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Organization of Hippocampal Cell Assemblies Based on Theta Phase Precession

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ABSTRACT: The factors that control the spatial tuning of hippocampal neurons are incompletely understood, and there is no generally agreed upon definition of what constitutes a "place field". One factor that must be considered is the phenomenon of "phase precession". As a rat passes through the place field of a particular hippocampal neuron, its spikes shift to earlier phases of the theta rhythm. Except for the special cases discussed herein, the phase shift never exceeds 360°. Moreover, under conditions in which place field sizes change dynamically, precession rate is tightly coupled with the place field size, suggesting that a single cycle of theta phase precession could be used to define unitary place field boundaries. Theta phase precession implies that the "cell assembly" of active hippocampal neurons changes systematically over the course of a single theta cycle. A given cell can exhibit more than one place field in a given environment, each field showing the same pattern of 360° of phase precession. The existence of multiple fields implies that one cell can participate in multiple cell assemblies within the same environment. We show here that place fields, defined as a single cycle of phase precession, can overlap spatially, with the result that the cell fires with spikes clustered at two different phases over the theta cycles in which the fields overlap. Thus, the same neuron can participate in different cell assemblies within a single theta cycle. © 2006 Wiley-Liss, Inc.

KEY WORDS: attractor; oscillation; place cell; rat; theta rhythm

INTRODUCTION

Neuronal recordings from the hippocampus of rats reveal that when a rat explores an environment, pyramidal (O'Keefe and Dostrovsky, 1971; O'Keefe and Conway, 1978) and granule cells (Jung and McNaughton, 1993) show patterned neural activity that is highly correlated with a rat's position in space (that is, the "place field" of the cell). Approximately 30–50% of all CA1 pyramidal cells in the dorsal hippocampus exhibit place-specific firing in a given environment, 124 cm in length and 62 cm in width (e.g., Wilson and McNaughton, 1993). The composite cell activity covers the environment relatively uniformly and, when the firing patterns of many hippocampal neurons are recorded simultaneously, it is possible to reconstruct the position of a rat within an environment from the place firing patterns alone (Wilson and McNaughton, 1993; Zhang et al., 1998). Approximately 2–5% of all dorsal CA1 neurons are active within a 20–25 cm region of space (estimated from Wilson and McNaughton, 1993). The subset of coactive neurons whose place fields highly overlap can be loosely defined as a 'cell assembly' (see Discussion). A portion of place cells will exhibit multiple place fields within an environment (e.g., O'Keefe and Conway, 1978; Shen et al., 1997), indicating that a single neuron can be part of multiple cell assemblies during an epoch of behavior.

Despite numerous published studies that have examined the effects of various conditions upon place field size, there is no widely agreed-upon standard regarding the definition of place field boundaries. Many researchers have defined place field size as that portion of the environment in which the cell's firing rate exceeds an arbitrarily defined criterion, such as three standard deviations above the overall mean firing rate (Thompson and Best, 1990), or some other specified threshold firing rate increase (Muller et al., 1987). Because spike timing in the hippocampus is modulated in a consistent manner with respect to local field potential oscillations at various frequencies (Buzsaki et al., 1983; O'Keefe and Recce, 1993; Skaggs et al., 1996), it is possible that the relationship of spike timing to network oscillations could provide additional information that may provide a less arbitrary definition of place field boundaries.

For most place fields, it is clear that there is a relatively monotonic shift of the timing of spikes relative to the local theta rhythm as a rat traverses the field (but see Yamaguchi et al., 2002). Regardless of the direction from which the animal enters the field, the first spikes appear late in the theta cycle and shift progressively backwards in relative phase as the rat traverses the field. As the rat leaves the field, spikes appear early in the last theta cycle expressing spikes. For the majority of place fields, this "theta phase precession" (O'Keefe and Recce, 1993) covers approximately 360° of phase shift, but not more. This observation appears to hold true in spite of variations in place field size along the septotemporal axis (Maurer et al., 2005), and in spite of changes in place field size brought about by experience-dependent effects (Ekstrom et al., 2001), aging (Shen et al., 1997), or by manipulations of self-motion signals that result in large changes of place field size (Terrazas et al., 2005). The apparent consistency of

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the phase shift, in the face of these induced or constitutive place field size differences, suggests that a cycle of precession is in some way deeply linked to the basic mechanism of expression of place fields and may be used as a means of operationally defining a unitary place field.

METHODS

Animals and Surgical Procedures

Neurophysiological studies were conducted on three Brown Norway/Fisher 344 hybrid male rats between 8 and 12 months old. The rats were housed individually and maintained on a 12:12 light–dark cycle. Recordings took place during the dark phase of the cycle. Surgery was conducted according to National Institutes of Health guidelines for rodents and approved IACUC protocols. Prior to surgery, the rats were administered bicillin (30,000 units i.m. in each hind limb). The rats were implanted, under isoflurane anesthesia, with an array of 14 separately moveable microdrives ("Hyperdrive"). This device, implantation methods, and the parallel recording technique have been described in detail elsewhere (Gothard et al., 1996). Briefly, each microdrive consisted of a drive screw coupled by a nut to a guide cannula. Twelve guide cannulae contained tetrodes (McNaughton et al., 1983; Recce and O'Keefe, 1989), which are four-channel electrodes constructed by twisting together four strands of insulated 13-μm nichrome wire (H.P. Reid, Neptune, NJ). Two additional tetrodes with their individual wires shorted together served as an indifferent reference and an EEG recording probe. A full turn of the screw advanced the tetrode 318 μm. For all three rats, recordings were made sequentially from the middle (5.7 posterior, 5.0 lateral to bregma) and septal (3.0 posterior, 1.4 lateral to bregma) regions. This was accomplished by directing all 14 probes first to the middle region and then physically moving the drive to a previously prepared and sealed craniotomy over the septal pole. The implant was cemented in place with dental acrylic anchored by dental screws. After surgery, rats were post operatively administered 26 mg of acetaminophen (Children's Tylenol Elixir, McNeil, PA). They also received 2.7 mg/ml acetaminophen in the drinking water for 1–3 days after surgery and oral ampicillin (Bicillin, Wyeth Laboratories, Madison, NJ) on a 10-days-on/10-days-off regimen for the duration of the experiment.

Neurophysiology

The tetrodes were lowered after surgery into the hippocampus, allowed to stabilize for several days just above the CA1 hippocampal subregion, and then gradually advanced into the CA1 stratum pyramidale. The neutral reference electrode was located in or near the corpus callosum. The EEG probe was used to record theta field activity from the vicinity of the hippocampal fissure. The four channels of each tetrode were each attached to a separate channel of a 50-channel unity-gain headstage (Neuralynx, Tucson, AZ). A multiwire cable connected the headstage to digitally programmable amplifiers (Neuralynx,). The spike signals were amplified by a factor of 1,000–5,000, bandpass-filtered between 600 Hz and 6 kHz, and transmitted to the Cheetah Data Acquisition system (Neuralynx,). Signals were digitized at 32 kHz and events that reached a predetermined threshold

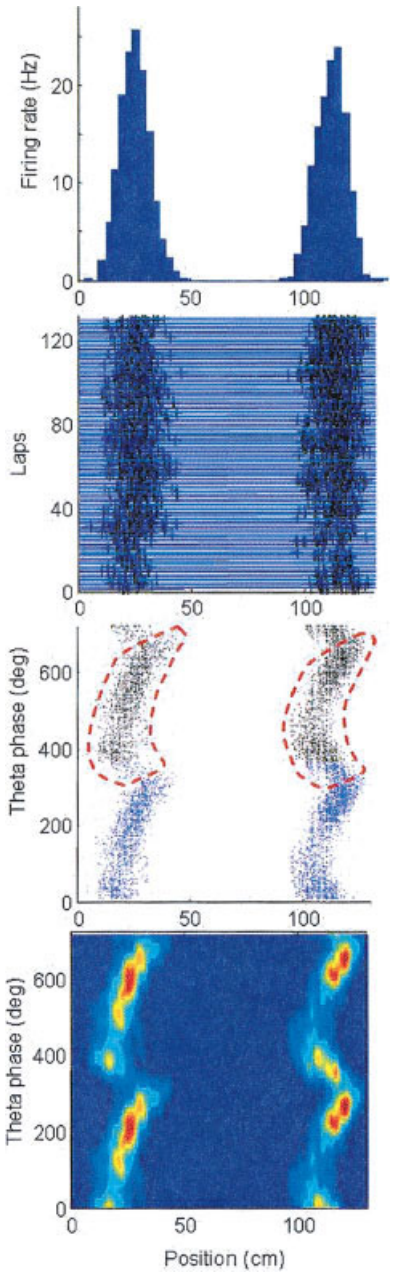

FIGURE 1. Two nonoverlapping place fields from a single CA1 neuron recorded while the rat was running on the small circular track. All plots were constructed such that the rats' trajectory is from right to left. The place field boundaries were determined using theta phase information. The top panel shows the mean firing rate vs. position histogram for both place fields. The second panel is a raster plot of spike vs. position for each lap. The third panel shows the phase by position plot for both fields. The red dotted line indicates the boundaries of the place fields as determined by the inclusion criteria described in Methods. Finally, the bottom panel shows the occupancy normalized density plot for phase by position. Note, that two cycles of theta phase precession are plotted for the bottom two panels.

TABLE 1.

Numbers of Recorded Pyramidal Neurons Making Up the Database for the Current Report for All Three Rats

	Dorsal hippocampus large track	Dorsal hippocampus small track	Dorsal hippocampus both tracks	Middle hippocampus large track	Middle hippocampus small track	Middle hippocampus both tracks
Total sessions	15	15	15	16	16	16
Total cells	554	554	554	386	386	386
Cells without fields	178	314	121	235	250	175
Cells with at least 1 field	376	240	433	151	136	211

were recorded for a duration of 1 ms. Spikes were sorted off-line on the basis of the amplitude and principal components from the four tetrode channels by means of a semiautomatic clustering algorithm (BBClust, author: P. Lipa, University of Arizona; KlustaKwik, author: K.D. Harris, Rutgers-Newark). The resulting classification was corrected and refined manually with custom-written software (MClust, author: A.D. Redish, University of Minnesota), resulting in a spike-train time-series for each of the well-isolated cells. No attempt was made to match cells from one daily session to the next, and, therefore, the numbers of recorded cells reported does not take into account possible recordings from the same cells on consecutive days; however, because the electrode positions were frequently adjusted from one day to the next, recordings from the same cell over days were probably relatively infrequent. Putative pyramidal neurons were identified by means of the standard parameters of firing rate, burstiness, and spike waveform (Ranck, 1973).

Theta activity was recorded from a separate probe that was positioned ~0.5 mm below the CA1 pyramidal layer, near the hippocampal fissure. The location of the theta recording electrode changed between the dorsal and middle regions of the hippocampus along with the recording microdrive. EEG signals were bandpass filtered between 1 and 300 Hz and sampled at 2.4 kHz. The EEG signals were amplified on the headstage with unity gain and then again with variable gain amplifiers (up to 5 K).

Several diodes were mounted on the headstage to allow position tracking. The position of the diode array was detected by a video camera placed directly above the experimental apparatus and recorded with a sampling frequency of 60 Hz. The sampling resolution was such that a pixel was ~0.3 cm.

Behavior

The animals were food deprived to about 85% of their *ad libitum* weight. During this time period, the rats were trained to run on circular tracks for food reinforcement. Two different tracks were used, one 167.5 cm in circumference, and the other 382 cm in circumference. Rats ran unidirectionally around the smaller track with food delivered at one point, and bidirectionally around the large track, which was partitioned by a barrier at one point. Food was given on either side of the barrier and at the 180° opposite point. The two-track design was adopted to increase the yield of independent place fields from a given recorded ensemble and for other reasons not relevant to this study. Rats ran each track for ~20 min, resulting in a variable number of laps per session. Rats ran two sessions per day, one on the large track and one on the small track (in counterbalanced order), with each running session flanked by a rest period in the "nest." Data from the rest periods were used to assess baseline firing and cell stability.

Analyses

Place field diagrams were constructed by plotting the circular trajectories of the animals on a linear scale. For the large track, clockwise and counterclockwise trajectories were plotted separately. Spikes were plotted onto the position of the rat using a 3-cm bin width for both tracks. Spike-phase vs. rat position plot, as well as occupancy-normalized phase vs. position spike-density plot, was constructed. To construct the phase of firing vs. position plots, each spike was assigned a nominal phase, according to the fraction of the time between the preceding and following

TABLE 2.

Numbers of Total Fields, Fields Excluded, and Fields Analyzed Making Up the Database for the Current Report for All Three Rats

	Dorsal hippocampus large track	Dorsal hippocampus small track	Middle hippocampus large track	Middle hippocampus small track
Total fields	1321	667	590	320
Fields excluded	287	162	252	132
Fields analyzed	1034	505	338	188

The greater proportion of excluded fields in the middle hippocampus reflects the larger size of the fields with the consequent increased likelihood of overlap of the place field with the food well.

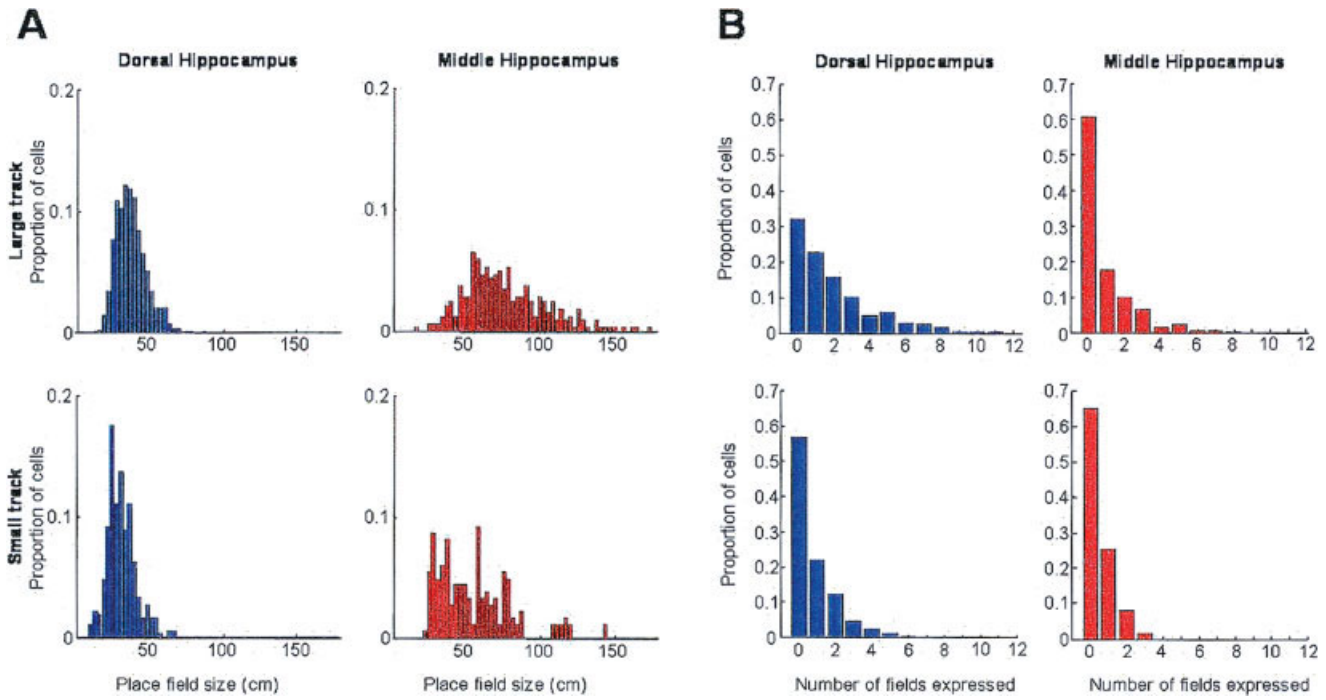

FIGURE 2

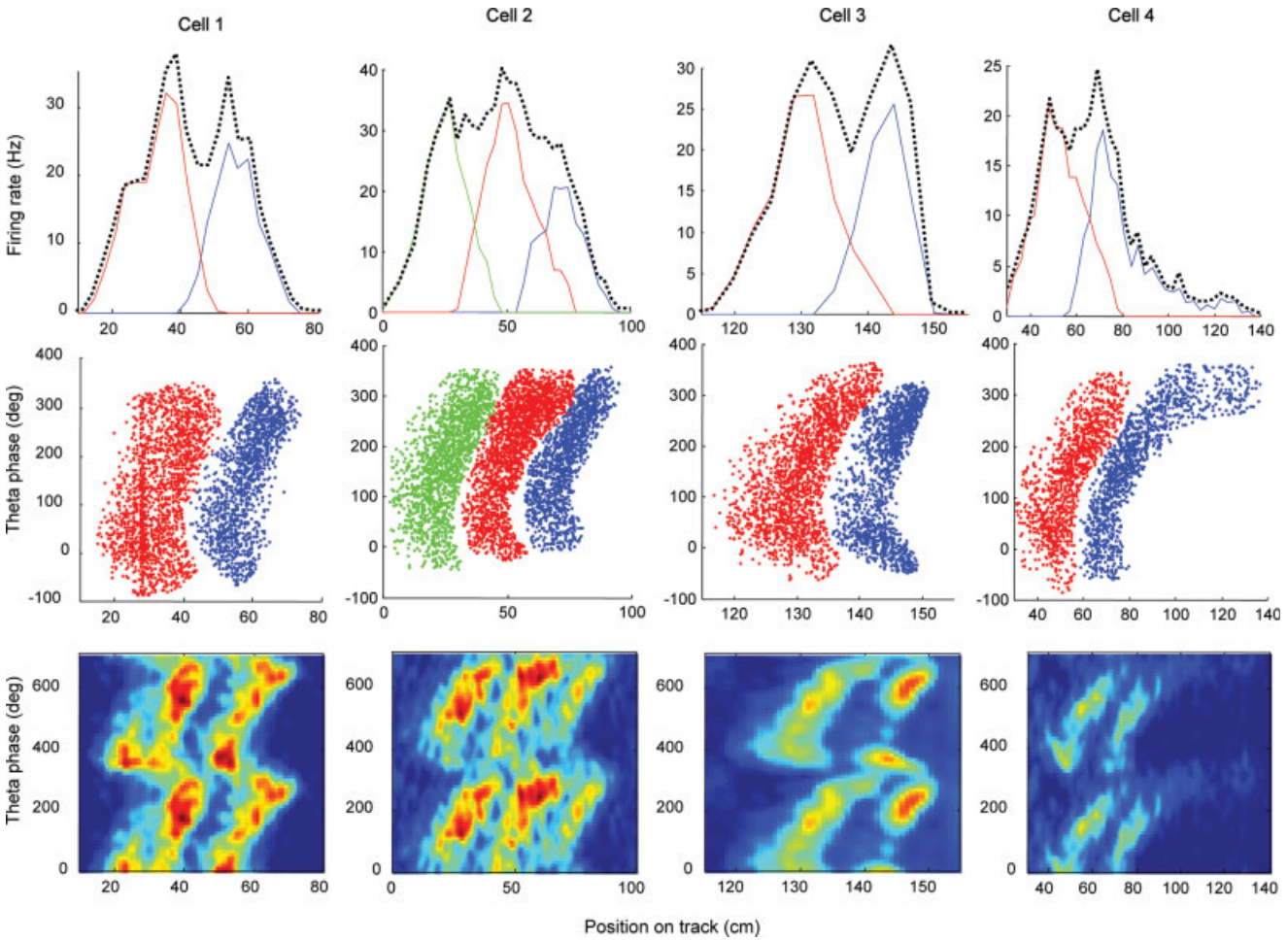

FIGURE 3

theta peaks at which it occurred. Precisely, the phase assigned to an event at time t was $360 \times (t - t_0)/(t_1 - t_0)$, where t_0 and t_1 are the times of the preceding and following peaks of the digitally filtered (6–11 Hz) reference EEG signal. Note that the phase is always a number between 0 and 360.

Place field boundaries were assigned with reference to the phase vs. position plots, according to the definition that a single place field involves a single cycle (360°) phase precession. The start location of a field was defined as the location at which spikes fired late in theta phase (~360°) and began a cycle of precession. The end location was determined to be the location of spikes with the smallest phase angle (~0°). Spikes belonging to a given place field were flagged by manually drawing boundaries around the relevant spikes in the phase vs. position plot. If a place field had two or more clear cycles of precession, it was split into multiple fields, so that each field would only contain spikes associated with a single cycle of precession. In some cases, two or more cycles of phase precession overlapped spatially. Using the manually cut spikes from each phase vs. position field, the sizes of the fields were derived by taking the difference between the maximum and minimum spike position in the field (Fig. 1).

When place fields overlapped with a food dish, it was excluded from consideration, because these fields exhibit an abrupt halt in their precession or no salient relationship with the theta rhythm (data not shown), preventing a quantitative analysis of place field size and slope of precession. In addition, neurons that did not exhibit spatial selectivity for at least half of the laps were eliminated from the analysis.

To ensure that the presence of multiple place fields on a single track was due to the firing characteristics of the neuron and not to spike sorting errors, measures of spike sorting quality for the cells with overlapping fields were compared with cells with one field, or two or three nonoverlapping fields. If the presence of multiple fields was an artifact arising from poor cell isolation, then those cells with multiple fields should have generally poorer cluster quality measures relative to other cells in the dataset. The two measures of cluster quality chosen for this analysis were Isolation Distance and the "L_{ratio}," calculated using two spike features, energy and the first principle component of the waveform (Schmitzer-Torbert et al., 2005). The L_{ratio} is an estimate of the likelihood that spikes near the outer edge of the isolated cluster should be included in the isolated cluster, while Isolation Distance measures the distance of the spikes within the isolated cluster to other data points outside of the cluster. These two measures have produced robust estimates of cluster quality on cells isolated in datasets containing simultaneous intra- and extracellular recordings and from various simulations run on real and artificial data (Schmitzer-Torbert et al., 2005).

Theta cycle-time histograms were constructed for a portion of the cells exhibiting overlapping place fields. For these cells, the spikes from a given 360° cycle of theta phase precession were manually cut so that spikes from different place fields were separated from each other. Theta cycle-time histograms were then generated by choosing a point near the maximum overlap between the two fields. All spikes for each phase precession cycle (i.e., place field) were then aligned in terms of its theta phase and the number of cycles before or after the rat passed the chosen point on each lap. These histograms were constructed as previously described (Skaggs et al., 1996).

RESULTS

Phase Precession Definition of Unitary Place Fields

Tables 1 and 2 provide the total number of cells analyzed and the total number of fields included in the analysis.

A total of 554 pyramidal cells were recorded in the dorsal hippocampus and 386 were recorded in the middle hippocam-

FIGURE 2. (A) Frequency distributions of place field size for dorsal (blue) and middle (red) CA1 on the large (top panels) and small (bottom panels) track. Place field sizes were determined using the phase precession definition (see Methods). The mean field size for the dorsal hippocampus was 37.5 cm on the large track and 29.0 cm on the small track. In the middle hippocampus, the mean place field size was 78.6 and 55.8 cm for the large and small tracks, respectively. The size of place fields was significantly different between the dorsal and middle CA1 on both the large and the small track. Moreover, there was a significant difference in the size of place fields between the large and small tracks in both the dorsal and middle hippocampus. (B) The proportion of cells in dorsal (blue) and middle (red) CA1 with n = 0,1,2,3 . . . place fields on the small track (top panels) or large track (bottom panels). All place fields were determined using the phase precession definition. Thus, a neuron with two spatially overlapping cycles of theta phase precession was considered to have two place fields. In the dorsal hippocampus more neurons have place fields on the two different tracks and are more likely to have multiple fields compared with the middle hippocampus. In both dorsal and middle CA1, as the size of the environment increases more neurons express place fields and are more likely to express multiple place fields.

FIGURE 3. Examples of firing rate distributions and theta phase information by position from four different neurons that had overlapping place fields. The direction of the rat's movement was from right to left. The top panels show the firing rate distributions by position. The firing rates for the different fields from the same cell are shown in different colors (blue, red, green), while the black dotted line is the unsorted mean firing rate vs. position. Note that the number of place fields expressed by a single neuron corresponds to the number of firing rate peaks (or in some cases shoulders). The middle panels show the theta phase (ordinate) by position (abscissa) information for the place fields of each neuron, after manually selecting the place field boundaries based on 360° of theta phase precession (see Methods and Fig. 1). Each dot corresponds to a spike, and spikes from different place fields from the same cell are shown in different colors. The bottom panels show the smoothed density plots of the phase by position information for the spikes from neurons shown in the top two panels. No spikes are cut out. Red indicates the highest spike density, while dark blue indicates the lowest. The theta phase is plotted over two cycles. Cells 1–3 are from the dorsal region of different rats, and Cell 4 is from the middle region.

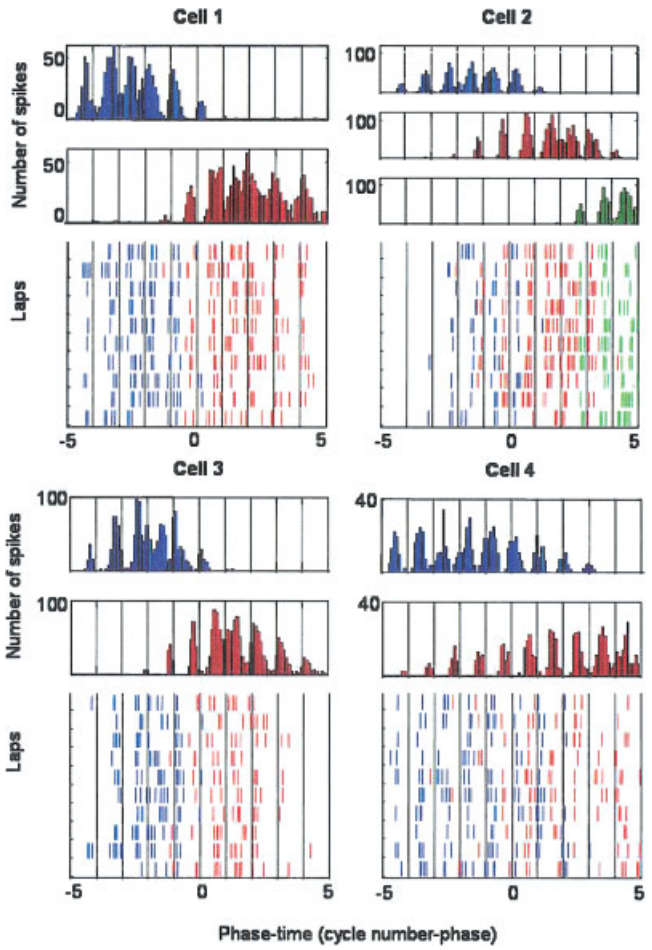

FIGURE 4. The top panels show theta cycle time histograms (Skaggs et al., 1996) constructed for the four cells with overlapping place fields shown in Figure 3. Histograms were constructed by selecting a point near the maximum overlap between the two fields, finding the nearest peak in the theta rhythm, setting that point to zero and aligning each spike in terms of its theta phase for the five theta cycles before or after the rat passed the chosen point (bin size = 0.1 theta cycles). The black vertical lines delineate the different theta cycles. Note that in each example, the centers of the bursts for each field precess steadily leftward with respect to the tick marks (Skaggs et al., 1996). For each example, 10 representative consecutive laps were chosen and spike rasters, in cycle-phase time, were generated (bottom panels). Spikes from different place fields are shown in different colors (blue, red, green). For each of these examples, the spike rasters show that within a lap, spikes from different place fields occur during single cycles of theta. Hence, the overlap in the average place fields is not due to any lap by lap fluctuation between one field and the other.

pus. Among these neurons, 121 of the dorsal cells and 175 of the middle cells did not express a place field on either track. Many cells, however, exhibited more than one field on a given track (Fig. 2). The majority of place fields exhibited clear theta phase precession, with the total phase change within 360°.

In dorsal CA1, 1,034 place fields met the inclusion criteria. Of the cases of multiple fields for a given cell on the large track, there were 87 instances in which two fields overlapped spatially in terms of phase precession ("double" fields) and firing rate, and 16 cases in which three fields overlapped spatially ("triple" fields). The characteristics of "double" and "triple" place fields are illustrated in further detail in Figures 3 and 4. On the small track, a total of 505 place fields were included in the analysis. Of these fields, there were 75 "double" fields and 15 "triple" fields.

Fewer place fields were observed on both the large and small tracks, respectively, in the middle hippocampal CA1 region. On the large track, a total of 338 place fields satisfied the inclusion criteria. Of these, 24 fields exhibited a double overlap, while six exhibited a triple overlap. On the small track, 188 fields fulfilled the inclusion criteria. Of these fields, 29 were doubles, and four were triples. Table 3 shows the number of fields in dorsal and middle CA1 with a single, double, or triple overlapped phase shift for all three rats on the large and small tracks.

When place field size was quantified using the phase precession definition, the mean field size for the dorsal hippocampus was 37.5 (±10.33 SD) cm on the large track and 29.0 (±9.59 SD) cm on the small track. In the middle hippocampus, the mean place field size was 78.6 (±27.80 SD) cm and 55.8 (±25.46 SD) cm for the large and small tracks, respectively. Figure 2A shows the frequency distributions of place field size on the large and small tracks for dorsal and middle CA1.

A repeated measures factorial analysis of variance (ANOVA) indicated that there was a significant difference in the size of place fields between the large and small tracks in both the dorsal and middle hippocampus, using the phase precession definition of place field size ($F = 52.32$, $P < 0.01$). There was also a signifi-

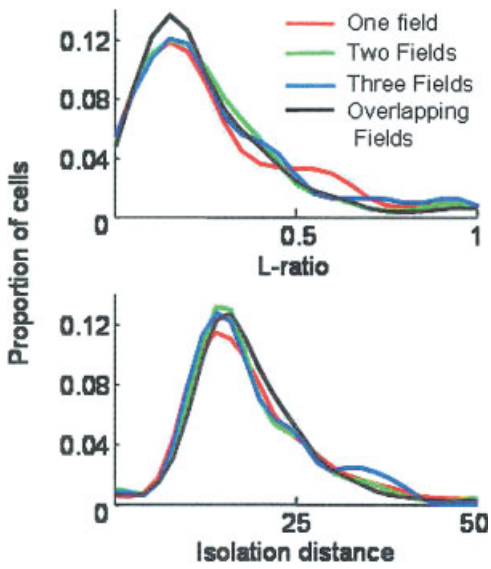

FIGURE 5. The smoothed frequency distributions of the L_{ratio} (top panel) and the isolation distance (bottom panel) scores for spike waveform clusters from neurons with a single place field and neurons with multiple place fields. The red, green, and blue traces represent frequency distributions for neurons with one, two, or three nonoverlapping place fields, respectively. The black trace shows the proportion of neurons with a given L_{ratio} and isolation distance for cells with place fields that overlap in space. There were no significant differences among the frequency distributions of either type, indicating that the isolation quality of the spikes from cells with multiple and overlapped fields is similar to that for cells with single fields.

TABLE 3.

Number of Place Fields for Each Rat on the Different Tracks for the Dorsal and Middle CA1 with Single Phase Shifts, or Overlapped Double or Triple Theta Phase Shifts

	Cycles of precession								
	Rat 1			Rat 2			Rat 3		
	1	2	3	1	2	3	1	2	3
Large track dorsal CA1	106 (67)	38 (24)	15 (9)	524 (84)	78 (13)	21 (3)	182 (72)	58 (23)	12 (5)
Large track middle CA1	70 (90)	8 (10)	0 (0)	104 (79)	18 (14)	9 (7)	98 (76)	22 (17)	9 (7)
Small track dorsal CA1	55 (61)	24 (26)	12 (13)	180 (59)	102 (33)	24 (8)	75 (70)	24 (22)	9 (8)
Small track middle CA1	32 (100)	0 (0)	0 (0)	40 (52)	28 (36)	9 (12)	46 (58)	30 (37)	3 (5)

The values given inside the parentheses are percentages.

cant difference in the size of place fields between the dorsal and middle CA1 on both the large ($P < 0.001$) and the small ($P < 0.001$) track. As reported previously, place fields from neurons in the dorsal hippocampus were significantly smaller than the place fields of neurons recorded from the middle hippocampus (Jung et al., 1994; Kjelstrup et al., 2003; Maurer et al., 2005).

For all four conditions combined (dorsal place fields – large track, dorsal place fields – small track, middle place fields – large track, and middle place fields – small track), place field size was significantly correlated with the rate of precession ($r^2 = 0.57$; $P < 0.001$). Moreover, the rate of precession was significantly different between all four conditions ($P < 0.05$ for all post hoc comparisons). Table 4 shows the mean and standard error for the rate of precession for the four conditions.

The overlap of place fields from a given cell ("doubles" and "triples") was determined from the superimposition of phase vs. position plots for all spikes emitted over all laps. It is possible, however, that within a single lap, the cell only fires spikes in one or the other of the place fields. To explore this possibility, plots were constructed of the firing relative to successive theta cycles within each lap (theta-time plots; see Methods), with the origin of the plot taken as the cycle occurring at the position of maximum overlap between the two fields (Fig. 4). For the majority of laps, spikes belonging to both fields occurred within at least one theta cycle within the region of space in which the fields overlapped. Hence, the field overlap is not a result of the place field center shifting from lap to lap. Rather, the spikes in the overlap regions occur as two clusters centered early and late in a single theta cycle.

TABLE 4.

Rate of Theta Phase Precession by CA1 Recording Location and Track Size

Condition	Rate of theta phase precession (cm/°)	Std. error of the mean
Large track dorsal CA1	0.147	0.013
Small track dorsal CA1	0.108	0.002
Large track middle CA1	0.284	0.008
Small track middle CA1	0.218	0.019

Quality of Cell Isolation

One obvious explanation of the apparent overlap of two cycles of phase precession at the same position in space is that the cells exhibiting overlap were poorly isolated. Thus, the overlap may reflect spikes from different neurons. Indeed, the same caveat may be raised for all cases in which more than one field was expressed by a given cell, regardless of overlap. This issue was addressed by an assessment of cluster quality, using the L_{ratio} and isolation distance described in Methods. Cluster quality measures for the cells with multiple fields were at least as good as the cluster quality measures obtained for other cells in the analysis. Figure 5 shows the isolation distance and L_{ratio} values for the clusters from cells with a single place field, two nonoverlapping place fields, three nonoverlapping place fields, and the clusters from cells with overlapping place fields. Each distribution was compared with each of the corresponding distributions using the Kolmogorov–Smirnov test. There were no significant effects of the number of fields or field overlap on either measure of isolation quality (Isolation distance: smallest $P > 0.14$; L_{ratio}: smallest $P > 0.36$; Fig. 5; Table 5).

TABLE 5.

Descriptive Statistics of the Distributions of Cluster Quality Based on the Number of Place Fields Expressed

	Mean	Standard deviation
Isolation distance		
Overlapping place fields	19.3	10.1
One place field	18.9	9.7
Two place fields	19.5	10.0
Three place fields	19.7	11.5
L_{ratio}		
Overlapping place fields	0.29	0.29
One place field	0.29	0.29
Two place fields	0.28	0.30
Three place fields	0.28	0.30

DISCUSSION

As a rat passes through a hippocampal neuron's place field, the spikes systematically fire at earlier phases of the theta rhythm. The rate of theta phase precession is tightly coupled to place field size, larger place fields exhibiting slower precession (Shen et al., 1997; Ekstrom et al., 2001; Terrazas et al., 2005). As pointed out by O'Keefe and Recce (1993), phase precession itself implies that within the place field, the firing rate of the neuron is modulated at a frequency slightly higher than that of the theta oscillation of the local field potential, with the number of theta cycles required for a 360° phase shift increasing as the difference in modulation frequency is reduced. This was confirmed by Maurer et al. (2005) who demonstrated that the intrinsic modulation frequency within the larger fields expressed by middle hippocampal cells was slower than for the smaller fields of the dorsal hippocampal cells. These considerations were confirmed in the present analysis, in which there was a strong linear relationship between the inverse slope of the phase vs. position function and place field size, both within and between regions. Overall, such data indicate that theta phase shift is deeply linked to the expression of place-specific activity in hippocampal cells and could, therefore, be usefully incorporated into a definition for unitary place fields. For example, in our data, defining fields as a single cycle of phase precession significantly reduced the variation in place field size compared with other methods (analysis not shown).

The idea that phase precession arises simply from the interaction of an ongoing intrinsic oscillation frequency with the background theta field potential, however, implies that spikes within a unitary place field always cluster unimodally around a single phase. It cannot account for the observed apparent overlap of two cycles of phase precession within a common region of space, with the consequent appearance of bimodal distributions of spikes at different phases within a single theta cycle. It is of interest, therefore, to consider the two main classes of existing hypotheses for the origin of phase precession in terms of their ability to account for this phenomenon.

The first class of model can loosely be characterized as accounting for phase precession by intrinsic membrane currents interacting with periodic inputs (O'Keefe and Recce, 1993; Kamondi et al., 1998; Yamaguchi and McNaughton, 1998; Bose et al., 2000; Magee, 2001). For example, Lengyel et al. (2003) proposed a model in which there are two major oscillation components. One oscillator is the somatic hyperpolarizing oscillation, modulated by the theta rhythm and generated by either the septal inputs or the hippocampal interneuronal network. The other oscillator is the dendritic membrane potential oscillation, and it is caused by a variety of sensory inputs via the entorhinal cortex and modulated by a velocity signal. The dendritic membrane oscillation, when outside of a cell's firing field, will be antiphase to the theta rhythm and therefore also antiphase with the somatic oscillation. Therefore, the net excitation of the combined oscillations will be zero. As the rat enters a cell's place field, there is an increase in dendritic input, gradually shifting the dendritic membrane oscillation into phase with the somatic oscillation. The more the somatic oscillation is in phase with the dendritic oscillation, the higher probability that the cell will fire. As the rat leaves the place field, the dendritic oscillation shifts back into antiphase with the somatic oscillation (Lengyel et al., 2003). The observation that a single cell can express overlapping place fields, however, poses a significant problem for this model and others of its class. As a rat enters the first of the overlapping fields, the cell would oscillate at a frequency slightly faster than the hippocampal theta rhythm. When the rat enters a location occupied by both fields, however, the neuron would be required to instantaneously double its frequency of intrinsic oscillation.

The other class of explanation for theta phase precession involves the dynamics of networks with asymmetric recurrent connections (Jensen and Lisman, 1996; Tsodyks et al., 1996). These models account for phase precession through systems-level interactions, in which a recurrent network of neurons receives two main inputs: a weakly location-specific external input and an intrinsic (i.e., recurrent) input that connects the cell with other neurons in the network. As shown by Tsodyks and Sejnowski (1995), in the presence of thresholding inhibition, such a network can exhibit highly spatially selective cells through "attractor" (Amit, 1989) or "cell-assembly" (Hebb, 1949) dynamics, in which those cells with the maximum external input at a given location mutually excite one another. As the rat moves through space, activity shifts through a continuum of attractor states whose membership changes with location. In such models, the phase precession is a result of the asymmetric spread of activation within the network over the course of one theta cycle. At the beginning of a theta cycle, the attractor with the strongest external input is activated. These cells then activate attractors whose maxima are located further ahead of the rat in its direction of travel as a consequence of asymmetric connectivity among successive attractors, as suggested by Hebb's (1949) phase sequence concept. This effect might be facilitated by selective inhibition of the external inputs (Wallenstein and Hasselmo, 1997). The accumulation of inhibition within the theta cycle, terminates further propagation of the wave of activity at the end of each cycle. Thus, the first spikes as the rat enters the traditional 'place field' arise late in the theta cycle, because they are actually driven by the forward sweeping intrinsic activity, and within each theta cycle the network passes through several different attractors. The population activity at the beginning of the cycle is essentially uncorrelated with the activity at the end of the cycle. This class of models can easily explain why, when place fields expand during repeated route following (Mehta et al., 1997), as a consequence of NMDA receptor dependent plasticity, phase precession continues to be restricted to 360° (Ekstrom et al., 2001). The increased asymmetric synaptic drive causes the network to shift further within each theta cycle. Note that this shift is accompanied by a *slowing* of the intrinsic burst frequency. Such models also account for why, following complete suppression of network activity by electrically induced postsynaptic inhibition, firing resumes at the correct phase as the inhibition wears off (Zugaro et al., 2005). This class of model, however, does require that a mechanism exists to accelerate the shift among successive attractors as the animal's running speed increases, so as to maintain the size of the classical place

field relatively invariant with changes in running speed. Assuming that the net excitation received by a postsynaptic neuron is related to the product of instantaneous firing rate and synaptic efficacy, the known increase in firing rate as a function of running speed (McNaughton et al., 1983; Ekstrom et al., 2001; Maurer et al., 2005; Terrazas et al., 2005) could easily accomplish this. Terrazas et al. (2005) found that manipulations that changed the gain of the firing rate vs. speed function resulted in corresponding changes in place field size, and Maurer et al. (2005) showed that in the middle region of the hippocampus, where place fields are larger than in the dorsal region, the slope of this function is correspondingly reduced.

Note that this class of model leads to the perhaps surprising conclusion that the spatial extent of the attractor to which a place cell belongs as a consequence of its external input is extremely small, about the distance that a rat moves in a fraction of a theta cycle at normal running speed (i.e., perhaps as small as a few millimeters). The apparent size of the much larger "classical" place field is a consequence of the network driven 'look ahead' phenomenon within each theta cycle, which is also the basis of the phase precession.

If a given cell can belong to more than one attractor within an environment, and if, as just discussed, the rat typically passes through several attractors within the timescale of one theta cycle, then there is no reason to suppose that the same cell cannot belong to more than one attractor within one theta cycle. The current observation of overlapping cycles of phase precession is consistent with this conclusion.

Given the current evidence that place fields are much smaller than previously imagined, the capacity of the hippocampus to resolve small differences in location would be correspondingly greater. When combining this idea with the phenomenon of rate remapping (Leutgeb et al., 2005), which is due to variation in the input at a given location, then the hypothetical "index" code generated by the hippocampus for the purpose of linking together neocortical ensembles is correspondingly more selective. Therefore, the hippocampus can provide a much finer grain resolution to episodic memory than perhaps previously imagined.

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