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Gradual Translocation of Spatial Correlates of Neuronal Firing in the Hippocampus towards Prospective Reward Locations

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Summary

In a continuous T-maze alternation task, CA1 complex-spike neurons in the hippocampus differentially fire as the rat traverses overlapping segments of the maze (i.e., stem) repeatedly via alternate routes. Temporal dynamics of this phenomenon were further investigated in the current study. Rats learned the alternation task from the first day of acquisition and the differential firing pattern in the stem was observed accordingly. More importantly, we report a phenomenon in which spatial correlates of CA1 neuronal ensembles gradually changed from their original firing locations, shifting toward prospective goal locations in the continuous T-maze alternation task. The relative locations of simultaneously recorded firing fields, however, were preserved within the ensemble spatial representation during this shifting. The within-session shifts in preferred firing locations in the absence of any changes in the environment suggest that certain cognitive factors can significantly alter the location-bound coding scheme of hippocampal neurons.

Introduction

The role of the hippocampal memory system in remembering discrete episodic events in a particular environment is well-recognized (Eichenbaum, 2000; McClelland, 1998; O'Keefe and Nadel, 1978; Vargha-Kadem et al., 1997). It has been proposed that the spatial information of events is represented by complex-spike neurons ("place cells") in the hippocampus since they exhibit strong firing correlates with particular locations ("place field;" O'Keefe and Dostrovsky, 1971). However, it is largely unknown how this location-bound coding scheme in the hippocampus interacts with nonspatial factors such as task demands and/or the reinforcement schedule for remembering significant events, especially in complex memory tasks.

Although it has been demonstrated that the location-bound coding scheme of complex-spike neurons in the hippocampus is influenced by changes in the physical context of the environment (Anderson and Jeffery, 2003; Gothard et al., 1996; Lee et al., 2004a; Leutgeb et al., 2004; Muller and Kubie, 1987; O'Keefe and Nadel, 1978; Tanila et al., 1997), prior studies have also shown that changes in the physical context are not necessary to alter the spatially biased firing pattern. For example, reward or goal appears to be an important influence; place cells change their firing characteristics when the pattern of reinforcement is altered in the same environment (Breese et al., 1989; Fyhn et al., 2002; Kobayashi et al., 1997; Markus et al., 1995). In addition, task demands modulate the location-bound coding scheme in the absence of physical changes in the environment (Bower et al., 2005; Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). For example, Wood et al. (2000) have shown that as a rat traverses the stem of a modified T-maze continuously while alternating between different goal locations, CA1 complex-spike neurons fire more strongly in association with a particular trial type (i.e., left-to-right or right-to-left trials) of the alternation task.

Although hippocampal neurons can conditionally fire when the rat traverses the stem of the T-maze (Wood et al., 2000) during continuous alternation, the time course of such conditional firing has not been reported. Here we report that hippocampal neurons develop the trial type-dependent, differential firing in the stem of the T-maze from the first day of acquisition of the task. Furthermore, examination of the firing patterns over multiple trials revealed that the spatial firing correlates of CA1 complex-spike neurons gradually shifted forward across trials, via the stem, toward prospective goal locations within a recording session. The results suggest that the shift in a reference frame bound to physical objects (Gothard et al., 1996) is not necessary to produce a systematic shift in firing locations of hippocampal neurons. Instead, the results imply that a goal-oriented, cognitive reference frame can significantly influence the location-bound firing characteristics of the hippocampal neurons, especially when animals need to parse a given physical space into multiple cognitive maps according to the mnemonic task demands.

Results

Daily Time Course of the Conditional Spatial Firing

We recorded from complex-spike neurons (n = 290) in the dorsal CA1 of the hippocampus while rats (n = 4) acquired and performed the continuous T-maze alternation task (see Experimental Procedures; Table 1). Among these neurons, 118 complex-spike neurons met the unit selection criteria [$\geq$100 spikes with a statistically significant ($p < 0.01$) information score (Skaggs et al., 1993, 1996) of 0.5 or higher] of our study, and the analysis was performed only for these units. In the previous study (Wood et al., 2000), the alternation rule was initially acquired using a forced choice paradigm with blockades. In the current study, however, rats acquired the alternation rule in the absence of blockades (see Experimental Procedures). Rats acquired the task within the first 10–15 trials on the first day of the acquisition of the task and made very few errors once they learned the task (see Figure S1 in the Supplemental Data). Individual trials of the alternation task were

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Table 1. Distribution of Single Units across Days and Rats

	Day 1	Day 2	Day 3	Day 4
Rat 1	11 (21)	13 (17)	9 (15)	13 (22)
Rat 2	2 (6)	5 (6)	6 (7)	3 (3)
Rat 3	*n/a*	*n/a*	2 (2)	7 (10)
Rat 4	3 (5)	1 (1)	3 (3)	*n/a*

The number of single units that met the unit selection criteria on the stem of the maze (see Experimental Procedures), recorded from different subjects across 4 days (the total number of complex-spike neurons with $\geq$100 spikes and a statistically significant [$p < 0.01$] information score of 0.5 or higher) is shown in parentheses; *n/a* indicates no recording data due to technical difficulties or poor performance.

parsed into left-to-right (L-R) and right-to-left (R-L) trial types (Figure 1A). As reported previously (Wood et al., 2000), hippocampal neurons fired differentially for L-R and R-L trials in the alternation task as the rat traversed the stem of the continuous T-maze (Figure 1B). Approximately 70% of neurons that represented the stem of the maze (n = 53/78; recording days 1 to 4 combined) sharply differentiated their firing rates (i.e., trial type differentiation index $\geq$ 0.5; Experimental Procedures) in the stem (Figure 1C), based on trial type information. Those neurons that showed trial type-specific firing on the stem were identified from recording day 1 from the first day of the acquisition of the alternation task (D1 in Figure 1C). The distributions of the conditional firing pattern on the stem across recording days 1 to 4 (Figure 1C, insets) were not significantly different from each other ($p > 0.1$, Kruskal-Wallis test, an equivalent of ANOVA in nonparametric testing), suggesting that the differential firing for L-R and R-L trials develops in the hippocampus quickly as the rat acquires the task.

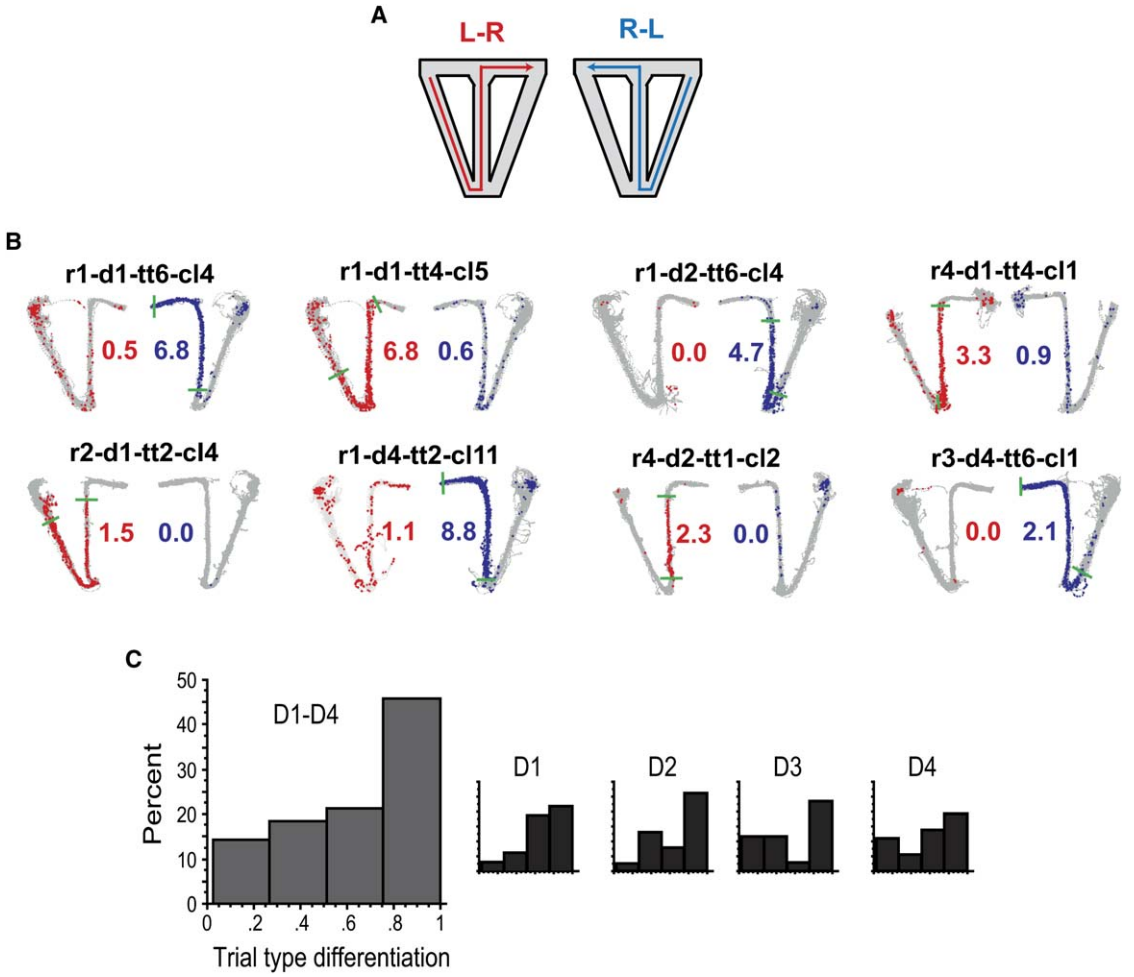

Figure 1. Trial Type-Specific Firing of CA1 Neurons in the Continuous T-Maze Alternation Task

(A) Illustration of the animal's trajectory associated with left-to-right (L-R; red) and right-to-left (R-L; blue) trials. The two trajectories are shown separately only for illustrative purpose.

(B) Representative examples of CA1 spatial firing fields selectively active on the stem for either L-R or R-L trial type of the alternation task. Firing fields for a single CA1 neuron are shown following the illustrative scheme shown in (A). Gray, position data for each trial type; red and blue, position data associated with spikes for L-R and R-L trial type, respectively. Numbers associated with the stem denote average firing rate (Hz) on the stem associated with each trial type. Green lines associated with the firing fields indicate field boundaries.

(C) (Top) The distribution of the amount of trial type-dependent firing on the stem (trial type differentiation index; 0 = no differentiation, 1 = perfect differentiation; see Experimental Procedures) across 4 days. Note the greater proportion of neurons that exhibited highly differential firing rates on the stem for trial types. (Bottom) The same distribution parsed based on each daily recording session. Note the highly biased distribution on all days including day 1 (D1) and no significant difference across 4 days (D1 to D4; $p > 0.1$, Kruskal-Wallis test).

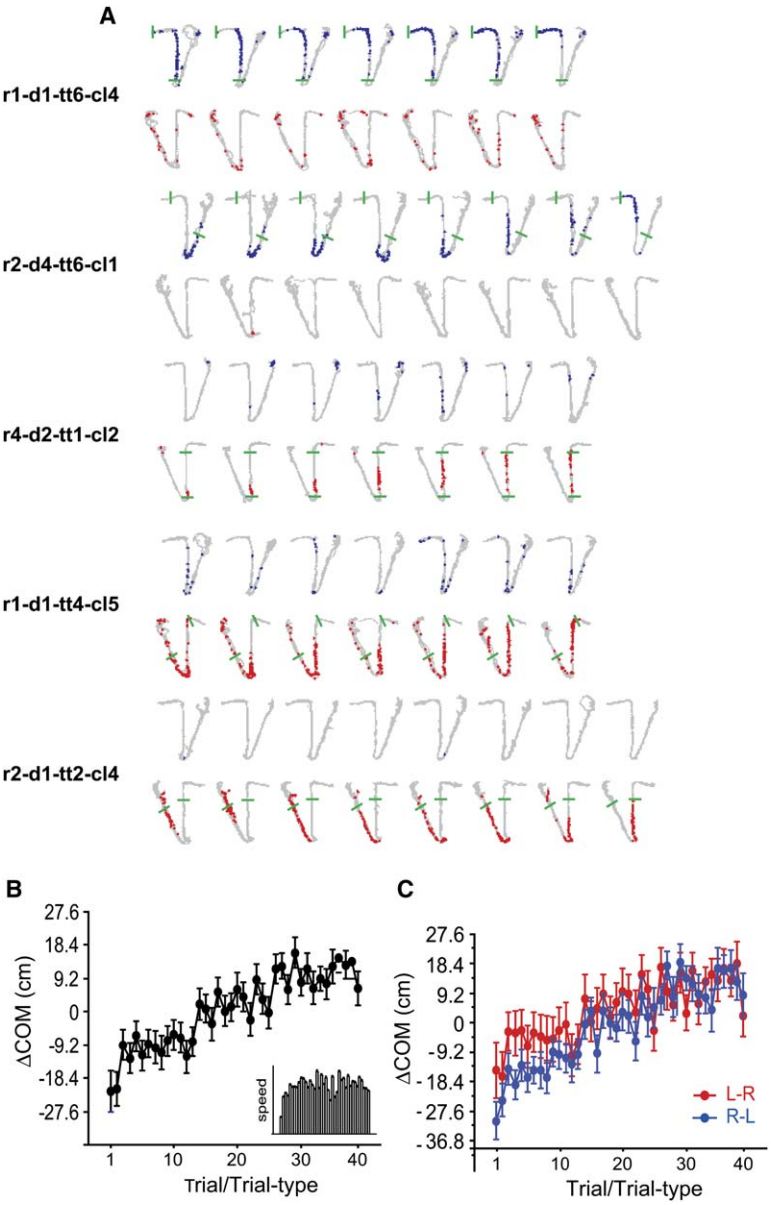

Figure 2. Forward Shifting of Spatial Representations of Hippocampal Neurons during the Continuous Alternation Task

(A) Representative examples of CA1 neurons that shifted their spatial representations forward via the stem of the maze across trials, plotted horizontally in sequential 5-trial blocks (L-R [red] and R-L [blue] fields for each neuron shown separately as in Figure 1). Green lines denote field boundaries of the firing fields.

(B) Forward shifting of preferred firing locations. The ΔCOM represents the location of an individual neuron's firing field relative to its average field location (ΔCOM = 0), calculated based on all the trials combined. Inset, speed profile across trials. Note the asymptotic speed profile after the first ~10 trials. Error bars = SEM.

(C) Similar forward shifts of the Center of Mass (COM) across trials between the two trial types [red, L-R; blue, R-L]. ΔCOM = difference in COM between the spatial representations based on all trials versus the spatial correlates for individual trials. Error bars = SEM.

Forward Shifting of Spatial Representations toward Goal Locations

Although there was no significant development of the conditional spatial firing in the stem across days (Figure 1C), it is possible that such development might be observed across trials within a given day's recording session. To investigate this possibility, all trials from each day's alternation session were divided into continuous 5-trial blocks for each trial type for those neurons representing the stem of the maze (based on the average firing fields across all trials as shown in Figure 1B). Figure 2A shows that CA1 complex-spike neurons fire differentially in association with the trial types of the alternation task from the onset of the task, all within the same recording session. During this within-session analysis, however, an unexpected firing pattern was observed; the spatial correlates of the complex-spike neurons gradually shifted *forward* toward upcoming reward locations associated with particular trial types (Figure 2A). Some neurons initially formed their firing fields on the oblique return arm and then shifted the fields further into the stem (neurons r2-d4-tt6-cl1, r1-d1-tt4-cl5, and r2-d1-tt2-cl4 in Figure 2A). Other neurons originally represented the stem of the maze and moved gradually toward a particular reward arm that was perpendicularly attached to the stem (r1-d1-tt6-cl4 and r4-d2-tt1-cl2 in Figure 2A).

To quantitatively characterize this forward translocation of spatial correlates, the center of mass (COM) of each trial's firing rate distribution was calculated for each neuron relative to the COM of the neuron's firing rate distribution from the entire set of trials (Mehta et al., 1997; Lee et al., 2004b). A marked, linear forward COM-shift ($p < 0.0001$, linear regression) was observed across trials on average (Figure 2B). Forty correct trials of L-R and the same number of correct trials of R-L

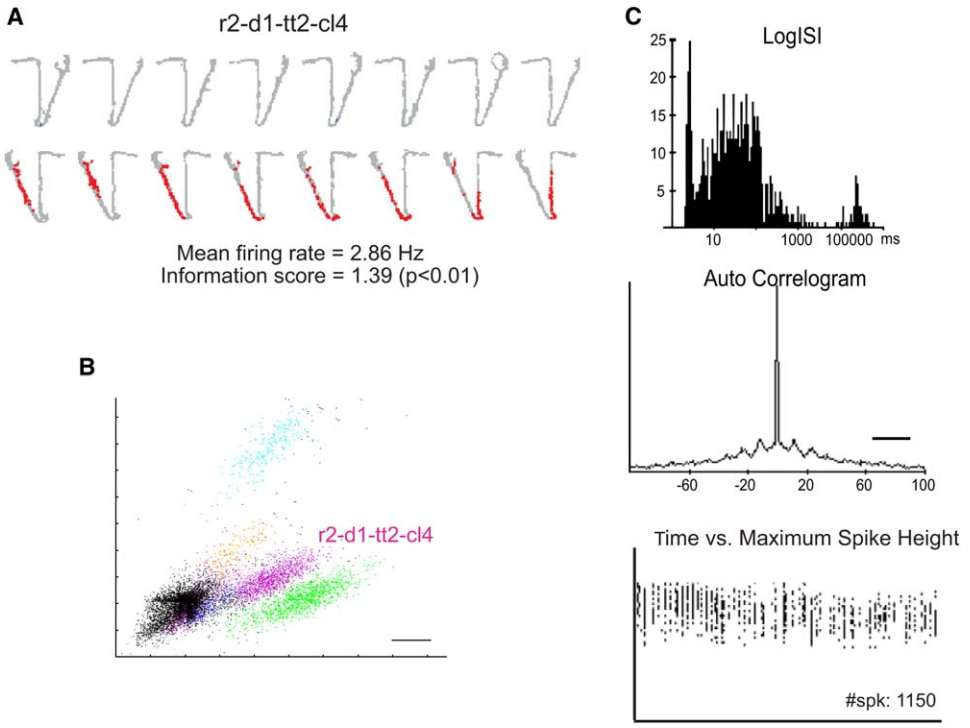

Figure 3. Firing Characteristics of a CA1 Complex-Spike Neuron

Unit isolation quality of a representative single unit that demonstrated large shifts (A) along the maze during the alternation task. (B) The distribution of spike heights of the same single unit shown in (A) on a 2D cluster-cutting space is shown (pink; each point represents the peak amplitude of a spike recorded on two of the channels of the tetrode; scale bars = 30 μV). (C) An interspike interval histogram plot (LogISI), a spike-time autocorrelation histogram plot (autocorrelogram; scale bar = 250 ms), and the distribution of maximal spike height across time (Time vs. Maximum Spike Height; #spk = total number of spikes) are presented (the entire x axis represents the time spent in performing the alternation task). The prominent refractory period in the interspike interval histogram plots with the bursting firing pattern, the highest peak at the center (t = 0) of the spike-time autocorrelogram, and the constant level of maximal spike height collectively demonstrate that the forward shifting phenomenon was not due to an artifact based on poor unit isolation.

were combined in Figure 2B since the linear COM-shift occurred similarly between L-R and R-L trials (Figure 2C). The remarkable forward shifts in COM observed in the population of neuronal data were also identified within individual rats (Figure S2). The magnitude of the distance in forward field shifting was much greater in our study compared to the previous backward COM-shift phenomenon (Mehta et al., 1997, 2000), possibly due to the fact that the length of the track traveled for an alternate pair of L-R and R-L trials (Figure 1A) was significantly longer (approximately 450 cm, including the stem once) than the tracks used for prior studies. It is unlikely that running speed is responsible for the linear forward shift in COM; in contrast to the constant linear COM-shift across trials (Figure 2B and 2C), average running speed of animals increased only during the first ~10 trials by ~56% with no further linear increase afterwards (Figure 2B, inset). The forward shifting phenomenon is not due to artifacts in unit isolation since well-isolated single units that exhibited typical firing characteristics (e.g., bursting firing patterns in interspike-interval plots) of complex-spike neurons showed robust translocations of their firing fields (Figures 3 and 4). It is also unlikely that the field shifting was due to different routes taken on the stem since each rat traversed the stem through a fairly consistent route within a session, resulting in narrowly distributed position data on the stem (Figure S3). A significant, forward translocation of the spatial correlates was also observed robustly (1) when the degree of shift of each trial's field was normalized with the average field size based on the entire set of trials (Figure S4A) and (2) when the firing field location for each trial was calculated in reference to the center of the linearized T-maze (Maze ΔCOM in Figure S4B) rather than to the COM of the average firing field.

Previous studies have shown that spatial representations of hippocampal neurons expand backward (i.e., opposite to the rat's movement direction; Mehta et al., 1997) with the development of an asymmetric firing rate distribution (i.e., negative skewness) across time (Mehta et al., 2000). In our study, the shifting fields also became negatively skewed across trials (Figure 5A; $p < 0.0001$, linear regression). However, the development of negative skewness in CA1 was not accompanied by a systematic increase in field width during the forward shifting of firing fields ($p > 0.5$, linear regression; Figure S5). Importantly, when the spatial firing fields ($n = 42$) were recorded as the rats initially performed a unidirectional navigation along one half (i.e., the left side) of the modified T-maze, the COM of those fields appeared to shift backward (Figure 5B) as observed previously in hippocampal cells as rats repeat single-path routes on tracks (Mehta et al., 1997, 2000; Ekstrom et al., 2001). Specifically, on recording day 1 in our study, the rats first finished 15 laps of unidirectional runs on the left half of

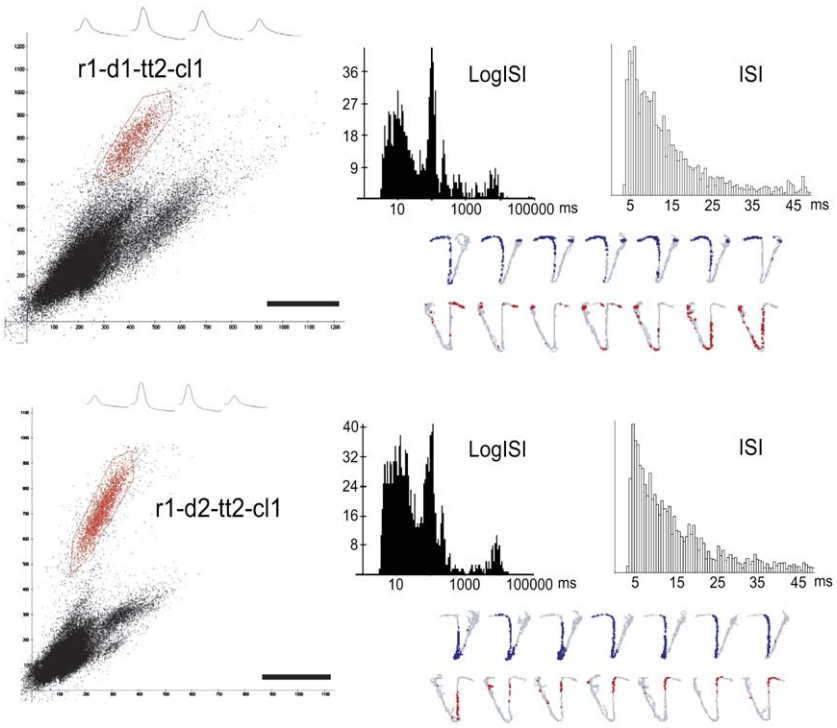

Figure 4. Unit Isolation Quality of Two Neurons

For each unit, its average spike waveform, distribution of spike heights on a 2D cluster-cutting space (scale bar = 100 μV), ISI plots (logarithmically scaled <100,000 ms for showing bursting firing patterns and nonlogarithmically scaled <50 ms to better demonstrate the refractory period), and translocating firing field over time, are shown.

the modified T-maze (i.e., familiar path) before they ran 15 laps of unidirectional runs on the right half of the maze (i.e., novel path) followed by the acquisition of the alternation task. When the shifts in field positions (ΔCOM) were plotted (n = 42) during the unidirectional runs on the left side (before the animals experienced that the stem was shared between two navigation paths, L-R and R-L), the backward shifting of firing field locations of CA1 neurons was observed (Figure 5B). This backward shifting of firing fields was observed before the rats were exposed to the alternation task, whereas forward shifting predominated immediately after the rats began to alternate routes, suggesting that the forward shifting results from the alternation task demands rather than any peculiarity of the T-maze environment.

Cohesive Shifting of Spatial Representations in Neuronal Ensembles

The forward COM-shift shown in Figure 2B was measured based on the population of neurons recorded from multiple subjects and recording days (Table 1). To better understand the network dynamics in the hippocampus, it is critical to know whether simultaneously recorded neurons shift their firing fields either cohesively or independently across trials. As shown in Figure 6A, an ensemble of neurons (n = 8) whose firing fields were simultaneously recorded shifted cohesively en masse across successive 5-trial blocks (see also Figure S6). When an ensemble COM was measured for each 5-trial block (based on the COMs measured for individual firing fields within the ensemble), a linear COM-shift was observed across trials at the ensemble level (Figure 6B). Furthermore, during the coherent ensemble shifting toward prospective reward locations, each neuron's firing field maintained a relatively constant position within the shifting ensemble representation (Figure 6C and 6D).

Stationary Fields outside the Stem Region

In contrast to the forward shifting firing fields, some complex-spike neurons (n = 40/118) in CA1 appear to maintain their firing fields stationary at fixed locations on the maze across all trials (Figure 7A). The average firing-rate distributions of these neurons across all trials were observed mostly in either the reward arm and/or return arm of the maze (Figure 7A). When the spatial firing fields were parsed based on continuous 5-trial blocks for these neurons, no significant forward shifting was noticeable (Figure 7B). Instead, the firing fields tended to remain stationary (Figure 7C) similar to those observed in hippocampal neurons in animals foraging in open fields (O'Keefe and Nadel, 1978; Muller et al., 1987), in contrast to the neurons whose firing fields migrated toward the reward locations (Figure 2).

Discussion

Forward Translocation of Spatial Representations in the Hippocampus

The current results provide experimental evidence showing the *forward* shifting of spatial representations of hippocampal neurons as a result of cognitive demands in the absence of any alternation in available cues. Previous literature (Blum and Abbott, 1996;

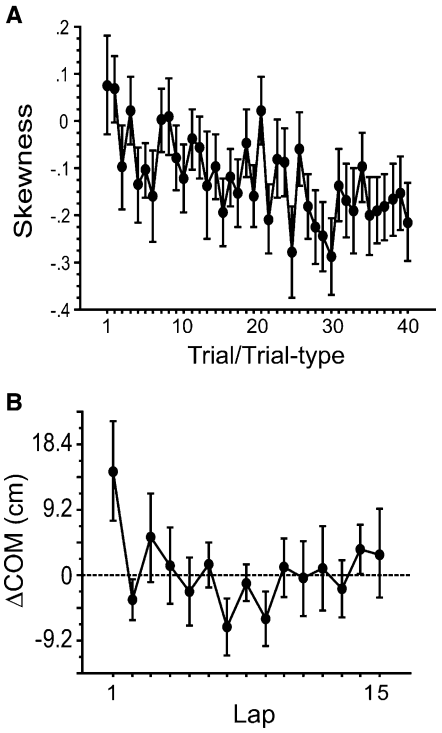

Figure 5. Development of Negative Skewness in Forward Shifting Fields during Alternation and Backward Shifting of Fields during Unidirectional Runs

(A) Development of negative skewness in the neurons representing the stem on average ($p < .0001$, linear regression). Skewness was defined as the ratio of the third moment of the spatial firing rate distribution divided by the cube of standard deviation.
(B) Trend of backward COM-shift in unidirectional laps.

Ekstrom et al., 2001; Mehta et al., 1997, 2000; Lee et al., 2004b) has suggested that the hippocampal representation of space shifts *backward* with experience. However, the results from our study suggest that the shifting direction of hippocampal spatial representations might be determined in a more complicated fashion depending upon task demands. In addition, prior investigators observed changes in the location of hippocampal firing fields, but only following abrupt changes in task demands (Markus et al., 1995), translocation of a reward locus (Breese et al., 1989; Kobayashi et al., 1997), or movement of critical environmental stimuli (Gothard et al., 1996; Rosenzweig et al., 2003). In contrast, the shifting of firing fields toward prospective reward locations in our study occurred gradually *within* a recording session, during which there were no changes in task demands or environmental cues.

Potential Task Components underlying the Forward Shifting Phenomenon

It is currently unclear what components of the continuous T-maze alternation task produced the dynamic changes in firing locations of CA1 neurons. However, we provide the following as possible explanations:

First, prior experimental evidence (Eichenbaum et al., 1987; Breese et al., 1989; Kobayashi et al., 1997) suggests that reward might play a significant role in the forward shifting phenomenon reported here. Those studies have shown that the location of reward exerts a significant amount of control over where the complex-spike neurons in the hippocampus fire in the environment. In our study, the reward appeared alternatively in different, yet fixed, locations in the maze. It is noteworthy that CA1 neurons stopped shifting their firing fields once their fields reached the reward arm at the end of which the rat consumed food rewards. A firing field that continued to shift forward to the return arm after reaching the end of the reward arm (where food reward was consumed) was never observed in the current study. Therefore, it appears that the reward locations serve as "final destinations" of the CA1 spatial representations shifting forward in the continuous T-maze, similar to the reward location-dependent shifts of field locations in prior studies (Breese et al., 1989; Kobayashi et al., 1997).

Although the exact mechanisms of how the alternation of reward locations produces the forward field-shifting phenomenon is unclear, it might be possible that the gradient of state values (prediction of reward) spreading from reward locations (Hasselmo, 2005) becomes stronger over trials, spreading backward along the correct navigation paths (L-R or R-L) leading to the reward locations. If the firing field shift is influenced significantly by the reward gradient in the environment (Foster et al., 2000; Hasselmo, 2005), this may produce the forward shifting of field locations in the alternation task. Reward alone, however, cannot fully account for the shifting phenomenon since the forward shifting often occurred more strongly in association with one of the reward locations (either L-R or R-L) rather than equally for both reward locations. Thus, a combined influence from the value function associated with different spatial locations in a given environment (Schultz et al., 1997; Foster et al., 2000) and task demands (i.e., systematic alternation between reward locations) seems to underlie the gradual forward shifting phenomenon.

Second, a systematic translocation of neuronal firing fields *within* a recording session, similar to the result reported here, has been reported in a start box translocation paradigm (Gothard et al., 1996), in which the rat shuttled between a fixed reward location on a linear track and a start box placed at variable positions on the track. Although environmental cues other than the shifting start box position remained constant, Gothard et al. (1996) reported that some firing fields shifted their locations as a function of the distance from the shifting start box. Although no movable start box was used in our study, the forward shifting phenomenon might occur if the animal's perceived starting point for a navigation path (L-R or R-L) moved gradually toward a prospective reward location across trials. The advantage of such a coding scheme, however, is unclear. One possibility is that, by mapping systematically shifted locations on the maze for different trials, the hippocampus might form trial-unique representations (spatially and temporally) of partially overlapping navigation paths (i.e., L-R versus R-L paths sharing the stem) across different trials. Although the task does not require differentiating, for example, the 1st and the 20th L-R trials, it is plausible that the hippocampal network performs this type of orthogonalization whenever overlapping inputs from the environment are represented. This network property might underlie the function of the hippocampus in

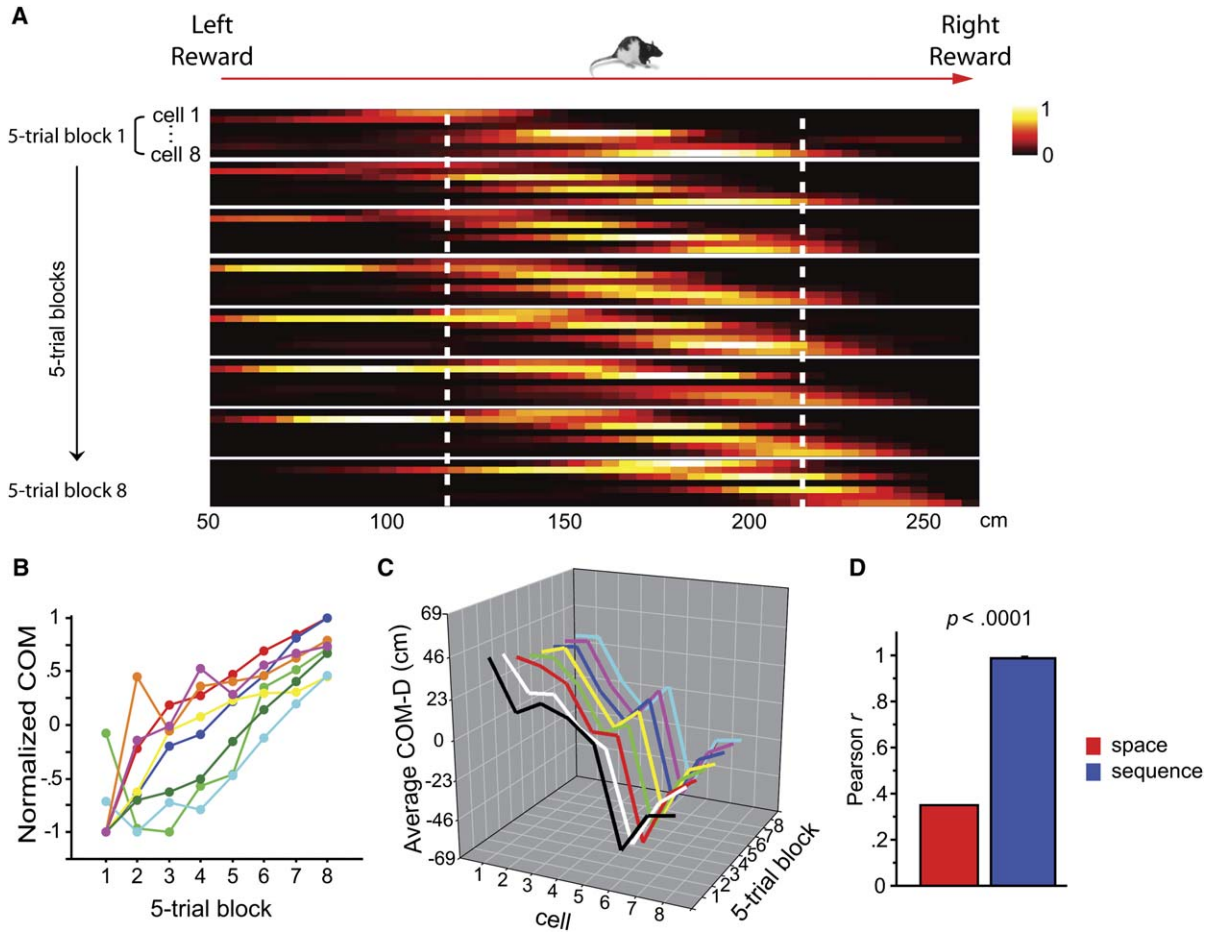

Figure 6. Translocation of Ensemble Spatial Representations

(A) An example of ensemble spatial representation shifting forward across successive 5-trial blocks (L-R trial type), based on eight simultaneously recorded CA1 neurons within a recording session. Each ensemble spatial representation/block is composed of the normalized firing rate distributions (minimum and maximum firing rates of 0 and 1, respectively) of simultaneously recorded neurons (each row represents a single neuron's normalized firing rate distribution) on a linearized maze (dashed white lines denote the boundaries of the stem; <50 cm not shown due to the lack of fields).

(B) Forward shifts in ensemble spatial representations. Each colored line corresponds to the shift in ensemble COM (normalized in each ensemble data set; see Experimental Procedures) across 5-trial blocks for each ensemble data set [eight ensemble data sets; cyan line for the ensemble shown in (A)]. Note the general property of forward COM-shift at the ensemble level.

(C) Preservation of the relative positions of individual firing fields within the ensemble representations shown in (A). Each colored line shows the pattern of the average distance (Average COM-D) of each cell's location (measured in COM) from the COM of other cells in the ensemble.

(D) Comparison of average spatial versus sequence correlations between adjacent trial blocks of ensemble spatial representations (based on eight ensemble data sets). To calculate the spatial correlation, the matrix for the ensemble firing rate distribution of each 5-trial block, as shown in (A), was correlated with the matrix for the adjacent ensemble firing rate distribution. To calculate the sequence correlation, an "Average COM-D distribution" for each 5-trial block, as shown in (C), was correlated with that of the adjacent 5-trial block. Note a significant difference ($p < 0.0001$) between the spatial and sequence correlations. The Pearson product-moment correlation coefficients were transformed into z-scores using the equation $z = 0.5 \times \ln[(1 + r)/(1 - r)]$, in order to perform a paired t test. Error bars = SEM.

encoding similar experiences of our daily episodes differentially along the spatial and temporal dimensions.

A previous study (Lever et al., 2002) showed that complex-spike neurons in the hippocampus gradually shifted their firing locations between geometrically different environments. The forward shifting of firing fields across trials in our study might be related to the Lever et al. (2002) study. That is, CA1 complex-spike neurons might gradually differentiate the two environments (i.e., left side and right side) within the modified T-maze in our study while the rats performed the continuous alternation task, similar to the behavior of CA1 neurons between different environments in Lever et al. (2002). It is important to note, however, that the field shifting in the Lever et al. study occurred over days as the rats experienced different environments, whereas the forwarding shifting of firing fields in our study occurred within each day's recording session without any change in the environment.

Neural Mechanisms underlying the Forward COM-Shifts

An NMDA receptor-dependent, asymmetric plasticity mechanism has been suggested to underlie the translocation of spatial representations in the hippocampus. Specifically, it has been reported that neuronal firing fields in CA1 expand backward and become negatively skewed in shape as rats experience the same

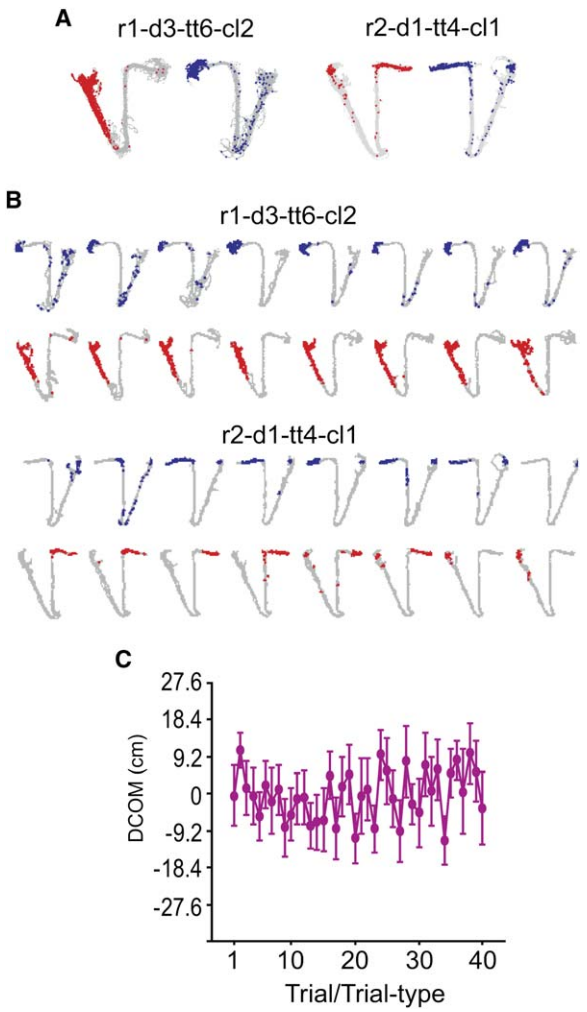

Figure 7. CA1 Firing Fields Representing Non-Stem Regions of the Maze

(A) Representative examples of non-stem firing fields often associated with the locations closer to reward locations of the maze.
(B) Stationary firing fields, shown in (A), parsed based on 5-trials blocks, exhibiting no apparent shifts in field locations across trials.
(C) Plot of change in COM of the CA1 complex-spike neurons whose firing fields were located outside the stem throughout all the trials in the alternation task. These neurons were identified according to the boundaries of their firing fields in the T-maze; if both start and end boundaries of a neuronal firing field were located ahead of bin 26 (bin 1 to 25) in the continuous 56 bins of the linearized maze or were located after bin 45 (bin 46 to 56), based on all the correct L-R or R-L trials *throughout the recording session*, the firing field was considered to not represent the stem. Note that these neurons did not demonstrate a systematic forward shift in ΔCOM.

environment repeatedly (Blum and Abbott, 1996; Ekstrom et al., 2001; Mehta et al., 1997, 2000). However, the results from the current study and other previous studies (Frank et al., 2002; Dragoi and Buzsáki, 2006; Dragoi et al., 2003; Huxter et al., 2003; Lee et al., 2004b) suggest that different experimental conditions may result in more complex patterns of plastic changes in the firing rate distribution of CA1 neurons. In our study, for example, the spatial firing rate distribution of shifting neurons skewed more negatively over trials as reported previously (Mehta et al., 2000; Ekstrom et al., 2001). However, although some neurons increased their field width across trials, on average, the CA1 neurons that shifted their firing distributions forward in the current study did not significantly increase their field size across trials.

Since the asymmetric strengthening of synapses between pre- and postsynaptic neurons should predict the backward shift of firing field positions over time (Blum and Abbott, 1996; Ekstrom et al., 2001; Mehta et al., 1997, 2000), it is currently unclear how to explain the forward shifting phenomenon solely based on the spike timing-dependent plasticity (STDP) mechanism. One possibility is that the STDP-based activation of sequentially connected CA1 neurons (Mehta et al., 2000) might occur strongly in an opposite spatial direction in certain phases (e.g., during and/or after consuming reward on each trial) of the continuous T-maze alternation task. In a recent report (Foster and Wilson, 2006), it has been shown that sequentially arranged place cells can be reactivated in a reverse temporal order in sharp waves at the reward location while the rats are awake, contrary to prior studies showing the opposite reactivation pattern in sharp waves during slow wave sleep (Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Lee and Wilson, 2002). Such reverse activation of neurons representing sequential locations of the maze might occur more strongly in the continuous T-maze alternation task compared to other nonmnemonic paradigms, especially during and/or immediately after the food consumption at the reward locations. The reverse activation might then depotentiate the asymmetrically potentiated, sequential connections among neurons and might even lead to a reversal in the direction of asymmetric strengthening among neurons, thus shifting the firing locations of individual neurons forward across many trials.

Alternatively, the forward shifts in CA1 spatial representations might result from the network interactions between the hippocampus and related cortical regions (e.g., postrhinal and entorhinal cortices), prompted by unique mnemonic demands of the continuous alternation task. Specifically, in our preliminary simulations (Figure 8A), the rat's current positional information (initially coded in the postrhinal-entorhinal network, then conveyed to CA1 directly or indirectly via CA3) is maintained in an individual CA1 neuron (via reverberatory circuits involving the medial entorhinal cortex) during the time of the arrival of the next positional information (Figure 8B). The CA1 neuron then becomes associated with the positional input for the location slightly ahead of the previously coded position. As the association with the previous location becomes competitively weakened after some trials, the CA1 neuron now responds to the new location in the maze and shows a forward shift of firing field for later trials (Figure 8B). Simultaneous recording of network dynamics in other areas (e.g., CA3 and entorhinal cortex) that interact with CA1 during the continuous T-maze alternation task is necessary to further investigate the neural mechanisms of the forward shifting phenomenon at the network level.

Daily Time Course of Trial Type-Dependent Firing on the Stem

The differential firing pattern of CA1 neurons on the stem was observed on the first day of the acquisition of the

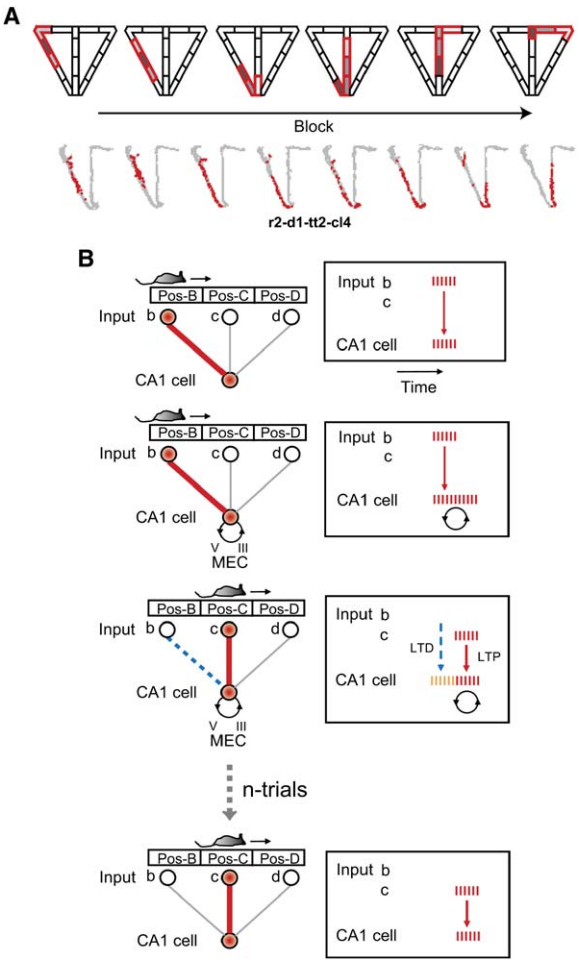

Figure 8. Results of Simulations of a Computational Model of the Forward Shifting Phenomenon in CA1

(A) Spatial representation (red boundaries) of a simulated CA1 neuron during L-R trials over the course of seven blocks (six trials/block). Initially the firing field is located on the beginning of the left return arm, but over time the field shifts toward the right reward zone via the stem. The translocation of the spatial representation physiologically recorded from a CA1 neuron (r2-d1-tt2-cl4) is shown below for comparison.

(B) Schematic of network that produces forward shifting shown in the simulation. Circles represent individual cells (red circles represent active cells, open circles represent nonactive cells at a particular step). "Input" units are composed of multiple components (i.e., postrhinal, medial entorhinal, and CA3), but not shown in the schematic diagram for simplification. Initially, afferent inputs from layer III of the medial entorhinal cortex (MEC) to CA1 convey the animal's current location information (Pos-B) to the CA1 cell (thick red line indicates strengthening of the synapses between the units). As the rat moves to the next location (Pos-C), the neuronal activity of CA1 neuron is maintained through activity in the recurrent network involving CA1 → MEC (layer V → layer III) → CA1 (denoted by the circular arrows associated with the CA1 cell and also by the prolonged spiking pattern of the CA1 cell in box). This persistence could also involve mechanisms for sustained activity in layer V of MEC. As the rat reaches the next location (Pos-C), the activation of the input unit (c) representing the shifted location (Pos-C) results in Hebbian strengthening (LTP) of the connection between the current position information (Pos-C) and the sustained CA1 neuronal activity. The strengthening of new afferent input (c) causes competitive weakening (LTD) of the previous afferent input (b) to the CA1 neuron (blue dashed line). The end result of this process after several trials is that the CA1 cells appear to shift their firing fields forward. In other words, the CA1 cell is no longer driven by the input (b), but is driven by the later input (c).

alternation task in our study, and no significant development was detected across days (Figure 1C). This result is different from that of a recent study (Bower et al., 2005) in which a lack of differential firing along a common navigation path was reported on the first day with a significant improvement afterwards in a similar behavioral paradigm ("skipped reward task"). Procedural differences might underlie the difference between the two studies. Specifically, in the Bower et al. (2005) study, rats were trained with a series of multiple behavioral paradigms, whereas only a single task was used in our study. Therefore, the daily development of the conditional firing pattern in the skipped reward task might be due to the interactive influence from other tasks. Another difference is that, in the Bower et al. (2005) study, rats navigated in a large open arena in which navigation paths were defined between visual objects (i.e., wooden clothespins) attached to the arena. In contrast, in the continuous T-maze, rats were guided by narrow tracks and did not run toward visual objects physically attached to the maze. Although directional navigation in an open platform endows the hippocampal neurons with more directionality than does random foraging in the same environment, the highest directionality of spatial tuning is observed when the rats are engaged in directional movements in a track environment (Markus et al., 1995). Therefore, CA1 neurons might be more directionally tuned in the continuous T-maze environment, and it may have led to a quicker development of the orthogonalized representation for the common navigation path in the sequential context in our study. Finally, memory load might have contributed to the slower development of conditional firing for the shared path in the Bower et al. (2005) study; rats learned to sequentially visit three different locations before they traversed the common path in the skipped reward task, whereas in our task, once a choice is made at the end of the stem in the continuous T-maze, animals were not given any other option than to go to the end of the reward arm, consume the reward, and return to the stem via the return arm.

Implications

Although underlying mechanisms are unclear, the current study provides convincing evidence that ensembles of neurons in the hippocampus can systematically alter their preferred firing locations significantly *within* a behavioral session in the absence of any changes in the environment. Stable firing properties of hippocampal neurons are well-recognized in relatively static environments (Muller et al., 1987; Thompson and Best, 1990). However, many memory tasks involving complex mnemonic strategies and/or cognitive factors have reported dynamic modulations of the place-specific coding scheme of hippocampal neurons (Bower et al., 2005; Breese et al., 1989; Ferbinteanu and Shapiro,

This process may be selective for the continuous alternation task because the hippocampal and associated cortical networks may maintain neural activity longer in this task to guide behavior, resulting in a progressive shift in the associations of cortical inputs with CA1 cell representations. The forward shift does not go past the reward location, because the memories are not retained past that location where the goal is achieved.

2003; Frank et al., 2000; Fyhn et al., 2002; Lenck-Santini et al., 2001; Kobayashi et al., 1997; Markus et al., 1995; Wood et al., 2000). Since it has been suggested that the hippocampal memory system plays a significant role in declarative or episodic memory (Eichenbaum, 2000; Squire, 1992; Vargha-Kadem et al., 1997), in which multiple nonspatial variables often change dynamically in a fixed spatial environment, it is critical to characterize in future studies how the location-bound activities interact in the hippocampus with other mnemonic factors in the environment in various memory tasks.

Experimental Procedures

Subjects

Four male Long-Evans rats (350–450 g; 5–10 months old) were used in the study. Their body weights were maintained at 80%–90% of their ad libitum weights with unrestricted access to water. The animals were housed individually in Plexiglas home cages and were maintained on a reversed light/dark cycle.

Surgery

National Institute of Health guidelines and approved Institutional Animal Care and Use Committee protocols were followed for all surgical procedures. Detailed surgical procedures can be found elsewhere (Lee et al., 2004b). Briefly, each animal was anesthetized with isoflurane, then implanted with a custom-made recording drive that allowed independent manipulations of five tetrodes for recording unit activities and one electrode for recording EEG from fissure. Each tetrode was composed of four fine nichrome wires (12 μm in diameter; Kanthal Palm Coast, FL) twisted together to form a single recording probe. Rats were orally administered 26 mg of acetaminophen in their drinking water bottles after surgery.

Apparatus

A modified T-maze was used for the study. The maze was composed of a traditional T-maze (116 × 107 cm, 10 cm in width) attached with return arms (112 cm, 10 cm in width) from both reward zones to the entry of the stem (Figure 1A). At the end of each "reward arm" (perpendicular to the stem), one or two black chocolate sprinkles were given as food reward. The maze was placed in a room where numerous extra-maze visual cues were available. The room was illuminated by a single 25 W incandescent bulb on the ceiling, centered over the maze. The recording equipment area was separated from the behavioral task area by black curtains on which visual cues were also available.

Behavioral Paradigm

Before surgery and recording, rats were pretrained to run unidirectional laps only on the left side of the continuous T-maze. The access to the opposite side was prevented by two black, wooden blocks placed at the start and end of the maze stem. One or two black chocolate sprinkles were given for every lap at the end of the reward arm. The unidirectional training continued while the recording probes were lowered to the CA1 pyramidal cell layer after the recovery from surgery.

On recording day 1, each animal first finished 15–20 laps of unidirectional runs on the familiar side (i.e., left) of the T-maze. After a brief delay period (5 min), the animal ran 15–20 laps of unidirectional runs on the novel side (i.e., right) of the T-maze. Access to the opposite side of the T-maze was blocked throughout the left- and right-side unidirectional runs. Finally, after the two unidirectional sessions, the blocks were removed and the animal was allowed to alternate between left and right arms of the T-maze to maximize the amount of food reward (~80 trials on average). Rats learned the alternation rule remarkably quickly (approximately within the first 10 trials) and produced very few errors once they acquired the task. Therefore, from recording day 2 to 4, rats were tested only with the alternation paradigm without preceding unidirectional sessions. On each recording day, pre-task and post-task sleep data were recorded to determine, off-line, the stability of recording during behavioral sessions.

Recording and Unit Isolation

Neural signals were first preamplified through unity-gain operational amplifiers. The signals were then amplified between 2000 and 10,000 times and were band-pass filtered (0.3–6 kHz; Neuralynx, Tucson, AZ). Neuronal signals exceeding a channel amplitude threshold were digitized and stored at 32 kHz across all channels of a given tetrode (Cheetah system, Neuralynx, Tucson, AZ). Only complex-spike units in the dorsal CA1 subfield were further analyzed. The rat's position data were also monitored at 30 Hz by recording positions of the array of light-emitting diodes (LEDs) connected to the drive on the rat's head during behavioral recording sessions. After the experiment, histological procedures were performed to verify electrode locations in the hippocampus as previously described (Lee et al., 2004a, 2004b). Single units were isolated based on the characteristics of waveforms recorded on the four channels of each tetrode (cluster-cutting technique) as previously described elsewhere (Lee et al., 2004a, 2004b). Only those units (n = 118/290) that fired $\geq$ 100 spikes with a statistically significant ($p < 0.01$) information score (Skaggs et al., 1993, 1996) of 0.5 or higher for each recording session were used for further analysis.

Data Analysis

T-Maze Data Parsing Based on Trial Types

To parse the continuous navigation of rats according to two trial types (i.e., left-to-right versus right-to-left) of the task, multiple zones (reward zones, reward arm zones, return arm zones, and stem) were overlaid on top of the position data and timestamps matching the entering and exiting events for those zones were marked. Based on the navigation sequence through those zones, the trials were parsed into four categories (i.e., left-to-right, right-to-left, left-to-left, and right-to-right). Only trials from the correct trial-categories (i.e., left-to-right and right-to-left) were analyzed. Position data and spatial correlates of neural spike data were parsed accordingly. Position data for spikes were included only if the instantaneous speed of the animal exceeded 0.25 m/s at the time of spiking, and those spikes in the reward zones were excluded from analysis.

T-Maze Linearization

The position data for each trial category were linearized separately for analysis. Specifically, for the left-to-right trial type, 56 contiguous bins were assigned from the left return arm to the right reward arm via the stem and vice versa for the right-to-left trial type. Position data were then assigned to the bins for each trial type to compose linearized position data. The firing rate for each bin was calculated by dividing the number of spikes fired while the rat occupied the bin by the amount of time spent in the bin. A linearized, firing rate distribution was smoothed by Gaussian kernel (full-width half-maxima at 4). The linearization algorithm did not include the reward locations (15 cm radius from the center of the food dish) due to the immobility frequently observed in those regions during and after the rats consumed the food reward.

Trial Type Differentiation Index

To quantify the amount of differential firing on the stem for different trial types for individual neurons, a trial type differentiation index was calculated as follows.

$$\text{Trial type differentiation index} = \left| \frac{FR_{LR} - FR_{RL}}{FR_{LR} + FR_{RL}} \right|$$

where FR_{LR} and FR_{RL} indicate average firing rate on the stem of the T-maze for all the left-to-right and right-to-left trials within a session, respectively (1.0 = perfect differentiation between the two trial types on the stem, whereas 0.0 = no differentiation). The differentiation index was calculated only for the neurons (n = 78) exhibiting > 1 Hz firing rates on average on the stem during the recording session.

Center of Mass

The shift in the firing field location for each neuron was monitored by calculating the center of mass as previously described elsewhere (Mehta et al., 1997, 2000; Lee et al., 2004b). Briefly, for each linearized firing rate distribution, when the mean firing rates for the four consecutive bins were lower than 10% of the peak firing rate of the distribution, the first bin of the consecutive bins served as a boundary of the firing rate distribution. Once both boundaries were found, the COM was calculated for the firing field distribution within the boundaries (COMAVG). For each trial, the COM for the

trial's firing rate distribution (COM_{trial}) was calculated within the boundaries of the average firing field. The difference (ΔCOM) between COM_{trial} and COM_{AVG} was then calculated to find the position of the trial-based firing field relative to the average firing field that was composed based on all trials.

Ensemble Analysis

Ensemble analysis was performed when a data set contained simultaneous recording of six neurons or more (average stem firing rate > 1 Hz). Ensemble data for each trial type were treated independently. A total of eight ensemble data sets were used for the ensemble analysis. Linearized firing rate distributions from individual trials for each cell in the ensemble were grouped into blocks of 5 trials. The grouped firing rate distributions were then normalized per each cell by maximal firing rate for the neuron throughout all the trials.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/51/5/■ ■ ■/DC1/.

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