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Research report

Detecting location-specific neuronal firing rate increases in the hippocampus of freely-moving monkeys

Nandor Ludvig[a,*], Hai M. Tang[a], Baiju C. Gohil[b], Juan M. Botero[c]

[a] Department of Physiology and Pharmacology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Box 31, Brooklyn, NY 11203, USA
[b] Department of Surgery, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[c] Department of Anesthesiology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

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Abstract

The spatial properties of the firing of hippocampal neurons have mainly been studied in (a) freely moving rodents, (b) non-human primates seated in a moveable primate chair with head fixed, and (c) epileptic patients subjected to virtual navigation. Although these studies have all revealed the ability of hippocampal neurons to generate spatially selective discharges, the detected firing patterns have been found to be considerably different, even conflicting, in many respects. The present cellular electrophysiological study employed squirrel monkeys (*Saimiri sciureus*), which moved freely on the walls and floor of a large test chamber. This permitted the examination of the spatial firing of hippocampal neurons in nearly ideal conditions, similar to those used in rodents, yet in a species that belongs to the primate Suborder Anthropoidea. The major findings were that: (1) a group of slow-firing complex-spike cells increased their basal, awake firing rate more than 20-fold, often above 30 spikes/s, when the monkey was in a particular location in the chamber, (2) these location-specific discharges occurred consistently, forming 4–25 s action potential volleys, and (3) fast-firing cells displayed no such electrical activity. Thus, during free movement in three dimensions, primate hippocampal complex-spike cells do generate high-frequency, location-specific action potential volleys. Since these cells are components of the medial temporal lobe memory system, their uncovered firing pattern may well be involved in the formation of declarative memories on places.

Theme: Neural basis of behavior
Topic: Learning and memory: physiology

Keywords: Squirrel monkey; Hippocampus; Complex-spike cell; Place cell; Medial temporal lobe memory system; Declarative memory

1. Introduction

Clinical studies in patients with bilateral surgical removal of the temporal lobe [19,36] and those in patients with ischemia-induced amnesia [31,38] revealed that the hippocampal formation plays a critical role in the formation of long-term memories on experiences. Experimental lesion studies in non-human primates confirmed these findings, leading to the development of the declarative memory concept [3,37]. Accordingly, the medial temporal lobe memory system that comprises the hippocampal formation and the perirhinal and parahippocampal cortices serves to consolidate consciously accessible (“declarative”) memories in other, neocortical areas [3,11,37]. Declarative memories include those on episodes, facts and verbal information, but also on places, locations and environments encountered during life [9,19,31,36]. In fact, one striking consequence of bilateral temporal lobectomy is the inability to form long-term spatial memories, which leads to severely impaired navigation in environments encountered after operation [36].

The cellular mechanisms that in the primate hippocampus mediate the formation declarative memories on places have not been clarified. It is plausible to assume that such spatial

* Corresponding author. Tel.: +1-718-270-1796; fax: +1-718-27-3103.
E-mail address: nludvig@downstate.edu (N. Ludvig).
URL: http://www.downstate.edu/pharmacology/ludvig.htm.

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memories are formed during free movement in the environment, when the human or animal is exposed to multimodal sensory stimuli from both local objects and distant landmarks, computing mentally their relative distances and directions and, if possible, interacting with them. Neuronal recordings in these experimental conditions have not, and could not, been made in humans. Remarkably, in rodents that are allowed to move freely in a relatively large space, most hippocampal complex-spike cells markedly increase their firing rate when the animal's body is in a particular location. These cells, discovered by O'Keefe and Dostrovsky in 1971 [26], have been named as "place cells", and their unique, location-specific firing pattern has been confirmed in rats and mice by numerous investigators [1,10,18,22,35] including us [13]. Among other pertinent data, it is now known that: (1) rodent place cells are predominantly complex-spike/pyramidal cells located in the hippocampus proper [21], (2) these cells are capable to increase their basal, awake firing rate of 0.1–2 spikes/s to as high as 30–50 spikes/s within a particular location [21], (3) their location-specific firing can be independent of the direction in which the animal's head is pointing [21], and (4) unrestrained, voluntary movement within the test area is necessary to fully activate the spatially specific discharges of these neurons [5]. Such hippocampal neuronal firing pattern, or its modified versions, may well promote in primates the neocortical consolidation of spatial declarative memories.

Place-cell type location-specific discharges, nevertheless, have not been conclusively shown in the monkey hippocampus. Although several studies performed in monkeys did reveal "place-related" neurons in the hippocampal formation [24,29,30], the place-related firing rate increases of these neurons did not exceed the range of 2–8 spikes/s. In fact, in similar experimental conditions other monkey studies found no place cells in the hippocampus at all [7,28,33,34]. Instead, in these latter experiments "spatial view cells" were detected, which tuned to respond when the monkey looked at a small part of the environment. It was not examined whether or not the recorded neurons were complex-spike/pyramidal cells. Notably, all of these studies were performed in monkeys restrained in a primate chair or motorized cab, with head fixed, although these restraining apparatuses were moveable in two dimensions either by the experimenter or the seated animal.

In a recent study in human epileptic patients, Ekstrom et al. [4] recorded temporal lobe neuronal activity while the subjects navigated in a "virtual town" presented on a computer screen. They concluded that "24% of cells in the hippocampus were bona fide place responsive cells". These data, albeit obtained in a seizure-generating/propagating neural circuitry, suggest that place-cell-type firing patterns are indeed generated in the primate hippocampus. Nevertheless, since this study was not performed in subjects that moved freely in a large space, the described "bona fide place responsive cells" were recorded in a fundamentally different experimental condition than the place cells found in rodents. This may explain, for example, why the absolute size of the areas covered by the firing rate increases of human place responsive cells was quite small, occupying only a restricted sector on the computer screen [4].

In order to bridge the gap between the neuron-recording data obtained in the hippocampus of freely moving rodents, chair-restrained monkeys, and humans viewing a computer-generated virtual town, we undertook the task of examining the electrical activity of hippocampal neurons in squirrel monkeys allowed to move freely, in three dimensions, in a relatively large space. The advantage of this experimental arrangement is that it creates conditions similar to those used to promote place cell firing in rodents, yet it offers hippocampal neuronal recording in an anthropoid primate. This, in turn, allows one to extrapolate the data, with the necessary caveats, to the physiology of the human medial temporal lobe. Since methodology for single-cell recording in freely moving monkeys had not been available, we developed a method for monitoring the electrical activity of single neurons in monkeys during free movement on the floor and walls of a large test chamber [16]. To induce fairly similar behaviors and stable movement patterns during the electrophysiological data collection, as this is critical for identifying spatially selective discharges, we employed a novel spatial memory task that keeps the monkey searching for food in baited food-ports interspersed among non-baited ones [17]. The sole specific aim of this study was to test whether or not high-frequency location-specific discharges, similar to those expressed by rodent place cells, are generated by monkey hippocampal neurons during free movement, as this, however limited, information can help to clarify the cellular mechanisms that mediate the formation of declarative memories on places in the primate temporal lobe.

2. Methods

2.1. Animals

Six squirrel monkeys of both sexes were used. These New World monkeys belong to the Suborder Anthropoidea. All except one 3-year-old animal were adults, with body weight varying from 0.75 to 1.10 kg. Two of the adult monkeys were 15 years old; the exact age of the remaining adults was not known. The animals were housed in the AAALAC-accredited facility of Downstate Medical Center, in large (1.5 × 1.5 × 2.2 m), interconnected cages within an enriched environment. This study was conducted according to the Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996), and was approved by the Institutional Animal Care and Use Committee at SUNY Downstate Medical Center (Protocol no. 12-126-02).

2.2. Behavioral training

The animals were trained to perform the eight-port task version of the spatial memory task we have recently described in detail [17]. Briefly, in this task the monkey learns to retrieve pieces of cereal from four baited food-ports interspersed among four non-baited ones. The ports are located on the walls of the test chamber, with one baited port on each wall. The food pellets are odorless and not visible for the monkey, so the animal should develop spatial memory for the location of the baited ports to execute the task efficiently. Each trial lasts until the monkey retrieves all food pellets from the baited ports, and each session comprises 10 trials. Incorrect Choice (IC) is defined as a reach by the monkey into a non-baited port at least once within a trial, whereas Correct Choice (CC) is defined as a reach by the monkey into a baited port at least once within a trial. From these values, spatial memory index (SMPI; ranging from 0.0 to 1.0) is calculated for each trial (as well as average SMPI for each session), as follows: SMPI=(CC − IC)/CC. Every aspect of this task, including food delivery and data acquisition, was controlled by a Monkey Spatial Memory Testing Apparatus (G-tech; Cortlandt Manor, NY). The animals performed this task both before and after the microelectrode implantation, and were introduced into the recording sessions when they reached an average SMPI index of 0.75 or higher. Accordingly, the monkeys became familiar with the test chamber and executed the spatial task with high accuracy by the time of the recordings.

2.3. Microelectrode assembly

The driveable microelectrode assembly [16] contained an array of 17 Nichrome microwires, each functioning as a single electrode. Each microwire was connected to one of the pins of an 18-pin Mill-Max socket; a grounding wire was connected to the 18th pin. Three special driving screws (Can-Do Services, Hopewell Junction, NY) were glued to this socket. The microwires (25-μm diameter each) were placed in a 26-G stainless steel tube, protruding to a 3-mm length with their tips spread over a 1-mm horizontal distance. Epoxy was applied in between the end of the tube and the initial segment of the microwire array to keep the tips of the wires spread without bending or clumping. (This was checked after each experiment by examining the removed assembly.) The recording tips of the microelectrode array were 26 mm below the bottom of the driving screws.

2.4. Surgical procedures

The microelectrode assembly was implanted into the brain of the monkey under general anesthesia. The anesthesia was induced with a mixture of intramuscular (i.m.) ketamine (11 mg/kg) and xylazine (0.5 mg/kg), and was maintained with 1.2–2% isoflurane in oxygen. After positioning the head of the monkey in the stereotaxic apparatus and exposing the skull, a 3-mm diameter craniotomy was made. The coordinates of the center of the hole, according to the atlas of Gergen and MacLean [8], were 0.2 mm anterior to the line between the ear bars and 8 mm right to the midline. Anchoring screws were placed in the bone. The microelectrode-array was introduced into the brain at a 7° angle through the center of the hole. The tip of the unit was positioned 19.5 mm below the brain surface. The grounding wire of the assembly was connected to an anchoring screw. The assembly was secured to the skull and the screws with dental cement, and a plastic ring and bone wax sealed the craniotomy. This was followed by inserting a custom-made i.m. catheter into the temporalis muscle. Finally, all components of the assembly, including the extramuscular portion of the i.m. catheter, were covered with a plastic protective cap. After surgery, the animal was placed in an individual cage for a week postoperative care, as described [16].

2.5. Experimental apparatus

The recording sessions took place in an experimental apparatus that integrated (a) the test chamber shown in Fig. 1, (b) the Monkey Spatial Memory Testing Apparatus, (c) an electrophysiological recording system we described previously [16], and (d) a video monitoring system. The

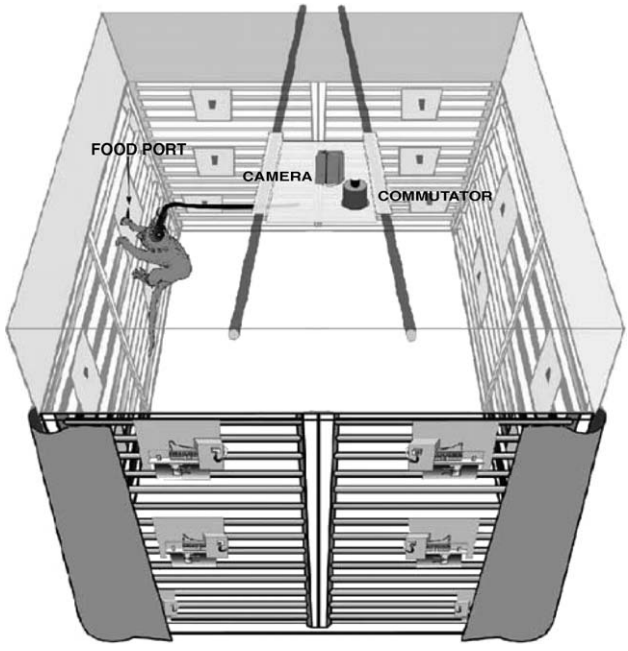

Fig. 1. Test chamber. The monkey moved freely around in the 1.5-ml × 1.5-m W × 1.2-m H area of the chamber composed of wooden bars, while collecting pieces of cereal from food-ports located on the walls. It is this area that is represented on the maps of Figs. 3, 4, 7, 8 and 9. Curtains blocked the animal's view to most of the laboratory. The monkey's behavior and spatial position were followed with a wide-angle-lens camera, as indicated. The recording cable was connected to a commutator next to this camera, via a special pulley/counterbalance assembly (not shown here for simplicity). This environment and its surroundings were unchanged throughout this study.

electrophysiological recording system comprised a special recording cable assembly equipped with a 0.25-mm inner diameter Tygon tubing, operational amplifiers, as well as a plastic protective tube to which a ring of red light-emitting diodes (LEDs) was secured. The cable and the attached tubing were connected, via a special pulley/counterbalance assembly (Can-Do Services), to an electric commutator-liquid swivel unit placed on the ceiling of the chamber. This setup was completed with AC differential amplifiers, oscilloscopes and the Discovery data acquisition system of DataWave Technologies (Longmont, CO). The video monitoring system included a wide-angle-lens camera secured to the ceiling, next to the commutator. With the use of this camera, the monkey's behavior was monitored and the position of the animal's body within the chamber was tracked. For this body-position tracking, the same Ebtronics device was used as in our prior rat place cell studies [13]. Thus, during recordings, the tracker followed the LEDs (secured to the cable-protecting tube close to the animal's head) and generated digitized *X* and *Y* coordinates for the location of this light source.

2.6. Extracellular recording sessions

These sessions started 2–3 weeks after microelectrode implantation. First, the monkey was seated in a primate chair. To eliminate stress, this procedure was performed after briefly exposing the animal to a mixture of nitrous oxide and sevoflurane in oxygen. Then the already awake monkey was transported to the test chamber. For a period of 30–50 min, the monkey was kept in the chair. This period was necessary for connecting the animal to the recording cable and for advancing the microelectrode assembly, in documented steps, to record single-neuronal electrical activity. Initially, the microelectrodes were advanced in 100–140-μm steps, and as they started to pick up action potentials, they were moved in smaller, 40–80-μm steps. The extracellular electrical signals were amplified (10,000 ×), filtered (low cut-off: 300 Hz; high cut-off: 10,000 Hz), displayed on oscilloscopes, and digitized and acquired at a 40,000-Hz sampling rate with the Discovery data acquisition system. When complex-spike cells [6,14,15], the targeted neurons, with amplitudes of 2.5× higher than the approximately 60-μV background noise were detected for at least 30 min, the monkey was released into the test chamber. For 2 h, the monkey rested on a perch in the chamber, without access to food. Then the perch was removed, the spatial memory testing apparatus was turned on, and data collection started. Thus, importantly, data were collected during similar behaviors, when the monkey was moving around retrieving food pellets from the baited ports. During data collection, the extracellular signals were stored on hard disk together with the body-positional data transferred to the Discovery system by the video-tracker at a 60-Hz rate. This later allowed us to determine the spatial properties of the action potentials. A recording session lasted from 6 to 24 h, comprising several 15–60-min data collection periods, depending on the monkey's behavior. Thus, when the monkey stopped performing and rested, data collection was not conducted. At the end of the session, the animal was sedated for 5–10 min by the infusion of 8–10 mg/kg ketamine via the i.m. catheter. This eliminated the stress of handling and allowed us to carefully disconnect the monkey from the recording cable. Then the animal returned to the home cage. The recording sessions were separated with 3–7-day intervals, and each monkey was subjected to four to eight sessions.

2.7. Histology

At the end of the study, the monkeys were euthanized with 200 mg/kg pentobarbital, i.m., for the necessary histological studies to identify the localization of the recording sites. After euthanasia, the animals were transcardially perfused with phosphate-buffered saline (PBS) followed by 10% formalin. From each monkey, the microelectrode-assembly was removed for subsequent examination. Then the brain was removed, frozen with liquid nitrogen and sectioned with a cryostat. Forty-micrometer-thick coronal sections were cut at the level of the hippocampus. The sections were Nissl-stained and examined with a light microscope. A SONY DKC500 digital camera system was used to generate photomicrographs on representative histological materials. The histological studies, along with the record on microelectrode-advancements, identified the track of the microelectrode-array and the recording sites (Fig. 2).

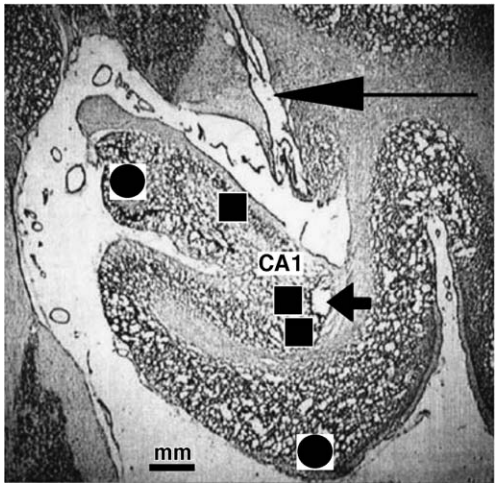

Fig. 2. Localizations of the recording sites. A Nissl-stained coronal section at the level of the right hippocampus is shown, from monkey #5. The area of the CA1 region is indicated. Arrow points to the track of the microelectrode; arrowhead points to the recording site that yielded location-specific neuronal firing in this animal. Squares indicate recording sites where similar spatial discharges were detected in monkeys #1, 2 and 6. The spheres indicate the recording sites where no location-specific neuronal activity was recorded in monkeys #3 and 4.

2.8. Data analysis

This report is based on data obtained in 28 recording sessions. In one monkey the recording site was localized in the dentate gyrus, while in another one the recording site was found to be in the entorhinal cortex (Fig. 2). In four monkeys, the recording site was correctly localized in the hippocampus proper (Fig. 2). The below described extensive data analysis was performed only on the data obtained in this latter group of animals. The CP Analysis program of DataWave Technologies and the advanced Mapmaker program of ESCO (Garden Grove, CA; formerly at Mt. Kisco, NY) were used, also employing the statistical analysis package of GraphPad Software (San Diego, CA).

The collected extracellular recordings were subjected to a multifaceted action potential discrimination protocol, similar to what was previously described [13]. First, the raw extracellular data were analyzed with the cluster-cutting procedure to discriminate single-neuronal action potentials. Second, the discriminated action potential waveforms were overlaid and examined. Third, interspike interval analysis was performed to further ensure the single neuronal origin of the discriminated action potentials. Fourth, firing rate histograms were generated to examine the occurrence of action potentials within the time frame of the data collection periods. This analysis also calculated the maximum firing rate for each analyzed neuron. Fifth, the action potential stream of each isolated neuron was played back to identify sustained (>4 s) action potential volleys with firing rate at least five times higher than the firing rate within the preceding, same-length recording segment.

To analyze the spatial properties of the discriminated action potentials, firing rate distribution maps were generated. The program for these maps, using both body-positional and action potential data, divided the floor and the walls of the test chamber to 90- and 180-cm^2 pixels, respectively, calculated the average firing rate (spikes/s or Hz) of the cell within the area of each pixel, assorted these firing rate values into color-coded ranges, calculated the mean of each of these ranges and filled the pixels of the map with the appropriate color. Yellow pixels indicated areas where the cell did not fire; green, light blue, dark blue and magenta pixels indicated increasingly higher firing frequencies; black pixels indicated areas where the firing rate of the cell was within the highest range. The basic algorithm has been described in detail [13]. In each of these maps, the central square represents the floor of the chamber, while the trapezoids represent the walls. This type of data presentation compensates for the limited view of the wide-angle-lens camera to the walls of the chamber.

Examination of the firing rate distribution maps allowed us to identify a group of maps where each map contained a single, distinctive cluster of at least 15 neighboring pixels with the three highest firing rate ranges. Thus, such a cluster comprised only black, magenta and dark blue pixels interconnected with each other. Each of these clusters, representing a unique location in the test chamber where the analyzed cell increased its firing rate, was further characterized by the following values: (a) the size of the location [cm^2] covered by the cluster, (b) the average firing rate/pixel [spikes/s]inside of this location, (c) the average firing rate/pixel [spikes/s] outside of this location, and (d) the ratio of the ‘‘inside-location’’ and ‘‘outside-location’’ values. For each cell that yielded at least one such firing rate distribution map, the consistency of the localized firing rate increases was also determined by comparing the cell’s spatial firing data across the entire 6–24-h recording session.

Considering the main characteristics of rat place cells [21], we defined location-specific(spatially selective) firing rate increase as a single-neuronal electrical activity that: (1) occurred in a location covering more than 3% of the total area explored by the monkey, (2) comprised inside-location discharges with an average frequency of more than five times higher than the average frequency of discharges outside of this location, (3) reached a maximum frequency of higher than 5 spikes/s, and (4) appeared repeatedly for more than 2 h during a recording session. Neurons that produced a firing pattern satisfying these four criteria were classified as ‘‘spatial cells’’. Neurons that lacked such a firing pattern were classified as ‘‘non-spatial cells’’. For these latter cells, average firing rate in the entire explored area in the test chamber (spikes/s/pixel) was determined. Statistical analyses of the generated variables, including maximum firing rates, were performed with paired and unpaired *t*-tests and one-way ANOVA, as appropriate.

Finally, staying time distribution maps were also generated for all data collection periods. These maps demonstrated the total time the monkey spent in the area of each pixel, as described [13]. This analysis method is useful for capturing such behaviors as place preference, prolonged resting, and sleep, as these behaviors are associated with increased staying time in a particular location.

3. Results

Altogether, 28 neurons with well-discriminated action potentials were recorded from the hippocampal CA1 and CA2 regions while the monkey moved around freely in the test chamber performing the behavioral task. These cells fell into three categories. The first category included slow-firing cells that generated location-specific (spatially selective) firing rate increases, as defined in Methods (Figs. 3–7). The second category included slow-firing cells that failed to generate such location-specific firing rate increases (Fig. 8). The third category included fast-firing cells that did not generate location-specific firing rate increases either (Fig. 9). Table 1 provides a summary of the electrophysiological properties of these neurons.

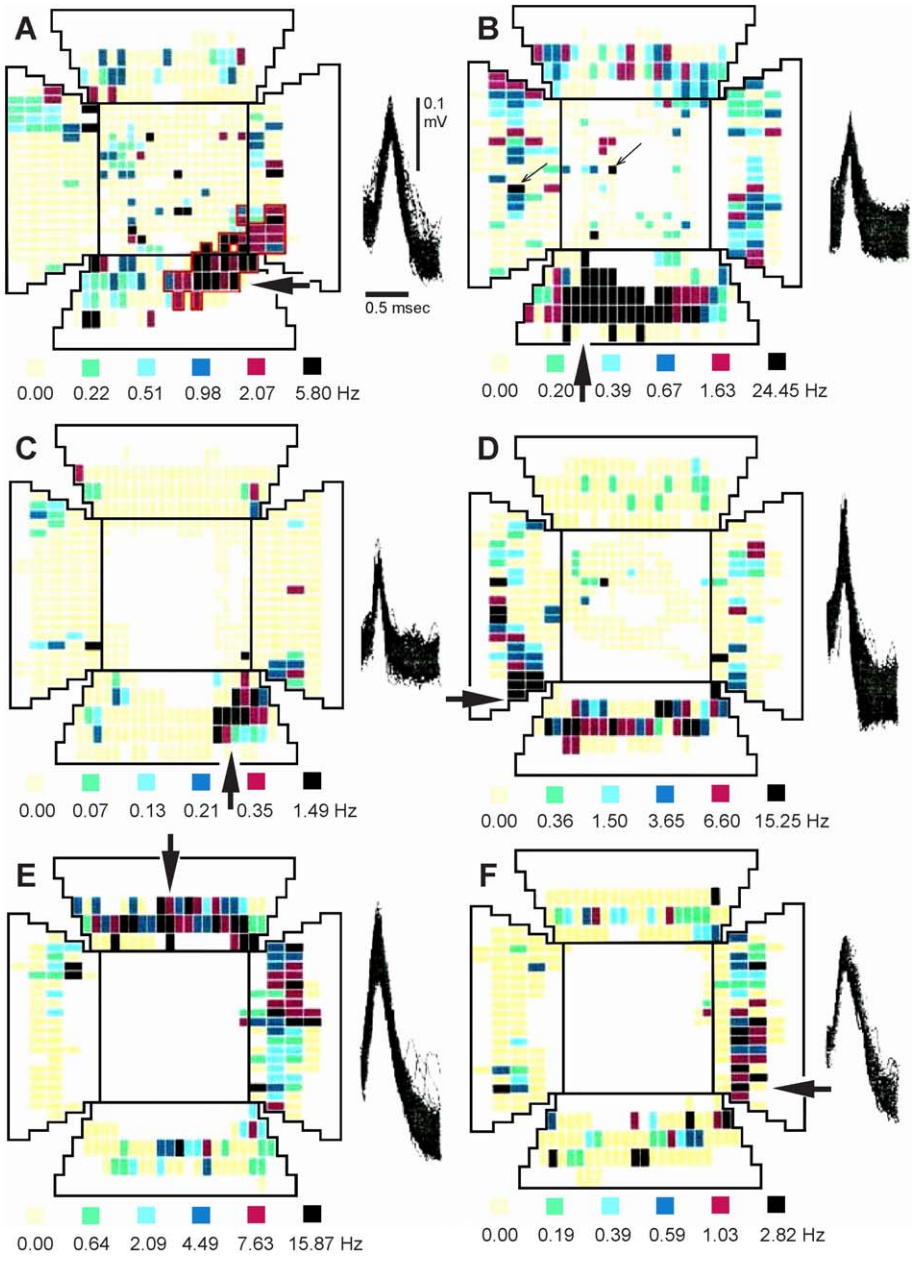

Fig. 3. Location-specific firing rate increases, as recorded in freely moving monkeys. Firing rate distribution maps are shown, with the corresponding overlaid action potential waveforms on the right side of each map (calibrations as indicated). The data for the maps were collected in monkey #1 (A), #2 (B and C), #5 (D and E) and #6 (F), during 15–60-min periods. Each pixel on the maps indicates the color-coded average firing rate of a discriminated slow-firing cell in the area of the pixel, as the monkey moved around in the test chamber, collecting food from the baited ports. Color codes are shown under each map: yellow = no firing; black = highest firing rate. White areas: locations not visited by the monkey. On each map, the central square represents the floor and the rectangles represent the walls. Note that each cell produced a marked firing rate increase in a particular, relatively large location in the chamber (indicated with arrowhead). The borders of one of these areas are indicated with red line in A. The generation sites of brief bursts recorded outside of these locations are indicated with thin arrows in B.

The slow-firing cells that generated location-specific discharges (spatial cells; $n = 9$) were complex-spike cells, like the one presented in Fig. 4A. All of these cells produced a distinctive firing pattern with the following characteristics: First, each of these neurons increased its firing rate significantly for prolonged (>4 s) periods only when the monkey's body was in a particular location in the test chamber (Figs. 3, 4 and 7). The average size of these locations was 5575.0 ± 856.7 cm^2 (Table 1), covering 4–35% of the total area explored by the monkey in the chamber. Within these locations, the cells generated sustained, 4–25-s action potential volleys (Fig. 5), manifesting an average spatial firing rate of 5.60 ± 1.98 spikes/s/pixel (Table 1). This value was 7–46 times higher (mean ± S.E.M. = 21.7 ± 4.5) than the average (basal) firing rate of the cells when the monkey's body was outside of these

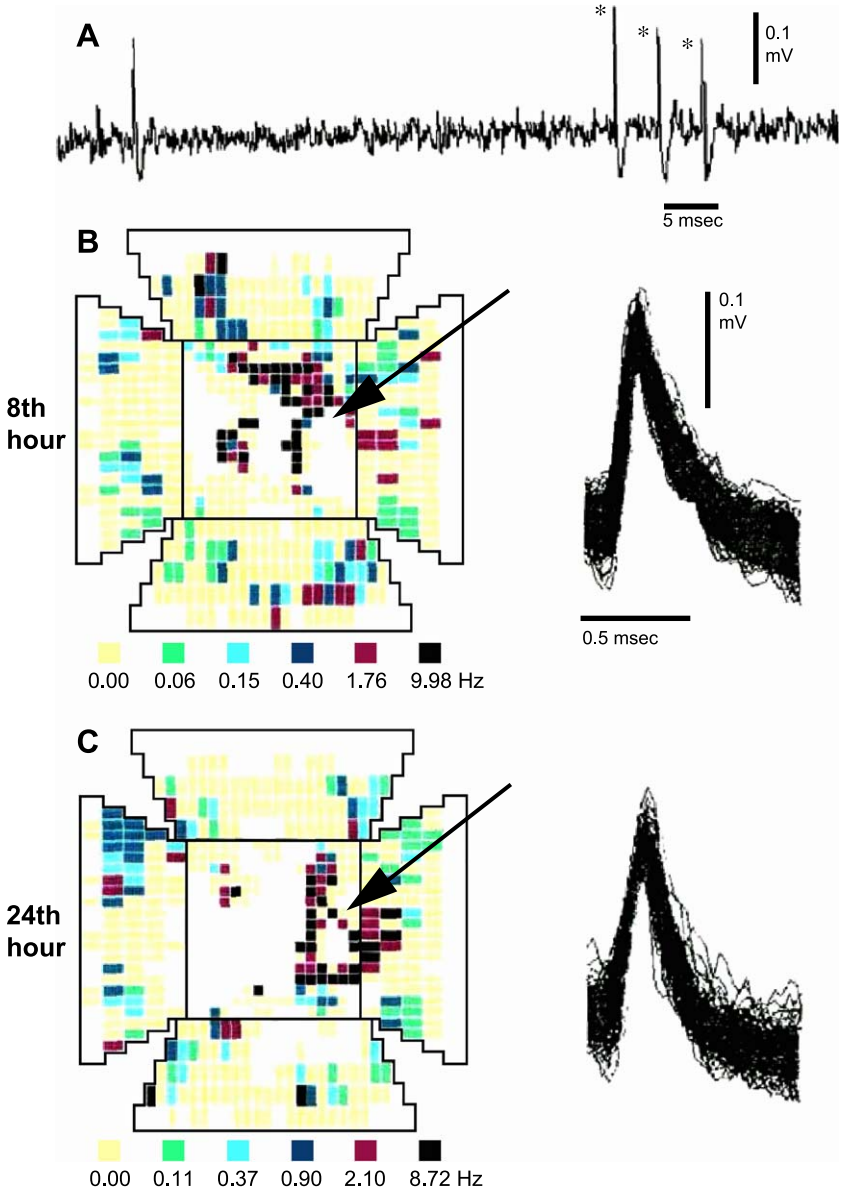

Fig. 4. Complex-spike pattern of the location-specific discharges. (A) Raw extracellular recording from monkey #2; note the complex-spike activity comprising three consecutive action potentials with descending amplitude (asterisks). Calibrations as indicated. (B and C) Firing rate distribution maps with corresponding overlaid action potentials (similar to those shown in Fig. 3) demonstrating the location-specific firing of the complex-spike cell of A. Data for both maps were collected in 60-min periods, in the 8th and 24th h of the recording session. Note the similar positions of the areas of firing rate increases on the floor (arrow) and that the action potential waveforms were virtually unchanged during the long-term recording session. Calibrations as indicated.

areas (0.29 ± 0.09 spikes/s/pixel; Table 1). Second, during the periods of location-specific firing rate increases, the maximum firing intensity of spatial cells reached 30 spikes/s (Fig. 6) or higher rates (e.g., 43 spikes/s by the cell represented in Fig. 3B). The maximum firing rate of these neurons was 21.18 ± 5.57 spikes/s (Table 1), reflecting their ability to produce high frequency discharges. Third, these firing rate increases occurred repeatedly and consistently throughout the 15–60-min data collection periods (Fig. 6). Fourth, as it was indicated by the staying time distribution maps, the location-specific firing rate increases of these cells were not due to preferential staying within a particular area in the test chamber, nor were they related to resting or sleep (Fig. 7). Indeed, during data collections, the monkeys moved around in the chamber, exploring the floor and visiting all four walls to retrieve food pellets from the baited ports.

The spatial position of the area of firing rate increases within the chamber varied from cell to cell, covering virtually the entire area explored by the monkeys. As shown in Fig. 3, the firing rate increases of cell A and C covered the south-east corner, that of cell B covered a large part of the south wall, that of cell D covered adjacent portions of the south and west walls, that of cell E covered adjacent portions of the north and

east walls, whereas cell F increased its firing on the east wall and the adjacent corner of the southern wall. Location-specific discharges that occurred on the floor are demonstrated in Fig. 4. Occasionally, these neurons generated brief, high-frequency action potential bursts outside of the locations of the above described firing rate increases (Fig. 3B). These outside-location bursts did not occur consistently in the same place and were usually restricted to small (<10-pixel) isolated areas. Thus, they did not meet with the criteria of location-specific firing, as defined in Methods. Of the nine slow-firing spatial cells, five maintained their location-specific electrical activity throughout the 6–24-h recording sessions. An example for stable location-specific firing maintained for as long as 24 h is shown in Fig. 4. Two cells ceased to generate spatially selective discharges by the second recording day, albeit their location-specific firing was stable in the first day (Fig. 3B/Fig. 7A and Fig. 3D/Fig. 7C). The stability of location-specific firing could not be appropriately determined in the two remaining cells, as they were detected in recording sessions with unusually long resting phases.

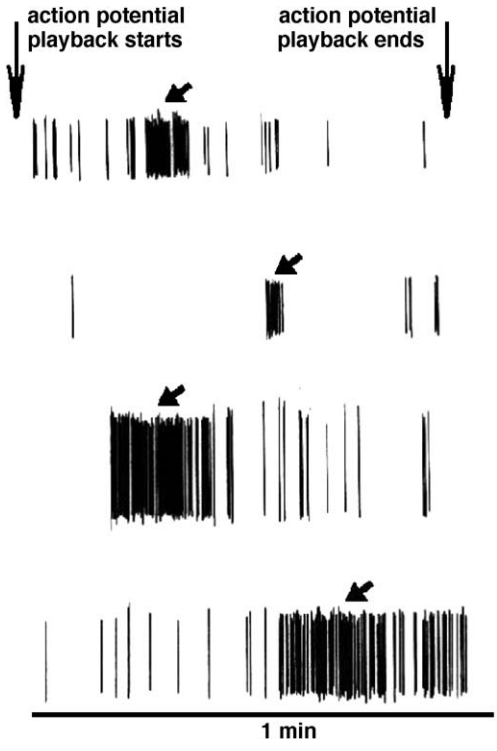

Fig. 5. Sustained, high-frequency action potential volleys during location-specific firing. Each trace shows a 1-min segment from the discriminated action potential stream of a single cell, with the arrowhead pointing to the high-frequency action potential volley displayed exclusively when the monkey's body was within a specific location. The spatial firing pattern and overlaid waveforms of cell 1 are shown in Fig. 3B, those of cell 2 is on Fig. 3D, those of cell 3 is on Fig. 3E, and those of cell 4 is on Fig. 4B and C. Note that while each cell produced its location-specific firing rate increase in a sustained (>4 s) manner, within the volley the frequency of action potentials varied.

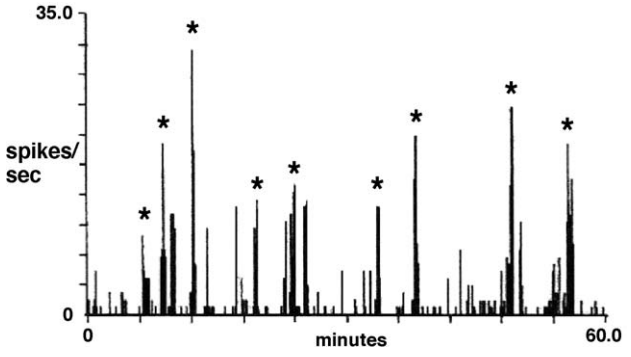

Fig. 6. Repetitive and consistent occurrence of the location-specific discharges. Firing rate histogram is shown; horizontal axis: recording time (min), vertical axis: firing frequency (spikes/s). The data for this histogram were collected from the spatial cell of Fig. 3B. Asterisks indicate the firing rate increases when the monkey's body was in the south wall of the chamber. Note that in between the periods of high-frequency discharges (when the monkey's body was in other areas of the chamber), the cell was either silent or generated a very low frequency firing. Also note the varying maximum frequencies of the asterisk-indicated firing rate increases.

The slow-firing cells that did not generate location-specific firing rate increases (slow-firing non-spatial cells; $n = 11$) were also complex-spike cells. These neurons produced low-frequency discharges that were independent of the monkey's position in the chamber (Fig. 8). The maximum firing rate of these complex-spike cells (7.15 ± 0.63 spikes/s) was significantly lower than that of the complex-spike cells with location-specific discharges (21.18 ± 5.57 spikes/s; Table 1). The fast-firing cells lacked any spatially specific firing pattern (Fig. 9). These neurons (fast-firing non-spatial cells; $n = 8$) produced single spikes. The average firing rate of fast-firing non-spatial cells in the entire area of the test chamber (5.61 ± 1.31 spikes/s/pixel) was significantly higher than that of the slow-firing non-spatial cells (0.36 ± 0.08 spikes/s/pixel; Table 1). The maximum firing rate of fast-firing cells and that of spatial cells, however, did not differ significantly (Table 1).

4. Discussion

This study revealed that during free movement in three dimensions, a group of slow-firing neurons in the monkey temporal lobe generates marked location-specific firing rate increases. Each of these cells was located in the hippocampus proper, was a complex-spike cell, and produced location-specific firing rate increases that often exceeded 30 spikes/s, occurred consistently, comprised 4–25-s action potential volleys, and covered 4–35% of the explored space in the test chamber. These characteristics are remarkably similar to those of place cells recorded in the rodent hippocampus [1,5,10,13,18,22,26,39]. Our data are also consistent with the studies that detected "place-related" neurons in monkeys seated in a moveable primate-chair [24,29,30] and are consistent with the discovery that during

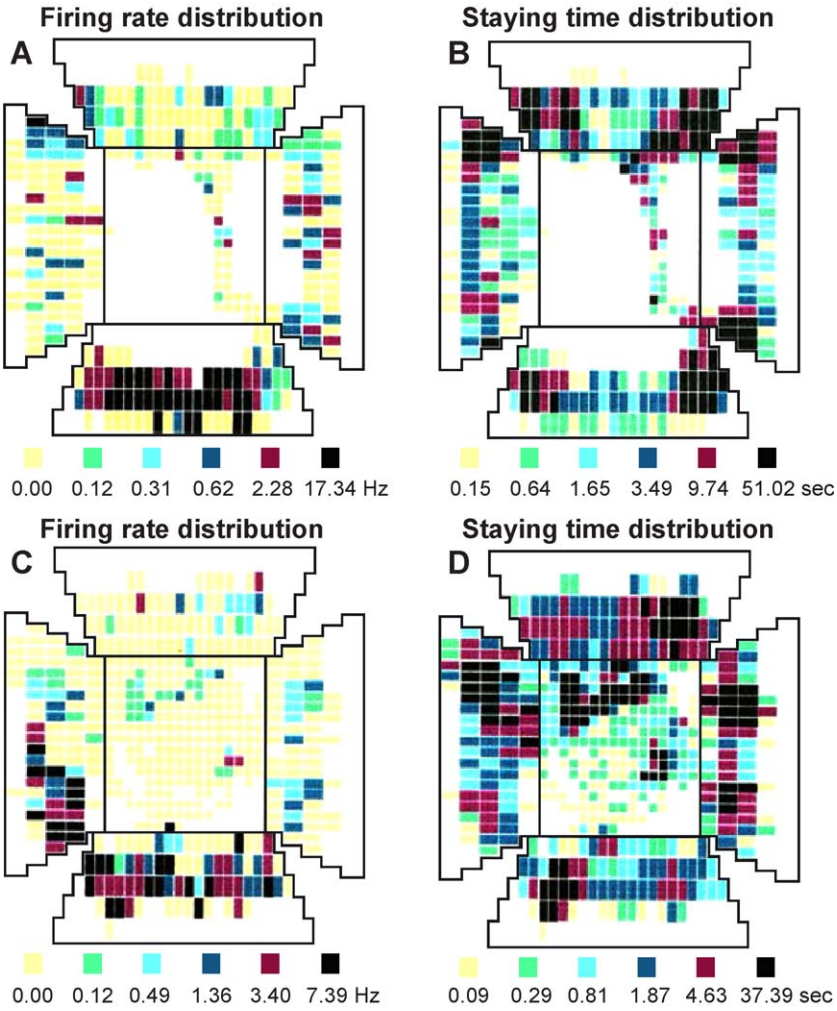

Fig. 7. Activity of the monkeys during the expression of location-specific discharges. Firing rate distribution maps (A and C) and staying time distribution maps (B and D) are shown. Each pixel in the staying time distribution maps indicates the total time the monkey spent in the area of the pixel during the data collection period. The data for A and B were obtained in the same, 60-min recording period from monkey #2 (4 h prior to the recording period represented in Fig. 3B). The data for C and D were obtained during the same, 60-min recording period from monkey #5 (3 h after the recording period represented in Fig. 3D). Note that in both data collection periods, the monkey visited the floor and spent considerable time on all four walls of the chamber (with retrieving food pellets from the baited ports). Yet, the cells recorded in these periods increased their firing rate only in a circumscribed spatial sector.

the performance of a virtual navigation task, a class of human hippocampal neurons expresses ‘‘place responsive’’ electrical activity [4].

Our findings indicate that if monkeys are allowed to move freely in three dimensions, the rate of the location-specific discharges of hippocampal neurons can be considerably higher than during passive or active movements under chair- and head-restraint [24,29,30]. This supports the conclusion of the rat study by Foster et al. [5] that free movement is necessary to fully activate the firing repertoire of place cells. In fact, when Ono and Nishijo [29] compared hippocampal neuronal firing in monkeys seated in a motorized cab moveable by both the animal and the experimenter, they found that ‘‘place-related activity proved to be obscure in the experimenter-controlled condition’’ [29]. In our opinion, free movement enables the monkey to act according to his/her will and eliminates restraint-induced stress, leading to a brain state where the hippocampus may receive an optimal input-configuration from the motivational and emotional systems with which it is richly interconnected [11]. This optimal input-configuration can potentiate the principal, spatial-information-carrying inputs from other areas of the cognitive system, activating the full repertoire of location-specific firing patterns in hippocampal neurons.

The present results also demonstrate that, unlike during navigation in virtual space, during movement in real space the location-specific discharges of primate hippocampal neurons are sustained over quite large (>5000 cm^2) environmental sectors. This suggests that the spatial firing properties of these cells in virtual and real space are not identical, and thus recordings in both experimental conditions will be necessary in future to fully understand the space-encoding mechanisms of primate hippocampal neurons.

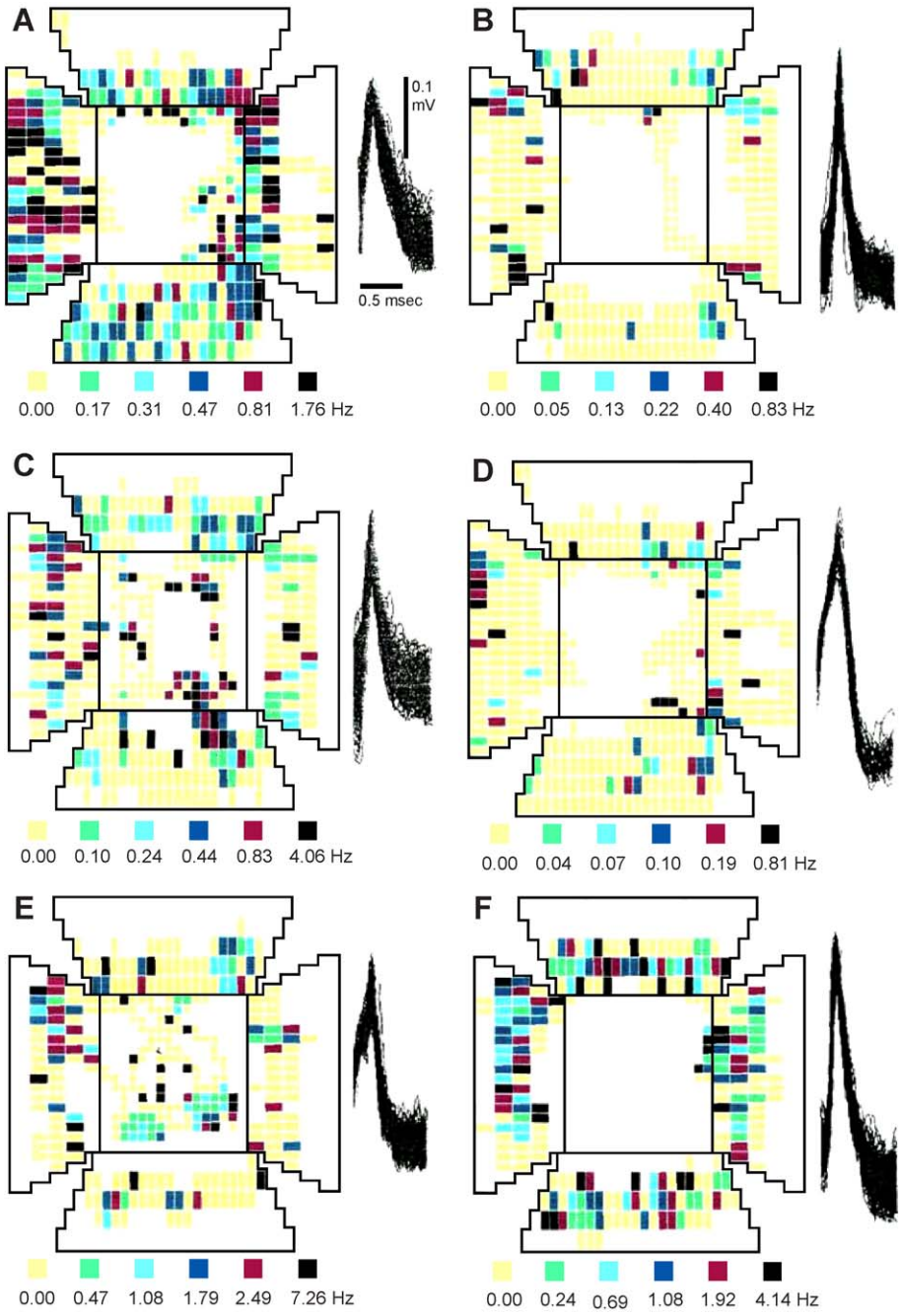

Fig. 8. Firing patterns of slow-firing non-spatial cells. Firing rate distribution maps and corresponding action potential waveforms are shown. The data for the maps were collected in 15–60-min periods from monkey #2 (A, B, C. D), #5 (E) and #6 (F). Note the diffuse, low-frequency firing of the cells all over the area of the chamber. Indeed, none of these electrophysiological patterns satisfied the four criteria of location-specific firing, as defined in Methods. Compare the maps with those of Figs. 3 and 4; note the profoundly different firing patterns.

It was not determined whether the observed location-specific discharges were induced by (1) local stimuli emanating from the particular location physically encountered by the monkey, by (2) distant stimuli emanating from objects and landmarks viewed by the monkey from that particular location, or by (3) both types of stimuli, as available for the animal in area of firing rate increase. In order to evaluate these alternatives, the eye movements of the monkeys, or at least direction of their face within the explored three-dimensional space should have been monitored simultaneously with the body-positional and cellular electrophysiological data. However, these monitoring techniques have not been available for us: they have yet to be developed. Indeed, considering the intriguing studies of Rolls and his colleagues on hippocampal spatial view cells in the monkey [7,33,34], the utilization of such advanced monitoring techniques in freely moving monkeys seems to be an urgent task.

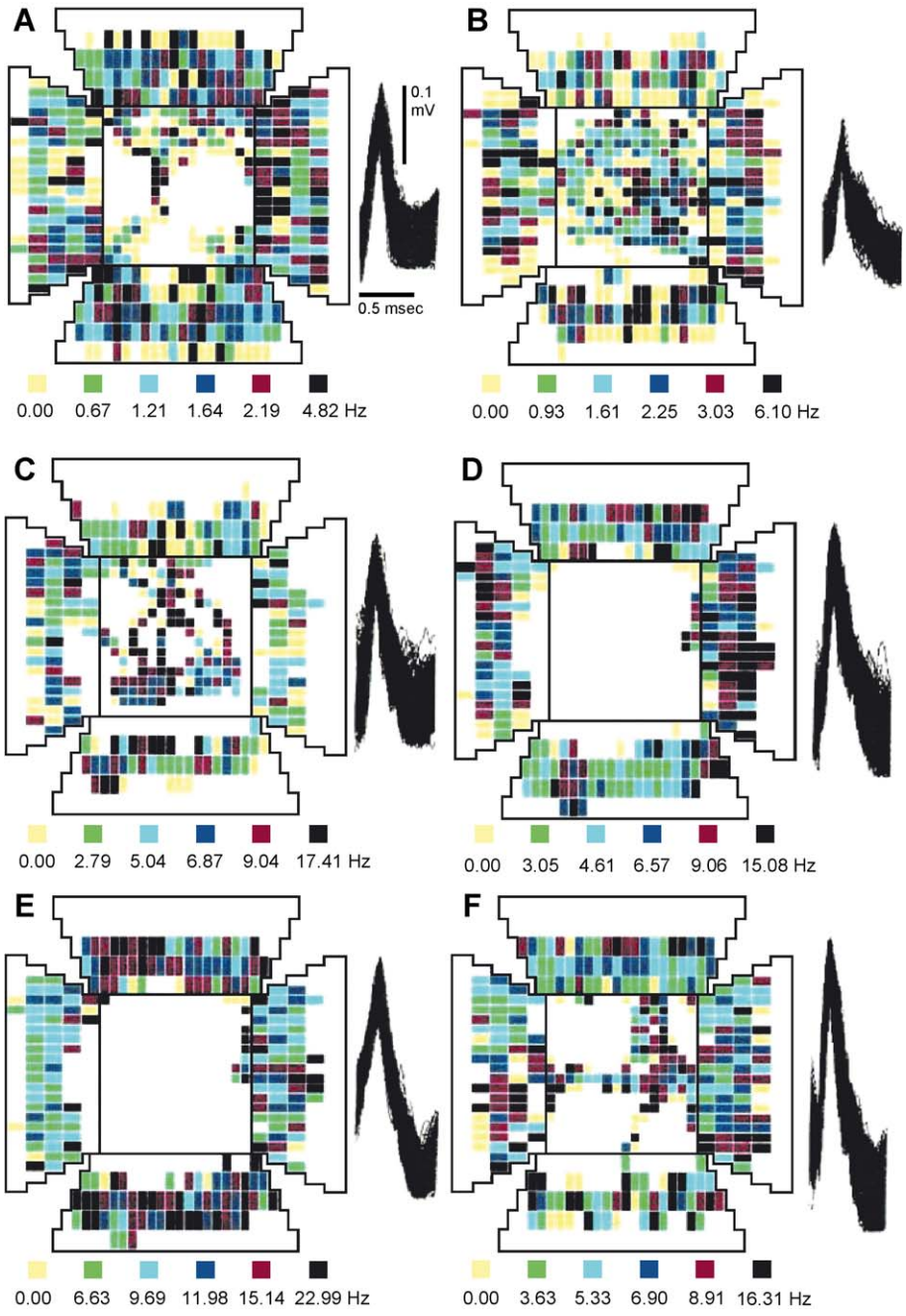

Fig. 9. Firing patterns of fast-firing non-spatial cells. Firing rate distribution maps and corresponding action potential waveforms are shown. The data for the maps were collected in 15–60-min periods from monkey #2 (A) #5 (B, C) and #6 (D, E, F). Note the diffuse, high-frequency firing of the cells all over the area of the chamber. Again, none of these electrophysiological patterns satisfied the four criteria of location-specific firing. Compare the maps with those of Figs. 3 and 4; note the profoundly different firing patterns.

A close inspection of the location-specific action potential volleys as they occurred repeatedly in time revealed that the temporal distribution of action potentials within a single volley was not uniform (Fig. 5) and the maximum frequencies of the successive volleys were highly variable (Fig. 6). It should be kept in mind, however, that the monkey's movement path, locomotion speed, views to the laboratory, mood and motivation to perform the task, handling of the retrieved food pellets, and his other behaviors were not, and could not be, exactly the same during every encounter of a particular location. Instead, these factors varied, however slightly, at each encounter, and it is these variations that probably exerted a modifying effect on the course of location-specific discharges. In fact, as it was shown by Wiener et al. [39], this is precisely the case with rodent hippocampal place cells, of which firing rate was also found to vary "systematically in relation to behavioral variables, including the speed, direction, and

Table 1
Basic statistics of the electrical activity of the three major cell types in the monkey hippocampus, as recorded during free movement in three dimensions

	Spatial cells ($n=9$)	Slow-firing non-spatial cells ($n=11$)	Fast-firing non-spatial cells ($n=8$)
Average firing rate/ pixel in the entire explored area	N/A	0.36 ± 0.08[a]	5.61 ± 1.31[b]
Maximum firing rate in the entire explored area	21.18 ± 5.57[c]	7.15 ± 0.63[d]	20.08 ± 2.43[e]
Average firing rate/ pixel inside the location of spatial firing	5.60 ± 1.98[f]	N/A	N/A
Average firing rate/ pixel outside the location of spatial firing	0.29 ± 0.09[g]	N/A	N/A
Area of location of spatial firing	5575.0 ± 855.7	N/A	N/A

The firing rates are given in spikes/s; the area of location-specific (spatial) firing is given in cm^2. Each value indicates mean ± S.E.M.
[a]vs. [b]$=p<0.01$; [c]vs. [d]$=p<0.05$; [c]vs. [e]$=p>0.05$; [d]vs. [e]$=p<0.05$; [f]vs. [g]$=p<0.05$.

turning angle of the rat as it moved through the place field".

Since the sole aim of this study was to clarify whether high-frequency, location-specific discharges are generated by monkey hippocampal neurons during free movement, the extent to which these discharges differ from those of rodent place cells was not examined. Nevertheless, it might be worth noting two curious features of the spatial cells we detected. First, they generated brief, non-spatial bursts outside of the location of their prolonged, spatially selective firing rate increases, even during movement. Rodent place cells can also increase their out-of-field firing rate, but these firing rate increases are usually due to resting or a "secondary" firing field [21]. Second, the absolute size of the areas where the monkey spatial cells increased their firing was quite large, often covering significant portions of two adjacent walls. The firing fields of rat place cells in our previous studies [13] were substantially smaller, about 100–1000 cm^2. While this difference can hardly be related to such methodological factors as the operation of the video-tracker or our action potential discrimination protocol, it can, indeed, be due to the different geometry of the test chambers we used for rats and monkeys.

In summary, employing experimental conditions similar to those used in rodent place cell studies, we demonstrated that a group of primate hippocampal complex-spike cells generates robust location-specific firing rate increases during free movement in a three-dimensional space. Since these cells were recorded in a brain region that is part of the medial temporal lobe declarative memory system in primates [3,11,37], we propose that these neurons, by the virtue of their high-frequency, location-specific action potential volleys, contribute to the formation of declarative memories on places. In fact, these hippocampus-promoted, spatial declarative memories may well be linked together in the neocortical association cortex, generating the cognitive map, as we previously hypothesized [13]. In essence, this hypothesis is consistent with both the declarative memory concept [2,3,9,11,37,40] and the notion that the hippocampus plays a crucial role in spatial cognition [12,18,20,23,25,27,32].

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