Cogn Neurodyn (2009) 3:165–175
DOI 10.1007/s11571-009-9081-5

RESEARCH ARTICLE

Behavioral state-dependent episodic representations in rat CA1 neuronal activity during spatial alternation

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Received: 7 October 2008 / Revised: 9 March 2009 / Accepted: 9 March 2009 / Published online: 1 April 2009

Abstract Hippocampus is considered crucial for episodic memory, as confirmed by recent findings of "episode-dependent place cells" in rodent studies, and is known to show differential activity between active exploration and quiet immobility. Most place-cell studies have focused on active periods, so the hippocampal involvement in episodic representations is less well understood. Here, we draw a typology of episode-dependent hippocampal activity among three behavioral periods, presumably governed by different molecular mechanisms: Active exploration with type 1 theta, quiet alertness with type 2 theta, and consummation with large amplitude irregular activity. Five rats were trained to perform a delayed spatial alternation task with a nose-poke paradigm and 12 tetrodes were implanted for single-unit recordings. We obtained 135 CA1 pyramidal cells and found that 75 of these fired mainly during active exploration, whereas 42 fired mainly during quiet alertness and 18 during consummation. In each type of neuron, we found episode-dependent activity: 51/75, 22/42, and 15/18, respectively. These findings extend our knowledge on the hippocampal involvement in episodic memory: Episode dependency also exists during immobile periods, and functionally dissociated cell assemblies are engaged in the maintenance of episodic information throughout different events in a task sequence.

Keywords Hippocampus · Spatial alternation · Memory · Rat · Nose-poke paradigm

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Introduction

Hippocampus is believed to play a crucial role in episodic memory (Eichenbaum 2000). In rodent studies, one line of empirical evidence shows episode-dependent place cells whose firing is not only spatially limited (i.e., place field) but also systematically changed by task episodes—from where the rat comes and/or to where it goes (Frank et al. 2000; Wood et al. 2000; Ferbinteanu and Shapiro 2003; Bower et al. 2005; Dayawansa et al. 2006; Lee et al. 2006; Smith and Mizumori 2006; Ainge et al. 2007a, b; Griffin et al. 2007).

On the other hand, it is also well known that hippocampal activity is significantly modulated by behavioral states such as active exploration versus quiet consummation which are thought to be controlled by different neurotransmitter mechanisms like acetylcholine, GABA, etc. (Bland and Oddie 2001; Hasselmo 1999, 2006). The hippocampal activity relating to different behavioral states exhibits several characteristic local field potentials (LFP): type 1 theta during active exploration, type 2 theta during

quiet alertness, and large amplitude irregular activity (LIA) during consummation (Vanderwolf 1969; Kramis et al. 1975; O'Keefe and Nadel 1978; Bland and Oddie 2001).

Although most place-cell studies focus on hippocampal activity during the active periods, one (memory-guided) task demand generally leads to many repetitions of stop and go. Thus episode coding by place cells may not be sufficient—a question arises whether and how episodic information is represented during immobile periods.

Several studies have reported episodic representations by hippocampal neurons during immobility. Foster and Wilson (2006) showed reversal replay of the previous movement sequence by place cells that fired during consummation. This result may indicate that place cells represent episodes irrespective of the original location in the place field. Ainge et al. (2007b) showed that hippocampal lesions specifically impaired the performance of a delayed but not continuous (no-delayed) spatial alternation task. This result may suggest that the hippocampal function in episode coding, as required for the successful performance of sequential behavior, differs according to the behavioral stage. That is, hippocampus may play a critical role in episodic representation specifically during the delay period, when the rat is relatively quiet. Several studies have described episode-dependent activity during the delay period. However, these studies relied on maze paradigms in which the rats were able to move around in a resting zone (Smith and Mizumori 2006; Ainge et al. 2007b). Such maze paradigms may not be suited to distinguish behavioral state-dependent hippocampal activities. Also, in many of maze paradigms, the reward zones were located at each end of the maze arms, making it difficult establish whether the hippocampal activity in these areas is due to the behavioral state (i.e., consummation) or the positional factor.

To address these issues, we established a delayed spatial alternation task with a nose-poke paradigm and one rewarding zone (briefly introduced in Takahashi et al. 2002, 2007). Using this paradigm, we were able to investigate hippocampal activity during the fixation period—representing a period of quiet alertness—and during the consummation period in the single rewarding zone, as well as during active periods, when the rat approached a nose-poke hole or the rewarding zone. Here, we present a typology of episode-dependent hippocampal activity throughout the different task events in delayed spatial alternation.

Methods

Animals

Five male Wistar/ST rats (4–6 months old; SLC, Hamamatsu, Japan) were used. They were housed individually with a 14:10 h light/dark cycle. Lights were on from 7:00 a.m. to 9:00 p.m. All trainings and recordings were conducted during the light phase of the cycle. Their body weights were maintained at >80% of their free-feeding weights by mild food deprivation. Water was freely available in their home cages. All procedures were approved by the Tamagawa University Animal Care and Use Committee, and in accordance with US National Institutes of Health guidelines for animal care.

Apparatus

Behavioral training and neuronal recording were conducted in an experimental chamber constructed of Plexiglas with black wall-papers to reduce the scattered reflection of lights on the headstage for position tracking (40 × 40 × 40 cm; Takahashi et al. 2002, 2007). The chamber was fitted within a sound-attenuating box and illuminated by a 15-W light bulb as a house light. Two nose-poke holes were located 10 cm right and left from the center on the front wall of the chamber. The third was located on the center of the rear wall. Each nose-poke hole was 2 cm in diameter, 2 cm deep, and 4 cm above the floor. Light emitting diodes (LED) at the rear of each hole were used as visual cues. Horizontal infrared photo-beam detectors in the hole were used to recode the nose pokes. A food dispenser (PD-25D; O'hara & Co., Ltd., Tokyo, Japan) delivered a 25 mg food pellet (O'hara & Co., Ltd., Tokyo, Japan) to a receptacle that was located 4 cm above the floor and at the middle of the front wall. A 0.5 s buzzer sound was presented at the time of reinforcer delivery. A CCD camera was mounted on the ceiling of the sound-attenuating box for monitoring and position tracking. All events were controlled by customized software developed Microsoft Visual C++ 6.0 on a Windows-based personal computer.

Behavioral task

The delayed spatial alternation task comprised the following sequence of events (see Fig. 1). A trial started when only the center hole light was illuminated. The rat was then required to make a nose-poke response, which had to be sustained for 1 s in the central hole (i.e., the fixation period). After 1 s sustained fixation, the central light was extinguished and after a delay of 1.5 s the right and left lights were illuminated simultaneously on the front wall. The rat had to alternate between choosing the right and left hole on a trial-by-trial basis, and was rewarded with a 25 mg pellet for each correct choice. If the rat made an erroneous response, no reward was given and the same trial repeated (i.e., a correction trial). The correct direction of the first trial of each session was determined randomly by the experimenter. The inter-trial interval was 10 s.

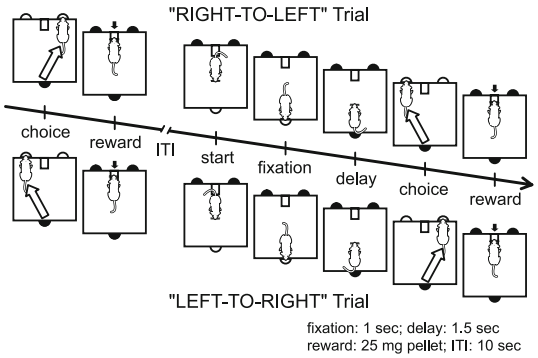

Fig. 1 Experimental design of the memory-guided spatial alternation task. The sequence depicted on top represents a RIGHT-TO-LEFT trial; the one below represents a LEFT-TO-RIGHT trial. *Open half circles* indicated illuminated nose-poke holes whereas *filled ones* indicate that the light is out

A training and recording session consisted of a maximum of 200 trials, not including correction trials, or until 60 min had elapsed. The training was completed when the rat could perform three consecutive sessions with an accuracy rate of more than 80%, and a total of at least 100 correct trials.

Electrodes and surgery

After the completion of behavioral training, each rat was anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (7 mg/kg, i.p.), and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Additional intramuscular injections of ketamine were given to maintain anesthesia. Twelve tetrodes made from four polyimide-insulated 12.7 μm nichrome twisted wires (Rediohm-800, Kenthal, Palm Coast, FL) and two reference wires were loaded into a 14-drive microdrive (Neuro-hyperdrive; David Kopf Instruments, Tujunga, CA) that allow independent vertical movement of each drive (Wilson and McNaughton 1993). Each tip of electrode wire was cut with sharp surgical scissors and gold plated to reduce its impedance to 300 kΩ at 1 kHz for multi-unit recording. A small oval craniotomy was performed on the right parietal bone and the dura was removed from the exposed area. The hyperdrive was then secured in place with dental cement supported by eight anchor screws and a ground screw. The center of the electrode bundle was positioned at coordinates 3.6 mm posterior to bregma and 2.2 mm lateral to midline for the dorsal CA1 recording in accordance with the brain atlas by Paxinos and Watson (2004). Immediately after surgery, all electrodes were advanced $\sim$1 mm. Rats were allowed at least five days to recover from surgery before resuming behavioral experiments.

Data recording

The neuronal data and the behavioral events were recorded with the Cheetah 160 Data Acquisition System (Neuralynx Inc., Bozeman, MT). The hyperdrive was connected to a headstage with 54 unity gain preamplifiers and ten LEDs for position tracking. For spike recording, tetrode channels were band-pass filtered (0.6–6 kHz), differentially amplified (×2,000–5,000), and digitized at 32 kHz. When the voltage on any of the four channels of a single tetrode exceeded a threshold set by the experimenter, a 1 ms window of the spike waveform on each of the four channels on the tetrode was recorded to disk and time-stamped with microsecond resolution. For local field potential (LFP) recording, the most prominent channel of each tetrode was picked out, band-pass filtered (1–475 Hz), differentially amplified (×2,000), digitized at 2 kHz, and stored to disk. For position tracking, the position of the ten LEDs on the headstage was detected by a CCD camera placed directly above the experimental chamber and their median point was calculated and recorded to disk at 60 Hz. The spatial sampling resolution was such that a pixel was approximately equivalent to 1 mm.

Each tetrode was advanced through parietal cortex toward the hippocampus from 80 to 320 μm per day while monitoring the unit activity with an audio amplifier when the animal was located on an adjusting table (30 cm in diameter, 1 m above the floor) and was either asleep or quietly resting. The reference electrodes were positioned in corpus callosum or silent white matter. As the cell body layer of CA1 was approached, 200–300 Hz oscillation (i.e., ripples; O'Keefe and Nadel 1978; Buzsáki 1986) could be observed. Then the tetrodes were advanced gradually into the cell body layer of CA1 until multiple single units were detected. The rat was then returned to its home cage. When the neuronal activity was still present after >2 h, it was judged to be stable and suitable for recording (Sakurai 1994, 1996).

At this time the recording session was conducted. Note that because units were extracellularly recorded, the same neurons may in principle have generated different waveforms over days, and the movement of other tetrodes may have affected the relative location of cells around the tetrode. For these reasons, it has been suggested that the precise number of recorded neurons cannot be known definitively (Knierim 2002; Ferbinteanu and Shapiro 2003). However, to prevent double counting of activity, we advanced each tetrode for a further >40 μm before returning the rat to its home cage after a recording session.

Data analysis

Our database included neurons from which activity was recorded during sessions of at least 100 trials at 80%

accuracy. Spikes were clustered off-line into putative neurons on the basis of their waveform properties using MClust 3.4 (Redish et al., http://redishlab.neuroscience.umn.edu/MClust/MClust.html) with automatic precluster-ing using KlustaKwik 1.7 (Harris, http://klustakwik.sourceforge.net/). The first to third principal components and the energy (i.e., sum of square values for each sampling points of the 1 ms waveform; Schmitzer-Torbert and Redish 2004) of their waveforms were used as the waveform features for spike sorting. To judge whether the calculated cluster actually consisted of only one neuron, we performed autocorrelation analysis for each cluster at 1 ms bin size to check the refractory period. If the autocorrelogram had no spike counts <2 ms, we regarded it a well-separated cluster and used it for further analyses.

A neuron was identified as a pyramidal cell if its waveform had a width of at least 270 μm (Smith and Mizumori 2006), if it showed a bimodal interspike interval distribution that reflected complex spike bursting (Bower et al. 2005), and if it had a low mean firing frequency over the entire recording session (<2 Hz).

We defined four task periods for data analysis: the reward period (2 s after the reward delivery), the consummation period (from the end of the reward period to the beginning of the approach to the central hole in the next trial), the approach period (from the beginning of the approach to the subsequent nose-poke response in the central hole), and the fixation period (1 s during fixation). The beginning of the approach was detected when the rat's position exceeded a threshold (8 cm from the front wall). We divided recording trials into two sequences: RIGHT-TO-LEFT trials in which the next spatial choice should be left and LEFT-TO-RIGHT trials in which the next spatial choice should be right. Excluded from analyses were data from error trials, correction trials, and trials in which the rat made premature fixations or nose-poke responses before the trial start.

Neuronal activity was considered to be specifically related to one of the four task periods if the firing frequency during that period (henceforth called "the target period") differed significantly from the session average excluding the target period in question (Wilcoxon signed rank test; $\alpha = 0.05$). The neuronal activity in each significant target period was judged to be episode-dependent if the firing frequency during the target period showed statistically significant difference between RIGHT-TO-LEFT and LEFT-TO-RIGHT trials (Mann-Whitney U-test; $\alpha = 0.05$).

To examine the role of the episode in the firing during each target period, we calculated a firing index similar to that of Lenck-Santini et al. (2001) was calculated: The index of episode-related firing frequency was $|fr(RL) - fr(LR)|/\{fr(LR) + fr(RL)\}$, where fr(RL) denotes the firing rate in RIGHT-TO-LEFT trials during the target period and fr(LR) denotes that in LEFT-TO-RIGHT trials during the target period. The indices from the four periods (reward, consummation, approach, and fixation) were compared for episode-dependent and -independent types separately, using one-way ANOVA, followed by Tukey's HSD for post-hoc contrasts.

Histology

After all recording had been completed, the rats were deeply anesthetized with an overdose of sodium pentobarbital (120 mg/kg, i.p.), and 30 μA anodal current was passed for 5 s through one channel for each of the 12 tetrodes. The rats were then perfused transcardially, initially with normal saline, subsequently with 10% formaline. Coronal section (50 μm) were cut with a cryostat (CM3050S, Leica Microsystems, Nussloch, Germany) and stained with cresyl violet. The locations of electrode tips and tracks in the brain were identified on the basis of a stereotaxic atlas (Paxinos and Watson 2004).

Results

Database

A total of 135 putative pyramidal CA1 units from five subjects were analyzed in the present study. The mean behavioral performance during 33 data recording sessions was 88.2 ± 6.5% in terms of correct choice ratio, and 128.0 ± 32.3 in terms of the number of trials completed per session (mean ± SD). Among the data set of 135 units, we checked how many neurons showed their strongest level of activity for any one task period. The numbers were as follows: 24 units showed their highest firing rate in the reward periond, 18 units in the consummation period, 51 units in the approach period, and 42 units in the fixation period. The characteristics of each type are described in detail below.

Reward-related unit

Out of 135 recorded units 24 (17.8%) showed the most significant increase of activity during the reward period, when the rat had performed a nose-poke response, heard the buzzer sound, turned its head toward the food receptacle, and quickly approached it. It took approximately 2 s in each trial; 20 of these 24 units (83.3%) exhibited episode-dependent activity. Of these units, 11 (55.0%) preferred RIGHT-TO-LEFT trials, whereas the other 9 (45.0%) preferred LEFT-TO-RIGHT trials. Figure 2 shows examples of this type of neuron: the upper panels provide the raster plots as well as the firing histograms of a unit that preferred the RIGHT-TO-LEFT sequence. The middle panels show a

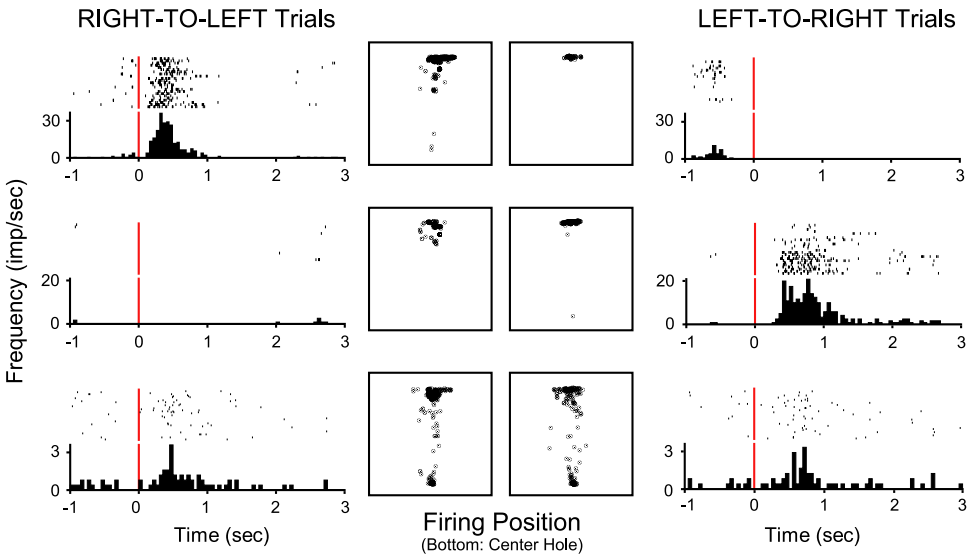

Fig. 2 Raster displays, firing rate histograms, and firing positions from three example neurons for reward-related neurons. The *upper panels* show a unit that preferred the RIGHT-TO-LEFT sequence. The *middle panels* show a unit that preferred the LEFT-TO-RIGHT sequence, and the *bottom panels* show an episode-independent type. Each raster display and histogram was aligned with the time of reward delivery ($t = 0$). The two figures depicted at the *center* of each row show the firing positions of each unit from the top view of the experimental chamber (from the onset of reward delivery to the offset of the central light). The central hole is shown at the *bottom* of each figure

unit that preferred the LEFT-TO-RIGHT sequence, and the bottom panels show an episode-independent type, whose firing during this period did not differ significantly between the two sequences. Each figure was aligned with the time of reward delivery ($t = 0$). The two figures depicted at the center of each row show the firing positions of each unit from the top view of the experimental chamber during the corresponding task period (from the onset of reward delivery to the offset of the central light). The central hole is shown at the bottom of each figure.

Consummation-related unit

Eighteen out of 135 recorded units (13.3%) showed the most significant increase of activity during the consummation period, when the rat was eating a reward pellet, and prepared for the next trial; 15 of these 18 units (83.3%) exhibited episode-dependent activity. Of these units, 10 (66.7%) preferred RIGHT-TO-LEFT trials, whereas the other 5 (33.3%) preferred LEFT-TO-RIGHT trials. Figure 3 shows examples of this type of neuron during the first

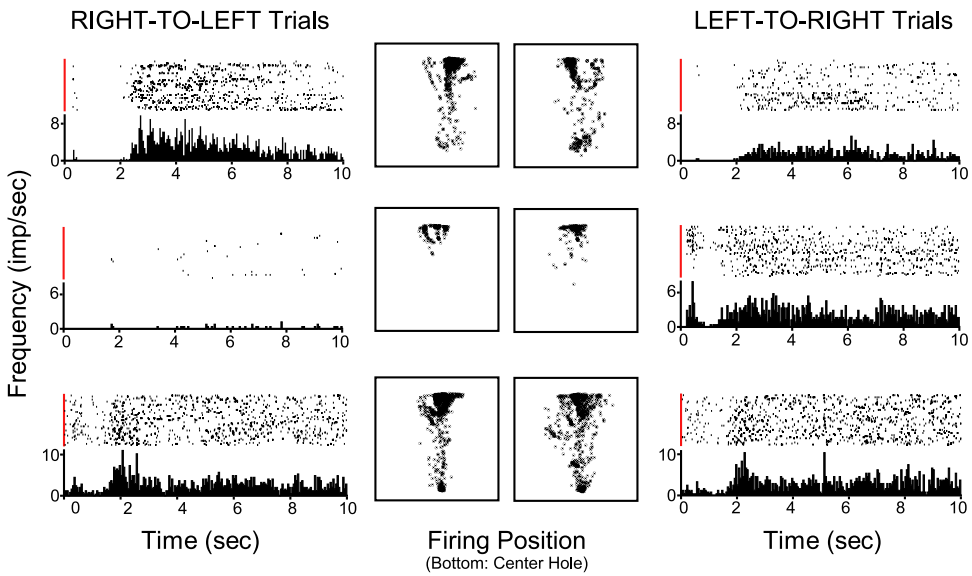

Fig. 3 Raster displays, firing rate histograms, and firing positions from three example neurons for consummation-related neurons. Each raster display and histogram show example activity during the first 10 s from reward delivery ($t = 0$). All parameters for the panels are similar to those of Fig. 2

10 s from reward delivery. All parameters for the panels are similar to those of Fig. 2.

Approach-related unit

Out of 135 recorded units 51 (37.8%) showed the most significant increase of activity during the approach period; 31 of these 51 units (60.8%) exhibited episode-dependent activity. Of these units, 15 (48.4%) preferred RIGHT-TO-LEFT trials, whereas the other 16 (51.6%) preferred LEFT-TO-RIGHT trials. Figure 4 shows examples of this type of neuron. All parameters for the panels are similar to those of Fig. 2 except for the alignment with the onset of subsequent central fixation ($t = 0$).

Fixation-related unit

Out of 135 recording units 42 (31.1%) showed the most significant increase of activity during the fixation period, when the rat kept its nose into the central hole and sustained it for 1 s; 22 of these 42 units (52.4%) exhibited episode-dependent activity. Of these units, 10 (45.5%) preferred RIGHT-TO-LEFT trials, whereas the other 12 (54.5%) preferred LEFT-TO-RIGHT trials. Figure 5 shows examples of this type of neuron. All the parameters for the panels are similar to those of Fig. 2, except for the alignment with the onset of the fixation ($t = 0$).

Firing selectivity index

Figure 6a shows the mean firing selectivity index (see Methods). Among the episode-dependent units, the one-way ANOVA detected statistical significance ($P < 0.001$) and the post-hoc Tukey's HSD comparisons detected statistical significance between the reward and approach period ($P < 0.001$), the reward and the fixation period ($P < 0.001$), the consummation and fixation period ($P < 0.001$), and the consummation and approach period ($P < 0.05$). These results indicate that the firing rates differed much more in the reward-related and consummation-related units (the selectivity indices >0.7), whereas they differed relatively less in the approach-related and the fixation-related units (the indices <0.5), suggesting that the episodic information is likely to be coded by a different neural assembly during the reward and consummation periods, whereas that during the approach and fixation periods is likely to be coded by the difference of firing rate in the same neurons. Figure 6b shows the mean firing selectivity index among episode-independent units. The one-way ANOVA did not detect statistical significance ($P > 0.05$).

Histology

Recording coordinates were reconstructed from the final location of each electrode tip and its track on the stained brain specimen, and were confirmed to lie in the CA1 area of the hippocampus. An example is shown in Fig. 7. The arrow mark indicates the final position of the electrode tip.

Discussion

In the present study, we concentrated on four task periods from when the rat received the reward to the end of the fixation, during which the rat has to maintain the current task sequence (i.e., episode) to perform the spatial alternation task correctly. The four task periods implied

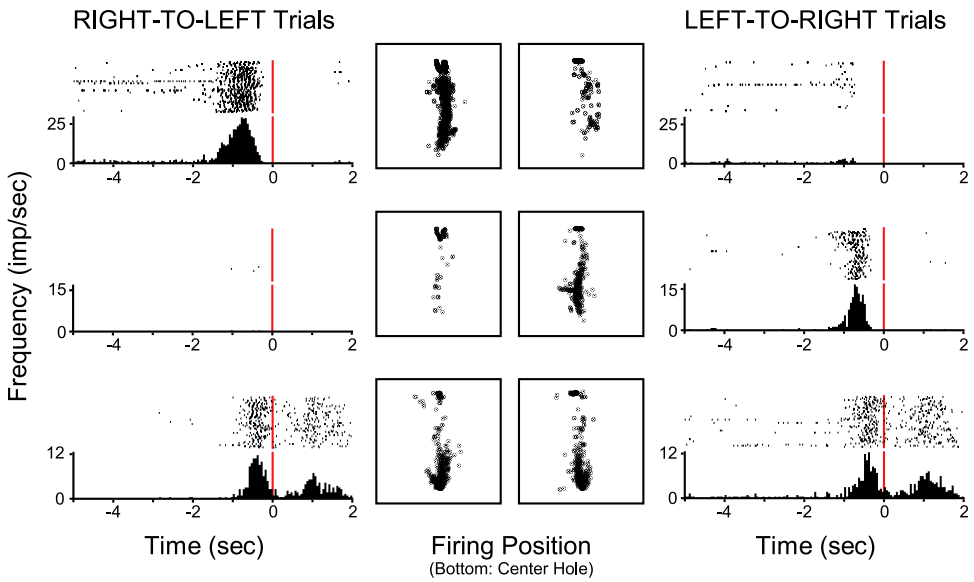

Fig. 4 Raster displays, firing rate histograms, and firing positions from three example neurons for approach-related neurons. All parameters for the panels are similar to those of Fig. 2 except for the alignment with the onset of subsequent central fixation ($t = 0$) in each raster display and histogram

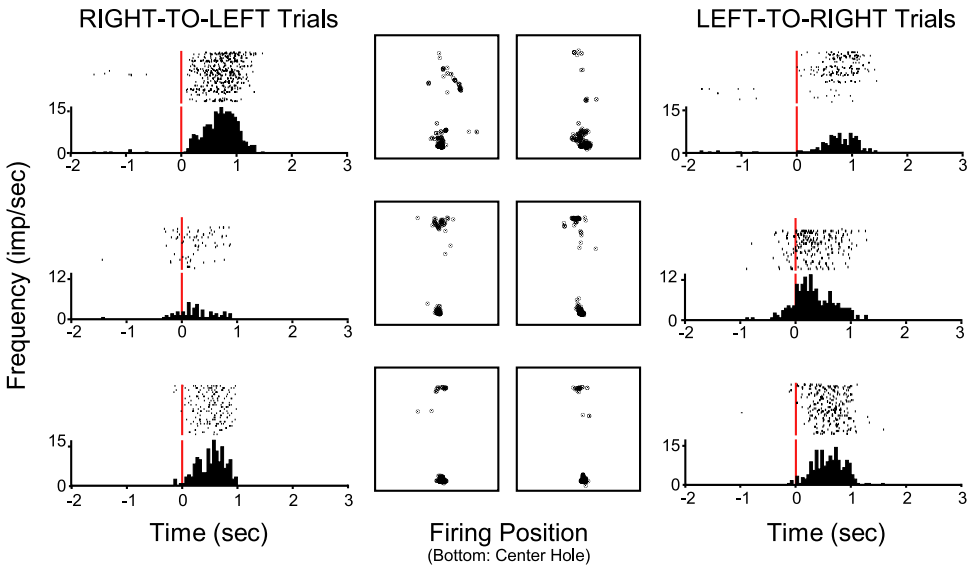

Fig. 5 Raster displays, firing rate histograms, and firing positions from three example neurons for fixation-related neurons. All parameters for the panels are similar to those of Fig. 2, except for the alignment with the onset of the fixation ($t = 0$) in each raster display and histogram

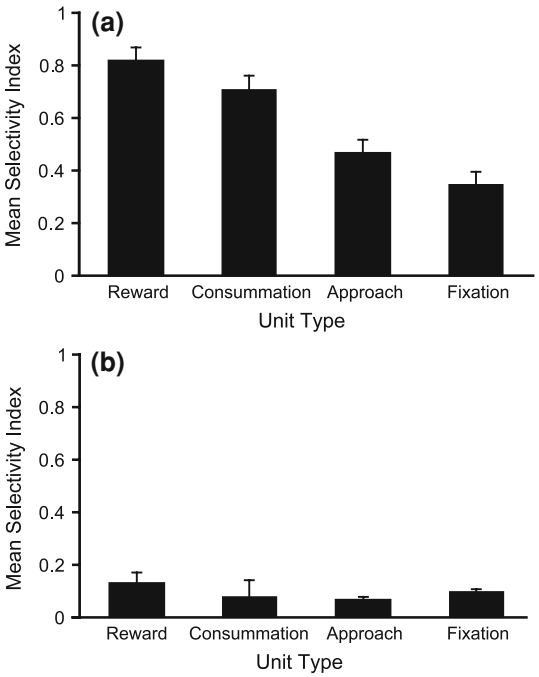

Fig. 6 Mean firing selectivity index. **a** The mean firing selectivity index among episode-dependent units (see section Methods). **b** The mean firing selectivity index among episode-independent units

different behavioral states: the reward period, the consummation period, the approach period, and the fixation period. In all four subperiods, we have obtained episode-dependent neuronal activity which changes its firing rate systematically as a function of the alternation sequence. Especially noteworthy among the current findings is the episode-dependent hippocampal activity during immobile periods (i.e., the consummation and the fixation periods). These observations extend previous findings concerning

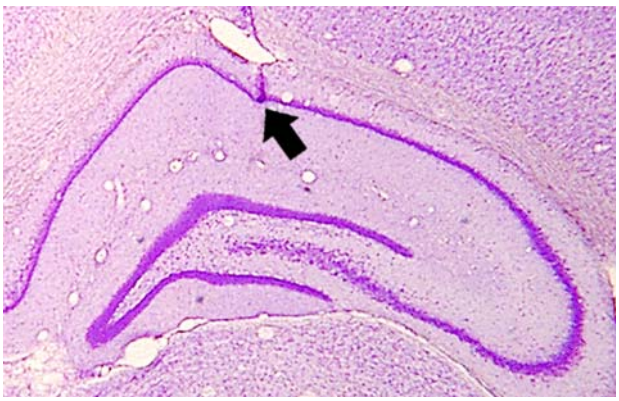

Fig. 7 Sample recording positions verified by histology. An *arrowhead* indicates a microlesion located in the dorsal CA1

spatial episode-dependent hippocampal activity in mobile periods (Frank et al. 2000; Wood et al. 2000; Ferbinteanu and Shapiro 2003; Bower et al. 2005; Dayawansa et al. 2006; Lee et al. 2006; Ainge et al. 2007a; Griffin et al. 2007). Thus, episode dependency exists not only in the active exploration periods but also in quiet, immobile periods.

Episode-dependent activity during the fixation period

We have found episode-dependent activity in the CA1 neurons during the fixation period. During this period in the current nose-poke paradigm, the positional factor could be stringently controlled and the movement velocity was kept at zero. Pastalkova et al. (2008) recently reported that episode-dependent hippocampal activity could be seen during the delay period when the rat ran in the running wheel at the same location, direction, and speed. The main

difference between their work and the current work was that the rat remained completely quiet during the delay period in the current study.

This difference is important because it is known that different hippocampal mechanisms control the firing in the network depending on the behavioral state. In situations when the subject remains immobile but waits for next event, the type 2 theta seems dominant (Kramis et al. 1975; Bland et al. 2006; Hok et al. 2007). With the current data set we have confirmed that the type 2 theta band activity significantly increased, whereas the type 1 theta band activity was reduced during the fixation period compared with active exploration. We also have confirmed that there was no LIA during this period (data not shown). Thus, the episode-dependent activity derived from a different hippocampal controlling mechanism compared with traditional place cells and Pastalkova and her colleagues' study, which are mainly observed under the type 1 theta band activity.

The key question from a result of Ainge et al. (2007b) was what the difference was between place cells whose place field was located in the "delay (resting) zone" and those whose place field was located in the central arm of their figure-8-shaped maze.

Our result may answer this question, suggesting that, to complete a delayed spatial alternation task, the episode-dependent hippocampal activity during the immobile period is necessary.

Episode-dependent activity during the consummation period

We also have obtained episode-dependent activity in CA1 neurons during the consummation period. The activity lasted from approximately 2 s after the reward delivery to the beginning of the approach toward the central hole in the next trial. In general, it is known that the theta band activity is reduced and LIA can be observed during this period (Vanderwolf 1969; O'Keefe and Nadel 1978; Buzsáki et al. 1983). In agreement with this, we observed LIA during the consummation period (data not shown). This hippocampal mechanism would be different from not only the active exploration (as examined in place-cell studies) but also the fixation period which contains no LIA as we discussed in the previous section. We should treat these two periods as separate types of immobility, though both are modulated by the episodic information.

Foster and Wilson (2006) reported a reversal replay of place cells' firings coincident with the sharp wave and ripple. As our apparatus was relatively small and had less explicit landmarks, we could not find sufficient "place cell" like activities to analyze such sequential replay during LIA. However, in the current study we did note that several unit activities during the consummation period with LIA were modulated by the episodic information.

There remain some other possibilities for such differential firings during this period because the rats' position and head-direction were not controlled in the consummation period in the current paradigm. Nevertheless these units should be considered different from traditional place cells that exhibit activity during mobile periods with type 1 theta and under relatively strong cholinergic suppression compared with quiet awake period (Marrosu et al. 1995; Hasselmo 2006).

Episode-dependent activity during active mobile periods

In our behavioral task using a nose-poke paradigm, we also have found episode-dependent hippocampal activity during active mobile periods, that is, the reward and approach periods. The phasic firing of hippocampus after the reward delivery was consistent with several pervious reports (Wiener et al. 1989; Hampson et al. 1993; and more). But we noted that the moving trajectory of the animal was quite different in RIGHT-TO-LEFT trials as compared with LEFT-TO-RIGHT trials during the reward period (see Fig. 2), thus the different firing property might simply reflect physical factors such as head-direction and/or position. The differential firing during the reward period might be caused by these differences. On the other hand, during the approach periods the trajectories of RIGHT-TO-LEFT and LEFT-TO-RIGHT trials were relatively similar (moving along the center line). Of course, compared with maze paradigms, these trajectories still differ from each other, thus our episode-dependent activity during this period may be due to positional differences (but the maximum differences are roughly <15 cm, and this is the normal width of maze corridors, suggesting the current trajectories may have the same spatial resolution).

Among previous studies that showed episode-dependent place cells, there are considerable discrepancies in the reports about the proportions of such units: Wood et al. (2000) reported that 22 out of 33 place cells (67%) showed episode dependency, whereas Hölscher et al. (2004) reported only 4 out of 45 place cells (9%) showed such tendency. Hölscher et al. (2004) proposed that episode-dependent hippocampal activity was not only a reflection of the sequence of experienced spatial information such as landmarks, but also that of the movement or behavior such as turning, running, etc. This hypothesis comes from the difference of training methods between Wood et al. (2000) and Hölscher et al. (2004). The former's training produced forced turns by setting a wooden block at turning points, whereas the latter's let the animal choose more freely (if it made a mistake, then the experimenter mildly adjusted the

direction manually, Hölscher et al. 2004; see also Bower et al. 2005 for the relationship between different proportions of episode-dependent place cells and training protocols). Our result may support this hypothesis because our apparatus provided very few external cues or landmarks—only three nose-poke holes, one food receptacle, and all walls were colored in black. In addition, our apparatus was relatively smaller (40 cm square) than that in the typical maze paradigm (1–2 m long). Therefore, the rat had to acquire a strategy for solving the spatial alternation not from the sequence of external cues but from that of egocentric movements or behaviors. This may explain why our proportion of approach-related units (61%) showed a similar value to that of Wood et al. (67%).

Summary and future directions

We observed episode-dependent activity in CA1 neurons not only during active exploration but also during quiet immobile periods. The current data address several issues that had remained unresolved in the literature. As we show the typology of hippocampal episode-dependent activity here, the next question will be how to integrate or incorporate these discrete episodic components into one consecutive episode. It is possible that the rat's decision (which direction to choose in the next trial) is already made when the rat starts to approach the central hole, so that it may not need to maintain such episodic information throughout the trial. However, as Ainge et al. (2007b) have shown, if the movement was interrupted by some delay, that is, the movement stops, then animals with lesioned hippocampus show significant impairment in their sequential choice. Therefore, the episodic information must be represented throughout the trials.

One possibility might be that prefrontal cortex (PFC) plays a role for such integration. Several studies reported episode-dependent activity in rat medial PFC during a spatial alternation task in a maze paradigm (Jung et al. 1998; Baeg et al. 2003; and more). Several reports have suggested anatomical and functional interaction between hippocampus and PFC (Sesack et al. 1989; Jay and Witter 1991; Fuster 1997; Siapas and Wilson 1998; Siapas et al. 2005). In line with these observations, it is conceivable that, during the consummation period, the rat makes a decision (updating the current task context) through the interaction between hippocampus and PFC. The decision signal, including information about movement sequences, would then be sent to some motor control structure as well as further decision-making circuits such as striatum, where the information would be maintained during active exploration (e.g., via distributed coding among hippocampus, striatum, and PFC circuits). However, when the rat stops its movement, the network responsible for maintaining episodic information during the active period is no longer able to hold such information. At this time, hippocampus plays an essential role to retrieve and transmit the sequence information in a quiet, immobile period, while being fully alert.

One candidate mechanism for this retrieval may be a kind of "phase coding" (e.g., Jensen and Lisman 1996, 2005; Igarashi et al. 2007; Wagatsuma and Yamaguchi 2007; Sato and Yamaguchi 2009), not based on type 1 theta but based on type 2 theta. We have confirmed that the unit firing rhythms during fixation are still modulated by the theta rhythm, and its peak frequency, calculated from the power analyses, was slightly faster than that of LFP, as is similar to the tendency during active exploration with type 1 theta. These similarities, and the finding that the theta phase precession can be observed while the rat is running at the same place (Pastalkova et al. 2008), imply that an analogous mechanism would also exist under type 2 theta condition. To investigate this "extended phase coding hypothesis"—especially theta phase precession and its dynamics during quiet alertness, future studies should introduce a longer duration of the stable fixation period—at least more than 2 s. Also, to reveal these mechanisms in detail, it will be fruitful to apply multi-site recording methods.

Acknowledgments This work was supported by grants from the 21st Century COE Program at Tamagawa University, Grants-in-Aid for Scientific Research (A) (19200014) and (17021036) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank H. Fujiwara, M. Kawai, and T. Ohashi for technical assistance.

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