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HIPPOCAMPAL PLACE CELL ACTIVITY DURING CHASING OF A MOVING OBJECT ASSOCIATED WITH REWARD IN RATS

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Abstract—Hippocampal place cells encode location of animals in the environment. However, it remains unknown whether the hippocampal place cells encode a continuously moving object in the environment. To investigate this topic, we analyzed the place cell activity of freely moving rats when a toy car was introduced into an arena. First, in a freely moving task without the car, the rats freely navigated inside the arena to earn an intracranial stimulation (ICS) reward for each 150 cm traveled. Second, they were divided into two groups and tested using two different tasks. In the car-dependent navigation (CDN) task, the car was placed inside the arena, and the rat received ICS if it chased and came within 20 cm of the car. In the car-independent navigation (CIN) task, the rat acquired ICS rewards if it traveled 150 cm regardless of its relation to the car. Place fields remapped more frequently in the CDN than the CIN tasks. In both the CDN and CIN tasks, the place cell activity inside the place fields displayed moderate tuning to the movement parameters of the rats and car, and the distance between the car and rats. However, tuning of the place cells to movement variables of the car was more selective in the CDN than the CIN tasks, while information regarding movement variables of the car represented by the place cell activity was larger in the CDN than the CIN task. These results indicated that place cell activity within the place fields represents not only an animal's own location but also the movement variables of another moving object if that object is associated with rewards. The present results provide new evidence that place cell activity conveys relevant information in a task even if this information is derived from other moving objects.

Key words: CA1, place cells, intracranial self-stimulation, reward, context.

The characteristics of hippocampal formation (HF) place cells have been widely analyzed over the last three decades. These analyses have revealed that the activity of the HF place cells increases when animals, including monkeys and humans, are in a specific location in the environment or navigate in a virtual town (O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978; McNaughton et al., 1983; Eichenbaum et al., 1987; Muller et al., 1987; Wiener et al., 1989; Quirk et al., 1990; Ono et al., 1991; Wilson and McNaughton, 1993; O'Keefe and Burgess, 1996; Nishijo et al., 1997; Knierim et al., 2000; Ekstrom et al., 2003; Hori et al., 2005; Siegel et al., 2006). The activity of these place cells encodes the current animal's location within a familiar environment, and areas in the environment associated with firing increment are called "place fields" (O'Keefe and Dostrovsky, 1971). The existence of place cells provides strong evidence of the important contribution of the HF to spatial information processing and memory (O'Keefe and Nadel, 1978).

Place cell activity is influenced by various environmental cues, including distal visual cues (O'Keefe and Conway, 1978; Muller and Kubie, 1987; Muller et al., 1987; O'Keefe and Speakman, 1987; Breese et al., 1989; Shapiro et al., 1997; Zinyuk et al., 2000; Knierim, 2002; Renaudineau et al., 2007), and by olfactory, vestibular, and proprioceptive inputs (Wiener et al., 1995; Save et al., 1998). Place cell activity is also modulated by behavioral parameters such as movement direction, speed, and turning angle of the animals (McNaughton et al., 1983; Wiener et al., 1989; Breese et al., 1989; Kobayashi et al., 1997). Neural correlates with behavioral variables increase in tasks in which the behaviors are more relevant (Kobayashi et al., 1997). The location of a goal or starting position for navigation and the manipulation of the reward also affect place cell activity (Gothard et al., 1996; Kobayashi et al., 1997, 2003; Hollup et al., 2001; Hölscher et al., 2003; Hok et al., 2007); in response to these changes, the place fields shift to different places or are completely remapped.

It is noted that, in these previous studies, the animals navigated to specific areas or to specific stationary objects (e.g. food pellets)—that is, the animals navigated within an environment that was constant for at least the duration of the trial. However, in the natural environment, animals chase and hunt small moving animals (rats) or run away from moving predators (cats). In such situations, the brains of animals must encode not only their own locations but also must continuously encode the locations of moving object(s). In the present study, we analyzed how HF place cells encoded the location and movement parameters (speed, direction, and tuning angle) of a continuously moving object. For that purpose, we designed two tasks. In one task, rats were awarded with a rewarding intracranial stimulation (ICS) if they chased and approached a toy car in an

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Abbreviations: ANOVA, analysis of variance; CA1, cornu ammonis 1; CDN, car-dependent navigation; CIN, car-independent navigation; FM, freely moving task; HF, hippocampal formation; ICS, intracranial stimulation; ICSS, intracranial self-stimulation.

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arena, and in the other, control task, rats received ICS by moving randomly in the arena irrespective of their relation to the toy car. HF place cell activity was analyzed and compared between the two tasks.

EXPERIMENTAL PROCEDURES

Subjects and surgery

Eleven male albino Wistar rats weighing 270–330 g (SLC, Hamamatsu, Japan) were used. The housing area was temperature-controlled at 23 °C and maintained on a 12-h light/dark cycle. The rats were individually housed with food and water available *ad libitum*. All rats were treated in strict compliance with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals, with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and with the Guidelines for the Care and Use of Laboratory Animals at the University of Toyama. We tried to minimize the number of animals used and their suffering.

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and implanted with a bipolar stimulating electrode (stainless steel insulated with enamel; 200 μm diameter) 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) according to the atlas of Paxinos and Watson (1986). A recording electrode assembly was stereotaxically implanted above the cornu ammonis 1 (CA1) layer of the dorsal part of the HF (3.0 mm caudal from the bregma, 2.0 mm lateral from the midline and 1.7 mm below the brain surface), and fixed on the skull by dental acrylic. The recording electrode assembly consisted of four tetrodes (polyimide-coated nichrome wires) (Gothard et al., 1996) with gold-plated tips (impedance, 200–400 kΩ) encased in a stainless steel cannula (30 gauge) and a microdrive. Each microdrive consisted of a screw coupled with a molded nut attached to the guide tube. A full turn of the screw advanced the electrodes by approximately 400 μm into the CA1 subfield of the HF.

Apparatus

Spatial behavior was investigated in a wooden arena (84 cm in diameter) with a 51 cm high wall, painted black on the inside (Fig. 1A). A piece of white cardboard, 51 cm in height×74 cm in width, was placed flat against the wall at the three o'clock position. The arena was enclosed by a circular black curtain (180 cm in diameter and 250 cm in height). Within this enclosed arena, a rat with a small red bulb on its head chased a toy car with a green bulb centered on its top. The toy car (10×6×9 cm) was manually controlled by a wireless remote controller, and could run with a maximum speed of 70 cm/s. The black-colored ceiling of the enclosure contained a video camera at the center connected an image analyzer (Video Tracker G260; OKK Inc., Tokyo, Japan). The image analyzer converted real video image signals to binary signals, and tracked the two-dimensional (horizontal) motion of the two small bulbs. A microcomputer received the X and Y coordinates of the position of the rat and toy car at 100 frames/s. The microcomputer program triggered the delivery of current for ICS when the rat's behaviors met a preset criterion. The experimenter monitored the location of the toy car, and moved it irregularly across the arena.

Behavioral procedures

Self-stimulation training. Before surgery, the rats were acclimated by handling. After recovery from surgery for implantation of the electrodes, the threshold level for intracranial self-stimulation (ICSS) was determined, and any rat for which the threshold exceeded 300 μA was excluded. The rats were screened to self-stimulate in an operant chamber (30×30×40 cm) equipped with a lever on one wall. Each lever press triggered the delivery of a 1.0 s train of 0.3 ms negative square pulses at 100 Hz. The current intensity for ICSS was determined to produce 40–70 lever presses/min. The rats were trained to self-stimulate daily in 15–30 min sessions for 1 week 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 arena. A total of 11 rats passed this screening.

Place task training

Free moving (FM) task without the toy car. After stable lever pressing was achieved, the rats were trained to perform a spatial behavior in the arena. Under the first condition, the ICS current was delivered when a preset cumulative distance was traveled by the rat. The initial distance was 80 cm, and this was increased progressively to 150 cm. The rats were usually trained for about 1 h/day for 2–4 days until they learned to travel the whole area of the arena continuously for at least 13 min with a distance of 150 cm for ICS delivery. They were then divided into two groups, and trained in the different place tasks (car-dependent (CDN) or car-independent navigation (CIN) tasks).

CDN task. Six rats (CDN group) were trained in this protocol to approach the car to acquire ICS rewards. The computer program delimited the circular area (20 cm radius) with the car in its center. An ICS reward was delivered if the rat entered this circular area, that is, if the distance between the car and the rat decreased to less than 20 cm by approaching the car. To disallow the rat continuously approaching and receiving ICS rewards, the computer program for ICS was made inactive for at least 10 s after each ICS delivery. The rats were usually trained for 20 min/day for 5–7 days until they learned to approach the car frequently.

CIN task. Five rats (CIN group) were trained in this protocol to travel in such a manner that they were awarded with ICS regardless of their relation to the car. In this group, rats were awarded with ICS if they traveled 150 cm irrespective of their distance from the car. Car movements were monitored through the CCD camera and controlled so that the car traveled throughout the entire arena.

In the recording sessions, the neuronal activity was recorded first in the FM task, and then in the CDN task (CDN group) or CIN task (CIN group). At the start of each session, the small electric bulb on the head of the rat was turned on, and a train of current for ICS was delivered to activate the rat. Each session was terminated after 50 rewards had been delivered or 13 min had elapsed, whichever occurred first.

Recording

After 2 weeks of training, the animals were put in a small box (25×25×40 cm) to check the stability of neuronal activity. The recording electrode assembly was slowly advanced to the CA1 pyramidal cell layer at about 20–80 μm per day in 20 μm steps across several days. If a complex spike cell was found, the stability of the cell recording was tested for 30–60 min.

The analog signals of neuronal activities, triggers for ICS, and X–Y coordinates of the rat and car were digitized and stored in a computer via a Multichannel Acquisition Processor (MAP) (Plexon Inc., Dallas, TX, USA) system. The amplified neuronal signals were digitized at a 40 kHz sampling rate, and 1.0-ms waveform segments of all events that crossed an experimenter-defined threshold were stored on a computer hard disk for off-line spike sorting. They were also recorded on a data recorder (RT-145T; TEAC, Tokyo, Japan). Multi-unit neuronal records were sorted into single units by their waveform components using the Offline Sorter program (Plexon Inc., see below). Superimposed waveforms of the isolated units were drawn to check their consistency through-