Place-related neuronal activity in the monkey parahippocampal gyrus and hippocampal formation during virtual navigation

(仮想空間移動課題におけるサル海馬体および海馬傍回ニューロンの場所応答性)

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Running head: PPA and hippocampal responses to place and view
Neuropsychological data in primates demonstrated a pivotal role of the hippocampal formation (HF) and parahippocampal gyrus (PH) in navigation and episodic memory. To investigate the role of HF and PH neurons in environmental scaling in primates, we recorded neuronal activities in the monkey HF and PH during virtual navigation (VN) and pointer translocation (PT) tasks. The monkeys had to navigate within three differently sized virtual spaces with the same spatial cues (VN task) or move a pointer on a screen (PT task) by manipulating a joystick to receive a reward. Of the 234 recorded neurons, 170 and 61 neurons displayed place-related activities in the VN and PT tasks, respectively. Significant differences were observed between the HF and PH neurons. The spatial similarity of place fields between the two different virtual spaces was lower in PH than in HF, while specificities of the neuronal responses to distal spatial cues were higher in PH than in HF. Spatial view information was predominately processed in posterior PH. The spatial scales (place field sizes) of the HF and PH neurons were reduced in the reduced virtual space, as shown in rodent place cells. These results suggest the complementary roles of HF (allocentric representation of landmarks) and PH (representation of the spatial layout of landmarks) in the recognition of a location during navigation.

Key words: hippocampus, parahippocampal gyrus, place cell, monkey
INTRODUCTION

Activity in the primate hippocampal formation (HF) increases during spatial tasks performed in both real and virtual environments (Aguirre et al., 1996; Maguire et al., 1998), and damage to HF produces severe deficits in memory tasks performed in a real or virtual space (Astur et al., 2002; Hampton et al., 2004). The findings of these studies are consistent with those of a cognitive map theory in which HF acts as a cognitive map of the environment and plays a central role in the formation of episodic memory (O’Keefe and Nadel, 1978). Consistent with this theory, the activities of some HF neurons (place cells) increase when the animal navigates within a particular place in the environment (O’Keefe and Dostrovsky, 1971; McNaughton et al., 1983; Muller and Kubie, 1987; Eichenbaum et al., 1990). Our previous study reported that place-related neurons were also present in the monkey HF in a virtual navigation (VN) task (Hori et al., 2005). These neurons have been found not only in monkeys (Ono et al., 1993; Matsumura et al., 1999; Ludvig et al., 2004) but also in humans (Ekstrom et al., 2003). The present study analyzed the responsiveness of neurons in monkeys to the deformation of a spatial boundary, which is an important characteristic of rodent place cells (O’Keefe and Burgess, 1996).

Previous virtual navigation studies conducted in humans demonstrated that the parahippocampal gyrus (PH) contains a region in its posterior part called the parahippocampal place area (PPA), which shows increased activity in response to scenes, such as photographs of landscapes (Epstein et al., 2003). Although monkey PH neurons displayed place-related activities (Matsumura et al., 1999), PH neurons responded to specific landmarks (Rolls and O’Mara, 1995; Ekstrom et al., 2003). Because PPA is sensitive to local scene geometry in a viewpoint-dependent manner (Epstein et al., 2003), the place-related activities of the PH
neurons might be attributed to their sensitivity to changes in scenes that result from changes in head orientation or in the location of the viewer. In this study, we compared the characteristics of the place-related neurons in the monkey HF and PH while the animal navigated within three differently sized virtual spaces with the same distal spatial cues to receive a juice reward. In these three virtual reality spaces, the animal looked at the same distal cues placed at different distances from different view angles. The present results indicated differences in spatial responsiveness between the HF and PH neurons, consistent with the complementary roles of HF and PH, which are characteristics essential for episodic memory.

MATERIALS AND METHODS

Animals

Two male adult monkeys (*Macaca fuscata*) weighing 6.5 and 7.5 kg were used in the experiment. The monkeys were housed individually in their home cages and supplied with monkey ration ad libitum. The animals were deprived of water in their home cages on the training and experimental days, but they could receive a liquid reward during the experimental session. Supplemental water and vegetables were given after each day’s session. To assess the monkeys’ health, their weight was routinely monitored. The experiment was conducted in strict compliance with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Guidelines for the Care and Use of Laboratory Animals at the University of Toyama.
Experimental apparatus

During the recording session, the animals were placed on a restraining chair, and their heads were painlessly fixed using acrylic U-shaped frames surgically implanted to the monkeys’ skulls. The frames acted as head movement restrainers. The chair consisted of an acrylic box with wheels in which the monkeys could be taken from their cages to the experimental room. Inside this box, the monkeys could sit comfortably to perform the task. In the experimental room, the chair was positioned 1.9 m away from a 1.5-m high × 1.9-m wide projector panel, which displayed three-dimensional (3D) polarized images projected by an LCD projector located behind and above the monkeys (Fig. 1A). The animals were trained to perform the task by looking at the screen using polarized lenses attached to the outer part of the chair; it was as if the monkeys were wearing 3D polarized glasses. During the task, the room lights were turned off.

A joystick, which the monkeys used to perform the task, was attached to the front wall of the chair. The animals could receive a liquid reward (sports drink). The liquid delivery was controlled using an electromagnetic valve connected to a tube projecting through the rear side of the monkey’s chair.

Behavioral paradigms

The animals were trained to perform two different tasks. The first task was a VN task (Fig. 2A), which required them to navigate (velocity, 1.0 m/s) in a 3D environment by manipulating the joystick. For this task, a large 3D open-field space (100 m diameter) was projected on the screen using a 3D software (EON Studio ver.2.5.2, EON Reality Inc., Irvine, CA, USA). Since previous studies reported that location could be represented by the distances and the directions of landmarks (Chen et al., 1987) and HF place cell activity was directly
influenced by the boundary of the environment (O’Keefe and Burgess, 1996; Jeffery, 2007), these parameters were manipulated in the present study. Using the same arrangement as that of the distal spatial cues, three different virtual spaces were created: control, expanded, and reduced (Fig. 2Aa–c). In the control VN task (Fig. 2Ab), the monkeys were allowed to move only in a limited 20-m diameter space located in the center of the open field and surrounded by a wall (0.3 m high). This central part of the virtual space will be henceforth referred to as the mobility area (Fig. 2B). The floor of the virtual space was green-colored, and there was no difference in the floor between inside and outside the mobility area. The mobility area included five reward areas (3.0 m diameter) that were symmetrically placed within the mobility area [North (N), East (E), South (S), West (W), and Center (C)]. The center of the four peripheral reward areas was located 6.0 m away from the center of the mobility area. The distal spatial cues (a tree, a building, a rock, and three posters) were located 5.0 m away from the wall surrounding the mobility area (control VN task). Figure 1B presents an example of the spatial arrangement of the control VN task (Fig. 2Ab). In the expanded VN task (Fig. 2Aa), only the arrangement of the spatial cues was expanded (the distal spatial cues were located 40 m away from the wall surrounding the mobility area), while the mobility area was of the same size as that in the control VN task (Fig. 2Ab). In the reduced VN task (Fig. 2Ac), the size of the mobility area (12 m diameter) and reward areas (1.8 m diameter) were reduced. The centers of the four peripheral reward areas were located 3.6 m away from the center of the mobility area. In this task, the distal spatial cues were located 3.0 m away from the wall surrounding the mobility area.

In these VN tasks, the tasks were initiated by projection of the virtual spaces on the screen, and the animals had to perform shuttle behavior in two different directions within the mobility area by manipulating the joystick: N-C-S-C and E-C-W-C. By repeating these
sequences, the monkeys could receive a liquid reward whenever they entered the reward areas in correct sequence. Because this experiment was a modified version of the Morris water maze, the monkeys could not see the reward areas, except on the first visit to each of the reward areas when a blue circle blinked three times in 2 s. As for the following visits, no visual signal was shown. In each trial, the monkeys could get a total of 15 rewards such that each neuron was tested at least once in each task, N-C-S-C and E-C-W-C, in all three versions (control, expanded, and reduced). Figure 1C shows an example of a movement trace in the control VN task. The movement trace shows that the monkey did not move straightly, but gradually changed movement direction to reach the reward areas. These findings indicate that the monkeys paid attention to the VR environment to control their navigation.

The second task was a pointer translocation (PT) task (Figure 2C) in which a pointer (11 cm diameter) and two reward circles (22 cm diameter; distance between the centers, 110 cm) appeared on the screen. The reward areas, which were the two reward circles, were located diametrically opposite to one another on the screen (right/left or up/down). The monkeys had to use the joystick to position the pointer inside the reward areas and receive a liquid reward (pointer speed, 0.2 m/s). After positioning the pointer inside one reward area, the monkeys were supposed to move it to the other reward area to continue the task and receive a liquid reward again. When the animals positioned the pointer inside the same reward area in which they had positioned the pointer previously, the animals could not receive any reward. There were no distal cues in this task. Only the pointer and reward areas were displayed on the screen.

Training

Initially, the monkeys were trained in the PT task in which they learned not only the task but also to operate the joystick. First, the animals had to move the pointer with a faster speed to
two different reward areas with a larger size. Initially, the joystick movements were physically restricted because of an acrylic plate placed below the joystick, allowing the movements only in vertical, horizontal, or diagonal directions depending on the position of the reward areas. As the monkeys became more skillful, the reward area size and the pointer speed were gradually decreased until the animals reached the final step in learning the task. The monkeys took 2–3 months to learn to move the joystick freely in all directions without any restriction. As for the task, when the monkeys could perform the PT task with a criterion of 95% correct responses, the animals were moved to the next level and training for the VN task began.

For the VN task training, the animals had to get used to the VN environment. First, as in the PT task, the monkeys manipulated a joystick under physical limitations created using an acrylic plate below the joystick, which allowed three movements simultaneously to the left, right, and front, so that the animals could learn that they must be facing the reward area to receive the reward. By movement of the joystick to the front, the monkey could move forward in the virtual space. By movement of the joystick to the right and left, the monkey could turn clockwise and anti-clockwise in the virtual space, respectively. With this setting, we could avoid accidental reward offering. In the absence of this limitation, the monkeys sometimes stopped paying attention and just held the joystick by usually pulling it back during training. The monkeys were first trained in the control version of the task, which was followed by the expanded version, and subsequently, the reduced version. The animals took approximately 8 months to perform the VN task with 95% correct responses.

**Surgery**

After the completion of the training period, a head movement restrainer (U-shaped acrylic frame) was implanted on the animals’ skulls. The surgical procedure was performed
under aseptic conditions. The animals were intramuscularly anesthetized with a combination of medetomizine hydrochloride (0.5 mg/kg) and ketamine hydrochloride (5.0 mg/kg). Using dental acrylic, the frame was anchored to tungsten bolts inserted in the skull. During surgery, heart and respiratory functions and rectal temperature were monitored (Lifescope 14; Nihon Kohden Corporation, Tokyo, Japan). A blanket heater was used to maintain body temperature at 36 ± 0.5 °C. To prevent infection, antibiotics were administered topically and systemically for 1 week after surgery. Two weeks after surgery, training was resumed with the animals’ heads fixed to the stereotaxic apparatus. Within 10 days, the performance criterion was attained once again. After this period, the animals were again intramuscularly anesthetized with medetomizine hydrochloride (0.5 mg/kg) and ketamine hydrochloride (5.0 mg/kg). A hole was opened in the animals’ skulls above the target area (i.e., HF and PH) to insert the electrode to be used for the recording sessions. A tungsten bar (0.5 mm diameter) was stereotaxically inserted exactly above HF as a marker, according to an atlas of M. fuscata (Kusama and Mabuchi, 1970). Then, X-ray photography and magnetic resonance imaging (MRI) were performed to estimate the coordinates of HF and PH (Hori et al., 2005).

**Recording procedures and data acquisition**

After the monkeys were placed in their monkey chairs, a glass-insulated tungsten microelectrode (Z = 0.5–1.5 MΩ at 1000 Hz) was stereotaxically inserted into various parts of HF and PH in a stepwise manner using a pulse motor-driven manipulator (SM-20; Narishige Scientific Instrument Lab, Tokyo, Japan). During the recording session, eye movements were monitored with electrooculograms (EOGs). These EOGs were recorded using Ag-AgCl electrodes placed on the lateral edges of the eyes. The experimental data, which included the analog signals of neuronal activities and EOGs, triggers for the juice reward, X–Y coordinates
of the monkeys in the virtual spaces, the pointer on the screen, and the joystick, were digitized and stored in a computer using a Multichannel Acquisition Processor system (Plexon Inc., Dallas, TX, USA). The amplified neuronal signals were digitized at a 40-kHz sampling rate, and 1.0-ms waveforms that crossed an experimenter-defined threshold were stored on a computer hard disk for offline spike sorting. The signals were also recorded on a data recorder (RT-145T; TEAC Corporation, Tokyo, Japan).

**Data analysis for definition of place-related neurons**

Single units were isolated by their waveform components (Offline Sorter; Plexon Inc). In addition, autocorrelation was computed for each neuron to confirm a refractory period of 1–2 ms. Superimposed waveforms of isolated units were redrawn to examine the invariability of the waveforms, and the data were then transferred to the NeuroExplorer program (Nex Technologies, Littleton, MA, USA) for further analysis.

Mean firing rate of each neuron was calculated by averaging firing rates across the 3 VN and PT tasks. The neurons in each brain region were classified into 2 groups, high frequency and low frequency, by using k-means clustering based on logarithm of the mean firing rates. The mobility area in each of the VN tasks was divided into $0.40 \times 0.40 \, \text{m}^2$ pixels. The mean firing rate for each pixel $x$ was calculated as:

\[
(x) = \frac{1}{n} \sum_{i=1}^{n} g(s_i, x) \int_{0}^{T} g(y(t), x) \, dt
\]

where $g$ is a Gaussian filter with 1.5 m half width, $n$ is the number of spikes, $s_i$ is the location of the $i$-th spike, $y(t)$ is the location of the monkey at time $t$, and $T$ is a duration of a
task (Fyhn et al., 2007; Kjelstrup et al., 2008). The pixels where the monkey spent less than 0.2 s were excluded from further analysis of the firing rate map. According to the methods of previous studies (Muller et al., 1987; Kobayashi et al., 1997; Matsumura et al., 1999), place fields, which were defined as the pixels in which the activities of the HF and PH neurons increased, were identified on the basis of the mean firing rates. Only place fields that had at least one pixel with a mean firing rate exceeding 2.0 times the mean firing rates and one adjacent pixel with a mean firing rate exceeding 1.5 times the mean firing rates were analyzed. The place fields could be expanded through any edge shared by two pixels meeting the criterion (greater than 1.5 times the mean firing rates). If one or more neighboring pixels satisfied the criterion, the field was expanded to include those 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 9 pixels. The place-related neurons in the VN tasks were defined as neurons that displayed the place field(s), as noted above, in at least one of the three VN tasks. Place-unrelated neurons were defined as neurons with no place fields across the three VN tasks. In the PT task, the pointer could be moved within the $1.5 \times 1.9$ m$^2$ mobility area on the screen. This mobility area on the screen was also divided into $4 \times 4$ cm$^2$ pixels. A firing rate map of the location of the pointer on the screen for each HF and PH neuron was obtained as in the VN task with 14 cm half width Gaussian filter and the pixels where the monkey spent less than 0.1 s were excluded from further analysis of the firing rate map. The place fields on the screen in the PT task were identified as in the VN tasks. The place-related and place unrelated neurons in the PT task were defined as neurons with and without place field(s) in the PT task. Because different parameters (pixel size and filter) were used for the VN and PT tasks in the above analyses, it is difficult to directly compare the percentages of the place-related
neurons between the VN and PT tasks. Therefore, we additionally analyzed place responses. Definition of “place-related neurons” supposes higher firing rates when the monkey is located in specific position(s). In case of place neurons, the monkey’s locations where the firing rates increase during the tasks should be localized. Therefore, the average distance between the nearest neighbor locations with higher firing rates was used as an index of spatial specificity. To compute this index, first, a task period was divided into 500-ms bins, and an average firing rate and monkey’s location in each bin was calculated. Then, the bins in which the firing rates were more than the mean + 2 SD were selected and the average nearest neighbor distance was calculated among the monkey’s locations in the selected bins. Statistical analysis of the average nearest neighbor distance was based on Monte Carlo simulation. The sample bin data were randomly selected from those in the whole task period (the number of selected bins was same as the actual ones). For each neuron in each task, 1000 simulated data, each of which consisted of the same number of randomly-selected bins, were generated. Given HF and PH neurons were defined as significant place-related neurons if their actual average nearest neighbor distances were smaller than 95 % of those of the simulated data. Then, the percentages of the significant place-related neurons were compared among the tasks.

**Parameters of place-related neurons**

To estimate the spatial scales (corresponding to place field sizes) of the responses of place-related neurons in the VN tasks, the spatial autocorrelation for the firing rate map of each place-related neuron in each VN task was calculated (Fyhn et al., 2007; Kjelstrup et al., 2008). With $\lambda(x, y)$ denoting the average firing rates of the neuron at location $(x, y)$, the autocorrelation between these fields with spatial lags of $\tau_x$ and $\tau_y$ was estimated as:
\[ r(x', y') = \frac{n \sum (x,y) (x - x', y - y') (x', y') (x - x', y - y')}{\sqrt{n \sum (x,y)^2} \sqrt{n \sum (x', y')^2}} \]

where the summation is over all n pixels in (x, y) for which rate was estimated for both \( \lambda(x, y) \) and \( \lambda(x - \tau_x, y - \tau_y) \). Autocorrelations were not estimated for lags of \( \tau_x \) and \( \tau_y \) where \( n < 20 \).

The area of the spatial autocorrelation function at 20% of the peak was used as an index for the spatial scale of the place neuron.

To estimate the spatial similarity of firing rate distributions among the three virtual spaces in the VN tasks (control, expanded, and reduced), pixel-to-pixel correlations (Dayawansa et al., 2006; Tran et al., 2008) were calculated using Pearson’s correlation coefficients. For pixel-to-pixel correspondence across the 3 VN tasks, the mobility area in the control and expanded VN tasks was divided into \( 0.40 \times 0.40 \) m\(^2 \) pixels, while the area in the reduced VN task was divided into \( 0.24 \times 0.24 \) m\(^2 \) pixels such that the number of pixels within the mobility area in the reduced VN task was the same as that in the control and expanded VN tasks.

To estimate the spatial selectivity of neuron firing to location of the monkey, information per spike (Skaggs et al., 1993) was calculated for the firing rate map of each place-related neuron in each task was calculated as:

\[
\text{Information per spike} = \frac{1}{n} \sum_{i=1}^{n} \log_2 \left( \frac{\lambda_i}{\lambda} \right)
\]

Where \( \lambda_i \) is the mean firing rate of a neuron in i-th pixel, \( \lambda \) is the overall mean firing rate and \( n \) is the total number of pixels in which rate was estimated.
Neuronal correlates to spatial cues

Previous studies reported that activities in HF and PH might be dependent on the scenes of views that the subjects were facing (Rolls and Xiang, 2005; Epstein, 2008) and that the activities of the HF place neurons were affected by the boundary of the environment, such as a wall, as well as extramaze spatial cues (Barry et al., 2006; Jeffery, 2007). Therefore, we analyzed the neuronal responses within the rectangle(s) circumscribing the place field(s) to the wall and distal spatial cues (Fig. 3A). It is noted that we analyzed only the place-related neurons in this analysis. The heading direction of the monkeys was simply defined as the angle of the direction in which the monkeys were facing (ΩD in Figure 3A). This direction was not necessarily identical to the movement direction.

Furthermore, we analyzed HF and PH neuronal responses in reference to the wall and distal spatial cues (i.e., landmarks). The location on the walls that the monkeys were facing [facing point (FP) in Figure 3A] was defined as the intersection between the walls and the line passing through the monkeys in the heading direction. FP on the walls was expressed as the angle (wall angle; ΩR, ΩC, and ΩE in the reduced, control and expanded virtual spaces, respectively) between the X-axis and the line connecting FP and the origin of the axes (Figure 3A). Wall angles in the expanded (ΩE) and control (ΩC) VN tasks were identical because the diameters of the walls were the same. The location of the spatial cues (FP) that the monkeys were facing was similarly defined as the angle (landmark angle; ΩR, ΩC, and ΩE in the reduced, control and expanded virtual spaces, respectively) between the X-axis and the line connecting the origin of the axes and the given FP on the circumscribed circle of the spatial cues (Figure 3B).

To compare the selectivity of each place neuron to each spatial variable, spatial
Spatial selectivity index (SI) was calculated as follows:

\[
\text{Spatial SI} = \frac{\text{Max R}}{\text{Mean of each response to each parameter analyzed}}
\]

where Max R was the maximum responses among the responses to all parameters analyzed. Spatial SI was computed using the data within the place fields. Spatial SI measures of selective responsiveness between the HF and PH place-related neurons were compared by 3-way ANOVA using task (control, reduced, and expanded VN tasks), region (HF and PH), and variable (heading direction, landmark angle, and wall angle) as factors.

To estimate the spatial selectivity of neuron firing to spatial view, information per spike (Skaggs et al., 1993) was calculated for the firing rate of each place-related neuron in the different landmark angle in each task was calculated as:

\[
\text{Information per spike} = \frac{\sum_{i=1}^{n} \lambda_i \log_2 \left( \frac{1}{\lambda} \right)}{n}
\]

Where \( \lambda_i \) is the mean firing rate of a neuron in i-th angle, \( \lambda \) is the overall mean firing rate and \( n \) is the total number of angles in which rate was estimated (\( n=8 \) in this study; see Figure 8B in the results).

**Neural correlates to the landmarks and locations of the monkeys**

Previous studies reported that not only place-related but also place-unrelated neurons in HF and PH responded to specific landmarks that subjects looked at (Rolls and O’Mara, 1995; Ekstrem et al., 2003). Therefore, specific landmarks viewed by the monkeys (view) and
locations of the monkeys (place) as well as the VN tasks (task) could modulate neuronal activity of place-related and/or place-unrelated neurons in the present study. We further analyzed whether binary presence or absence of specific landmark(s) and locations of the monkeys modulated HF and PH neuronal activity (Figure 3B). Firing rates of the all HF and PH neurons during different epochs of the VN tasks were compared by an ANOVA with 4 factors [view (4) x place (5) x movement direction (4) x task (3)]. The view factor coded for times when the monkey viewed north, east, south or west landmark(s) (i.e., when the north, east, south or west landmark(s) were displayed on the center of the screen) (Figure 3Ca). The place factor coded for times when the monkey was located in one of the 5 areas in the mobility area; a central circle with 3 m radius in the control and expanded VN tasks and 1.8 m radius in the reduced VN task (C), and 4 surrounding annuli [north (N), east (E), south (S) and west (W)] (Figure 3Cb). The movement direction factor coded for times when the monkey moving north (between -45 and 45°), east (between 45 to 135°), south (between 135 to 225°) or west (between 225 and 315°). Only the epochs that continued more than 500 ms were included in the analysis. In this analysis, since the data for all possible combinations of the factors were usually not sampled due to fixed navigation, the data were analyzed using a model without interaction.

**Neuronal correlates to behavioral variables**

For both the HF and PH neurons, behavioral correlates of the activities of the place-related neurons within the rectangle(s) circumscribing the place field(s) were analyzed according to Wiener et al. (1989). The variables analyzed were the instantaneous movement direction, the instantaneous turning angle of the movement, and the heading direction (Fig. 3B). The instantaneous movement direction at each location was calculated along the vector between sequential locations 100 ms before and after passing through the observed point ($\alpha'$). The
movement direction is not necessarily the same as the heading direction because the latter refers to the direction in which the monkeys were facing. The monkeys sometimes moved the joystick backward, while they themselves moved backward as they looked in the front. In this case, the movement direction was opposite to the heading direction. Thus, the movement direction refers to the direction in which the position of the animals changed.

The instantaneous turning angle of the movement at each location within the place field was estimated as the arc subtended by two vectors connecting a local point and the points 100 ms before and after passing through the observed point ($\beta^\circ$).

To compare the selectivity of each place neuron to behavioral variables, movement SI was calculated using the same formula as that used for the spatial SI. Movement SI measures of the selective responsiveness between the HF and PH neurons were compared by 3-way ANOVA using task (control, reduced, and expanded VN tasks), region (HF and PH), and variable (movement direction and turning angle) as factors.

**Histology**

After the last recording session, several small marking lesions were made in the HF and the PH by passing 20-30 µA of anodal current for 30 sec through an electrode placed stereotaxically. Subsequently the monkey was deeply anesthetized with an overdose of sodium pentobarbital (60 mg/kg, i.m.) and perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brains were removed from the skulls and cut into 50-µm sections through the HF. Sections were stained with cresyl violet, and sites of electrical lesions were determined microscopically. Anatomical boundaries of PH were based on a recent study by Saleem et al. (2007).

The location of each recording site was then calculated by comparing the stereotaxic
coordinates of recording sites with those of lesions. The positions of the HF and PH, and of the tungsten bar were checked by MRI during the experiments, and these photographs were compared with those of the marking electrodes to verify the calculated recording sites.

Locations of the HF and PH neurons in the both hemispheres of the two monkeys were compared on the basis of the shapes of the HF and PH, and re-plotted on the serial sections of the right HF and PH of one monkey from 16 mm (A16) to 6 mm anterior (A6) to the interaural line.

RESULTS

Place-related activities in the VN and PT tasks

The activities of 234 neurons were recorded in the monkey HF and PH during the performance of one or more of three VN and/or PT tasks. Of these 234 neurons, 182 neurons were tested in all four tasks. Of the 234 neurons, 170 (73%) and 61 (26%) neurons displayed place-related activities in the VN and PT tasks, respectively. Because previous studies reported a dichotomy of the HF neurons with low and high mean firing rates in monkeys (Eifuku et al., 1995; Matsumura et al., 1999) and in rats (Kubie et al., 1990; Jung and McNaughton, 1993), we divided the HF and PH neurons into two groups, i.e., high frequency and low frequency neurons, based on their mean firing rates. Figure 4A shows distributions of the mean firing rates in HF and PH. We applied k-means clustering to each distribution in HF and PH to classify the neurons into the high and low frequency neurons. Figure 4A indicates the resultant low and high frequency neurons, respectively. Gray and black bars indicate the place-related neurons in the low and high frequency neurons, respectively. Most place-unrelated neurons belonged to the
high frequency neurons in both HF and PH.

Figure 4B shows a comparison of the percentage of place-related neurons between HF and PH. The percentages of the place-related neurons in the total samples were significantly higher in PH than HF (Fisher’s exact probability test, $p < 0.01$) (Ba). When the high and low frequency neurons were separately analyzed (Bb), the percentage of the place-related neurons were significantly higher in the low frequency than high frequency neurons in both HF (Fisher’s exact probability test, $p < 0.001$) and PH (Fisher’s exact probability test, $p < 0.01$). These results replicate the results of the previous studies that reported a dichotomy of the HF neurons with low and high mean firing rates in monkeys (Eifuku et al., 1995; Matsumura et al., 1999) and rats (Kubie et al., 1990; Jung and McNaughton, 1993).

Figure 4C shows comparison of the percentages of the significant place-related neurons (for definition, see Methods) among the VN and PT tasks in HF and PH. In the total samples, the percentages of the significant place-related neurons were significantly lower in the PT task than the VN tasks in both HF and PH (Fisher’s exact probability test, $p < 0.05$) (Ca). When the high and low frequency neurons were separately analyzed (Cb), the percentages of the significant place-related neurons were significantly lower in the PT task than some of the VN tasks in both HF and PH except the low frequency neurons in HF (Fisher’s exact probability test, $p > 0.05$). These results replicate the results of previous studies in which more monkey HF and PH neurons responded during real navigation or VN than during the PT task performed on a computer display or screen (Matsumura et al., 1999; Hori et al., 2005).

**Spatial similarity of place-related activities across the three virtual spaces**

Figure 5 shows an example of a place-related neuron in HF that was tested in the three different VN tasks. The activities of the neuron increased around the central reward area in the
control (A) and reduced (C) virtual spaces in the VN task, and the spatial similarity of the place-related activities between these two virtual spaces was 0.60. However, the spatial similarity of the place-related activities between the control (A) and expanded (B) virtual spaces (0.14) and that between the reduced (C) and expanded (B) virtual spaces (-0.01) was low. In the PT task (D), this HF neuron was inactive and showed almost no activity.

Figure 6 shows an example of a PH neuron. The activities of the neuron increased around the West reward area in the control virtual space (A), while they increased around the South reward area in the expanded virtual space (B) in the VN task. Furthermore, this neuron did not display place-related activities in the reduced virtual space in the VN task (C) although the monkey navigated the same corresponding areas in the reduced virtual space, i.e., the place-related activities were different among these spaces. Thus, the spatial similarity of this neuron between the two spaces was relatively low. The spatial similarity between the control and expanded virtual spaces, between the control and reduced virtual spaces, and between the reduced and expanded virtual spaces was 0.39, 0.36, and -0.01, respectively. In the PT task (D), this PH neuron was also inactive and almost no activity was observed.

The spatial similarity was calculated using the data derived from the 146 place-related neurons that were tested in the three VN tasks. Figure 7A shows distribution of spatial similarity among the three different virtual spaces in the place-related neurons in PH and HF. Figure 7B shows a comparison of the mean spatial similarities of the HF and PH neurons with high similarity (> 0.4) between at least one of the virtual space pairs. The spatial similarity tended to be higher in HF than PH in the total samples (Mann–Whitney U test, p < 0.1) (Ba). In the low frequency neurons, the spatial similarity was significantly higher in HF than PH (Mann–Whitney U test, p < 0.05) (Bb). Figure 7C shows the results of the same analyses in individual combinations of the virtual spaces in the low and high frequency neurons. In the total
samples (Ca), the similarity between the control and reduced virtual spaces was higher in HF than PH (Mann–Whitney U test, p < 0.05). Furthermore, the similarity between the control and expanded virtual spaces was higher than those between the other combinations of the virtual spaces in PH (Mann–Whitney U test, p < 0.05). In HF, the similarity between the control and expanded virtual spaces was higher than that between the expanded and reduced virtual spaces (Mann–Whitney U test, p < 0.05). In the low frequency neurons (Cb), the similarity between the control and reduced virtual spaces was higher in HF than PH (Mann–Whitney U test, p < 0.05).

In the high frequency neurons in PH, the similarity between the control and expanded virtual spaces was higher than those between the other combinations of the virtual spaces (Mann–Whitney U test, p < 0.05). Furthermore, the similarity between the control and expanded virtual spaces was higher than that between the expanded and reduced virtual spaces (Mann–Whitney U test, p < 0.05). These results indicated that the similarity, especially that between the control and reduced virtual spaces, was higher in HF than PH.

Figure 7D shows the mean spatial scale of the HF and PH place-neurons across the 3 virtual spaces. In the total samples (Da), significant differences were observed in the spatial scale of the place-neurons among the three virtual spaces in HF (Kruskal–Wallis test, p < 0.01) and PH (Kruskal–Wallis test, p < 0.05). Multiple post-hoc comparisons indicated that the spatial scale of the HF place-related neurons were significantly smaller in the reduced virtual space than the control and expanded virtual spaces (Steel-Dwass test, p < 0.05), while the spatial scale of the PH place-related neurons were significantly smaller in the reduced than control virtual spaces (Steel-Dwass test, p < 0.05). When the low and high frequency neurons were separately analyzed (Db), the same results were true for the low frequency HF neurons.

Neural correlates to spatial variables
Figure 8 shows the activities of the place-related neuron during the control VN task in HF shown in Fig. 5 at various heading directions (A), landmark angles (B), and wall angles (C) inside the place field. The neuronal activities were significantly modulated by heading direction ($x^2 = 37.76$, df = 7, $p < 0.001$), landmark angle ($x^2 = 40.76$, df = 7, $p < 0.001$), and wall angle ($x^2 = 46.22$, df = 7, $p < 0.001$). Of the 349 place fields in the three VN tasks, 96 had significant heading direction-dependent responses (HF, 54; PH, 42), 99 had significant landmark angle-dependent responses (HF, 56; PH, 43), and 90 had significant wall angle-dependent responses (HF, 48; PH, 42). To quantitatively assess the selective tuning to each spatial variable, SI was calculated. This neuron showed relatively high SI (heading direction, 3.82; landmark angle, 3.94; and wall angle, 4.09).

Table 1 shows a summary of spatial SIs calculated from responses to spatial variables in the three VN tasks in HF and PH. The results of 3-way ANOVA indicated that the significant main effects of region [$F(1, 1029) = 7.316$, $p < 0.01$]. Other factors and interactions were not significant (data not shown). When the low frequency neurons were separately analyzed, a significant main effect of region [$F(1, 612) = 6.293$, $p < 0.05$] was also observed. In the high frequency neurons, only significant main effect of task was observed [$F(2, 399) = 3.393$, $p < 0.05$]. These results indicate that spatial SIs were significantly higher in PH than HF. That is, place-related activity was more influenced by spatial views in PH than HF.

Furthermore, we analyzed effects of the specific views and locations of the monkeys shown in Figure 3B on HF and PH neuronal activity. The neuronal activity with and without place-field(s) was analyzed by an ANOVA with 4 factors [view (4 views) x place (5 locations) x movement direction (4 directions) x task (3 VN tasks)]. Figure 9 shows comparison of the percentages of the neurons with significant main effects of view, place, movement direction, and task in HF and PH. In the total samples (A), the percentages of the neurons with the
significant main effect of view were larger in PH than HF (Fisher’s exact probability test, p < 0.05). When the low and high frequency neurons were separately analyzed (B), the same tendency was observed in the low frequency neurons (Fisher’s exact probability test, p < 0.1). These results indicate that the activities of the PH neurons were more selectively tuned to specific views of the landmarks, consistent with the results in Table 1.

**Neural correlates to movement variables**

Figure 8D and E shows the activities of the place-related neuron in HF shown in Fig. 5 at various movement directions (D) and turning angles (E) inside the place field. The neuronal activities were significantly modulated by movement direction ($\chi^2 = 23.04$, df = 7, p < 0.01) and were not significantly modulated by turning angle ($\chi^2 = 3.18$, df = 7, p = 0.87). Of the 349 place fields in the three VN tasks, 110 had significant movement direction-dependent responses (HF, 67; PH, 43) and 91 had significant turning angle-dependent responses (HF, 54; PH, 37). However, differences in movement SIs between HF and PH were not observed. Table 2 shows a summary of movement SIs across the three VN tasks in HF and PH. The results of 3-way ANOVA indicated that the main effects of region [$F(1, 686) = 1.457$, $p > 0.05$] was not significant, but only the main effect of task [$F(2, 686) = 3.545$, $p < 0.05$] was significant. Furthermore, no significant interactions were observed between region and task (data not shown). When the low and high frequency neurons were separately analyzed, a significant main effects of task [$F(2, 408) = 3.229$, $p < 0.05$] was also observed in the low frequency neurons, and in high frequency neurons, no significant main effect and interaction were found. These results indicate that movement SIs were not different between PH and HF. This finding is consistent with the results of the view (4) x place (5) x movement direction (4) x task (3) analysis of variance (Figure 9) in which there was no difference in percentages of the neurons.
with a significant main effect of movement direction between HF and PH.

**Recording sites**

A total of 182 HF and PH neurons were tested in the VN and PT tasks. Figure 10 illustrates the locations of the recording sites and categories of the HF and PH neurons recorded in the VN tasks. Figure 11 illustrates the locations of the recording sites and categories of the neurons recorded in the PT task. These recording sites are plotted on coronal sections of the right hemisphere. The place-related neurons (filled symbols) were located in the posterior parts of HF and PH in both the VN and PT tasks. Figure 12 shows a comparison of spatial information conveyed by the place-related neurons in the VN tasks among anterior and posterior parts of HF and PH. First, information for place was analyzed by a 2-way ANOVA with region (HF, PH) and AP (anterior, posterior) as factors (A). In the total samples of the place-related neurons (Aa), there were significant main effect of AP \[F(1, 345) = 13.42, p < 0.001\] and significant interaction between region and AP \[F(1, 345) = 6.83, p < 0.01\]. Post-hoc tests indicated that mean information was significantly larger in posterior PH than anterior and posterior HF and anterior PH (p < 0.01, 0.05, 0.001, respectively; Tukey test). When the high frequency neurons were separately analyzed, the same results were observed. There were significant main effects of region \[F(1, 135) = 5.66, p < 0.05\] and AP \[F(1, 135) = 12.69, p < 0.001\], and interaction between region and AP \[F(1, 135) = 5.86, p < 0.05\]. Post-hoc comparisons indicated that mean information was significantly larger in posterior PH than anterior and posterior HF and anterior PH (p < 0.001, 0.01, 0.01, respectively; Tukey test). In the low frequency neurons, there were significant main effects of AP \[F(1, 206) = 4.70, p < 0.05\], and interaction between region and AP \[F(1, 206) = 8.37 p < 0.01\]. Post-hoc comparisons indicated that mean information was significantly larger in posterior PH than
anterior PH (p < 0.01, Tukey test).

Second, information for spatial view was similarly analyzed by a 2-way ANOVA with region (HF, PH) and [anterior (A10-16), posterior (A6-8)] as factors (Figure 12B). In the total samples of the place-related neurons (Ba), there were significant main effect of AP [F(1, 345) = 22.76 p < 0.001] and significant interaction between region and AP [F(1, 345) = 5.54 p < 0.05]. Post-hoc tests indicated that mean information was significantly larger in posterior PH than anterior and posterior HF and anterior PH (p < 0.001, 0.05, 0.001, respectively; Tukey test). When the low frequency neurons were separately analyzed (Bb), the same results were observed. There were significant main effect of AP [F(1, 206) = 13.53 p < 0.001], and interaction between region and AP [F(1, 206) = 5.76 p < 0.05]. Post-hoc tests indicated that mean information was significantly larger in posterior PH than anterior and posterior HF and anterior PH (p < 0.01, 0.05, 0.001, respectively; Tukey test). In the high frequency neurons, there was no significant main effect nor interaction (data not shown). These results indicate that posterior PH is more deeply involved in spatial information processing than the other areas.

**DISCUSSION**

*Place-related activities of the HF and PH neurons*

In this study, the monkey HF and PH neurons displayed place-related activities in the virtual spaces in the VN tasks (Figs. 4-6). In primate HF, similar place-related activity has been reported in previous studies (Ono et al., 1993; Matsumura et al., 1999; Ekstrom et al., 2003; Ludvig et al., 2004). Human fMRI studies also reported that both PH and HF were activated during navigation (Maguire et al., 1997, 1998; Ghaem et al., 1997; Rosenbaum et al., 2004),
and that PH was activated whenever subjects viewed an image of a place (Epstein et al., 1999; Grön et al., 2000). A rodent study also reported place cells in the postrhinal cortex (Burwell and Hafeman, 2003), which is thought to be the rodent homologue of primate PH (Furtak et al., 2007). These place-related neurons were more frequently observed in the VN than PT tasks. These results are consistent with our previous studies, in which more HF and PH neurons responded during real navigation than during translocation of a pointer on a computer display in monkeys (Matsumura et al., 1999). These results suggest that a large scale environment is one of the important determinants to stimulate the HF and PH (Maguire et al., 1996; Matsumura et al., 1999).

Information for place conveyed by these place-related neurons was significantly higher in posterior PH than in HF (Fig. 12A). Here the monkeys were well trained and therefore could navigate within the mobility areas. HF activity typically emerged when subjects took novel short-cuts (Hartley et al., 2003) or used representations flexibly during navigation (Zhang and Ekstrom, 2012), and was typically not observed simply during canonical spatial navigation (Aguirre et al., 1996; Shipman and Astur, 2008). However, PH activity was not strongly affected by navigation task demands and complexity of the environment (Spiers and Maguire, 2007). This difference in the percentages of the place-related neurons between HF and PH might be attributed to this functional difference between HF and PH. Furthermore, information for place was higher in the posterior part than in the anterior part of PH (Fig. 12A). These results highlight the importance of the posterior part of PH including PPA in spatial coding.

**Place-related activities across the different virtual spaces in HF**

In the place-related neurons with high spatial similarity more than 0.4, the spatial similarity, especially that between the control and reduced virtual spaces, was higher in HF than
PH (Fig. 7). Relative spatial arrangement of the landmarks and wall was similar in both the control and reduced VN tasks. These results suggest that the place fields proportionally shrank in the reduced virtual space, and support the idea that HF is important for map-like representation of space in which relative positions of external cues are coded (O’Keefe and Nadel, 1978). These results indicate that the characteristics of the place-related neurons in primate HF correspond well with those in the rodent place cells.

The place fields of rodent HF place cells were directly influenced by the boundary of the environment that provided metric (distance) inputs to HF (O’Keefe and Burgess, 1996; Jeffery, 2007). In the present study using monkeys, the spatial scale was smaller in the reduced VN task than in the other VN tasks (Fig. 7D). This phenomenon is very similar to that observed in rodent place cells in which place fields expand or shrink based on maze sizes (distance between maze walls) (O’Keefe and Burgess, 1996). Furthermore, spatial scale in the present study using large virtual spaces is larger than those in the previous monkey studies using real navigation within relatively small areas (Ono et al., 1993; Matsumura et al., 1999; Ludvig et al., 2004). On the other hand, expansion of arrangement of the distal spatial cues in the expanded VN task did not affect spatial scale (Fig. 7D). These results suggest that the size of the mobility area, but not the size of the surrounding space (or distance from the distal spatial cues), regulate place-related activity. These findings suggest that primate place-related neurons have characteristics similar to rodent place cells.

For spatial navigation, 3 categories of stimuli [distal visual cues (O’Keefe and Dostrovsky, 1971; Muller and Kubie, 1987), self-motion cues (optic flow, proprioception, vestibular cues) (Gothard et al., 1996; Pastalkova et al., 2008), and other sensory cues (olfaction, audition, etc.)] are processed in the hippocampus. In the present study, only visual cues were presented; distal visual cues and optic flow can be coded for spatial navigation and place-
related neuronal activity. Recent studies reported that rodents can also navigate in the virtual environment (Hölscher et al., 2005; Chen et al., 2013; Ravassard et al., 2013). These studies using rodents in virtual environment indicated that place cell activity was dependent on both visual and movement (locomotion)-related information, and suggest that self-location is initially set (or reset) by visual information and is updated by movement-related information (or “path integration”) (Chen et al., 2013). Since primates have better visual system than rodents (Van Hooser et al., 2005), path integration might be more effectively coded using visual information in primates. These findings suggest that neural mechanisms for place-related neuronal activity in the HF might be similar in both primates and rodents, but visual information might take more important role in primates.

**View-sensitive and place-related activities in HF and PH**

Activity of some HF neurons was modulated by specific views (Fig. 9). Previous studies also reported that some primate HF neurons such as “view cells” responded to specific landmarks in the specific locations (Rolls and O’Mara, 1995; Georges-François et al., 1999; Ekstrom et al., 2003). These “view cells” might correspond to item-positions cells in rodent HF, in which the neurons increased firing in reference to specific objects in specific positions (Komorowski et al., 2009). However, it is noted that view-dependent activity was more prominent in the PH than HF (see below).

In this study, some PH place-related neurons also displayed landmark (wall) angle-dependent responses (Table 1, Fig. 8), which might correspond to view cells in previous studies (Rolls and O’Mara, 1995; Ekstrom et al., 2003). Furthermore, the PH neurons were more sensitive to specific views than the HF neurons; mean spatial SIs of the place-related neurons were higher in PH than HF (Table 1), and percentages of the neurons with a significant main
effect of view were higher in PH than HF (Fig. 9). Previous human study also reported that PH neurons were more sensitive to spatial view than HF neurons (Ekstrom et al., 2003). In addition, mean information for spatial view was higher in posterior PH than anterior PH, and also higher than anterior and posterior HF (Fig. 12B). These results indicated that posterior PH is important in coding specific views during navigation.

PPA (most posterior part of PH) is sensitive to changes in scenes induced by head orientation (Park and Chun, 2009) as well as by the movements of the viewer (Epstein et al., 2007a,b). The place-related activities in PH in this study suggested that PH encodes changes in the scene by the movements of the viewer, while the responses to specific landmarks and wall angles within the same place fields suggested that PH also encodes changes in scenes induced by head orientation. These results are generally consistent with the fact that PPA encodes scene geometry in an observer-centered (viewpoint specific) reference frame (Epstein, 2008) in which the walls and distal spatial cues constitute important elements of the spatial layout.

However, some PH neurons displayed response characteristics similar to those displayed by HF neurons (i.e., high spatial similarity and low spatial SI) (Fig. 7A). Responses to scenes in PPA became viewpoint-invariant when the scenes were repeated after a long lag (Epstein et al., 2005; Park and Chun, 2009), and the degree of this change was correlated with the navigational competence of the subjects (Epstein et al., 2005). Since the monkeys were well trained in this study, the presence of these neurons in PH might reflect changes in scene representation in PH that occur by repeated experience. These results are consistent with those of human neuropsychological studies suggesting that the posterior part of PH to be involved in route learning in the real world environment (Barrash et al., 2000), and that PH might be involved in the translation between egocentric (viewer centered) and allocentric frames of memory (Weniger et al., 2010). Finally, it is suggested that monkey PPA is only a part of the
posterior PH, and the rest of PH is involved in more general role in spatial functions (Epstein, 2008). Posterior PH in the present study corresponds to posterior half of area TF, while area TFO just posterior to area TF is suggested to correspond to human PPA (Saleem et al., 2007). Further studies are required to characterize monkey PPA neurons.

**Complementary roles of HF and PH during navigation**

In this study, the response characteristics of the PH neurons were significantly different from those of the HF neurons. First, the spatial similarity between the control and reduced virtual spaces was lower in PH than in HF. Second, SI was higher in the PH than HF. That is, place-related activity was more influenced by spatial views in PH than HF. Furthermore, information for spatial view was higher in posterior PH than in HF. Previous non-invasive and behavioral studies using human patients with HF damage reported that HF was involved in viewpoint-invariant (allocentric) spatial coding (King et al., 2002; Suthana et al., 2009; Goodrich-Hunsaker et al., 2010), and that fMRI signal intensities in HF were correlated with real-world distances between landmarks (Morgan et al., 2011). The above response characteristics of the HF neurons in this study support the idea that HF represents a cognitive map (O’Keefe and Nadel, 1978). In contrast, the characteristics of the PH neurons suggest that PH processes scene geometry in a viewpoint-dependent manner (for this discussion, see above). The present study results provide neurophysiological bases for a role of PH in viewer-centered representation. These results suggest that PH plays an important role in place recognition system that operates by template matching of viewpoint-dependent representations of landmarks in familiar environment. Consistent with this idea, behavioral studies suggest that human subjects navigate by updating their viewer-centered representation of landmarks during navigation to recognize their location (see review by Wang and Spelke, 2002). On the other
hand, viewer-independent (allocentric) representation allows recovery of distances and
directions between locations and flexible planning of routes (O’Keefe and Nadel, 1978;
Gallistel, 1990; Bennett, 1996). Recent behavioral studies suggest that both representations
exist in parallel and are joined to support navigation (Wang and Spelke, 2002; Mou et al., 2006).
Taken together, these findings suggest that both HF and PH complementarily contribute to
navigation, and these findings are consistent with those of non-invasive studies in which both
HF and PH were active during navigation.

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FIGURE LEGENDS

Fig. 1. Schematic illustrations of the experimental set up (A), an example of a view of the virtual space (B), and an example of a movement trace in the control VN task (C). Five circles indicate the reward areas. N, north; E, east; S, south; W, west.

Fig. 2. Schematic of the task paradigms of the virtual navigation (VN) and pointer translocation (PT) tasks.

A: Spatial arrangement of the three different VN tasks. In the expanded VN task (a), the arrangement of the spatial cues was expanded (the distal spatial cues were located 40 m away from the wall surrounding the mobility area), while the mobility area was of the same size as that in the control VN task (b). In the control VN task (b), the spatial cues were located 5.0 m away from the wall surrounding the mobility area. In the reduced VN task (c), the diameters of the movable and reward areas were reduced to 12.0 and 1.8 m, respectively. In this task, the distal spatial cues were located 3.0 m away from the wall.

B: Mobility area in the control VN task. A circular mobility area (20 m diameter) was surrounded by a wall (height, 0.3 m), and five reward areas (3.0 m diameter) were located symmetrically [North (N), East (E), South (S), West (W), Center (C)]. The monkey navigated toward the reward areas during the VN tasks. The blue line and red spots indicate the trail of the monkey and reward delivery, respectively. Since the reward areas were unmarked, the monkeys had to find the areas according to the spatial cues.

C: Screen views of the PT task. The gray circles indicate the reward areas (22 cm diameter).
Fig. 3. Schematic representation of spatial (A, C) and (B) movement parameters for analysis of neuronal correlates to various parameters.

A: Representation of spatial variables. The heading direction of the monkey was simply defined as the angle of the direction that the monkey was facing (θD) (a). The locations [facing points (FP)] on the walls that the monkey was facing were defined as the angle (wall angle; θR, θC, and θE in the reduced, control and expanded virtual spaces, respectively) between the X-axis and the line connecting the given FP and the origin of the axes. Wall angles in the expanded (θE) and control (θC) VN tasks were identical because the diameter of the wall was the same (a). The location of the landmark (FP) that the monkey was facing was defined as the angle (landmark angle; θR, θC, and θE in the reduced, control and expanded virtual spaces, respectively) between the X-axis and the line connecting the given FP and the origin of the axes in each virtual space (b). Y- and X-axes indicate north and east direction in the virtual space, respectively.

B: Representation of movement variables. The instantaneous movement direction (αn) and instantaneous turning angle (βn) of the movement. Points indicated by (n−1) × 100 ms and (n+1) × 100 ms represent sequential locations 100 ms before and after passing through the observed point (n) × 100 ms. The instantaneous movement direction at each location was calculated along the vector between the sequential locations 100 ms before and after passing through the observed point (αn). The instantaneous turning angle of the movement at each location within the place field was estimated as the arc subtended by two vectors connecting that a local point and the points 100 ms before and after passing through the observed point (βn).

C: Factorial distinction of the spatial variables. The view factor coded for times when the monkey viewed north, east, south or west landmark(s) (i.e., when the north, east, south or west landmark(s) were displayed on the center of the screen) (a). The place factor coded for
times when the monkey was located in one of the 5 areas in the mobility area; a central circle
with 3 m radius in the control and expanded VN tasks and 1.8 m radius in the reduced VN task
(C), and 4 surrounding annuli [north (N), east (E), south (S) and west (W)] (b).

Fig. 4. Distribution of the mean firing rates of HF and PH neurons (A) and comparison of the
percentages of the place-related neurons between HF and PH (B, C).

A: Each dashed line and each value above each dashed line indicate the border between
the high frequency and low frequency neurons based on k-means clustering.

B: Bar graphs comparing the percentages of the place-related neurons between HF and
PH in the total samples (a), and in the high and low frequency neurons (b). * , p < 0.05; ** , p
< 0.01; *** , p<0.001 (Fisher’s exact probability test).

C: Bar graphs comparing the percentages of the significant place-related neurons in HF
and PH during performance of the VN and PT tasks in the total samples (a), and in the high and
low frequency neurons (b). * , p < 0.05; ** , p<0.01 (Fisher’s exact probability test). Low freq,
low frequency neurons; High freq, high frequency neurons; PN, place-related neuron; unPN,
place-unrelated neuron.

Fig. 5. An example of a place-related neuron in HF.

A–C: The trails of the monkey (a) and the firing rate maps of the neuron within the
mobility area (b) in the control (A), expanded (B), and reduced (C) VN tasks. Values with
arrows indicate spatial similarity between the given spaces. The activities of the neuron
increased around the central reward area in the control and reduced VN tasks. The red circles
indicate the reward areas. N, north. Black pixels indicate that the monkeys did not visit these
pixels or only stayed there for a very short period (< 0.2 s).
D: The trails of the pointer (a) and the firing rate map of the neuron on the screen (b) in the PT task. In the PT task, this HF neuron was inactive and almost no activity was observed. The red circles indicate the reward areas. T, top of the screen. Black pixels indicate that the pointer did not visit these pixels or only stayed there for a very short period (< 0.1 s).

Fig. 6. An example of a place-related neuron in PH.

A-C: The trails of the monkey (a) and the firing rate maps of the neuron within the mobility area (b) in the control (A), expanded (B), and reduced (C) VN tasks. The activities of the neuron increased around the West reward area in the control VN task, while it increased around the South reward area in the expanded VNT.

D: The trails of the pointer (a) and the firing rate map of the neuron on the screen (b) in the PT task. The other descriptions are the same as those for Fig. 5.

Fig. 7. Comparison of the spatial similarity (A-C) and the spatial scale of the place fields (D).

A: Bar graphs comparing distribution of spatial similarity between HF and PH in the total samples (a), and in the high and low frequency place-related neurons (b).

B: Bar graphs comparing mean spatial similarity between PH and HF in the total samples of the place-related neurons with high spatial similarity higher than 0.4 in at least one of pairs (a), and in the high and low frequency place-related neurons with high spatial similarity higher than 0.4 at least one of pairs (b). +, p< 0.1; *, p < 0.05 (Mann–Whitney U-test).

C: Bar graphs comparing mean spatial similarity between PH and HF in the total samples of the place-related neurons with high spatial similarity higher than 0.4 in at least one of pairs (a), and in the high and low frequency place-related neurons with high spatial similarity higher than 0.4 at least one of pairs (b). The same data as B were compared among the three
combinations of the virtual spaces. *, p< 0.05; **, p< 0.01 (Mann–Whitney U-test); C, Control; E, Expanded; R, Reduced.

D: Bar graphs comparing spatial scale among the three different virtual spaces in the total samples of the place-related neurons (a), and in the high and low frequency place-related neurons (b) in the HF and PH. *, p<0.05; **, p<0.01 (Steel-Dwass test); C, Control; E, Expanded; R, Reduced.

Fig. 8. Activity of a place-related neuron in HF (the same neuron shown in Figure 5) at various heading directions (A), landmark angles (B), wall angles (C), movement directions (D), and turning angles (E) inside the place field.

SI, selectivity index; N, North; S, South; W, West; E, East; F, forward; B, backward; L, leftward; R, rightward.

Fig. 9. Comparison of responsiveness to view and place factors between HF and PH.

Bar graphs indicate the percentages of the neurons with significant a main effect of each factor in the total samples (A), and in the high and low frequency neurons (B). +, p<0.1; *, p<0.05 (Fisher’s exact probability test). View, view factor; Place, place factor; Move, movement direction factor; Task, task factor.

Fig. 10. Schematics of the locations of the recording sites and categories of the HF and PH neurons in the VN task.

F5 and F6 indicate locations of the neurons shown in Figs. 5 and 6, respectively. EC, entorhinal cortex; SUB, subiculum; DR, dentate gyrus. TH: area TH; TF: area TF.
Fig. 11. Schematics of the locations of the recording sites and categories of the HF and PH neurons in the PT task.

Other descriptions are the same as those for Fig. 10.

Fig. 12. Bar graphs comparing information conveyed by the place-related neurons among the anterior and posterior parts of HF and PH in the VN tasks.

A: Comparison of information amount for locations in the total samples of the place-related neurons (a) and in the high and low frequency place-related neurons (b).

B: Comparison of information amount for spatial view (landmark angle) in the total samples of the place-related neurons (a) and in the high and low frequency place-related neurons (b). Ant, anterior; Post, posterior; *, p < 0.05; **, p < 0.01; ***, p<0.001 (Tukey tests after 2-way ANOVA).
Fig. 1
A. VN
a. Expanded

b. Control

c. Reduced

B. Mobility area

C. PT

Fig. 2
A. Spatial variables
a. Heading direction ($\theta_d$) and wall angle

b. Landmark angle

B. Movement variables

C. Factorial distinction of spatial variables
a. View factor
b. Place factor

Fig. 3
Fig. 4
Fig. 5

A. VN (control)
   a. Trail
   b. Firing rate map

B. VN (expanded)
   a. Trail
   b. Firing rate map

C. VN (reduced)
   a. Trail
   b. Firing rate map

D. PT
   a. Trail
   b. Firing rate map
Fig. 6
Fig. 7

Aa. Total

Ba. Total

Ca. Total

Da. Total

Spatial similarity

Number of neurons

Spatial scale (m²)

Spatial scale (m²)

High freq

Low freq

High freq

Low freq

High freq

Low freq

High freq

Low freq

High freq

Low freq

High freq

Low freq

High freq

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Lowfreq
A. Heading direction  
B. Landmark angle  
C. Wall angle  
D. Moving direction  
E. Turning angle  

Fig. 8
Percentage of the neuron with significant main effect

A. Total

B. High freq

Lowfreq

HF

PH

Fig. 9
Fig. 11
A. Information of place

a. Total

B. Information of spatial view

Fig. 12
Table 1. Comparison of spatial selectivity index (SI) in terms of spatial variables in the 3 VN tasks between HF and PH.

<table>
<thead>
<tr>
<th>VN tasks</th>
<th>Variables</th>
<th>Regions</th>
<th>n</th>
<th>Spatial SI</th>
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<tbody>
<tr>
<td>Reduced</td>
<td>Heading direction</td>
<td>HF</td>
<td>59</td>
<td>2.20 ± 1.13</td>
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<tr>
<td></td>
<td></td>
<td>PH</td>
<td>41</td>
<td>2.18 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Landmark angle</td>
<td>HF</td>
<td>59</td>
<td>2.18 ± 1.05</td>
</tr>
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<td></td>
<td></td>
<td>PH</td>
<td>41</td>
<td>2.23 ± 0.95</td>
</tr>
<tr>
<td></td>
<td>Wall angle</td>
<td>HF</td>
<td>59</td>
<td>2.06 ± 0.80</td>
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<td>PH</td>
<td>41</td>
<td>2.26 ± 0.97</td>
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<tr>
<td>Control</td>
<td>Heading direction</td>
<td>HF</td>
<td>83</td>
<td>1.99 ± 1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PH</td>
<td>48</td>
<td>2.17 ± 1.10</td>
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<tr>
<td></td>
<td>Landmark angle</td>
<td>HF</td>
<td>83</td>
<td>1.95 ± 0.90</td>
</tr>
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<td></td>
<td></td>
<td>PH</td>
<td>48</td>
<td>2.20 ± 1.12</td>
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<td>Wall angle</td>
<td>HF</td>
<td>83</td>
<td>1.98 ± 0.88</td>
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<td></td>
<td></td>
<td>PH</td>
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<td>2.16 ± 1.06</td>
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<tr>
<td>Expanded</td>
<td>Heading direction</td>
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<td>1.96 ± 0.77</td>
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<td>PH</td>
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<td>PH</td>
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Table 2. Comparison of movement selectivity index (SI) in terms of movement variables in the 3 VN tasks between HF and PH.

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<tr>
<th>VN task</th>
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<th>Movement</th>
<th>SI</th>
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<td>PH</td>
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<td>PH</td>
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<td>Control</td>
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<td>PH</td>
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<td>1.80 ± 0.58</td>
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