the behavioral condition of the recording session. Place fields that overlap with a food dish often show an abrupt halt in theta phase precession and can show considerably less than 360° of precession. For this reason, place fields that overlapped with a food dish were excluded.

No attempt was made to match cells from one daily session to the next, and, therefore, the numbers of recorded cells reported does not take into account possible recordings from the same cells on consecutive days. Because none of the statistical analyses were calculated using single cells as the number of observations (see below), however, the potential overlap in the recorded cell population from one to the next is not likely to skew the results.

For analyses that were conducted on 4 rats, the mean of the dependent variable of interest was determined for each rat, and all statistics were calculated using a rat mean (*n* = 4) rather than the cell mean, which can inflate statistical power due to the large number of observations. For analyses that involved comparisons between epochs 1 and 2 of the object-no object and no object-objects conditions, that only 2 rats completed, means were calculated for each rat for the two different behavioral conditions (objects-no objects and no objects-objects) separately. This resulted in an *n* of 4. All statistical tests and *p* values were calculated using SPSS 9.0 (Chicago, Illinois), with alpha set at the 0.05 level.

Results

The Effects of Objects on the Probability of Place Field Expression and Firing Rate

The activity of 1036 pyramidal cells from the intermediate CA1 region was recorded in this experiment. A summary of the database from which the current results were derived is given in Table 2. Additionally, Table 2 shows the number of CA1 neurons without a place field versus neurons with one or more place fields. In both epochs 1 and 2, significantly more CA1 pyramidal cells had at least one place field during track-running conditions with objects compared to conditions without objects ($F_{[3,2]} = 9.65$, $p < 0.01$; repeated-measures ANOVA). Consistent with this observation, in the two rats that circumnavigated the track with objects in one epoch but no objects in the other epoch a significantly larger proportion of cells showed place field activity during the epochs with objects compared to epochs with no objects ($T_{[3]} = 3.46$, $p < 0.05$; paired-samples T test). This suggests that, within the same population of CA1 neurons, encountering objects on the track increased the probability of place field expression.

During the objects condition, in which objects appeared in both epochs but were novel during epoch 1 and familiar during epoch 2, there was no significant difference between the proportion of cells with at least one place field between epochs ($T_{[3]} = 0.53$, $p = 0.63$; paired-samples T test). Moreover, the proportion of CA1 neurons that had place field activity was not significantly different between any of the different behavioral conditions with objects ($F_{[3,2]} = 0.60$, $p = 0.62$; repeated-measures ANOVA). This indicates that novelty did not significantly influence the probability of place field expression and that the primary factor that increased the proportion of active CA1 neurons in the intermediate region of the hippocampus nearest the subicular border was the presence of objects on the track.

Figure 3A shows the mean proportion of cells with zero through eight place fields for conditions without objects (black) versus the behavioral conditions where the rats had to run past objects (grey). When there were no objects on the track, these proportions were comparable to those observed in other multiple-single unit recording studies from the intermediate CA1 region of the hippocampus (Jung et al., 1994; Maurer et al., 2006a). For behavioral conditions with objects on the track, however, there was an approximately 10–15% increase in the proportion of CA1 neurons expressing place fields, and this increase was statistically significant ($F_{[3,2]} = 9.65$, $p < 0.01$; repeated-measures ANOVA). Additionally, the mean number of place fields expressed by each individual CA1 pyramidal neuron that was active during track running was significantly greater for conditions with objects compared to conditions when the track was empty ($F_{[3,2]} = 4.56$, $p < 0.03$; repeated-measures ANOVA). Figure 3B shows the mean number of place fields expressed by the individual CA1 neurons that were active during track running. When the track was empty a single CA1 cell with place specific activity had an average of 1.6 place fields within an epoch of track running, but when the track contained objects each active neuron had an average of 2.1 place fields. Together these data indicate that when rats traverse a track with objects, the probability that an intermediate CA1 pyramidal cell will have a place field increases. Moreover, the neurons with place fields are more likely to have multiple fields within a single environment compared to when the track is empty.

One factor that could contribute to an increase in the probability for a CA1 neuron to express a place field in the behavioral conditions with objects would be an increase in the numbers of cells that had bi-directional fields. Typically, when a rat traverses a fixed path, a CA1 neuron with a place field will only exhibit elevated firing when the rat is running in one direction on the track (McNaughton et al., 1983a). One study has shown, however, that if a track is enriched with tactile cues, principal cells in dorsal CA1 show significantly increased

correlated activity between inbound and outbound journeys (Battaglia et al., 2004). In the current study, when the activity patterns between the clockwise and counterclockwise running directions were correlated, the Pearson's correlation coefficient between directions was 0.37 when the track was empty and 0.35 when the track contained objects, which was not significantly different ($T_{[3]} = 2.21$, $p = 0.11$; paired-samples T test of normalized Fisher's Z scores). Moreover, if significant directional selectivity is defined as having a Pearson's correlation coefficient of less than 0.6 between the firing rates during clockwise versus counterclockwise running, 80.6% of cells recorded during the no object conditions and 79.9% of cells recorded during the epochs with objects showed directional selectivity. Together, these data indicate that an increase in bi-directionality did not contribute to the observed increase in the probability of place field expression in the behavioral conditions with objects. The ~20% of neurons that had bi-directional place fields could be due to the more ventral region of the hippocampus from which these data were obtained. Specifically, relative to the dorsal hippocampus, neurons in more ventral regions are more likely to show significant activity in both running directions of a restricted route (Kjelstrup et al., 2008).

Table 3 shows the single-unit firing characteristics of the neurons recorded during each behavioral condition for epoch 1 and epoch 2. Although, a larger proportion of CA1 cells show place field activity during track-running conditions with objects, there was no significant difference in the overall behavioral firing rate for any of the different track-running conditions during epoch 1 or epoch 2 ($F_{[3,1]} = 1.18$, $p = 0.41$; repeated-measures ANOVA). This suggests that the presence of objects did not affect the global excitability of the intermediate CA1 neuron population. Additionally, there was not a significant effect of track running condition on the mean in-field firing rate during epoch 1 or epoch 2 ($F_{[3,2]} = 0.38$, $p = 0.92$; repeated-measures ANOVA). Finally, the mean firing rate did not vary systematically across laps for any of the behavioral conditions, as there was no significant difference between the first two laps and subsequent laps for any of the behavioral conditions in either epoch 1 or 2 ($p > 0.5$ for all comparisons; paired-samples T tests). This indicates that novelty did not significantly influence the overall firing rate of CA1 neurons. The mean firing rate by lap for the no objects, both epochs; objects, both epochs; and configuration change conditions is shown in Supplemental Figure 1.

Objects and CA1 map stability

The observation that 3-dimensional objects can increase the probability and number of place fields that a neuron will express suggests that objects might influence hippocampal map stability. Figure 4A shows a representative example of the activity of a single CA1 neuron when there are no objects on the track during epoch 1 (left panels), and when objects were on the track for epoch 2 (right panels). Conversely, Figure 4B shows a representative example of the activity of a single CA1 neuron when there were objects on the track during epoch 1 (left panels), and when the objects are removed for epoch 2 (right panels). In Figures 4A and B the top panels show the occupancy normalized firing rate histograms. The bottom panels show the raster plots of spikes by lap, and the bottom panel is the theta phase (y-axis) by position (x-axis). In both examples the spatial firing of the same CA1 neuron was different in the epoch with objects relative to the epoch with no objects. In Figure 4, position on the track (cm) relative to the barrier is the X axis. The negative values indicate that the rat was running in the counterclockwise direction. Firing in the clockwise direction for cells in Figure 4 A and B was not statistically above the overall mean firing rate.

In order to quantify whether or not CA1 place field activity showed global remapping (Leutgeb et al., 2004) between track-running epochs without objects and epochs with objects, within a single recording session, the correlated activity between epoch 1 and epoch 2 was calculated for each neuron. Specifically, the track was divided into 72 bins of ~4.6 cm and the firing rate of an individual neuron was calculated for each bin. Laps that the rats ran

in the counterclockwise direction were measured separately from laps where the running direction was clockwise; therefore, the firing rate was determined for a total of 144 bins resulting in a 144×1 firing rate vector. The Pearson's correlation coefficient between the epoch 1 and the epoch 2 firing rate vectors was then calculated for all CA1 neurons across the five different behavioral conditions (no objects, both epochs; objects, both epochs; configuration change; no objects-objects; and objects-no objects conditions). Figure 5 shows the proportion normalized frequency histograms of the correlation coefficients (r values) of the neurons recorded during each of the five track-running conditions. For the no objects, both epochs (Figure 5A) and the objects, both epochs conditions (Figure 5B) the mean correlations between epochs were 0.65 and 0.72, respectively. This indicates that even though two objects from epoch 1 were replaced with novel objects for epoch 2, this small alteration was not enough to induce a decrease in the between epoch correlation. One explanation for this may be that only a small portion of the recorded neurons had place fields near one of the objects that was replaced, and this was not a sufficient change to impact the mean correlation between epochs significantly. In contrast, the mean correlation for the no-objects, objects (Figure 5D) and objects-no objects (Figure 5E) conditions was 0.49 and 0.55, respectively.

To test whether or not behavioral condition had an effect on the mean correlation between epochs, the Pearson's correlation coefficient for each rat was first converted to a normally distributed Z value with Fisher's r to Z transformation. As anticipated, the CA1 activity between the epochs in which the track-running experience was identical was significantly more correlated than the activity between epochs when the track-running conditions changed (Figure 5F (#); $T_{[3]} = 3.60$, $p < 0.05$; paired samples T-test). Additionally, the mean correlation between epoch 1 and epoch 2 of the configuration change condition (Figure 5C) was 0.60, and intermediate in value between the "same" and "change" conditions. This indicates that at least some of the CA1 neurons' firing patterns were sensitive to a particular object, or to the conjunction of a specific object in a specific location. In contrast, other CA1 neurons continued to fire at the same spatial location even after a different object was placed at that location. Finally, the between epoch place field correlations for the configuration change condition was significantly different from the mean correlation measured during the objects, both epochs condition (Figure 5F (*); $T_{[3]} = 5.61$, $p < 0.05$; paired samples T-test). Figure 5F shows the mean correlation values between epoch 1 and epoch 2 for the different behavioral conditions. These data suggest that changing the location of objects caused some neurons to remap but others continued to fire in the same location on the track regardless of the identity of the nearest object. This suggests that there was a 'partial remapping' of CA1 neuron place fields (Lee et al., 2004; Skaggs and McNaughton, 1998). Because of the close proximity of the objects to each other (~30–40 cm depending on object size), it could not be determined whether or not the place fields nearest an object were the ones that showed global remapping. Specifically, the mean of size of place fields in the object conditions was ~55 cm (see next section), and therefore every place field was near an object. Nonetheless, the observation that at least partial remapping occurred when objects were added or removed from the track, and when object locations were shuffled, suggests that objects did have an influence on place field activity. Future experiments will address whether this relationship is causal by using a larger environment in which objects can be spaced at distances that are greater than the mean size of places fields in intermediate CA1. Therefore, when object location is manipulated it will be possible to measure the extent to which place fields follow individual objects.

Another possible variable that could change with the addition, removal or shuffling of objects could be the within field firing rate. It has been hypothesized that this 'rate remapping' may encode distinct episodes that occur within the same environment (Leutgeb et al., 2005b). Because the Pearson's correlation coefficient used above is robust against

changes in firing rate within fields, the change in firing rate between epoch 1 and epoch 2 was compared across the behavioral conditions for cells with stable place fields between epochs (Pearson's correlation coefficients above 0.6). Figures 6A–C show the change in firing rate between epochs 1 and 2 plotted against the Pearson's correlation coefficient. There was no significant difference in the firing rate change from epoch 1 to epoch 2 between any of the behavioral conditions ($F_{[3,4]} = 3.92$, $p = 0.11$; repeated-measures ANOVA). This suggests that firing rate-remapping did not occur in conditions in which changes were made to the objects between epochs. Although rate remapping has been reported for experimental conditions in which the environment remains the same but local cues change (Leutgeb et al., 2005a; Leutgeb et al., 2005b), and when different objects appear in the same spatial location (Komorowski et al., 2009; Manns and Eichenbaum, 2009), this phenomenon is most robustly observed in the CA3 subregion of the hippocampus (Leutgeb et al., 2005a; Leutgeb et al., 2005b). Rate remapping may not have been observed in the current experiment because all pyramidal cell recordings were obtained from CA1, and all other studies that have reported significant rate remapping included neurons recorded in CA3 (Komorowski et al., 2009; Leutgeb et al., 2005a; Leutgeb et al., 2005b; Manns and Eichenbaum, 2009). Alternatively, it is also possible that the studies showing rate remapping in some CA1 neurons (Komorowski et al., 2009; Leutgeb et al., 2005b; Manns and Eichenbaum, 2009) were recorded from a more proximal region of the dorsal hippocampus, relative to the present experiment. This additional anatomical difference could also partially account for the divergent results.

The Effect of Objects on Intermediate CA1 Pyramidal Cell Field Size

Figure 7A shows a representative example of the activity of a CA1 neuron when no objects were on the track. Figure 7B shows the activity of a different CA1 neuron recorded from the same tetrode in the same rat when the animal was required to circumnavigate the track with objects. Notice that the neuron recorded during the no objects condition (Figure 7A) exhibits activity over larger portion of the track relative to the cell recorded during the condition with objects (Figure 7B), which is particularly evident in the occupancy normalized firing rate histograms (Figure 7; top panels). Moreover, the place field size was consistent throughout all laps, as can be observed in the lap by lap spike raster plots (Figure 7; bottom panels). The example neuron recorded from a no-objects condition was active for more than an 80 cm area of the track compared to only approximately 40 cm for the example neuron recorded during a behavioral condition with objects on the track.

In order to quantify a potential effect of objects on place field size, the spatial extent of fields was determined by measuring the distance that a rat traversed in order for a CA1 neuron's spikes to precess 360° relative to the theta rhythm (see Methods). This analysis revealed that the mean size of place fields for behavioral conditions with no objects on the track was ~80 cm, but the presence of objects on the track significantly reduced the mean size of place fields to ~55 cm ($T_{[3]} = 5.68$, $p < 0.01$; paired-samples T-test). Figure 8A shows the mean size of place fields for conditions with no objects versus conditions with objects.

When both epochs 1 and 2 contained objects or when both epochs did not have objects on the track, the size of place fields was not significantly different between the first epoch of track running and the second ($T_{[3]} = 0.71$, $p = 0.53$; paired-samples T-test). Moreover, place field size was not significantly different between behavioral conditions with novel objects and conditions with familiar objects ($F_{[2,3]} = 2.02$, $p = 0.25$; repeated-measures ANOVA). Together these data indicate that although novelty did not appear to affect place field size, enriching a track with objects led to a significant reduction in the size of CA1 place fields. Figure 8B shows the proportion normalized frequency distribution of place field size when rats traversed a bare track (black) and when the track contained objects (grey). This figure

shows that the range of place field sizes between behavioral conditions with and without objects is similar, which suggests that adding objects to the track does not change the spatial scale of place field size in intermediate CA1. Rather, it appears from Figure 8B that when rats traverse a track with objects, the proportion of small place fields increases and the frequency of large place fields decreases.

The above analyses, however, compared the size of place fields for neurons that were recorded on different days. Thus, with these data, it could not be determined if the same population of neurons can have large place fields when no objects are present but smaller place fields when objects are added to the environment. In order to investigate this possibility, the size of place fields were compared between epoch 1 and epoch 2 for the two rats that performed the objects-no objects and the no objects-objects behavioral conditions. Because many place fields showed global remapping between object versus no object epochs (see Figure 5D and E), it was not possible to determine whether the size of an individual place field, at a given location, was modulated by the presence of an object. In the population of recorded CA1 neurons that re-mapped, the size of the place fields in the condition with objects was significantly smaller than in the condition with no objects ($T_{[3]} = 6.14$, $p < 0.05$; paired-samples T test). These data are consistent with the interpretation that field size is smaller when objects are on the track relative to when the track is empty. Place field size was also quantified using a rate-based definition (the area of the track that the firing rate exceeded 10% of the maximum for at least 5 consecutive 3.84 cm bins), which yielded similar results to the phase precession definition (data not shown).

Importantly, for the behavioral procedures where both epochs of track running had objects on the track or both epochs did not have objects on the track, any change in place field size between epochs was minimal and not statistically significantly different ($F_{[1,3]} = 4.52$, $p = 0.12$; repeated-measures ANOVA). Figure 9 shows the absolute value of the mean difference in place field size between epoch 1 and epoch 2 for the five different behavioral procedures. These data, together with the observation that 3-dimensional objects can affect place map stability (Figure 5), indicate that when objects are introduced or removed from the track, CA1 place fields globally remap. Additionally, when objects are on the track, the mean place field size for the hippocampal map is smaller relative to when the track is bare. In contrast, when objects were on the track for both epochs, or if there were no objects present for both epochs, the size of place fields remained relatively stable between the two epochs of track running.

Consistent with the observation that place fields were smaller in conditions in which the track contained objects relative to when the track was empty, the rate of theta phase precession (i.e., the mean slope of the distance versus phase plots) was different between object and no object conditions. Specifically, the rate of theta phase precession was 3.8 deg/cm in the objects conditions, and 2.4 deg/cm in the no objects conditions (Figure 10A). This difference was statistically significant ($T_{[3]} = 6.94$, $p < 0.01$; paired-samples T test). In contrast, the dispersion of phase precession (i.e., the correlation of phase and distance) did not differ significantly between the no objects and objects conditions (Figure 10B; $T_{[3]} = 0.59$, $p = 0.60$; paired-samples T test).

Additionally, the mean phase shift was compared between the no objects and objects conditions to ensure that the decrease in place field size during the conditions with objects could not be explained by a rat's tendency to not run through the center of the place field when objects were on the track. Specifically, if a rat runs through the edge rather than the center of a field, the total phase shift for a single pass would be less (Skaggs et al., 1996). Figure 11 shows the mean phase shift by passes through a field and the overall mean phase shift for epoch 1 (Figure 11A) and epoch 2 (Figure 11B). During epochs 1 and 2 the mean

phase shift was approximately 180 degrees for both the no objects and objects conditions, and there was no significant difference in phase shift between conditions ($T_{[3]} = 0.61$, $p = 0.55$; paired-sample T test). This phase shift of 180 degrees is similar to previous reports (Harris et al., 2002; Huxter et al., 2008; Mizuseki et al., 2009), and indicates that the rats ran through the center of place fields in both behavioral conditions.

Running Velocity and Path Variability

Rats have a natural tendency to explore objects, or other stimuli, that are novel (Ennaceur and Delacour, 1988). In the current experiment, this naturally occurring behavior could lead to slower overall velocities in behavioral conditions with novel objects, as the rats' running would be interrupted by periods of exploration. Additionally, it is also possible that familiar objects could interfere with the rats' ability to run, and thereby confound the current results. To examine these possibilities we compared the running velocity for the different behavioral conditions with objects versus conditions where the track was bare. Figure 12A shows the mean velocity for all the behavioral conditions when the track was empty (no objects; black) versus the conditions with objects (grey). Overall, there was no significant difference in the rats' running velocities when objects were on the track compared to the no object conditions ($T_{[3]} = 1.48$, $p = 0.24$; student's T test). Additionally, we compared the running velocity of rats during conditions with no objects, novel objects, familiar objects, and familiar objects in novel locations (see Supplemental Figure 2). There was no significant difference in the mean running velocity between these conditions ($F_{[3,3]} = 0.97$, $p = 0.44$; repeated-measures ANOVA), which suggests that the current results were not confounded by differences in running velocity.

In order to examine if novel objects on the track increased exploration and lead to slower velocities, the mean running speed of the rats during the first 2 laps and the last 2 laps were compared over the different behavioral conditions. Figure 12B shows the mean running velocity during laps 1–2 (white) and laps 19–20 (shaded) for conditions with no objects (no objects-both epochs, and no objects-objects), conditions with novel objects (objects, both epochs, and objects-no objects), and the configuration change condition. A comparison between the first and the last two laps was selected because during the novel objects conditions only laps 1–2 had a mean velocity that was significantly different from laps 19–20 ($T_{[3]} = 4.55$, $p < 0.05$; paired-samples T test; $p > 0.1$ for all other comparisons). Moreover, when the running speed during the novel objects conditions was compared to the mean velocity during the no objects conditions it was only during laps 1–2 that the rats ran more than 2 standard deviations slower than the average velocity when the track was empty. The running speed for all laps for the different behavioral conditions is shown in Supplemental Figure 2. Figure 12C shows the mean running velocity during epoch 2 for laps 1–2 (white) and laps 19–20 (shaded) during conditions with no objects in epoch 2 (no objects-both epochs, and objects-no objects), conditions with novel objects (no objects-objects), conditions with familiar objects (objects, both epochs), and the configuration change condition. Statistical analysis revealed that, when all conditions were analyzed together, the rats ran significantly more slowly during laps 1–2 relative to laps 19–20 ($F_{[1,22]} = 32.80$, $p < 0.001$). Post hoc analysis indicates that the difference in running velocity between the first two laps and last two laps was significantly different for conditions with novel objects (epoch 1 of the objects, both epochs condition; epoch 1 of the objects-no objects condition; epoch 2 of the no objects-objects condition; $p < 0.05$ for all comparisons), and for epoch 2 of the configuration change where familiar objects were placed in a novel spatial arrangement ($p < 0.05$). During all behavioral conditions without objects, or with familiar objects in familiar positions on the track, there was no significant difference in running speed between laps 1–2 and laps 19–20 ($p > 0.1$ for all comparisons). Taken together these data are consistent with the idea that rats spent more time exploring objects

when they were the most novel during laps 1–2 of epoch 1, or when familiar objects were placed in novel locations. Once the objects were familiar, however, their presence on the track did not significantly impede the rats' ability to run for a food reward. Therefore, any potential effect of behavioral condition on CA1 pyramidal cell activity cannot be explained by overall differences in velocity.

Another variable that could affect the current results would be differences in the paths that the rats took around the track in the object versus the no object conditions. In order to test whether or not this was a considerable confound “rho” was calculated for the different behavioral conditions. This parameter reflects the distance from the spectral center of the sphere, defined by the edge of the circular track, on a spherical coordinate system. Rho was calculated from the raw video tracker data with the formula: $\rho^2 = X^2 + Y^2$, where X and Y are the position of the rat in Cartesian coordinates (Maurer et al., 2006a; Maurer et al., 2006b; Maurer et al., 2005). A difference in the mean rho between conditions would indicate that the path length varied, while a difference in the standard deviation of rho would suggest that the path variability was affected by objects. Representative examples of the rats' path around the track for the object-no objects, and no objects-objects conditions are shown in Supplemental Figure 3. There was considerable overlap in the path between epochs with and without objects. Additionally, Supplemental Figure 4 shows representative examples of the spiking of CA1 neurons interpolated on a rat's path (and corresponding occupancy normalized rate map) for conditions with and without objects. Moreover, statistical analysis revealed that neither the path length ($T_{[3]} = 1.92$, $p = 0.15$; paired-samples T test) nor the path variability ($T_{[3]} = 0.04$, $p = 0.97$; paired-samples T test) were significantly different when the track was empty versus when the track contained objects, indicating that these variables did not contribute significantly to the present results.

Discussion

The main novel observation from the present experiment is that the activity characteristics of CA1 pyramidal neurons that receive direct input from the perirhinal and lateral entorhinal cortices are different under conditions in which rats traverse an empty track, versus when the track contains salient visual, tactile, and olfactory features. Specifically, compared to when the track was bare, tracks with 3-dimensional objects result in several significant changes in the spiking pattern of these neurons: 1) an increase in the probability for a cell to express a place field (Table 2; Figure 3A); 2) an increase in the mean number of place fields per neuron of those active on the track (Figure 3B); 3) remapping (Figure 5); and 4) a decrease in the mean size of place fields (Figure 8). Although there was a substantial decrease in the proportion of large place fields (>75 cm), and a dramatic increase in small place fields (<40 cm) in the object conditions, the minimum and maximum values for place field sizes was comparable between conditions in this intermediate dorsal/ventral region of the hippocampus (Figure 8B). Since the observed change was a shift in distribution towards smaller place fields, this suggests that the overall spatial scale (Jung et al., 1994; Maurer et al., 2005) of this subregion of CA1 was preserved in the object conditions. Finally, the mean and in-field firing rates during behavior were not different between object and no-object conditions (Table 3), suggesting that the global excitability of distal CA1 is not affected by adding prominent 3-dimensional stimuli to the track.

When rats traversed an empty 335 cm track, approximately 35% of the CA1 neurons recorded in this experiment expressed place fields. This is consistent with the report that approximately 39% of neurons in intermediate CA1 were active on an empty 380 cm track (Maurer et al., 2006a). When objects were added to the track in the current experiment, approximately 48% of distal CA1 neurons in the intermediate hippocampus showed place specific firing (see Figure 3A). Because both novel and familiar objects conditions resulted

in increased place field expression, the degree of familiarity of objects is not likely to explain the increased number of neurons active within an environment.

The present results stand in apparent contrast to those reported by Battaglia et al. (2004) who also compared CA1 activity in 'cue-rich' versus 'cue-poor' environments. In this study, the cue-rich condition led to an increase in the numbers of neurons with bi-directional place fields but the increased number of active neurons in the cue-rich conditions relative to the cue poor conditions did not reach statistical significance (Battaglia et al., 2004). Several possible explanations for these apparent discrepancies can be offered. The recording location in Battaglia et al. (2004) differed from the current experiment both in the location along the dorsal/ventral axis of the hippocampus, and with respect to proximity to the subiculum. Moreover, the types of sensory input used in Battaglia et al. (2004) consisted of different tactile cues placed on the track rather than the 3-dimensional objects used in the present experiment. It is unclear exactly how these different types of stimuli would impact the results obtained in the present experiment and in Battaglia et al., (2004), but at least two hypotheses can be offered regarding how anatomical differences in the locations of recorded neurons could account for different experimental outcomes. First, because the CA1 recordings in Battaglia et al., (2004) were obtained from cells further from subiculum, it is possible that some of the neurons analyzed in their study did not receive direct input from the perirhinal and lateral entorhinal cortices. Neurons that receive a larger portion of medial entorhinal cortical input may not be as responsive to tactile and visual information. Second, the CA1 neurons examined in the Battaglia et al. (2004) study were recorded in a more dorsal region of the hippocampus relative to the current experiment. Because higher proportions of neurons are active in dorsal compared to more ventral hippocampal regions (Jung et al., 1994; Maurer et al., 2005), it is possible that the cue-poor condition in Battaglia et al., (2004) was close to the "ceiling". In the present experiment a smaller proportion of cells are active when the track is empty, and thus the recruitment of additional active cells in the conditions with objects may have been easier to obtain.

Although the percent of active neurons increased, and the mean size of place fields decreased between the no-object and the object conditions, there was no overall increase in the global excitability. That is, the mean and in-field firing rates of CA1 neurons during behavior in both conditions were similar (Table 3). This is consistent with the observation that although place fields were smaller when objects were on the track (Figure 8), each active CA1 neuron was more likely to express multiple place fields relative to when the track was bare (Figure 4B, ~2.3 versus ~1.6 place fields/cell). This suggests that the total area of the track over which a given neuron was active was similar between the no object and object conditions. In fact, when mean place field size was multiplied by the mean number of place fields per active neuron (i.e., the total area over which a place cell fired) this value was ~129 cm for the no-object conditions and ~131 cm for the object conditions, which was not statistically different ($p = 0.94$). Thus, there was a conservation of the amount of space over which a given CA1 neuron fired. A possible consequence of this property is that it may serve to maintain a constant level of excitation in the CA1 neuron population when behavioral conditions change, possibility resulting from homeostatic maintenance of synaptic gain within the hippocampus (Buzsaki et al., 2002).

Importantly, because the increased probability of place field expression was accompanied by a decrease in place field size, these data indicate that the proportion of CA1 neurons active at a given point in space was unchanged between object and no object conditions. Typically 3–5% of CA1 pyramidal cells are active within a discrete region of an environment (estimated from Wilson and McNaughton, 1993; Maurer et al., 2006). Although the probability of place field expression is lower in more ventral regions of CA1, the size of the fields is also larger and this maintains the same proportion of active place fields at any point

in space (Maurer et al., 2006a; Skaggs and McNaughton, 1992). Thus, given a fixed environment size, a difference in place field expression should always correspond to change in place field size. In this view, the current differences observed between the object and no object conditions is consistent with the differences in field size and expression described for the dorsal and ventral hippocampus in the absence of objects (Maurer et al., 2006a). Together, these data indicate that the CA1 network has a mechanism for maintaining a low probability (<5%) of neuronal activity at any single point in space, which may serve to maximize storage capacity.

A number of theories take into account the input required to update the spatial firing of CA1 pyramidal cells (Burgess and O'Keefe, 1996; Samsonovich and McNaughton, 1997). It is postulated that hippocampal activity is tightly coupled to voluntary motion (Samsonovich and McNaughton, 1997), and that variations in the gain of a movement-speed signal to the hippocampus lead to changes in spatial selectivity (for review, see McNaughton et al., 2006; Terrazas et al., 2005). According to this 'path integration' model, the decrease in the responsiveness of ventral CA1 neurons to increasing velocity is the basis for the systematic variation in spatial scaling along the dorsoventral axis of the hippocampus (Maurer et al., 2005). While the path integration model accurately accounts for spatial scaling in the medial entorhinal cortex (Hafting et al., 2005; for review, see McNaughton et al., 2006), it may not completely account for the changes in place field size in intermediate CA1 observed in the present study. It is clear from the current data that place field size within the intermediate portion of distal CA1 can be decreased by providing additional non-spatial information. Moreover, when the object locations were shuffled between epochs of track running, place field activity was significantly less correlated than when the objects remained in the same spatial configuration between epochs (Figure 5F). Together, these data indicate that 3-dimensional objects can influence CA1 spatial representations.

Previous reports of the effects of objects on the location of CA1 place field activity have produced conflicting results, however (Cressant et al., 1997; Cressant et al., 1999; Rivard et al., 2004). Whether these discrepancies are due to the proximity of objects to the boundaries of the environment (Cressant et al., 1999), or to differences in the locations of recorded CA1 neurons remains to be determined. Nonetheless, the current observation that discrete stimuli can influence place field activity is consistent with previous reports that have shown that some place fields follow individual tactile cues when a maze is rotated (e.g., Brown and Skaggs, 2002; Lee et al., 2004; Shapiro et al., 1997), and selectively fire at olfactory cues in a given spatial location (e.g., Komorowski et al., 2009; Muzzio et al., 2009). Moreover, while spatial location appears to be the primary factor that influences the activity of a place field, there is increasing evidence that objects can have an impact on where in an environment a hippocampal pyramidal cell is active (Manns and Eichenbaum, 2009). Therefore, both non-spatial input, presumably arising from lateral entorhinal and perirhinal cortices, and path integration information can operate in concert to adjust place field activity patterns in the hippocampus. The current recording location differs from previous studies both in the proximal/distal axis of CA1, as well as in the dorsal/ventral axis. Thus, it is possible that either or both of these anatomical variables contribute to the present results. Importantly, however, the lateral entorhinal and perirhinal cortices project along the entire extent of the dorsal/ventral axis of CA1 (Amaral and Witter, 1995). Because it is only the distal portion of CA1 that receives direct input from these brain structures (Amaral and Witter, 1995), the most parsimonious explanation for the current results is that the critical variable accounting for the effect of objects on the activity patterns of the CA1 neurons is the distal implant coordinates.

The observation that adding 3-dimensional objects to an environment increases the number of active neurons in a CA1 ensemble, and decreases the mean size of place fields could

provide insight into how spatial and non-spatial information is integrated in the hippocampus to enable memory consolidation (e.g., Moscovitch et al., 2005; Nadel and Moscovitch, 1997; Squire and Zola-Morgan, 1991). It is generally agreed that the neocortex serves as the permanent store of long-term semantic memories (McClelland et al., 1995; Squire and Zola-Morgan, 1991), but the overall sparse interconnectivity of the neocortex (about 10^{-6} on average) makes it difficult to sustain the associations necessary for the rapid formation of new memories directly. Thus, the hippocampus could serve as an indirect link to enable rapid associations among different areas of the neocortex (Marr, 1971). According to this general theory, during the initial encoding of a memory, output from the hippocampus becomes associated with patterns of activity across distinct neocortical areas and thus provides an index code for each memory (e.g., Teyler and DiScenna, 1986; Teyler and Rudy, 2007). If substantially more feature information is added to a familiar environment, and more areas of sensory cortex are activated during behavior, the hippocampal index may need to be altered in order to represent the additional sensory input. The increased numbers of active CA1 neurons and the decrease in place field size could therefore reflect the incorporation of object information into memory. In this view, non-spatial input from the perirhinal and lateral entorhinal cortices may be critical for determining the firing characteristics of distal CA1 neurons that receive direct projections from these regions. Moreover, the influence that these two distinct types of information have on hippocampal activity patterns can account for the involvement of the hippocampus in recollection (Fortin et al., 2004) and episodic memory (e.g., Eichenbaum et al., 2007; Nadel and Hardt, 2004; Nadel et al., 1985), both of which require the association of ‘what’ stimulus was encountered with ‘where’ it was experienced.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Amaral, D.; Witter, M. Hippocampal Formation. In: G, P., editor. The Rat Nervous System. 2nd ed.. San Diego: Academic Press; 1995. p. 443-486.
- Battaglia FP, Sutherland GR, McNaughton BL. Local sensory cues and place cell directionality: additional evidence of prospective coding in the hippocampus. *J Neurosci*. 2004; 24(19):4541–4550. [PubMed: 15140925]
- Brown JE, Skaggs WE. Concordant and discordant coding of spatial location in populations of hippocampal CA1 pyramidal cells. *J Neurophysiol*. 2002; 88(4):1605–1613. [PubMed: 12364491]
- Brun VH, Leutgeb S, Wu HQ, Schwarcz R, Witter MP, Moser EI, Moser MB. Impaired spatial representation in CA1 after lesion of direct input from entorhinal cortex. *Neuron*. 2008; 57(2):290–302. [PubMed: 18215625]
- Burgess N, O'Keefe J. Neuronal computations underlying the firing of place cells and their role in navigation. *Hippocampus*. 1996; 6(6):749–762. [PubMed: 9034860]
- Burwell RD. The parahippocampal region: corticocortical connectivity. *Ann N Y Acad Sci*. 2000; 911:25–42. [PubMed: 10911865]
- Burwell RD, Amaral DG. Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *J Comp Neurol*. 1998; 398(2):179–205. [PubMed: 9700566]
- Buzsaki G, Csicsvari J, Dragoi G, Harris K, Henze D, Hirase H. Homeostatic maintenance of neuronal excitability by burst discharges in vivo. *Cereb Cortex*. 2002; 12(9):893–899. [PubMed: 12183388]

- Cressant A, Muller RU, Poucet B. Failure of centrally placed objects to control the firing fields of hippocampal place cells. *J Neurosci.* 1997; 17(7):2531–2542. [PubMed: 9065513]
- Cressant A, Muller RU, Poucet B. Further study of the control of place cell firing by intra-apparatus objects. *Hippocampus.* 1999; 9(4):423–431. [PubMed: 10495023]
- Eichenbaum H, Yonelinas AP, Ranganath C. The medial temporal lobe and recognition memory. *Annu Rev Neurosci.* 2007; 30:123–152. [PubMed: 17417939]
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res.* 1988; 31(1):47–59. [PubMed: 3228475]
- Fortin NJ, Wright SP, Eichenbaum H. Recollection-like memory retrieval in rats is dependent on the hippocampus. *Nature.* 2004; 431(7005):188–191. [PubMed: 15356631]
- Foster TC, Castro CA, McNaughton BL. Spatial selectivity of rat hippocampal neurons: dependence on preparedness for movement. *Science.* 1989; 244(4912):1580–1582. [PubMed: 2740902]
- Furtak SC, Wei SM, Agster KL, Burwell RD. Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus.* 2007; 17(9):709–722. [PubMed: 17604355]
- Fyhn M, Hafting T, Treves A, Moser MB, Moser EI. Hippocampal remapping and grid realignment in entorhinal cortex. *Nature.* 2007; 446(7132):190–194. [PubMed: 17322902]
- Fyhn M, Molden S, Witter MP, Moser EI, Moser MB. Spatial representation in the entorhinal cortex. *Science.* 2004; 305(5688):1258–1264. [PubMed: 15333832]
- Gothard KM, Skaggs WE, Moore KM, McNaughton BL. Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. *J Neurosci.* 1996; 16(2):823–835. [PubMed: 8551362]
- Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. *Nature.* 2005; 436(7052):801–806. [PubMed: 15965463]
- Hargreaves EL, Rao G, Lee I, Knierim JJ. Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science.* 2005; 308(5729):1792–1794. [PubMed: 15961670]
- Harris KD, Henze DA, Hirase H, Leinekugel X, Dragoi G, Czurko A, Buzsaki G. Spike train dynamics predicts theta-related phase precession in hippocampal pyramidal cells. *Nature.* 2002; 417(6890):738–741. [PubMed: 12066184]
- Huxter JR, Senior TJ, Allen K, Csicsvari J. Theta phase-specific codes for two-dimensional position, trajectory and heading in the hippocampus. *Nat Neurosci.* 2008; 11(5):587–594. [PubMed: 18425124]
- Jung MW, Wiener SI, McNaughton BL. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J Neurosci.* 1994; 14(12):7347–7356. [PubMed: 7996180]
- Kjelstrup KB, Solstad T, Brun VH, Hafting T, Leutgeb S, Witter MP, Moser EI, Moser MB. Finite scale of spatial representation in the hippocampus. *Science.* 2008; 321(5885):140–143. [PubMed: 18599792]
- Komorowski RW, Manns JR, Eichenbaum H. Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. *J Neurosci.* 2009; 29(31):9918–9929. [PubMed: 19657042]
- Lee I, Yoganarasimha D, Rao G, Knierim JJ. Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature.* 2004; 430(6998):456–459. [PubMed: 15229614]
- Leutgeb JK, Leutgeb S, Treves A, Meyer R, Barnes CA, McNaughton BL, Moser MB, Moser EI. Progressive transformation of hippocampal neuronal representations in "morphed" environments. *Neuron.* 2005a; 48(2):345–358. [PubMed: 16242413]
- Leutgeb S, Leutgeb JK, Barnes CA, Moser EI, McNaughton BL, Moser MB. Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. *Science.* 2005b; 309(5734):619–623. [PubMed: 16040709]
- Leutgeb S, Leutgeb JK, Treves A, Moser MB, Moser EI. Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science.* 2004; 305(5688):1295–1298. [PubMed: 15272123]
- Manns JR, Eichenbaum H. A cognitive map for object memory in the hippocampus. *Learn Mem.* 2009; 16(10):616–624. [PubMed: 19794187]

- Marr D. Simple memory: a theory for archicortex. *Philos Trans R Soc Lond B Biol Sci.* 1971; 262(841):23–81. [PubMed: 4399412]
- Maurer AP, Cowen SL, Burke SN, Barnes CA, McNaughton BL. Organization of hippocampal cell assemblies based on theta phase precession. *Hippocampus.* 2006a; 16(9):785–794. [PubMed: 16921501]
- Maurer AP, Cowen SL, Burke SN, Barnes CA, McNaughton BL. Phase precession in hippocampal interneurons showing strong functional coupling to individual pyramidal cells. *J Neurosci.* 2006b; 26(52):13485–13492. [PubMed: 17192431]
- Maurer AP, Vanhoads SR, Sutherland GR, Lipa P, McNaughton BL. Self-motion and the origin of differential spatial scaling along the septo-temporal axis of the hippocampus. *Hippocampus.* 2005; 15(7):841–852. [PubMed: 16145692]
- McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev.* 1995; 102(3):419–457. [PubMed: 7624455]
- McNaughton BL, Barnes CA, O'Keefe J. The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp Brain Res.* 1983a; 52(1):41–49. [PubMed: 6628596]
- McNaughton BL, Battaglia FP, Jensen O, Moser EI, Moser MB. Path integration and the neural basis of the 'cognitive map'. *Nat Rev Neurosci.* 2006; 7(8):663–678. [PubMed: 16858394]
- McNaughton BL, O'Keefe J, Barnes CA. The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. *J Neurosci Methods.* 1983b; 8(4):391–397. [PubMed: 6621101]
- Mishkin M, Vargha-Khadem F, Gadian DG. Amnesia and the organization of the hippocampal system. *Hippocampus.* 1998; 8(3):212–216. [PubMed: 9662136]
- Mizuseki K, Sirota A, Pastalkova E, Buzsaki G. Theta oscillations provide temporal windows for local circuit computation in the entorhinal-hippocampal loop. *Neuron.* 2009; 64(2):267–280. [PubMed: 19874793]
- Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G, et al. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *J Anat.* 2005; 207(1):35–66. [PubMed: 16011544]
- Muller RU, Kubie JL. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J Neurosci.* 1987; 7(7):1951–1968. [PubMed: 3612226]
- Muller RU, Kubie JL, Ranck JB Jr. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *J Neurosci.* 1987; 7(7):1935–1950. [PubMed: 3612225]
- Muzzio IA, Levita L, Kulkarni J, Monaco J, Kentros C, Stead M, Abbott LF, Kandel ER. Attention enhances the retrieval and stability of visuospatial and olfactory representations in the dorsal hippocampus. *PLoS Biol.* 2009; 7(6):e1000140. [PubMed: 19564903]
- Myhrer T, Andersen JM. Interference with visual memory in rats following infusion of the functional NMDA receptor antagonist, HA-966, into temporal regions. *Eur J Pharmacol.* 2001; 428(3):323–330. [PubMed: 11689190]
- Naber PA, Witter MP, Lopez da Silva FH. Perirhinal cortex input to the hippocampus in the rat: evidence for parallel pathways, both direct and indirect. A combined physiological and anatomical study. *Eur J Neurosci.* 1999; 11(11):4119–4133. [PubMed: 10583500]
- Nadel L, Hardt O. The spatial brain. *Neuropsychology.* 2004; 18(3):473–476. [PubMed: 15291725]
- Nadel L, Moscovitch M. Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol.* 1997; 7(2):217–227. [PubMed: 9142752]
- Nadel, L.; Wilner, J.; Kurz, EM. Cognitive maps and environmental context. In: Tomie, PDBA., editor. *Context and Learning.* Hillsdale, NJ: Lawrence Earlbaum; 1985. p. 385-406.
- Norman G, Eacott MJ. Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behav Neurosci.* 2005; 119(2):557–566. [PubMed: 15839802]
- O'Keefe J. Place units in the hippocampus of the freely moving rat. *Exp Neurol.* 1976; 51(1):78–109. [PubMed: 1261644]

- O'Keefe J, Conway DH. Hippocampal place units in the freely moving rat: why they fire where they fire. *Exp Brain Res*. 1978; 31(4):573–590. [PubMed: 658182]
- O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res*. 1971; 34(1):171–175. [PubMed: 5124915]
- O'Keefe J, Recce ML. Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus*. 1993; 3(3):317–330. [PubMed: 8353611]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press; 1998.
- Ranck JB Jr. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. *Exp Neurol*. 1973; 41(2):461–531. [PubMed: 4355646]
- Recce ML, O'Keefe J. The tetrode: an improved technique for multiunit extracellular recording. *Soc Neurosci Abstr*. 1989; 15:1250.
- Rivard B, Li Y, Lenck-Santini PP, Poucet B, Muller RU. Representation of objects in space by two classes of hippocampal pyramidal cells. *J Gen Physiol*. 2004; 124(1):9–25. [PubMed: 15197223]
- Samsonovich A, McNaughton BL. Path integration and cognitive mapping in a continuous attractor neural network model. *J Neurosci*. 1997; 17(15):5900–5920. [PubMed: 9221787]
- Shapiro ML, Tanila H, Eichenbaum H. Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli. *Hippocampus*. 1997; 7(6):624–642. [PubMed: 9443059]
- Skaggs WE, McNaughton BL. Computational approaches to hippocampal function. *Curr Opin Neurobiol*. 1992; 2(2):209–211. [PubMed: 1638156]
- Skaggs WE, McNaughton BL. Spatial firing properties of hippocampal CA1 populations in an environment containing two visually identical regions. *J Neurosci*. 1998; 18(20):8455–8466. [PubMed: 9763488]
- Skaggs WE, McNaughton BL, Wilson MA, Barnes CA. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus*. 1996; 6(2):149–172. [PubMed: 8797016]
- Solstad T, Moser EI, Einevoll GT. From grid cells to place cells: a mathematical model. *Hippocampus*. 2006; 16(12):1026–1031. [PubMed: 17094145]
- Squire LR, Zola-Morgan S. The medial temporal lobe memory system. *Science*. 1991; 253(5026):1380–1386. [PubMed: 1896849]
- Steffenach HA, Witter M, Moser MB, Moser EI. Spatial memory in the rat requires the dorsolateral band of the entorhinal cortex. *Neuron*. 2005; 45(2):301–313. [PubMed: 15664181]
- Terrazas A, Krause M, Lipa P, Gothard KM, Barnes CA, McNaughton BL. Self-motion and the hippocampal spatial metric. *J Neurosci*. 2005; 25(35):8085–8096. [PubMed: 16135766]
- Teyler TJ, DiScenna P. The hippocampal memory indexing theory. *Behav Neurosci*. 1986; 100(2):147–154. [PubMed: 3008780]
- Teyler TJ, Rudy JW. The hippocampal indexing theory and episodic memory: updating the index. *Hippocampus*. 2007; 17(12):1158–1169. [PubMed: 17696170]
- Vanderwolf CH. Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol*. 1969; 26(4):407–418. [PubMed: 4183562]
- Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. *Science*. 1994; 265(5172):676–679. [PubMed: 8036517]
- Winters BD, Bussey TJ. Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci*. 2005; 25(1):52–61. [PubMed: 15634766]

Figure 1. Behavioral procedures used for electrophysiological recordings

The track used for behavior during all electrophysiological recordings. Rats were required to run 20 laps bi-directionally (10 counterclockwise, 10 clockwise) for a food reward. **(A)** Rewards were given in two food dishes located on opposite sides of a barrier (indicated by squares), at the position where the rat was required to turn around. The "X" indicates the location of the pot that the rat was placed in during rest episodes. **(B)** Examples of behavioral procedures where objects were placed on the track. Numbers indicate the approximate positions of the different objects. In the "objects-both epochs" condition, 8 novel objects were placed at discrete locations around the track for the first epoch of behavior (top panel), and the rat had to run past the objects to obtain the food reward. During the second epoch of behavior (bottom panel), 6 of the 8 objects used in epoch 1 were placed on the track at the same location as in epoch 1, while 2 of the 8 objects were removed and substituted with 2 novel objects (in this case objects 3 and 5 were replaced with objects 9 and 10 as indicated by the grey boxes). The "configuration change" condition occurred on the day following the objects-both epochs condition. For this behavioral procedure, the same objects used in epoch 1 from the previous day were again placed on the track at the same location as in epoch 1, and the rat ran 20 laps (top panel). During the second epoch of track-running, the positions of the eight familiar objects were pseudo-randomly shuffled such that no object was at the same position between epoch 1 and 2 (bottom panel).

Figure 2. Tetrode tract reconstructions for the intermediate portion of CA1 close to the subiculum border (“distal CA1”)

(A) A nissl stained coronal section of the right hemisphere of a rat that participated in the current experiment. This section is approximately -5.3 mm posterior to bregma and shows 3 tracks, made by the tetrode recording probes, that reached the pyramidal cell layer (circles) of the distal region of intermediate CA1 in rat 8879. (B) The areas of distal CA1 from which pyramidal neuron recordings were obtained. The ovals show the regions where marker lesions were observed following histological verification (Figure adapted from Paxinos and Watson, 1998). The CA1 regions where recordings were obtained for each rat are indicated by the rat number.

Figure 3. Place field expression across behavioral conditions

(**A**) The proportion of cells in intermediate CA1 with $n = 0, 1, 2, 3, 4, 5, 6, 7$ or 8 place fields when there were no objects on the track (black) versus conditions with objects on the track (grey). A significantly larger proportion of intermediate CA1 cells expressed place fields during track running conditions with objects ($F_{[3,2]} = 9.65$, $p < 0.01$). (**B**) The mean and standard error (SEM) for the number of place fields per active neuron during track-running epochs with no objects (black) versus the conditions with objects (grey). The CA1 neurons that showed a significant firing rate increase during the track running epochs expressed significantly more place fields within an epoch of behavior when objects were placed on the track relative to when the track was empty ($F_{[3,2]} = 4.56$, $p < 0.03$; repeated-measures

ANOVA). All place fields were determined using the phase precession definition (Maurer et al., 2006a).

Figure 4. The effect of objects on place field expression in single CA1 neurons

Representative examples of the activity of single CA1 neurons between object and no object epochs. In both (A) and (B) the X axes are position on the track (cm) relative to the barrier, which is at position 0 and -335. The rat was running from left to right, and the negative values for position indicate that the animal's heading was in the counterclockwise direction. The top panels show the occupancy normalized firing rate histograms and the bottom panels are raster plots of spikes by lap. Finally, the black asterisks indicate the positions of the objects. (A) In this example there were no objects on the track during epoch 1 (left panel), and then objects were placed on the track for epoch 2 (right panel). This neuron only had place field activity near the food dish (far left area) in the absence of objects. When objects

were added to the track, however, the cell expressed three place fields. **(B)** A representative example of the activity of a CA1 neuron when objects were on the track during epoch 1 (left panel) but the objects were removed for epoch 2 (right panel). In this example, the neuron was active at three discrete locations and near the food dish when objects were present. When the objects were removed, however, only one of the three regions of high firing near objects remained.

Figure 5. The effect of objects on inter-epoch global remapping in CA1

Frequency distribution of activity correlations (r) between epoch 1 and epoch 2 for CA1 neurons in the (A) no objects, both epochs, (B) objects, both epochs, (C) configuration change, (D) no objects-objects, and (E) objects-no objects conditions. Each distribution was normalized by the number of neurons recorded during a given condition. (F) Mean r value for the five different behavioral conditions. The between epoch activity patterns of CA1 place fields for behavioral procedures where the treatment was the same for both epochs was significantly more correlated compared to when the procedures changed between epochs (#; $T_{[3]} = 3.60$, $p < 0.05$; paired-samples T test). The configuration change condition showed an intermediate r value between the “no change” and “change” conditions. Moreover this condition was significantly less correlated across epochs of track running than the objects, both epochs condition (* $T_{[3]} = 5.61$; $p < 0.05$; Tukey HSD).

Figure 6. No evidence for rate-remapping in CA1 pyramidal cell activity between epochs 1 and 2
 For a single neuron to demonstrate 'rate-remapping' between epochs 1 and 2, both the between epoch correlation coefficient and firing rate change should be high. This would be indicative of a cell that showed activity at the same location but changed its rate to represent an alteration to the environment (Leutgeb et al., 2005b). The difference in firing rate between epochs for every pyramidal cell with a least one place field in either epoch plotted against the neuron's Pearson's correlation coefficient for CA1 cells that did not show global remapping ($r > 0.6$) in the (A) Same conditions (no objects, both epochs and Objects, both epochs) (B) Configuration change, and (C) Change conditions (No objects-objects and Objects-no objects conditions). In all plots, cells with a between epoch firing rate change at

least 2 standard deviations from the mean rate change for all cells are indicated by the open circles. In all conditions less than 9% of CA1 neurons with stable place fields between epochs showed a significant firing rate change. Moreover, the rate change between epochs 1 and 2 was not significantly different between any of the behavioral conditions ($F_{[3,4]} = 3.92$, $p = 0.11$; repeated-measures ANOVA).

Figure 7. Representative examples of activity for CA1 neurons recorded during epoch 1

In both (A) and (B), the X-axes are the linearized position on the track relative to the barrier (in cm), the rat was running from left to right, and the negative values indicate that the rat's heading was counterclockwise. The top panels are the firing rate histograms for both cells, and the bottom panels are the raster plot of spikes by lap. (A) One cell was recorded when there were no objects on the track, (B) while the other neuron was recorded from the same rat on a different day when objects were placed on the track during epoch1. In (B) the asterisks indicate the position of objects.

Figure 8. The effect of objects on place field size

(A) The mean size of place fields for behavioral procedures with no objects on the track (black) versus conditions with objects (grey). The presence of objects on the track significantly reduced the mean size of place fields ($T_{[3]} = 5.68$, $p < 0.01$; paired-samples T-test). Error bars represent $\pm$ one standard error of the mean. (B) The frequency distribution of place field size when rats traversed an empty track (black) versus when the track had objects on it (grey). Both frequency distributions were normalized by the total number of place fields in each condition and smoothed with a hanning window of 5. Although the range of place field size is similar between groups, when the track was enriched with objects

a larger proportion of place fields were small and there was a reduction in the number of large place fields.

Figure 9. Place field size is stable when objects are not added or removed

The magnitude of the difference in place field size between epochs 1 and 2. Adding or removing objects from the track between the two epochs of track running significantly changed the size of place fields ($T_{[3]} = 6.14$, $p < 0.05$; paired-samples T test). For the behavioral procedures where both epochs had objects on the track or both epochs did not have objects on the track, there was no significant difference in place field size between epochs ($F_{[1,3]} = 4.52$, $p = 0.12$; repeated-measures ANOVA). Error bars represent $\pm$ one standard error of the mean.

Figure 10. The slope and dispersion of theta phase precession in the no objects and objects conditions

(A) Representative examples of theta phase by position plots for two neurons recorded from the same rat. In both panels the X axes are the position on the track normalized by the center of mass of the spikes, and the Y axes are theta phase normalized so that the peak phase is 360. The left panel shows the activity of a cell during a no object condition and the right panel is the activity of a cell from an object condition. The Pearson's correlation coefficient of phase by position is indicated for each cell, and the black lines represent the least-squares line of best fit. (B) The mean slope of theta phase as a function of distance (i.e., the rate of phase precession) for the no objects (black) and the objects (grey) conditions. The rate of phase precession was significantly greater in the objects conditions ($T_{[3]} = 6.94$, $p < 0.01$; paired-samples T test), which is consistent with the smaller field size. (C) The dispersion of theta phase precession, as measured by the Pearson's correlation of the phase versus distance plots, was not different in the no objects (black) and objects (grey) conditions (Figure 11B; $T_{[3]} = 0.59$, $p = 0.60$; paired-samples T test). This indicates that the

relationship between phase and position was not affected by the objects. Error bars represent $\pm$ one standard error of the mean.

Figure 11. Objects on the track did not impede rats from running through the centers of place fields

The mean phase difference between the first spike and the last spike fired (i.e., total phase shift) for the first 5 passes through a place field during epoch 1 (A) and epoch 2 (B). A decrease in the total phase shift would indicate that a rat was not running through the place field centers. There was not a significant difference in the phase shift between the no objects (black) and objects (grey) conditions ($T_{[3]} = 0.61$, $p = 0.55$; paired-sample T test), suggesting that in both conditions rats were traversing the centers of place fields. Error bars represent $\pm$ one standard error of the mean.

Figure 12. Running velocity during the different behavioral conditions

(A) The mean running velocity for conditions without objects (black) and conditions with objects (grey). The rats' running velocities when objects were on the track were not significantly different compared to the no object conditions ($T_{[3]} = 1.48$, $p = 0.24$; student's T test). (B) The mean running velocity during epoch 1 for laps 1–2 (unfilled) and laps 19–20 (filled) for conditions with no objects, conditions with novel objects, and the configuration change condition. (C) The mean running velocity during epoch 2 for laps 1–2 (unfilled) and laps 19–20 (filled) for conditions with no objects, conditions with novel objects, conditions with familiar objects, and the configuration change condition. For all behavioral conditions, when both epochs were analyzed together, the rats ran significantly more slowly during laps

1–2 relative to laps 19–20 ($F_{[1,22]} = 32.80$, $p < 0.001$). Individual post hoc comparisons indicated that the difference in running velocity between the first two laps and last two laps was for epochs with novel objects (epoch 1 of the objects, both epochs condition; epoch 1 of the objects-no objects condition; epoch 2 of the no objects-objects condition; $p < 0.05$ for all comparisons). Additionally, the rats' running velocity was significantly slower during laps 1–2 relative to laps 19–20 for epoch 2 of the configuration change where familiar objects were placed in a novel spatial arrangement ($p < 0.05$).

Table 1

Procedures used under the five behavioral conditions

Condition	Epoch 1	Delay	Epoch 2	# of rats
No objects, both epochs	No objects	20 min	No objects	4
Objects, both epochs	8 novel objects	20 min	6 familiar objects from epoch 1, and 2 novel objects	4
Configuration change	8 familiar objects, in familiar locations	20 min	8 familiar objects with all object locations different from epoch 1	4
No objects-objects	No objects	20 min	8 novel objects	2
Objects-no objects	8 novel objects	20 min	No objects	2

Table 2

Numbers of place fields in recorded intermediate CA1 pyramidal cells

	No objects, both epochs	Objects, both epochs	Configuration change	No objects objects	Objects-no objects	Total
Total recording sessions	20	14	14	8	8	64
Total cells	321	170	190	194	161	1036
Epoch	1	2	1	2	1	2
Cells without place fields	214	213	126	113	101	91
Cells with at least 1 place field	107	108	80	86	77	93
Proportion with ≥ 1 place field	0.33	0.34	0.42	0.45	0.48	0.38

Table 3

Mean firing rates for intermediate CA1 pyramidal cells

Epoch	No objects, both epochs		Objects, both epochs		Configuration change		No objects- objects		Objects- no objects	
	1	2	1	2	1	2	1	2	1	2
Mean all behavior firing rate (Hz)	2.1 ± 0.3	2.4 ± 0.4	1.8 ± 0.3	2.0 ± 0.3	2.0 ± 0.5	1.9 ± 0.4	1.6 ± 0.6	1.6 ± 0.6	1.6 ± 0.6	1.6 ± 0.6
Mean in-field firing rate (Hz)	14.9 ± 6.3	11.1 ± 2.4	17.86 ± 8.41	13.4 ± 2.9	16.8 ± 7.7	11.3 ± 1.9	14.7 ± 1.7	17.2 ± 8.5	10.2 ± 5.5	21.5 ± 4.3