CONTRIBUTION OF HIPPOCAMPAL PLACE CELL ACTIVITY TO LEARNING AND FORMATION OF GOAL-DIRECTED NAVIGATION IN RATS

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Abstract—Although extensive behavioral studies have demonstrated that hippocampal lesions impair navigation toward specific places, the role of hippocampal neuronal activity in the development of efficient navigation during place learning remains unknown. The aim of the present study was to investigate how hippocampal neuronal activity changes as rats learn to navigate efficiently to acquire rewards in an open field. Rats were pre-trained in a random reward task where intracranial self-stimulation rewards were provided at random locations. Then, the rats were trained in a novel place task where they were rewarded at two specific locations as they repeatedly shuttled between them. Hippocampal neuronal activity was recorded during the course of learning of the place task. The rats learned reward sites within several sessions, and gradually developed efficient navigation strategies throughout the learning sessions. Some hippocampal neurons gradually changed spatial firing as the learning proceeded, and discharged robustly near the reward sites when efficient navigation was established. Over the learning sessions, the neuronal activity was highly correlated to formation of efficient shuttling trajectories between the reward sites. At the end of the experiment, spatial firing patterns of the hippocampal neurons were re-examined in the random reward task. The specific spatial firing patterns of the neurons were preserved if the rats navigated, as if they expected to find rewards at the previously valid locations. However, those specific spatial firing patterns were not observed in rats pursuing random trajectories.

These results suggest that hippocampal neurons have a crucial role in formation of an efficient navigation. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

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Navigation toward a specific place in the environment is crucial for survival for animals as well as humans. Behavioral experiments in rats have shown that the hippocampal-formation (HF) damage severely impairs navigation and place learning (O'Keefe and Nadel, 1978; Olton et al., 1979; Jarrard, 1993). Neuropsychological studies showed patients with damage in the medial temporal lobe including the HF and the parahippocampal gyrus had deficits in topographical orientation (Maguire et al., 1996; Abrahams et al., 1997). Recent neuroimaging studies have also shown that activity in these regions increases during navigation in a virtual reality space (Maguire et al., 1998) and recalling actual routes (Maguire et al., 1997; Mellet et al., 2000). All of these studies indicated that the HF has a crucial role for spatial navigation, not just for spatial recognition.

The discovery of the place cell in the HF (O'Keefe and Dostrovsky, 1971) provided a neurophysiological approach to examine the role of the HF in spatial functions, and subsequent studies revealed characteristics of spatial representation in the HF (Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Eichenbaum et al., 1989; McNaughton et al., 1996). The place cell of the rodent HF is characterized by location-specific firing (O'Keefe and Conway, 1978; Eichenbaum et al, 1987; McHugh et al, 1996; Skaggs and McNaughton, 1998), and comparable HF neuron responses were also reported in monkeys (Ono et al., 1991, 1993; Nishijo et al., 1997; Matsumura et al., 1999). Place-cell activity is controlled by a salient cue or multiple spatial cues (e.g., O'Keefe and Conway, 1978; Muller and Kubie, 1987; O'Keefe and Burgess, 1996), and remains stable over a long period (Muller et al., 1987; Thompson and Best, 1990). These studies suggest that place-cell activity reflects a spatial representation of an environment.

However, alterations of behavioral as well as spatial variables induce plastic changes in place-cell firing. Place-cell activity changes in an experience-dependent manner (Bostock et al., 1991; Wilson and McNaughton, 1993; Mehta et al., 1997, 2000). Alteration of reward-place associations also induces changes in locations of place fields (Breese et al., 1989; Kobayashi et al., 1997). Furthermore, Markus et al. (1995) indicate that the place cell flexibly changes its spatial firing as rats learn to navigate to specific locations. However, it remains unclear how spatial firing of place cells contributes to development of navigation directed to specific goals during place learning.

For the understanding of functions of the HF, it is important to investigate the actual role of place cells in spatial navigation (Bures et al., 1997a, 1997b; Zinyuk et al., 2000; Lenck-Santini et al., 2001). A purpose of the present study is to elucidate relationships between activity of HF neurons and goal-directed behaviors acquired dur-

ing learning of a novel place task. Some of these data have been reported in abstract form (Kobayashi et al., 1991; Nishijo et al., 1999).

EXPERIMENTAL PROCEDURES

Subjects

Thirteen male albino Wistar rats (Sankyo Lab Service, Hamamatsu, Japan) were used. The rats weighed 270–320 g at the time of surgery, and were individually housed in a room with constant temperature and had food and water freely available. Efforts were made to minimize the number of animals used and their suffering. All rats were treated in strict compliance with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Guideline for the Care and Use of Laboratory Animals in Toyama Medical and Pharmaceutical University.

Surgery

Each rat was anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and implanted bilaterally with monopolar stimulating electrodes (enamel insulated, 200-μm diameter, stainless steel) aimed at the medial forebrain bundle at the level of the lateral hypothalamus (4.3 mm caudal from the bregma, 1.6 mm lateral from the midline, and 8.8–9.0 mm ventral from the skull surface), and a recording electrode assembly above the CA1 layer of the dorsal HF (3.6 mm caudal from the bregma, 3.0 mm lateral from the midline and 2.8 mm below the skull surface) according to the atlas of Paxinos and Watson (1986). This coordination allowed us to record neuronal activity not only in the CA1 but also CA3 layers. The recording electrode assembly consisted of a bundle of four or eight wires (Formvar insulated, 20-μm diameter, nichrome) encased in a stainless steel cannula (25 gauge), a small platform, and two screws. Stimulating electrodes and a recording electrode assembly were attached to the skull with dental acrylic and stainless steel screws, one of which also served as the indifferent electrode for stimulation.

Apparatus

Spatial behavior was investigated in a 150-cm-diameter circular open field with a 45-cm-high wall, painted black on the inside. The open field was enclosed by a black curtain (180 cm in diameter and 200 cm high). The ceiling of the enclosure contained four small speakers mounted near the circumference, spaced 90° apart, four light bulbs individually mounted near the inner edge of each speaker, and a video camera (Behavioral Tracking Analyzer, BTA-2A, Muromachi Kikai, Tokyo, Japan) at the center. Usually a light bulb was lit at the three o'clock position, and a speaker continuously emitted white noise at the nine o'clock position. A small light bulb was mounted on the head of the rat. The video camera converted a real video image signal to a binary signal, and tracked the horizontal (two-dimensional) motion of the small bulb. A laboratory microcomputer (PC-9801, NEC, Tokyo, Japan) received the X and Y coordinates of the position of the head through an RS-232C serial port at 20 frames/s. A program delimited circular areas (reward sites) in the open field, and triggered the delivery of intracranial self-stimulation (ICSS) when the rat entered the reward site. The experimenter monitored the locations of the reward site and the rat on a display screen, but no distinctive cues marked the reward site for the rat.

Behavioral Procedures

Self-stimulation screening After at least 1 week recovery from surgery, the rats were screened to self-stimulate in an oper-

ant chamber (30×30×33 cm) equipped with a lever on one wall. Each lever press triggered the delivery of a 0.5-s train of 0.3-ms negative square-wave pulses at 100 Hz. The current intensity for ICSS was determined to produce 40–70 lever presses/min in the operant chamber. A stimulating electrode, which produced stable lever pressing at a low current intensity was selected for each rat. The rats were trained to self-stimulate in daily 30–60-min sessions for 5–10 days until stable lever pressing was achieved. The current intensity, which was determined in this period for each rat, ranged from 100 to 300 μA, and was used throughout the following place tasks in the open field.

Task training and testing protocol in open field. The rats were pre-trained to perform the following spatial behavior in the open field. ICSS was delivered when the cumulative distance traveled by the rat reached a given distance. The initial distance was 80 cm, and this was increased progressively to 120 or 160 cm. The rats were usually trained for about 1 h/day for 2–4 days until they learned to travel the open field continuously. They were then trained in the random-reward place-search task (RRPST), In this protocol a reward site (72-cm diameter) was delimited; its center was chosen at random within a square circumscribed around the open field (Fig. 1Aa). The rat was rewarded with ICSS when it entered the reward site, which was then made inactive. After a 5-s interval, the reward site was moved to a different location and reactivated.

After the rats obtained at least 30 rewards in each session of successive three sessions of the RRPST, the recording electrode assembly was advanced at about 20–80 μm per day in 20-μm steps. The electrodes in each rat were checked while the rat performed the RRPST. A purpose of the present study was to elucidate activity of HF neurons during new learning of goal-directed behaviors. Then, each animal could only be examined once. If a complex spike cell was found, and stable recording was obtained, data for this rat were collected in 1 day as follows. Initially data were recorded in a several sessions of the RRPST, and then five to 10 sessions of place learning task (PLT) were run. In the PLT, two 40-cm-diameter reward sites were located diametrically opposite to one another in the open field at separation of 70 cm (Fig. 1Ab). In each session the first entrance into the north reward site was always rewarded. Then, the rat was rewarded in both reward sites on the condition that it had previously visited the other one and remained within the reward site for 1 s. This delay was intended to ensure that the rat identified the reward site as such, and was not rewarded for simply arriving at the reward site by automatic locomotion.

At the start of a session, the small electric bulb on the head of the rat was turned on, and an ICSS train was delivered to prime the rat. Each session was terminated after 50 rewards had been delivered or 10 min had elapsed, whichever occurred first.

Recording procedures and data acquisition

Neuronal activity was passed through a high-input impedance preamplifier made of a dual-channel field-effect transistor (2SK389, Toshiba Electric, Tokyo, Japan) or a FET-input op-amp (AD824, Analog Devices, Norwood, MA, USA), amplified by a main amplifier, and then fed into time amplitude window discriminators (BAK Electronics, Germantown, MD, USA) to convert to standardized pulses. During ICSS, neuronal activity was isolated from the stimulus artifact by the time-window discriminator. Differential recording with these electrodes and recording electrode assembly has been shown to yield stable, single neuronal activity (Sasaki et al., 1983; McNaughton et al., 1989). The activity of a neuron was recorded as a single neuronal activity if it had a peak amplitude at least 2.0 times greater than noise, and the amplitude, duration (spike width), and waveform were confirmed to be constant by visual examination on a storage oscilloscope (Fig. 1B). In the cases in which one electrode had multiple neurons, single
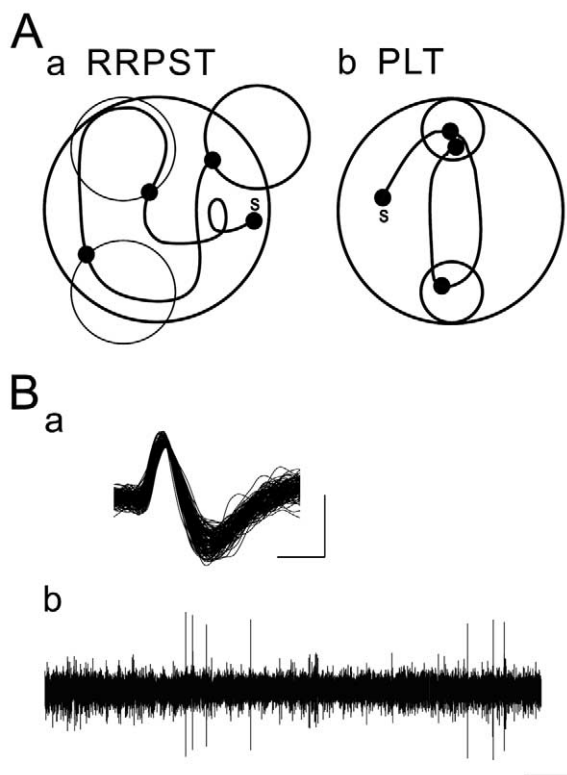
Fig. 1. Behavioral tasks and examples of place cell activity. (Aa) Random reward place search task (RRPST). A computer program delimited a circular reward site (small thick-line circle) at some randomly selected coordinate. The rat was rewarded with intracranial self-stimulation when it entered the reward site, which was then made inactive (changed to thin-line circle). After a 5-s interval, the reward site was moved to a different location and reactivated. (Ab) Place-learning task (PLT). The rat received rewards in two target areas (thick-line circles) when it returned to one reward site after a visit to the other reward site. There was a 1-s delay between arrival and reward delivery. S, location of rat at start of session. Large dots, locations of reward delivery. (Ba) Superimposed waveforms for the activity of a hippocampal place cell. Vertical and horizontal bars, calibration for amplitude (0.1 mV) and time (0.2 ms), respectively. (Bb) An example of neuronal activity of the place cell. Vertical and horizontal bars, calibration for amplitude (0.1 mV) and time (0.5 s), respectively.

neuronal activities were identified and recorded by means of separation with the time amplitude window discriminator (Sakurai, 1996). Data sets were discarded if the neurons were judged to be poorly isolated. The microcomputer summed the spikes (standardized pulses) over 50-ms intervals and combined these data with the X and Y coordinates of the head of the rat to construct a distribution map of the mean firing rate as a function of the position of the rat.

Data representation and analysis

To represent the firing rate distribution in the open field, firing-rate maps consisting of 4.5×4.5-cm pixels were constructed. Dividing the total number of spikes by the cumulative dwell time in each pixel for the entire session yielded the firing-rate map. Pixels where the rat visited but a given neuron did not fire were represented by gray pixels. Pixels with greater than zero firing rate were sorted into ascending order and divided into five color-scale categories. The values used as boundaries of the five color categories increased at a step of half of mean firing rate, and pixels with

the highest firing categories (greater than twice the mean firing rate) were shown as red pixels. To permit comparisons among firing-rate distributions across several sessions for a given cell, the rate categories determined for the first map were retained for the subsequent maps.

To estimate similarity of trajectories and firing-rate distributions in each session to those in the final session of the PLT, pixel-to-pixel correlations (Sharp et al., 1990) were calculated using the dwell-time map and firing-rate map (see above), respectively. For the dwell-time map, a smoothing algorithm was applied in that the value of a given pixel, the values of the four adjacent pixels that share an edge with it, and the values of the four adjacent pixels that share only one corner with the center were divided by four, eight, 16, respectively, and then summed to generate the map to calculate trajectory similarity. The rat gradually spent more and more time near and within the reward site during learning the PLT, which falsely increased pixel-to-pixel correlation for trajectory similarity. Therefore, to accurately estimate trajectory similarity, the pixels near and within the reward site were excluded from the calculation. To estimate the similarity of firing-rate distributions, firing-rate maps consisting of 9.0×9.0-cm pixels were generated. The same smoothing algorithm used for the trajectory similarity analysis was applied to these maps. Then, pixel-to-pixel correlation was calculated using only common pixels between the final session of the PLT and each session.

Place fields were delineated by a process used previously (Muller et al., 1987; Breese et al., 1989; Kobayashi et al., 1997). Clusters of 4.5×4.5-cm pixels with firing rates exceeding twice the mean firing rate were identified. All pixels that did not satisfy this criterion were eliminated. A place field could be continued through any edge shared by two pixels meeting the criterion, but not through corners. If one or more neighboring pixels satisfied the criterion, the field was expanded to include the pixel(s). Each added pixel was then tested for the presence of a neighboring pixel that met the criterion. When no neighboring pixel satisfied the criterion, the limit of the field was identified. The minimum size for a place field was set at nine pixels. Boundaries of a place field were established by constructing a rectangle that had one diagonal connecting the minimum X and Y coordinates with the maximum X and Y coordinates.

Histology

After the recording electrodes were advanced below the HF, the locations of the recording and stimulating electrodes were identified histologically. The rats were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the stimulating site was marked by an iron deposit, created by passing a 20-μA positive current through the stimulating electrode for 30 s. The recording sites were marked by an electrolytic lesion, created by passing a 20-μA negative current through the recording electrodes for 30 s. For marking of the stimulating and recording sites, the stainless steel screws to attach the recording electrode assembly to the skull were used as the indifferent electrodes. Each rat was then perfused through the heart with 50 ml of 0.9% saline containing heparin followed by 200 ml of 10% buffered formalin containing 2% potassium ferrocyanide. The brain was removed and fixed in formalin for at least 48 h. Sections of 75 μm were cut on a freezing microtome and were then stained with Cresyl Violet.

RESULTS

Behavioral performance

Figure 2 summarizes the results of behavioral performance of 13 rats during learning of the PLT. The rats acquired 19.54 ± 4.68 (mean$\pm$S.E.M.) rewards in the first session, and 46.54 ± 3.14 rewards in the last session of the
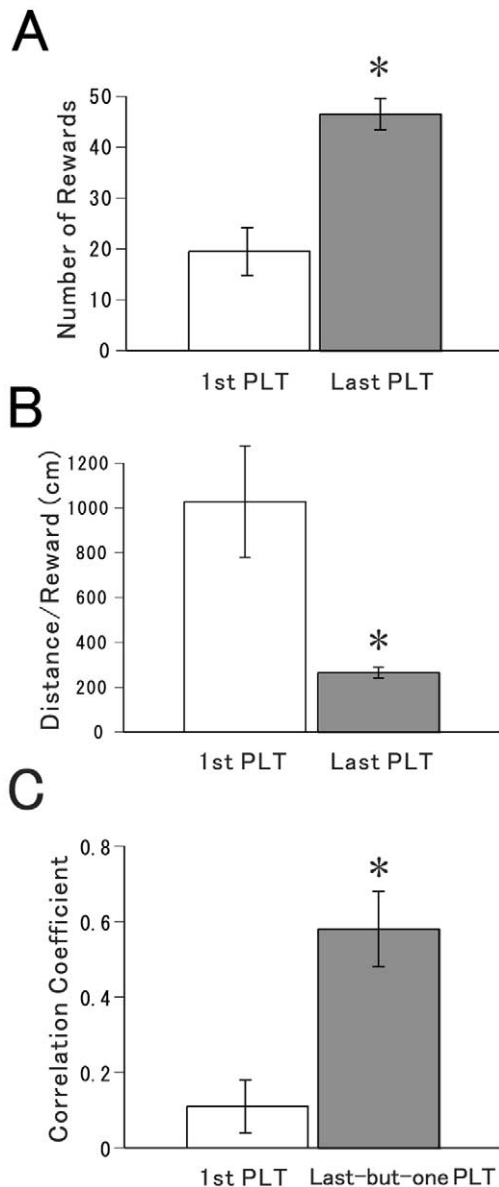
Fig. 2. Behavioral performance for learning the place-learning task PLT. (A) mean number of rewards obtained in the first and last session of the PLT. (B) Efficiency of obtaining rewards in the first and last session of the PLT. Efficiency was calculated as dividing traveled distance by total number of rewards in each session. (C) Trajectory similarity. Trajectory similarity was calculated as correlation coefficients between the first and last sessions and between the last-but-one and last sessions of the PLT (mean±S.E.M.; *$P<0.01$; t-test, $n=13$).

PLT (Fig. 2A). There was a significant improvement in the number of rewards between the first and last sessions of the PLT (Student's t-test, $P<0.01$). The efficiency of acquiring rewards was quantified by dividing the traveled distance by the total number of rewards that the rats acquired in each session (Fig. 2B). The rats traveled 1028.4±249.2 cm per reward in the first session, and 265.9±24.4 cm per reward in the last session of the PLT. There was a significant improvement in the traveled distance per reward between the first and last sessions of the

PLT (Student's t-test, $P<0.01$), indicating that the rats acquired rewards more efficiently in the last session. Trajectories of the rats also greatly changed across the sessions of the PLT (Fig. 2C). The trajectory similarity between the first and last sessions of the PLT was low ($r_{\text{trajectory}}=0.11\pm0.07$), whereas the trajectory in the session immediately before the last session more resembled that in the last session ($r_{\text{trajectory}}=0.58\pm0.10$) (Student's t-test, $P<0.01$). These results indicate that the rats learned the PLT well, and, as learning proceeded, the pattern of trajectories also changed for efficient reward acquisition.

Changes in firing rate distributions during learning the PLT

The activity of 14 complex-spike cells was recorded from 13 rats. Each complex-spike cell was initially recorded in a several sessions of the RRPST and the following five to 10 sessions of the PLT. Data for each rat were collected in 1 day. To estimate whether or not spatial firing patterns changed after learning the PLT and to characterize the neurons, we used correlation coefficients (r_{rate}) between firing-rate distributions in the last session of the RRPST before PLT learning and the last session of the PLT. Neurons which had high r_{rate} values ($r_{\text{rate}}\geq0.4$) were considered to have maintained the same spatial firing patterns, whereas neurons which had low r_{rate} values ($r_{\text{rate}}<0.4$) were defined as having changed spatial firing patterns after learning the PLT. Four neurons (29%) belonged to the first group (mean $r_{\text{rate}}=0.48\pm0.06$), and 10 neurons (71%) belonged to the second group (mean $r_{\text{rate}}=0.03\pm0.05$).

Figure 3 shows an example of trajectories and spatial firing patterns of a CA1 neuron which maintained the spatial firing patterns in the RRPST after the PLT was learned. Activity of the neuron increased when the rat passed through the location around one to two o'clock in the RRPST before the PLT (Fig. 3A). This spatial firing pattern was preserved over subsequent learning sessions (Fig. 3B–E). The spatial firing pattern in the last session of the PLT learning (Fig. 3E) resembled that in the RRPST before learning ($r_{\text{rate}}=0.65$). Furthermore, the spatial firing pattern in the RRPST after learning the PLT (Fig. 3F) was also similar to that in the last session of PLT learning ($r_{\text{rate}}=0.61$).

Figure 4 shows an example of trajectories and spatial firing patterns of a CA1 neuron which changed spatial firing patterns in the RRPST after PLT learning. Spatial firing patterns were diffuse in the RRPST before learning the PLT and in the initial three sessions of the PLT (Fig. 4A–C). In the fifth session of the PLT, the rat was clearly orienting toward two reward sites, and activity increased when the rat was near or within the reward site at 12 o'clock location (Fig. 4D). In the final session of the PLT (Fig. 4E), spatial firing near the reward site at 12 o'clock location was more evident, and activity of this neuron increased before reward delivery and decreased after the delivery. In the RRPST after learning the PLT, both the goal-directed shuttling behavior and spatial firing pattern acquired in the PLT learning session were preserved (Fig.
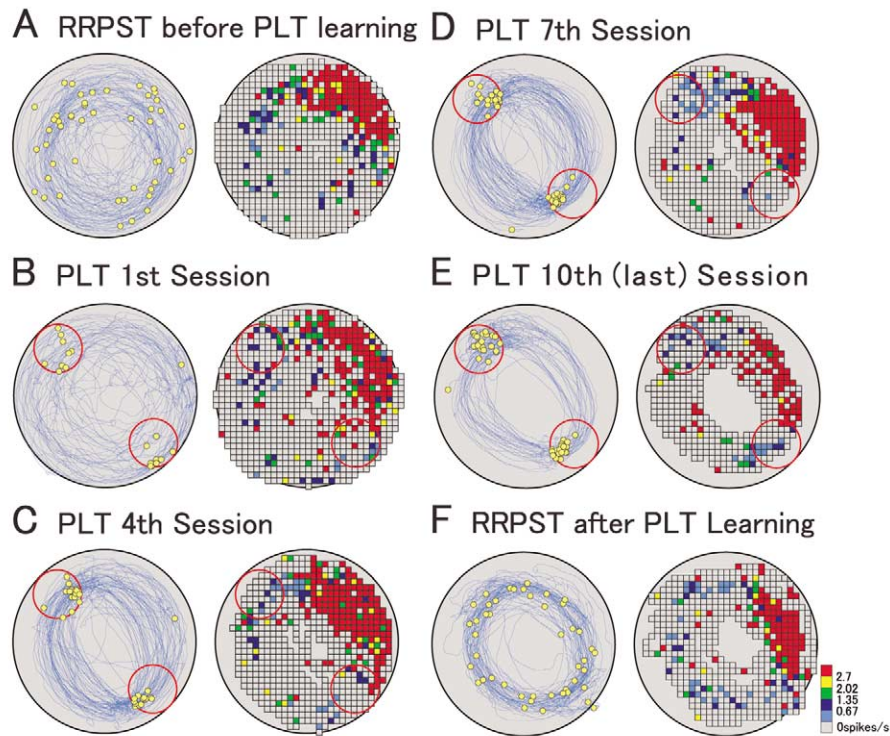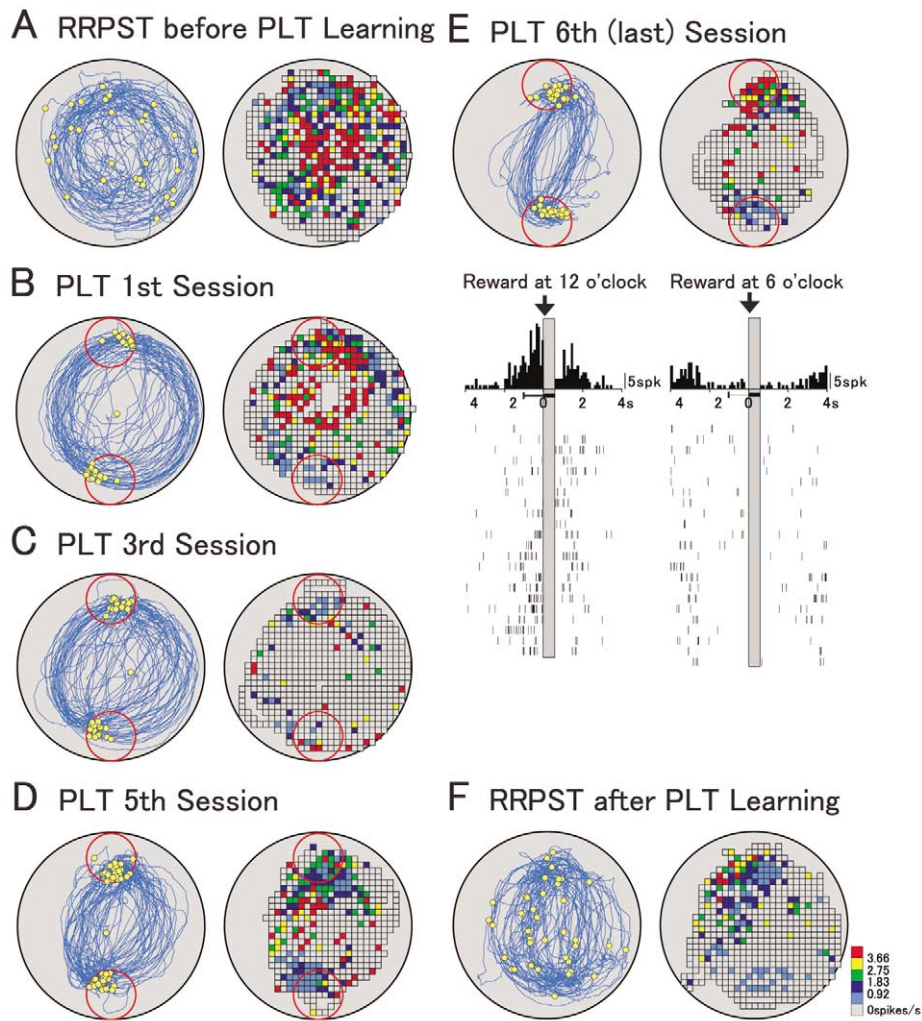
Fig. 3. An example of trajectories and firing-rate distribution of a hippocampal-formation neuron whose spatial firing pattern did not change during the learning of the place-learning task (PLT). (A) Random-reward place-search task (RRPST) before learning. (B) First session of the PLT. (C) Fourth session of the PLT. (D) Seventh session of the PLT. (E) Tenth session of the PLT. (F) RRPST after the learning of the PLT. This neuron had a place field at the 1 to 2 o'clock location along the cylinder wall in the RRPST before the PLT was learned (A). The sense of rotation of the rat was indefinite in the first PLT session, but became counterclockwise from the fourth session. This spatial firing pattern was maintained over subsequent learning sessions (B–E) and in the RRPST after learning (F). Blue lines, trajectories of rat. Large yellow dots, locations of reward delivery. Red circles, locations of reward sites. Pixels with greater than zero firing rate were divided into five color-scale categories, and the values used as boundaries of these categories are shown in calibrations. Pixels where the rat visited and the cell never fired are represented by gray pixels. A–F are data from the same neuron.

4F). The trajectory in the RRPST after the PLT was highly correlated to that in the last session of the PLT (see below in detail). Figure 5 shows changes in number of rewards and efficiency of acquiring rewards (A), and the trajectory and firing rate distribution similarity of the same neuron (B) as a function of session. The rat was already able to acquire over 30 rewards in the initial three sessions, and earned 50 rewards in the last two sessions of the PLT. On the other hand, traveled distance per reward linearly decreased as learning proceeded (Fig. 5A). Both the trajectory similarity ($r_{trajectory}$) and the firing rate similarity (r_{rate}) increased as learning proceeded (Fig. 5B), and the values of these similarities were highly correlated ($r=0.84$, $P<0.05$). It should be noted that high values of $r_{trajectory}$ as well as r_{rate} were preserved in the RRPST after sessions of the PLT, indicating that both behavioral and spatial firing patterns in this session resembled those in the last session of the PLT learning as shown in Fig. 4E and F.

Another example of PLT learning-related changes in trajectories and spatial firing patterns of a CA3 neuron is shown in Fig. 6. The spatial firing pattern was diffuse in the RRPST before PLT learning (Fig. 6A). In the initial sessions of the PLT, spatial firing patterns were indistinct or unstable (Fig. 6B and C). However, in the last two sessions

of the PLT, where the rat clearly directed its course toward the goal locations, the neuronal activity increased when the rat was near or within the reward site at the 12 o'clock location (Fig. 6D and E). In contrast with the neuron shown in Fig. 4, these behavioral and spatial firing patterns were not preserved in a subsequent RRPST session (Fig. 6F); the rat did not direct its course toward the reward sites, and spatial firing pattern was diffuse. Figure 7 shows the time course of number of rewards earned and efficiency of acquiring reward (A), and the trajectory and firing-rate similarity (B) of this neuron across the sessions in the PLT. The rat acquired rewards gradually in the initial five sessions of the PLT, and near maximum numbers (50) of rewards in subsequent sessions. Coherent with this, traveled distance per reward rapidly decreased in the initial three sessions, and continued to gradually decrease in subsequent sessions (Fig. 7A). There were consistent changes in the trajectory similarity ($r_{trajectory}$) and firing-rate similarity (r_{rate}) (Fig. 7B), which resulted in the high correlation coefficient ($r=0.7$, $P<0.05$) between these two variables. Note that both $r_{trajectory}$ and r_{rate} in the RRPST following PLT learning consistently decreased as compared to those in the final PLT session. This finding reflected the fact that behavioral and spatial firing patterns in

Fig. 4. An example of trajectories and firing-rate distribution of a hippocampal-formation neuron whose spatial firing pattern changed as the rat learned the place-learning task (PLT). (A) Random-reward place-search task (RRPST) before learning. (B) First session of the PLT. (C) Third session of the PLT. (D) Fifth session of the PLT. (E) Sixth and last session of the PLT. Histogram of average firing rates and raster plots in this session (below), which are time locked to onset of intracranial self-stimulation (ICSS) at reward sites at 12 or 6 o'clock positions. The gray region denotes the duration of ICSS, during which spikes were not recorded because of potential artifacts. Thin lines below abscissas, delay periods between arrival at reward site and ICSS. Thick lines below abscissas, duration of ICSS. (F) RRPST after the learning of the PLT. This neuron had no place field in the RRPST before the rat learned the PLT (A) and in the first through third sessions of the PLT (B, C). A place field near the reward site at the 12 o'clock position appeared in the fifth and sixth (last) session of the PLT (D, E). In the RRPST session after PLT learning, the rat persisted in shuttling between the reward sites as if they expected to find rewards at the previously learned locations. The place field established during the PLT learning was maintained (F). A–F are data from the same neuron. Other conventions as for Fig. 3.

this session did not resemble those in the last session of the PLT learning as shown in Fig. 6E and F.

Relationship between trajectories and firing-rate distributions in the RRPST after PLT learning

Of 10 complex-spike cells which changed the spatial firing patterns after PLT learning, seven neurons were tested again with the RRPST as shown in Figs. 4–7. Of these seven neurons, four (57.1%) had reliable firing fields in the RRPST after PLT learning. To analyze the contribution of spatial firing of the seven complex-spike cells to specific trajectory patterns, similarity of trajectories ($r_{trajectory}$) and firing-rate distributions (r_{rate}) to those in the final session of the PLT was analyzed in the RRPST after PLT learning.

Figure 8 illustrates the relationship between the $r_{trajectory}$ and r_{rate} of the seven neurons. Two neurons displayed high values of both r_{rate} and $r_{trajectory}$ ($r \geqq 0.4$), indicating that the behavioral and spatial firing patterns were maintained in the RRPST after PLT, while three had low values of both r_{rate} and $r_{trajectory}$ ($r < 0.4$), indicating that the behavioral and spatial firing patterns were not preserved. The analysis of variance indicated that the linear regression line fit to the data was significant [$F(2,4) = 7.44$, $P < 0.05$; r^2 (adjusted squared multiple correlation) $= 0.6$]. The $r_{trajectory}$ increased linearly when the r_{rate} increased, i.e., spatial firing in the RRPST after the PLT was similar to spatial firing in the last session of the PLT when the rats displayed similar trajectories in the RRPST to those in the last ses-
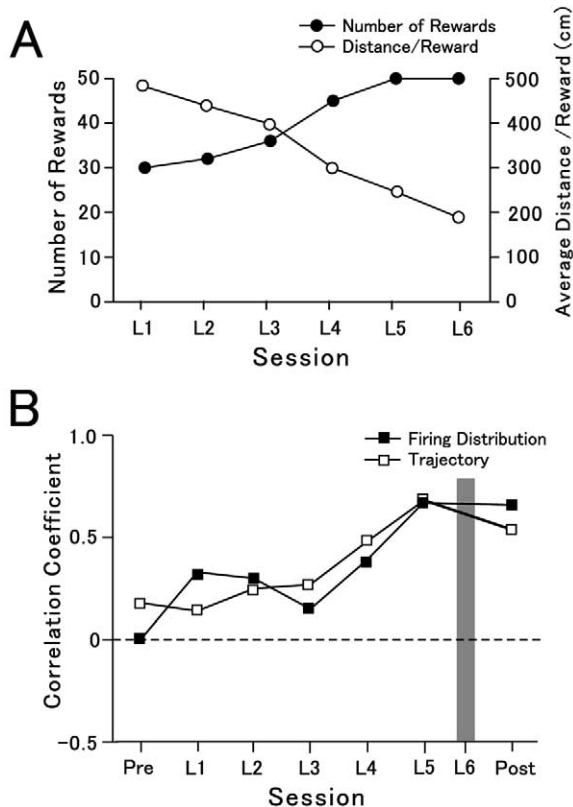
Fig. 5. Behavioral performance and changes of trajectory and firing distribution of the data shown in Fig. 4 as a function of the learning session. (A) Number of rewards and the efficiency of acquiring rewards (distance/reward) in each session. (B) Correlation coefficients of trajectory and firing distribution which were calculated between each session and the final session of the place-learning task (PLT). The gray region denotes the final session of the PLT, where both correlation coefficients were 1. There were consistent changes in correlation coefficients of trajectory and firing distribution, which resulted in the significant correlation coefficient ($r=0.84$, $P<0.05$) between these two variables, and both correlation coefficients maintained high values in the random-reward place-search task after the learning session.

sion of the PLT. This indicates causal relations between the spatial firing of the HF neurons and the movement patterns during navigation.

Recording sites

The approximate anatomical locations where the place neurons were recorded were determined histologically from small lesions made in HF after recording. Nine complex-spike cells recorded were located in the CA1 and five complex-spike cells in CA3 subfields of HF, respectively.

DISCUSSION

The present study investigated changes in the spatial firing of HF principal neurons during learning of a novel place task as measured by the efficiency of navigation strategies. We found that spatial firing of the HF neurons changed as learning proceeded. That is, changes of the HF neuron activity were well correlated to changes in rats' shuttling behaviors between reward sites. Furthermore,

spatial correlates established during learning of the PLT were preserved in the RRPST after the PLT to the degree that rats maintained the same goal-directed navigation in the RRPST as appeared in the PLT.

Correlates of the HF neuron activity to learning

Previous studies have suggested at least two distinct functions of the HF in spatial behaviors, identification of locations in an environment and planning of trajectories to goals (Morris et al., 1990; Bannerman et al., 1995; Whishaw et al., 1995; Whishaw and Jarrard, 1996; Hollup et al., 2001a). The present results showed that the rats became capable of acquiring maximal numbers of rewards at a fixed location in the initial few sessions of the PLT, and, in contrast, efficiency in acquiring rewards more slowly increased throughout all sessions of it. In the last session, the rats displayed the shortest route and shuttled between the two reward sites. Recent studies in our laboratory showed that HF lesions due to transient ischemia significantly delayed learning of this PLT (Zhong et al., 2000; Hori et al., 2002). Rats with HF lesions displayed circuitous routes along a wall to reach reward sites and could not acquire rewards as efficiently as sham-operated rats. Behavioral observations in the present study along with those of these previous studies support the idea that rats can adequately learn reward-place associations in an initial stage of PLT learning, but, on the other hand, learning of efficient routes to acquire rewards develops more slowly and requires the intact HF. In the present study, the HF neuron activity changed as the latter type of learning proceeded. Therefore, it is suggested that plastic changes of HF neuronal activity contribute to the formation of efficient navigation strategies to goals rather than learning of place-reward associations.

Previous experiments reported that place-cell activity has plasticity which develops in an experience-dependent manner (Bostock et al., 1991; Wilson and McNaughton, 1993; Mehta et al., 1997), and the plasticity of the place cell responses depends on NMDA receptors (Kentros et al., 1998). Extensive studies have shown that long-term potentiation, a leading candidate as a cellular mechanism for mnemonic processes (Bliss and Collingridge, 1993), requires activation of NMDA receptors, and their blockade impairs performance of spatial memory tasks (Morris and Frey, 1997; Cain, 1997). Recently, Mehta et al. (2000) showed that repetition of unidirectional trajectories through the place field changed its shape, and they suggested that this experience-dependent modulation depends on NMDA receptors. Further studies are required to investigate whether the plasticity of the HF firing shown in the present experiment is also NMDA-dependent.

Role of the HF neuron activity in navigation

Previous studies as well as the present one have reported that an alteration of reward-place contingencies leads to shifts in the location of the place fields of some HF neurons (Breese et al., 1989; Markus et al., 1995; Kobayashi et al., 1997; present results). However, Speakman and O'Keefe (1990) showed that majority of place fields were unaltered
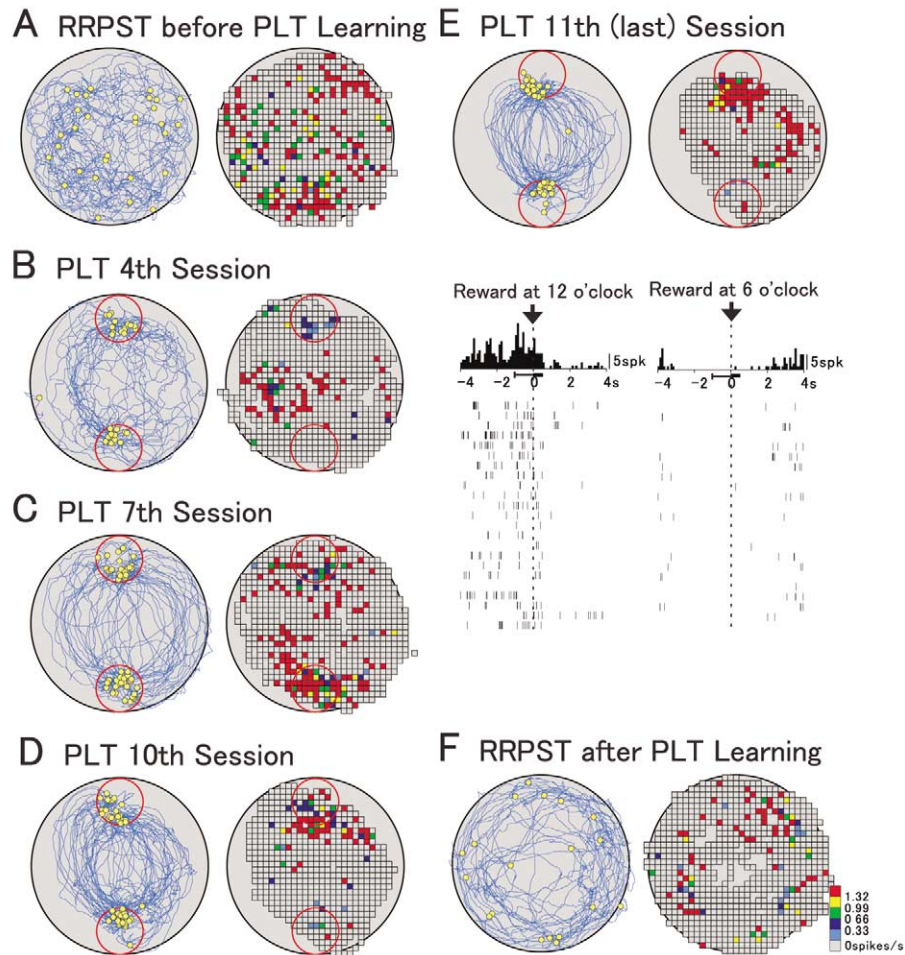
Fig. 6. Another example of trajectories and firing-rate distribution of a place cell whose spatial firing pattern changed as the rat learned the PLT. (A) Random-reward place-search task (RRPST) before learning. (B) Fourth session of the place-learning task (PLT). (C) Seventh session of the PLT. (D) Tenth session of the PLT. (E) Eleventh and last session of the PLT. The dashed line denotes the onset of intracranial self-stimulation. (F) RRPST after the learning of the PLT. This neuron had no place field in the RRPST before the rat learned the PLT (A). In seventh session of the PLT, a weak place field near the reward site at 6 o'clock appeared (C). In tenth and eleventh (last) session, the neuron showed a place field near the reward site at 12 o'clock (D, E). In the RRPST after the learning, the rat navigated randomly in the open field and the place field established during the learning was degraded (F). A–F are data from the same neuron. Other conventions as for Fig. 4.

after relocation of a reward site. Speakman and O'Keefe (1990) tested rats on a plus-shaped maze, and thus, behaviors of the rats were restricted to straight movements on the arms and turns on the central platform. In the plus-shaped maze, it might not be necessary for rats to compute a precise route to a goal, and information concerning the goal location might require only the selection of an arm from the central platform. By contrast, in an open-field experiment, rats must plan a more precise route from any given position to a goal, and correct it along the trajectory if wrong directions are selected on the way (Breese et al., 1989; Markus et al., 1995; Kobayashi et al., 1997). Thus, it is likely that spatial information processing for navigation to a goal is more important in an open field than a plus-shaped maze. Previous studies have also shown that the place cell activity is influenced by the structure of the maze where the rats are navigating (McNaughton et al., 1983; Muller et al., 1994). Furthermore, some HF neurons discharge when rats approach goals

(Eichenbaum et al., 1987; Gothard et al., 1996; Hollup et al., 2001b). Such HF neuronal responses might be closely related to navigation to a goal in an open field rather than simply to passive representation of a spatial environment.

Role of reward contingency in goal-directed navigation

The present study shows that some HF neurons shift their spatial correlates along with establishment of efficient shuttling. These HF neurons robustly fired as the animal arrived at only one of the two reward sites, and continued firing during the 1-s delay before reward delivery, and decreased rapidly after the delivery. Our previous study reported comparable characteristics of place cells in the same PLT (Kobayashi et al., 1997). If the HF neurons encoded only reward contingency or reward approach behavior *per se*, increased firing should be observed at both reward sites. However, robust firings were observed at
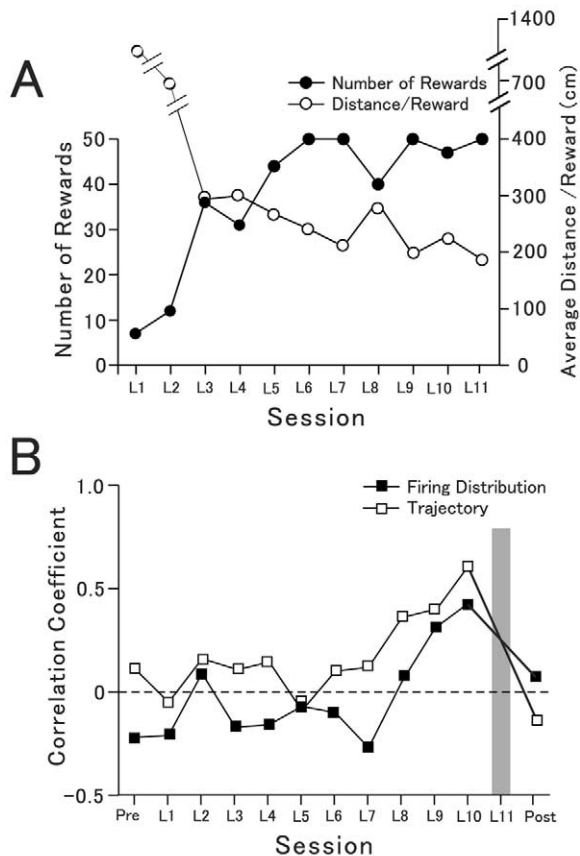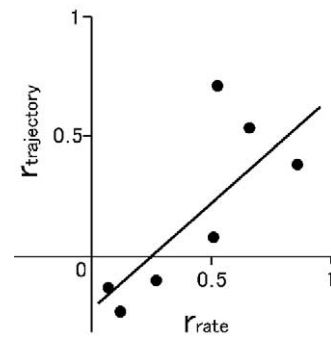
Fig. 7. Behavioral performance and changes of trajectory and firing distribution of the data shown in Fig. 6 as a function of the learning session. (A) Number of rewards per session and the efficiency of acquiring rewards (average distance/reward) in each session. (B) Correlation coefficients of trajectory and firing distribution which were calculated between each session and the final session of the place-learning task (PLT). The gray region denotes the final session of the PLT, where both correlation coefficients were 1. There were consistent changes in correlation coefficients of trajectory and firing distribution, which resulted in the significant correlation coefficient ($r=0.7$, $P<0.05$) between these two variables, and both correlation coefficients decreased in the random reward place-search task after the learning session.

Fig. 8. Trajectory similarity versus firing-rate distribution similarity in the random-reward place-search task (RRPST) after place-learning task (PLT) learning. Similarity of trajectories ($r_{\text{trajectory}}$) and firing-rate distributions (r_{rate}) in the RRPST after PLT learning were calculated to those in the final session of the PLT, and relationship between these variables was plotted. There was a significant positive correlation between the $r_{\text{trajectory}}$ and r_{rate} in the simple linear regression analysis ($P<0.05$).

tion in the PLT but not in the RRPST. However, task dependency of the HF neuronal activity in the present task is not restricted to movement-correlated activity. In similar tasks HF neurons continued to fire until the rat acquired rewards if delays were imposed prior to reward delivery (Kobayashi et al., 1997). Furthermore, some HF neurons showed spatial firing near the reward sites in the present experiment, consistent with previous studies (Breese et al., 1989; Hollup et al., 2001b). These results suggest that activity of some HF neurons reflects critical variables required for task solving such as structuring behavior to deal with varying reward contingency or an attitude to a given situation where reward is available after specific behaviors.

Theoretical consideration of HF involvement in navigation

The present experiment showed that spatial firing of HF neurons changed in correlation with learning of the PLT. Firing patterns of the HF neurons represent the environment, that is a cognitive map (O'Keefe and Nadel, 1978). In this view, the present results could be interpreted as creation of a novel representation of the environment. An alternative interpretation to this account, however, is that the firing changes were attributed to changes in trajectories which were acquired during learning. A recent theory of hippocampal representation by Eichenbaum et al. (1999) suggests that coding of spatial locations by HF place cells emerges from a fundamental representation of behavioral episodes, leading to the idea that spatial firing of place cells reflects a specific action of the rat at a specific location. The present experiment supports the second interpretation, because changes of spatial firing established during learning disappeared if the rat did not take the routes which had been acquired.

A direct route to a goal can be computed by taking the difference between a vector which describes the rat's current positional orientation and a vector which gives the position of the goal (Collett et al., 1986; McNaughton et al., 1994), and these vectors are encoded by the place-cell

only one of the two reward sites. These results suggest that the HF neuronal activity is related to reward contingency in a particular place.

In the RRPST following PLT learning, spatial firings were preserved only if the rats displayed PLT-like navigation behaviors, as if they expected to find rewards at the previously valid locations. However, the spatial firing was altered from this pattern in rats which randomly navigated at this time. These results are consistent with previous reports that spatial correlates of HF neurons were dependent on the structure of the task (Eichenbaum et al., 1987; Wiener et al., 1989; Markus et al., 1995). The task dependency of place-cell activity in the present study could be interpreted as directional dependency of the place-cell activity (McNaughton et al., 1983; Wiener et al., 1989; Muller et al., 1994; Kobayashi et al., 1997), because rats typically passed through the place field in a specific direc-

activity because spatial firing patterns of the place cell represent a certain distance and an allocentric bearing from a landmark (McNaughton et al., 1994). Subtraction of a vector representing a current position from a vector encoding a reward site might provide a direct route to a goal, and then rats could shuttle efficiently between the reward sites. Recently, Frank et al. (2000) demonstrated that the neuron in the HF and entorhinal cortex differentially fired at a given location depending on the past or future path of rats. Furthermore, these characteristics were more evident in the HF as well as the deep layers of the entorhinal cortex, which receives the primary outputs of the HF (Witter et al., 1989), than its superficial layers. These results suggest that planning of an entire route from a starting point to a goal is computed within the HF, and the output of the computation could be provided through the deep layers of the entorhinal cortex to other related structures. Vector subtraction between a start point and a goal might be performed within the HF.

CONCLUSIONS

The present study showed that plasticity of the HF neuron activity contributed to the formation of efficient navigation strategies to goals, and suggests that the activity of HF neurons might not merely serve to represent the current position of animals, but be associated with variables which have a major behavioral significance to solve a task. The HF neuron activity might represent the manner how animals have a significant relation to an environment as well as physical structures which consist of the environment.

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