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Hippocampal Episode Fields Develop With Learning

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ABSTRACT: Several recent studies have shown that hippocampal neurons fire during the delay period in between trials and that these firing patterns differ when different behaviors are required, suggesting that the neuronal responses may be involved in maintaining the memories needed for the upcoming trial. In particular, one study found that hippocampal neurons reliably fired at particular times, referred to as ''episode fields'' (EFs), during the delay period of a spatial alternation task (Pastalkova et al. (2008) Science 321:1322–1327). The firing of these neurons resulted in distinct sequential firing patterns on left and right turn trials, and these firing patterns could be used to predict the upcoming behavioral response. In this study, we examined neuronal firing during the delay period of a hippocampus-dependent plus maze task, which involved learning to approach two different reward locations (east and west), and we examined the development of these firing patterns with learning. As in the previous study, hippocampal neurons exhibited discrete periods of elevated firing during the delay (EFs) and the firing patterns were distinct on the east and west trials. Moreover, these firing patterns emerged and began to differentiate the east and west conditions during the first training session and continued to develop as the rats learned the task. The finding of similar firing patterns in different tasks suggests that the EFs are a robust phenomenon, which may occur whenever subjects must maintain distinct memory representations during a delay period. Additionally, in the previous study (Pastalkova et al. (2008) Science 321:1322–1327), the distinct firing patterns could have been due to the differing goal locations, behavioral responses (left or right turns), or trajectories. In this study, neuronal firing varied with the goal location regardless of the trajectories or responses, suggesting that the firing patterns encode the behavioral context rather than specific behaviors. © 2010 Wiley Periodicals, Inc.

KEY WORDS: hippocampus; delay; context; place cell; episode field

INTRODUCTION

The hippocampus is importantly involved in memory processes. One line of evidence supporting this idea is the observation that hippocampal neurons respond differently in the same environment under different memory conditions. For example, hippocampal neurons exhibit highly differentiated spatial firing on the stem of a T-maze before right and left turn trials (Wood et al., 2000). Similarly, spatial firing changed dramatically when rats learned to approach different goal locations on a plus maze (Ferbinteanu and Shapiro, 2003; Smith and Mizumori, 2006b). These differential firing patterns were expressed even though the spatial environment had not changed, suggesting that the hippocampus encodes these different experiences that occur in the same place.

Interestingly, recent reports have shown that hippocampal firing also occurs during the delay period in between trials and that these responses also differentiate trial type. For example, neuronal responses differentiated left and right turn trials on a T-maze (Ainge et al., 2007) and the go east and go west conditions of a plus maze task (Smith and Mizumori, 2006b). These results are consistent with findings indicating that the hippocampus is needed when subjects must maintain memory representations across a delay period (O'Keefe and Nadel, 1978; Morris et al., 1982; Squire and Zola-Morgan, 1991) and suggest that differential firing during the delay period may be involved in memory for recent or upcoming trials.

More recently, Pastalkova et al. (2008) showed that hippocampal neurons exhibit strikingly consistent firing patterns during the delay period of a spatial alternation task. Each neuron reliably fired during a discrete period of time, referred to as an "episode field" (EF) by the authors, resulting in the sequential firing of a population of neurons that covered the entire duration of the delay period. Remarkably, these delay-related temporal firing patterns differed on left and right turn trials and they were sufficiently distinct that they could be used to predict the upcoming behavioral response. This finding suggests that the delay-related temporal firing patterns may play an important role in maintaining memory across the delay period.

In this study, we examined delay-related neuronal firing to determine whether similar temporal firing patterns could be found in a hippocampus-dependent plus maze task that we have used previously (Smith and Mizumori, 2006a,b). In this task, the rats learned to approach the east arm for reward during the first half of each session and the west arm during the second half. Each condition (east and west) involved unpredictable start locations so that the rats could not simply remember particular behavioral responses (e.g., right or left turns) or trajectories, but instead had to remember the reward locations.

The rats in the previous study (Pastalkova et al., 2008) were trained to run in an exercise wheel throughout the delay period because continuous loco-

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motion ensured continuous theta activity, which may play an important role in hippocampal firing sequences (Dragoi and Buzsaki, 2006). In this study, we sought to determine whether continuous locomotion was needed to produce the delay-related firing patterns. The rats were placed on a platform adjacent to the maze where they could move freely, but they did not run continuously.

Previously, we have shown that hippocampal spatial and event-related neuronal responses develop with learning in the plus maze task (Smith and Mizumori, 2006b). In this study, we examined delay-related neuronal firing throughout acquisition of the task and during a pretraining control condition to determine whether the temporal firing patterns develop with learning.

METHODS

Subjects and Surgical Procedures

The subjects were 14 food-restricted (80–85% of free feeding weight) adult male Long–Evans rats (Simonsen, CA). Movable stereotrode recording electrodes, fabricated by twisting together two 25-μm lacquer-coated tungsten wires (McNaughton et al., 1983), were stereotaxically positioned just above the CA1 field of the hippocampus (2.5–4.5 mm posterior to bregma, 2.5 mm lateral, and 1.7 mm ventral). A 114-μm Teflon-coated stainless steel wire was implanted in the corpus callosum to serve as a reference electrode. The rats were anesthetized with sodium pentobarbital (40 mg/kg). They were also given atropine sulfate (0.2 mg/kg) to prevent respiratory congestion, an antibiotic (5 mg/kg Baytril), and an analgesic (5 mg/kg ketoprofen). All procedures complied with guidelines established by the University of Washington Animal Care and Use Committee.

Behavioral Training

The rats were trained to retrieve rewards (two drops of chocolate milk) from the east arm of a plus maze during the first half of each training session and from the west arm during the second half of the sessions. The environment and the specific motor behaviors (e.g., locomotion and right and left turns) were equivalent across conditions. Thus, any differential firing patterns in the go east and go west conditions cannot be attributed to these factors.

The maze occupied a circular area, enclosed by curtains, with objects placed around the perimeter to serve as visual cues [for details, see Yeshenko et al. (2004)]. Trials began when the rats were placed on the maze facing outward at the end of an arm. The rats were allowed to search the maze until they located and consumed the reward. The rats were then placed on a 5.5 × 25-cm platform adjacent to the maze for an intertrial delay period, during which time the experimenter pretended to bait each of the four arms of the maze, but actually baited only the appropriate arm for the upcoming trial. The duration of the delay varied from trial to trial and among experimenters, but typically lasted 18–25 s (mean = 23.35 ± 0.35 s). During the delay period, the rats were allowed to move freely about on the platform. However, the small size of the platform prevented running or continuous locomotion. The position of the delay platform was constant throughout training. For this study, the analyses were focused on the delay period in between trials.

Before beginning regular training sessions, the rats were given a preliminary training session during which baseline neuronal and behavioral data were collected. During this session, the rats started each trial on a randomly designated arm and searched for rewards located on a different randomly designated arm. The rats were given two blocks of 10 training trials, separated by 30 s of darkness. Because the dark period in between blocks and the period after the final trial of each were not used, this yielded 18 intertrial delay periods, 9 for each block. The training procedures and behavioral requirements did not differ between the two blocks of trials. Thus, we did not expect to observe differential firing patterns in the two blocks of this random reward session. Instead, this session served as a control condition during which the rats engaged in similar behaviors and received rewards, but the rats did not have to remember the go east and go west conditions of the subsequent regular training sessions.

After the random foraging control session, the rats were given daily training sessions consisting of two blocks of 15 trials each. This yielded 28 intertrial delay periods, 14 for each block. During the first block of every training session, the reward was always placed at the end of the east arm. During the second block, the reward was always placed at the end of the west arm. The start positions for each trial were randomly designated from among the three nonreward arms. The two blocks were separated by 30 s of darkness to cue the rats that the second block was about to begin. Training continued with the same two reward locations presented each day until the rats attained a behavioral criterion of at least 75% correct choices. After achieving this criterion, the rats were given 2–10 additional training sessions for the collection of neuronal data during asymptotic performance.

Data Collection

For this study, we examined data that were collected as part of a study investigating spatial and event-related responses of hippocampal neurons while the rats ran the trials (Smith and Mizumori, 2006b). In this study, we examined the firing of these neurons during the intertrial delay period. Neuronal spike data and video data were collected with the Cheetah Data Acquisition System (Neuralynx, Bozeman, MT). Before training, the recording probes were lowered in 40-μm increments until isolatable units were encountered. The electrodes were advanced to obtain new units when records were lost. The electrodes were also advanced to obtain new units between asymptotic performance sessions to improve the yield. Signals from the electrodes were amplified 2,000–10,000 times, filtered at 600 Hz and 6 kHz, and digitized. All waveforms exceeding a user-defined threshold along with their time of occurrence were stored to disk for offline analysis. Standard spike sorting techniques were used to separate the multiunit records into component single units (MClust, A. D. Redish). Waveform features used for sorting included spike amplitude, spike width, waveform principle components, and waveform area. Additional template-matching algorithms were used to facilitate sorting. For this study, we examined the firing of hippocampal pyramidal neurons (spike widths greater than 270 μs and average firing frequency less that 5 Hz, mean spike width = 328 μs, mean firing frequency = 2.2 Hz). Local field potentials (LFPs) were also recorded from the electrodes in six of the subjects (19 sessions), sampled continuously at 2 kHz, filtered at 1–50 Hz, and stored to disk. The rat's position and direction of travel were monitored by digitized video (sampled at 30 Hz) of an LED array attached to the rat's head. Video data were used to establish the beginning and end of the delay period, which were defined as the time of the rat's arrival and removal from the platform.

Analysis of Delay-Related Firing Patterns

The goals of these analyses were to (a) identify those neurons that exhibited discrete periods of elevated firing during the intertrial delay period, referred to as EFs, (b) determine whether the firing of neurons with EFs differed in the go east and go west conditions, and (c) to determine whether these firing patterns developed as the rats learned the task. We adapted the procedures of the previous study (Pastalkova et al., 2008) to identify those neurons that had EFs. Briefly, the spike trains were smoothed with a Gaussian [standard deviation (SD) = 100 ms] and grouped according to trial type (go east or go west). Neurons were classified as having an EF during the intertrial delay period if they (a) had a peak firing rate of at least 5 Hz, (b) had a peak firing rate at least three standard deviations above the mean, and (c) had an EF duration (defined as a period of elevated firing greater than 10% of the maximum firing rate) of less than half the duration of the delay period.

We also computed a measure of the temporal deviation for each neuron that had an EF in at least one block (see Supporting Information for details). This measure reflects how well the period of elevated firing (i.e., the EF) was aligned from one trial to the next. To obtain this measure, we correlated the firing rate profile from each trial with the average of all other trials in the block. The spike train for the trial was then shifted in time (with wrap around) in 1-ms increments until the best correlation was achieved. The time shift that resulted in the best correlation was recorded, and this procedure was repeated for each trial of a given type (east or west). The median time shift served as our temporal deviation measure. The median, rather than the mean, was used so that the temporal deviation measure would not be overly sensitive to individual trials with large deviations from the average. This temporal deviation measure reflects the consistency of the trial-to-trial firing rate profiles and therefore serves as a measure of how reliably the neurons fired within a circumscribed period during the intertrial delay.

We also used the temporal deviation measure to determine whether the firing patterns observed in the go east condition differed from those in the go west condition. In the previous analysis, we computed the average temporal deviation of the trials within a block. For this analysis, we computed the temporal deviation between each trial of one block and the average of the trials from the other block. We then compared the temporal deviation scores obtained within each trial type to those obtained across trial types with a two-sided Wilcoxon signed rank test (see Supporting Information for details). Any neuron with significantly different ($P < 0.05$) within- and across-trial type temporal deviation scores was classified as having differential firing in the two conditions.

Analysis of Learning-Related Development of Neuronal Firing Patterns

To examine the learning-related development of the neuronal firing patterns, we compared the neuronal responses during the random foraging control session with the neuronal responses during regular training sessions. Any block differences in neuronal responding that develop with training, but were not present during the random foraging control session, can be attributed to the rats learning to discriminate the go east and go west conditions. To accomplish this, we examined data from the random foraging session, the first acquisition session, the middle training session, and asymptotic performance sessions. The middle training session was simply the session that was half way through the duration of training for each rat. For example, for a rat that required seven sessions to reach the criterion, the fourth training session served as the middle session.

To determine whether trial type-specific responses developed with learning, we compared the percentage of neurons that exhibited significantly different responses during the east and west trials at each stage of training to a chi-square test. To determine whether EFs became more consistent with training, the temporal deviation scores were submitted to an analysis of variance (ANOVA) with training stage as a factor. Similarly, the EF durations were submitted to an ANOVA with training stage as a factor.

Spatial Firing During Trial Performance

The spatial firing (place field) data have been reported previously (Smith and Mizumori, 2006b), but are included here to compare place fields and EFs and to determine whether the two kinds of responses are generated by overlapping neuronal populations. Neurons were classified as having a place field if they (a) fired in at least four adjacent pixels (2.5×2.5 cm^2) but less than half of the maze area, (b) had a within-field firing rate at least twice that of the firing rate outside the field, and (c) fired during more than 50% of the passes through the field.

It was important to ensure that EF-related firing during the delay period could not be attributed to spatial firing (i.e., a place field) on the delay platform. It is unlikely that the rats arrived at a particular location on the platform at the same latency from the start of the delay period on each trial and then

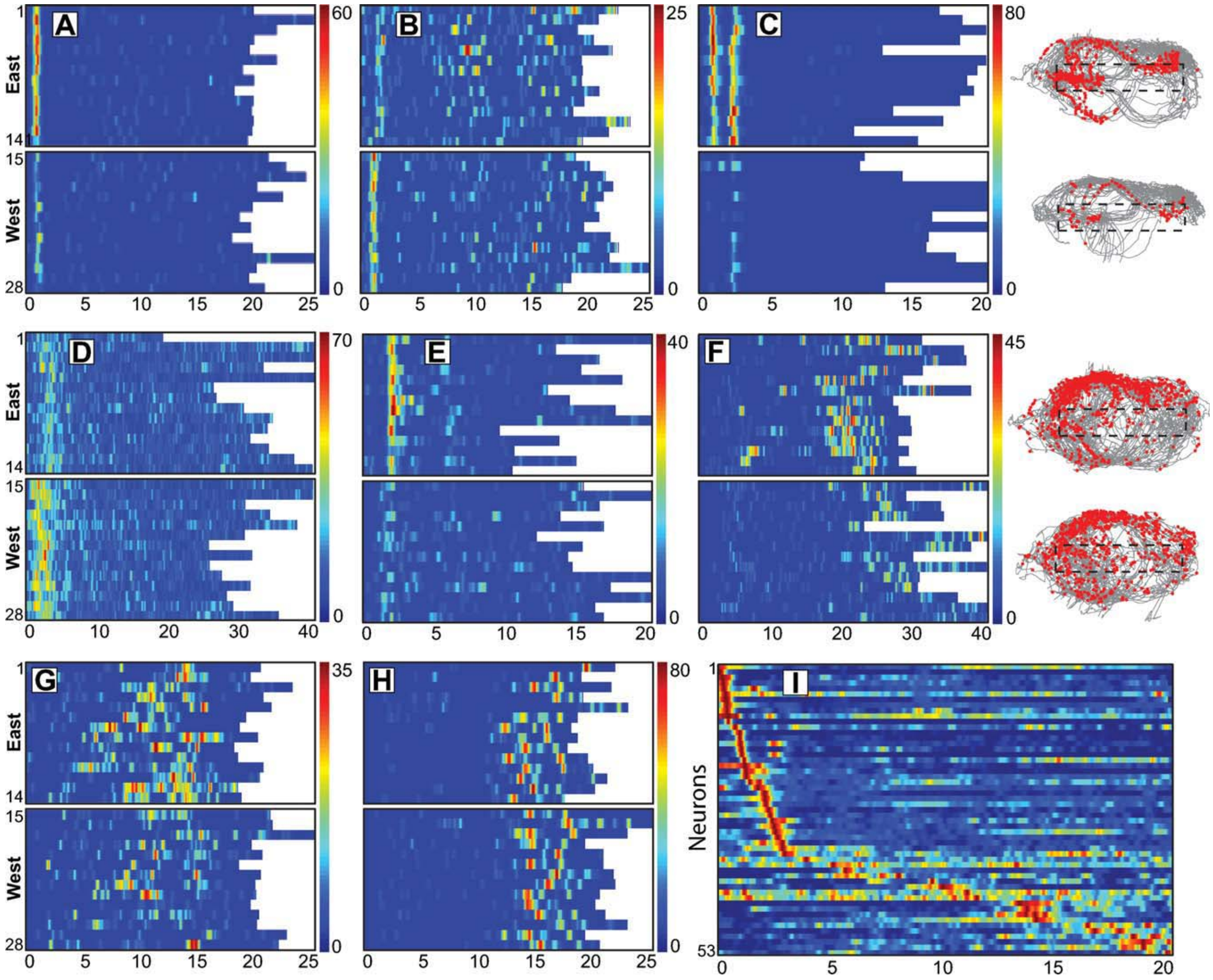

FIGURE 1. Hippocampal EFs after learning. Plots A–H show examples of individual neurons that exhibited EFs during the intertrial delay period, while the rats were performing at asymptote. Each plot was constructed from the data of a single neuron recorded during a single session. For each plot, the trial-by-trial firing of the neuron is shown, one trial per row. The first fourteen trials (the go east condition) are shown in the top half of the plot, while the second fourteen trials (the go west condition) are shown in the bottom half. Different neurons fired at different times throughout the delay period. Time (s) from the start of the variable delay period is shown for each plot. Also shown are examples (plots C and F) of the movement paths of the rats head (gray lines) and neuronal firing (red dots) during the delay period. The platform is indicated by the dashed rectangle. Plot I shows the firing of 53 neurons, recorded from 41 sessions in 12 rats. Each row illustrates the firing of a single neuron, averaged across the 14 trials of the go east condition, arranged according to the time of firing to illustrate the distribution of firing across the duration of the intertrial delay period.

did not enter the area at other times. Nevertheless, to ensure that EF-related firing during the delay period could not be attributed to spatial firing on the delay platform, we only included neurons in the EF analysis that did not meet the criterion for having a place field (firing in less than half the platform area, firing rate at least twice the firing outside the putative place field, and firing during more than 50% of the passes through the field). With many of the neurons, place fields could be ruled out because firing was not localized, but occurred all over the platform. In other cases, the firing was not uniformly distributed across the platform area but a place field could be ruled out because the neurons did not reliably fire in any particular location. For example, the firing depicted in Figure 1F occurred primarily in the upper left part of the platform. However, unlike typical place cells, the neuron did not fire on most of the passes through that region but instead only fired on 36% (21/58) of the passes.

LFP Analysis

LFPs were recorded from several of the rats in this study (19 records from six different rats). LFP records from electrodes that had pyramidal neurons were filtered at 6–12 Hz to examine theta band activity. For each record, the average theta power was computed separately for the trials and the intertrial delay period. The power spectrum density analysis was carried out using the multitaper method (Matlab, Mathworks; Jarvis and Mitra, 2001). The data of the trials and the delay period were compared with a paired samples *t*-test.

RESULTS

Behavior

The rats achieved the performance criterion in an average of 6.7 sessions. Choice accuracy improved significantly with training ($F[3,39] = 72.47$, $P < 0.001$). As expected, the rats chose the rewarded arm at chance levels during the random foraging control session (35.71% correct, compared to chance performance of 33.3%, one-sample *t*-test, $t_{(13)} = 1.01$, $P = 0.33$) and they reached an asymptote of 79.6% correct, on average.

EF Characteristics During Asymptotic Performance

Hippocampal neurons reliably exhibited discrete periods of firing during the intertrial delay period (i.e., EFs, Fig. 1), which were very similar to those reported by Pastalkova et al. (2008). After the rats had learned the task, 58 of the 301 neurons recorded at asymptote (19.3%) exhibited EFs. Remarkably, these temporal firing patterns were different in the go east and go west conditions. Of the 58 neurons that exhibited EFs during asymptotic performance, 28 (48.3%) exhibited significantly different firing patterns in the two conditions. Many of these firing periods were remarkably consistent from one trial to the next (e.g., Fig. 1A). The average temporal deviation was 1.87 s [±0.20 standard error of mean (SEM), range: 0.02–12.73 s]. The average duration of the EFs was 2.79 s ± 0.10 SEM (min = 0.74, max = 9.7).

As in the previous study (Pastalkova et al., 2008), the EFs could occur at any time during the intertrial delay period. However, the prevalence of the EFs and their characteristics varied across the duration of the delay period. EFs were much more likely to occur during the first 5 s of the delay period than during later times in the delay period. During the first quarter of the intertrial delay period, 73.1% of the EFs occurred, whereas during second, third, and fourth quarters of the delay period, 8.9, 3.8, and 14.1% of the EFs occurred, respectively. The EFs that occurred early in the delay period were of shorter duration than those that occurred later (3.0 ± 0.35 s during the first half of the delay, compared to 8.1 ± 1.45 s during the second half). The EFs that occurred early in the delay period were also more likely to differentiate the go east and go west conditions than those that occurred late in the delay period. 58.1% of the EFs that occurred during the first half of the delay period differed significantly across the conditions, compared to 20.0% during the second half. Figure 1I shows the firing of 53 neurons recorded from several different rats and training sessions, aligned according to the time of the EFs. The similarity of these data to those reported by Pastalkova et al. (2008), in which many neurons were recorded from a single rat, suggests that the present data reflect the same underlying neural processes.

Learning-Related Development of EFs

Over all stages of training, 103 of the 526 neurons (19.58%) exhibited EFs. EFs were discernable at the outset of training (Fig. 2). Fifteen of the 105 hippocampal neurons (14.3%) recorded during the random foraging control session exhibited EFs that met our criteria. However, they were significantly less reliable during the random foraging session than during subsequent regular training sessions. Post hoc tests following a significant main effect of training stage ($F[3,165] = 3.41$, $P < 0.05$) indicated that the temporal deviation scores were significantly higher during random foraging than during all other training stages (all $P < 0.05$). Thus, the EFs became significantly more reliable during the first training session (mean temporal deviation = 1.30 s, compared to 3.47 s during the random foraging session). Although the percentage of neurons that exhibited EFs, regardless of whether they differentiated the east and west conditions, increased from 14.3% (15 of 105 neurons) during random foraging to 19.3% (58 of 301 neurons) during asymptotic performance, this increase was not significant (${\chi_{(3)}}^2 = 6.11$, $P = 0.11$).

As mentioned above, EFs were present from the outset of training and the overall percentage of neurons with EFs did not change. However, the neurons began to differentiate the go east and go west conditions during the first training session, and the percentage of neurons with differential responses increased with training. During the first session, 20.8% (15 of 72) of the neurons exhibited EFs and 26.7% (4 of the 15 neurons with EFs) of these EFs were significantly different in the go east and go west conditions. By the middle training session, the percentage of neurons that exhibited significantly different EFs in the go east and go west conditions had increased to 53.3% (8 of the 15 neurons with EFs). The percentage of neurons with these differential EFs increased significantly with training ($\chi^2_{(3)}= 8.25$, $P < 0.05$, Fig. 2A).

EFs and Place Fields

Not surprisingly, many pyramidal neurons exhibited spatially localized firing (place fields) on the maze. Over all stages of training, 205 of the 526 pyramidal neurons (39.0%) exhibited place fields, compared to 103 neurons (19.6%) that exhibited EFs. Of the 103 neurons that had an EF during the delay period, 46 (44.7%) also had a place field on the maze. Thus, the spatial and temporal firing patterns seen in this study were produced by different, but overlapping populations of neurons.

To determine whether there was any relationship between the EFs and place fields, we compared firing during the delay period and the trials in those neurons that had an EF and a place field during asymptotic performance. Consistent with the results of Pastalkova et al. (2008), the firing rates during the

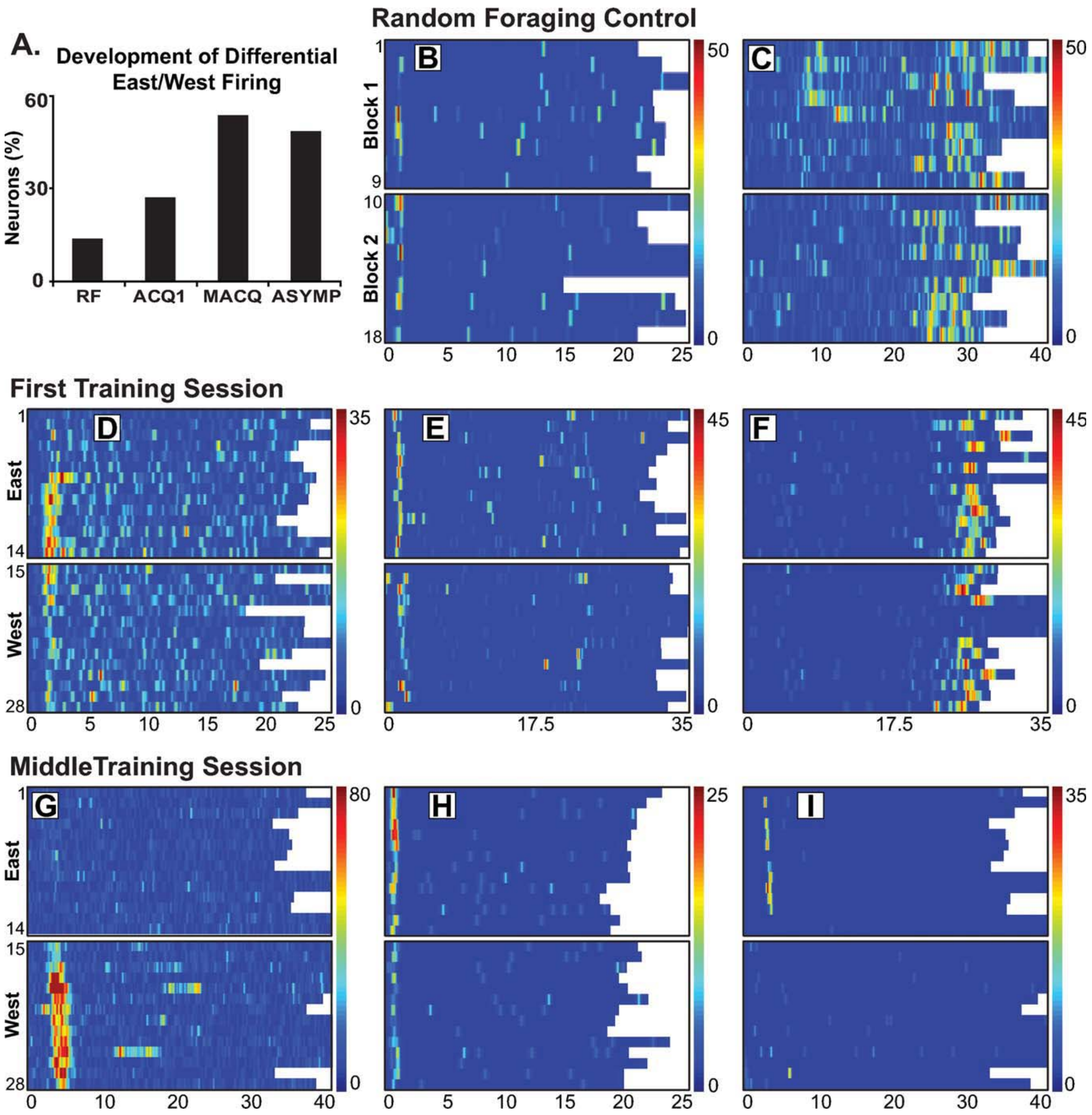

FIGURE 2. **Development of EFs during learning. Plot A shows the percentage of hippocampal neurons that exhibited significantly different EFs in the go east and go west conditions for each stage of training. Plots B and C illustrate the firing of two neurons recorded during the random foraging control session, when the EFs were significantly less reliable than during subsequent regular training sessions. Plots D–F and plots G–I illustrate EFs recorded during the first training session and the session midway through training, respectively.**

trials and during the delay period were not correlated ($r = -0.16$, $P = 0.44$). The firing rate during the delay was also not correlated with firing on the goal arm ($r = 0.07$, $P = 0.52$). Additionally, the size of the EFs (duration) was not correlated with the size (area) of the place fields ($r = -0.18$, $P = 0.37$). We also examined whether the time of firing during the delay period was associated with the time of firing during the trials. To accomplish this, we binned the EFs and place fields into four categories according to their time or place of occurrence. For example, a neuron that had an EF during the first quarter of the delay period and a place field near a start position on the maze was given a value of 1 for the EF and 1 for the place field. A neuron that had an EF during the second quarter of the delay period and a place field near the reward location on the maze was given a value of 2 for the EF and 4 for the place field. We constructed a 4 × 4 table (the 4 quarters of delay by

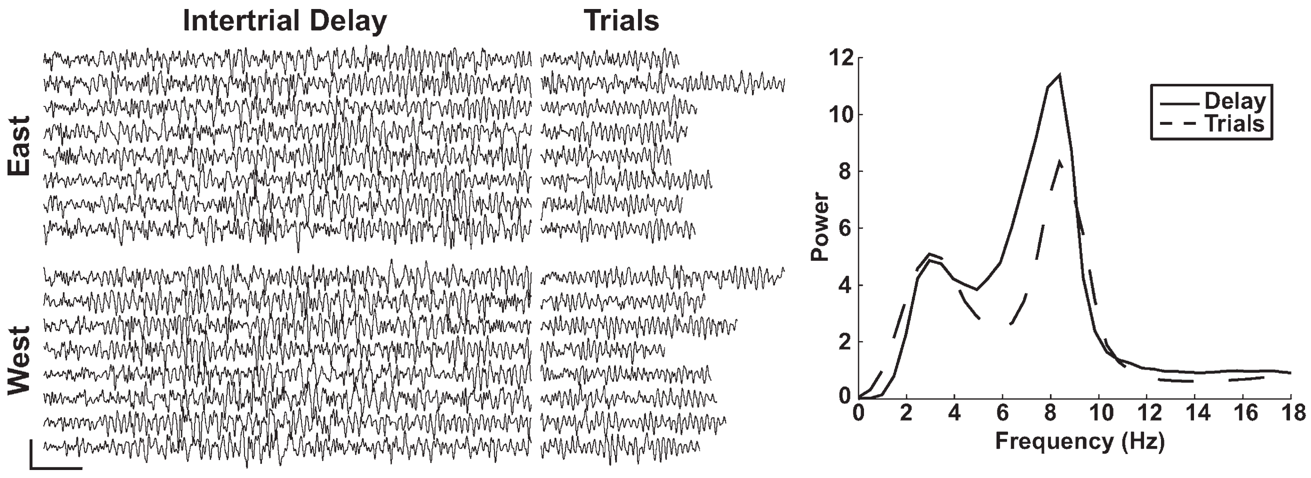

FIGURE 3. Representative LFP traces. Traces, filtered at 0–20 Hz, are shown for the intertrial delay period and during the running of the trials for a representative subject. For simplicity, traces are only shown for the last eight trials of the go east condition and the first eight trials of the go west condition, and only the first 10 s of intertrial delay period are shown. The scale bars indicates 1 s and 1 mV. The average power spectra, in arbitrary units, for the LFPs recorded during the delay period (dotted) and the trials (dashed) are shown in the plot on the right.

the 4 quarters of the trial) and performed a chi-square test for independence, which indicated that the time of firing during the delay and during the trials were not related ($\chi^2_{(9)} = 6.73$, $P = 0.67$). Overall, these comparisons suggest that there was no clear relationship between the EFs and the place fields.

EFs and the Theta Rhythm

The firing of hippocampal place cells is systematically related to the theta oscillations seen in the LFP. Pastalkova et al. (2008) reported that the firing during the EFs is related to theta in a manner similar to that of place fields, including theta phase precession and theta compression. These findings suggest that the theta rhythm may be an important feature of the EFs.

In this study, the rats were placed on a small platform during the intertrial delay. Although they could not run consistently, as they did during the trials, the rats were free to move about on the platform (Fig. 1C,F). The theta rhythm was readily detectable during the delay. In fact, the theta power (6–12 Hz) was significantly higher during the delay than during the trials (6.17 ± 0.64 dB during the delay, compared to 5.24 ± 0.62 during the trials, $t_{(18)} = 3.82$, $P < 0.001$, Fig. 3).

The greater theta power seen during the delay could not be attributed to greater movement during the delay period, relative to the trials. The rats' velocity and acceleration were significantly greater during the trials, when the rats were running the maze, than during the delay period (velocity: 30.38 ± 0.66 cm/s during the trials, compared to 9.27 ± 0.32 during the intertrial delay, $t_{(62)} = 45.09$, $P < 0.001$; acceleration: 47.29 ± 1.15 cm/s^2 during the trials, compared to 28.83 ± 0.65 during the intertrial delay, $t_{(62)} = 21.37$, $P < 0.001$).

Previous authors (e.g., Bland, 1986) have identified two different forms of the theta rhythm, type 1 (8–12 Hz) and type 2 (4–8 Hz), associated with locomotion and nonlocomotor arousal and sensory processing, respectively. Thus, we separately analyzed theta power in these two frequency bands and found that the type 2 theta power was significantly greater during the delay period, relative to the trials (4–8 Hz, $t_{(18)} = 3.72$, $P < 0.05$). However, there was no difference between the delay period and the trials for type 1 theta (8–12 Hz, $t_{(18)} = 1.22$, $P = 0.24$).

DISCUSSION

Hippocampal neurons exhibited discrete periods of elevated firing during the delay period, and these firing patterns differed on go east and go west trials. These results confirm the previous finding of EFs (Pastalkova et al., 2008) and extend the findings in several important ways. The present results indicate that distinct delay-related firing patterns began to develop during the first day of training, relative to the random foraging control condition, and that the differential firing patterns developed as subjects learned to discriminate the go east and go west conditions. Additionally, we used a blocked alternation procedure instead of trial-by-trial alternation, and our task took place on a plus maze with variable start locations rather than a figure 8 maze with a consistent start location. Our training procedure did not require that the rats run in an exercise wheel during the delay, as in the previous study. Instead, they spent the intertrial delay on a small platform adjacent to the maze. The fact that we observed similar temporal firing patterns despite the differences between the two tasks suggests that EFs are a robust phenomenon that may occur whenever subjects must maintain a memory through a delay period to distinguish different kinds of trials.

Although EFs were present at the outset, during the random foraging control session, they became significantly more reliable as soon as regular training sessions began. Thus, the EFs became sharper and more well defined when a memory demand was placed the subjects, with the switch from random foraging to the east/west discrimination task. Importantly, the EF firing patterns did not differ in the two blocks of the random foraging control session. Instead, the EFs began to differentiate the go east and go west conditions during the first training session, and these differential firing patterns continued to develop as the rats learned to discriminate the go east and go west trials (Fig. 2A).

Interestingly, the characteristics of the EF population in the present study differed across the duration of the delay period. It appeared that the EFs of the previous study (Pastalkova et al., 2008) were more prevalent at the beginning of the delay period and the later EFs had a greater duration than the earlier EFs. This pattern was apparent and may have been exaggerated in our data. Although EFs occurred throughout the delay period, the majority of them (73%) occurred in first quarter of the delay (5–7 s). Additionally, the EFs during the first half of the delay had a much shorter duration, about half the duration of the EFs occurring during the second half, and they were more likely to differentiate the go east and go west conditions. Although the reasons for this are not clear, one possibility is that the firing patterns become more variable with the accumulation of timing errors across the delay period, which could be exacerbated by the unpredictable delay duration in the present task. Another possibility is that this reflects an intrinsic property of the hippocampal network. Interestingly, a computational model of learning in CA3 predicted similar firing patterns, with shorter firing periods and a greater number of neurons firing during the early part of a trace interval (Levy et al., 2005). Under this model, EF durations increase as CA3 neurons approach a stable state and neuronal activity changes more slowly.

Pastalkova et al. (2008) simultaneously recorded many neurons and were able to show that hippocampal neurons fire in a sequence that covers the entire delay period. In contrast, we recorded comparatively fewer neurons from each of several subjects. Therefore, we cannot directly confirm the presence of firing sequences in individual rats. Nevertheless, hippocampal neurons consistently fired during restricted time periods during the delay, and the sampled neurons fired at different times, which covered the entire delay period, just as in the previous study. In fact, the distribution of the fields from several rats (Fig. 1I) was remarkably similar to the distribution from a single rat in the previous study. These similarities suggest that both studies reflect the same phenomenon.

In our task, the rats spent the intertrial delay period on a small platform adjacent to the maze, rather than a running wheel. Previous authors have suggested that the ongoing theta rhythm may be an important organizing mechanism for the sequential firing patterns seen during the offline replay of place cell firing (Dragoi and Buzsaki, 2006) and for the sequential firing seen during the delay period (Pastalkova et al., 2008). In this study, the theta rhythm was clearly present during the intertrial delay even though the rats did not run continuously (Fig. 3). Interestingly, type 2 theta power was greater during the intertrial delay period than during the trials. Previous reports have shown that type 2 theta can occur during periods of little or no movement, particularly in relation to arousal and attention (Givens, 1996; Seager et al., 2002; Bland et al., 2007; Takahashi et al., 2009). In this study, the rats often engaged in behaviors associated with arousal (e.g., rearing, pacing back and forth, circling). Thus, the enhanced theta seen during the delay period may have been related to heightened arousal.

Relevance of the Temporal Firing Patterns to Memory

The differential firing patterns developed as subjects learned to distinguish the go east and go west conditions, but not in a control condition that did not involve different memory demands (the random foraging condition and the control conditions of the previous study) and hippocampal lesions impair performance of these tasks (Smith and Mizumori, 2006a; Pastalkova et al., 2008). These results clearly suggest that the delay-related firing patterns are relevant to memory. Thus, the present results join previous findings (Smith and Mizumori, 2006b; Ainge et al., 2007; Pastalkova et al., 2008) in establishing a link between neuronal firing during the delay and the ability to remember and perform different behavioral responses. However, the precise role of these temporal firing patterns is not clear. An obvious possibility is that they are involved in maintaining the memory for the specific behavioral response (e.g., right or left turn) or the appropriate movement trajectory for the upcoming trial. However, the data of this study and theoretical considerations suggest that this is unlikely.

The firing patterns were highly consistent from one trial to the next, but the specific behavioral responses and path trajectories varied across trials because the rats started from different positions (see Supporting Information Fig. S1). For example, starting from the north arm required a left turn response in the go east condition, whereas starting from the south arm required a right turn response and starting from the west arm required a straight run. Thus, if the firing patterns were related to the specific behavioral responses or trajectories, we would expect three distinct firing patterns in each condition (east and west). We found no evidence of this. Additionally, the start positions for the trials were unpredictable and so the firing patterns could not have represented upcoming responses.

Theoretical considerations also suggest that these firing patterns are unlikely to be memory representations of upcoming or recent behavioral responses. A basic principle of memory theory is that memories are expressed through activity in the population of neurons, which were active during the original experience. Perhaps the most striking example of this idea is the experience-dependent replay of hippocampal place cell firing. In these studies, the same neurons that fired while rats ran on a maze spontaneously fire in the same sequence (or reverse sequence) during subsequent pauses in behavior and during sleep (Wilson and McNaughton, 1994; Diba and Buzsaki, 2007; Ji and Wilson, 2007). Additionally, recent studies involving neuronal recording in humans have shown that hippocampal neurons that responded to video clips were also active during the subsequent free recall of those items (Gelbard-Sagiv et al., 2008).

In contrast to this idea, our data suggest that the delay-related firing patterns were not a replay of the spatial firing patterns seen during performance of the trials. The delay and spatial firing patterns were produced by substantially different neuronal populations. Even in those cases where an individual neuron did exhibit an EF and a place field, there was no clear relationship between the two kinds of responses. The firing rates during the delay and the trials were not correlated nor were the size of the EFs and place fields. Moreover, the timing of the EFs and the place fields were not related. Neurons that had EFs early (or late) in the delay period did not preferentially have place fields near the beginning (or end) of the trials. This suggests that the memory function of these firing patterns is different from the memory function of sequential replay of place cells that were active during the task.

Although it is unlikely that the temporal firing patterns represent the memory for the specific behavioral response or trajectory, it is possible that they maintain the memory for the reward location. However, it is not clear why the encoding of a reward location would require a temporally extended sequential population code when simpler coding mechanisms would probably be sufficient. For example, neurons in the dorsolateral prefrontal cortex of monkeys appear to encode working memory representations for spatial locations across a delay by means of sustained firing throughout the delay period (Funahashi et al., 1989). Sustained delay-related firing, which could be involved in memory maintenance, has also been seen in the hippocampus of rats during a spatial alternation task (Takahashi et al., 2009).

For consistency, we have adopted the "EF" terminology of Pastalkova et al. (2008). However, it is not our intent to suggest that EFs encode individual memory episodes in the same way that place fields have been thought to encode specific locations. Nor does this suggest that "episode cells" are more closely associated with episodic memory processes than place cells or other kinds of hippocampal responses. Although these firing patterns may well be involved in episodic memory processes, it remains to be determined exactly which experiences these firing patterns represent. The fact that the firing patterns are very similar across trials of the same type but differ across the east and west conditions suggests that they are more directly relevant to distinguishing these conditions than distinguishing unique, single trial experiences from each other.

At asymptote in the present task, about one-fifth of hippocampal neurons exhibited EFs and about half of these neurons (9.3% of the total neurons recorded) fired differentially in the east and west conditions. Interestingly, this kind of heterogeneous mixture of "differentiating" and "nondifferentiating" neurons is also commonly seen in the spatial firing patterns observed in similar tasks (Wood et al., 2000; Ferbinteanu and Shapiro, 2003; Smith et al., 2003) and may be related to the phenomenon of "partial remapping" (Skaggs and McNaughton, 1998; Mizumori et al., 2007). At first glance, the percentage of neurons that differentiated the east and west conditions of this study may seem small. However, the EFs are only one of several kinds of memory-relevant responses that develop as the rats learn this task. Hippocampal neurons also exhibit strikingly different spatial firing patterns and differential responses to the reward (Smith and Mizumori, 2006b). Thus, hippocampal neurons are engaged by various aspects of this task, and they appear to be particularly relevant to differentiating the east and west conditions.

The delay-related firing patterns seen here and the well-known spatial firing patterns of hippocampal neurons are clearly related to behavioral performance in tasks such as the present one, and lesion studies confirm that the hippocampus is needed for these tasks (Smith and Mizumori, 2006a; Ainge et al., 2007; Pastalkova et al., 2008). Indeed, there is an extensive literature showing that hippocampal firing patterns are highly sensitive to the task demands present in a given learning situation. For example, changes in the behavioral task demands result in changes in the spatial firing patterns, even when the spatial geometry has not changed (e.g., Markus et al., 1995; Wood et al., 2000; Ferbinteanu and Shapiro, 2003; Smith and Mizumori, 2006b), and different firing patterns can occur while a rat is engaged in the same behavior in the same location if the behavioral strategy or the motivation has changed (Yeshenko et al., 2004; Kennedy and Shapiro, 2009). Previously, we have suggested that these differing task demands, goals, and motivations constitute a kind of behavioral context and that the highly distinctive spatial firing patterns could provide a means of encoding and differentiating these contexts (Smith and Mizumori, 2006a; Smith, 2008). The delay-related firing patterns seen here could also contribute to this process. If so, these context representations unfold as the rats pass through place fields while they perform the behavioral responses and, as the present results indicate, as they maintain the memory for the current behavioral context through a delay period.

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REFERENCES

Ainge JA, van der Meer MA, Langston RF, Wood ER. 2007. Exploring the role of context-dependent hippocampal activity in spatial alternation behavior. Hippocampus 17:988–1002.

Bland BH. 1986. The physiology and pharmacology of hippocampal formation theta rhythms. Prog Neurobiol 26:1–54.

Bland BH, Derie-Gillespie D, Mestek P, Jackson J, Crooks R, Cormican A. 2007. To move or not: Previous experience in a runway avoidance task determines the appearance of hippocampal type 2 sensory processing theta. Behav Brain Res 179:299–304.

Diba K, Buzsaki G. 2007. Forward and reverse hippocampal place-cell sequences during ripples. Nat Neurosci 10:1241–1242.

Dragoi G, Buzsaki G. 2006. Temporal encoding of place sequences by hippocampal cell assemblies. Neuron 50:145–157.

Ferbinteanu J, Shapiro ML. 2003. Prospective and retrospective memory coding in the hippocampus. Neuron 40:1227–1239.

Funahashi S, Bruce CJ, Goldman-Rakic PS. 1989. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61:331–349.

Gelbard-Sagiv H, Mukamel R, Harel M, Malach R, Fried I. 2008. Internally generated reactivation of single neurons in human hippocampus during free recall. Science 322:96–101.

Givens B. 1996. Stimulus-evoked resetting of the dentate theta rhythm: Relation to working memory. Neuroreport 8:159–163.

Jarvis MR, Mitra PP. 2001. Sampling properties of the spectrum and coherency of sequences of action potentials. Neural Comput 13:717–749.

Ji D, Wilson MA. 2007. Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10:100–107.

Kennedy PJ, Shapiro ML. 2009. Motivational states activate distinct hippocampal representations to guide goal-directed behaviors. Proc Natl Acad Sci USA 106:10805–10810.

Levy WB, Sanyal A, Rodriguez P, Sullivan DW, Wu XB. 2005. The formation of neural codes in the hippocampus: trace conditioning as a prototypical paradigm for studying the random recoding hypothesis. Biol Cybern 92:409–426.

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

McNaughton BL, O'Keefe J, Barnes CA. 1983. The stereotrode: A new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. J Neurosci Methods 8:391–397.

Mizumori SJ, Smith DM, Puryear CB. 2007. Hippocampal and neocortical interactions during context discrimination: Electrophysiological evidence from the rat. Hippocampus 17:851–862.

Morris RG, Garrud P, Rawlins JM, O'Keefe J. 1982. Place navigation is impaired in rats with hippocampal lesions. Nature 297:681–683.

O'Keefe J, Nadel L. 1978. The Hippocampus as a Cognitive Map. Oxford, UK: Clarendon Press.

Pastalkova E, Itskov V, Amarasingham A, Buzsaki G. 2008. Internally generated cell assembly sequences in the rat hippocampus. Science 321:1322–1327.

Seager MA, Johnson LD, Chabot ES, Asaka Y, Berry SD. 2002. Oscillatory brain states and learning: Impact of hippocampal theta-contingent training. Proc Natl Acad Sci USA 99:1616–1620.

Skaggs WE, McNaughton BL. 1998. Spatial firing properties of hippocampal CA1 populations in an environment containing two visually identical regions. J Neurosci 18:8455–8466.

Smith DM. 2008. The hippocampus, context processing and episodic memory. In: Dere E, Easton A, Nadel L, Huston JP, editors.Handbook of Behavioral Neuroscience, Vol 18: Handbook of Episodic Memory. Amsterdam, The Netherlands: Elsevier. pp 465–481.

Smith DM, Mizumori SJ. 2006a. Hippocampal place cells, context, and episodic memory. Hippocampus 16:716–729.

Smith DM, Mizumori SJY. 2006b. Learning-related development of context-specific neuronal responses to places and events: The hippocampal role in context processing. J Neurosci 26:3154–3163.

Smith DM, Kolarick D, Mizumori SJY. 2003. Hippocampal neurons develop context-specific firing patterns during spatial learning. Soc Neurosci Abstr Program No. 677.11.

Squire LR, Zola-Morgan S. 1991. The medial temporal lobe memory system. Science 253:1380–1386.

Takahashi M, Lauwereyns J, Sakurai Y, Tsukada M. 2009. Behavioral state-dependent episodic representations in rat CA1 neuronal activity during spatial alternation. Cogn Neurodyn 3:165–175.

Wilson MA, McNaughton BL. 1994. Reactivation of hippocampal ensemble memories during sleep. Science 265:676–679.

Wood ER, Dudchenko PA, Robitsek RJ, Eichenbaum H. 2000. Hippocampal neurons encode information about different types of memory episodes occurring in the same location. Neuron 27:623–633.

Yeshenko O, Guazzelli A, Mizumori SJ. 2004. Context-dependent reorganization of spatial and movement representations by simultaneously recorded hippocampal and striatal neurons during performance of allocentric and egocentric tasks. Behav Neurosci 118:751–769.