ABSTRACT: Traditionally, most of the information processing of neural networks is thought to be carried out by excitatory cells. Likewise, recent evidence for temporal coding comes from the study of the detailed firing patterns of excitatory neurons. In the CA1 region of the rat hippocampus, pyramidal cells discharge selectively when the animal is in specific locations in its environment, and exhibit a precise relationship with the ongoing rhythmic activity of the network (phase precession). We demonstrate that during a spatial exploratory behavior on a linear track, inhibitory interneurons also show spatial selectivity and phase precession dynamics. We found that the firing rate of interneurons is modulated reliably up and down around an ongoing baseline activity level for specific locations in the environment, producing robust place-specific increases or decreases in discharge. On some sections of the track, the range of theta phases shifts progressively to earlier parts of the theta cycle as the rat advances, so that a negative correlation between phase and position could be demonstrated. Unlike pyramidal cells, phase and rate were not strongly correlated. We discuss the influence of the intrinsic firing properties of interneurons on a model of phase precession, as well as the influence of the detailed shape of the inhibitory oscillation. These results indicate that spatial selectivity and phase precession in CA1 are not properties restricted to pyramidal cells. Rather, they may be a more general expression of a common interaction between the different inputs impinging on both excitatory and inhibitory cells in CA1 and the intrinsic characteristics of those cells. Furthermore, they suggest that the role of interneurons may extend beyond a global damping of the network by participating in a finely-tuned local processing with the pyramidal cells.

KEY WORDS: GABAergic neurons; theta rhythm; tetrode recordings; coding; place cell

INTRODUCTION

Neural networks are composed of excitatory and inhibitory neurons. In the hippocampus, as in the cortex, the vast majority of inhibitory neurons have dendrites and axons that stay locally within the structure (Ramon y Cajal, 1911; Freund and Buzsaki, 1996). By contrast, the axons of excitatory pyramidal cells project to downstream targets where they convey all the information about the output of the network. This confinement of interneurons to local networks, as well as their relatively low proportion, has contributed to the widespread idea that interneurons merely modulate the global activity of the network through nonspecific suppression of excitability, preventing the uncontrolled firing that would inevitably occur in a purely excitatory feedback network.

However, a growing body of evidence suggests that the role of inhibitory neurons might in fact be much more complex than a simple dampening of activity. Anatomical and biochemical studies have revealed an intriguing diversity of interneurons unmatched by the excitatory cells (Freund and Buzsaki, 1996; Kawaguchi and Kubota, 1996; Gupta et al., 2000; Somogyi and Klausberger, 2005). In sensory cortices, electrophysiological recordings in the anesthetized animal have shown that inhibitory neurons have receptive fields with sharp selectivity qualitatively similar to those of excitatory neurons, and that only careful quantification allows to draw differences between the two groups (see for example Azouz et al., 1997; Bruno and Simons, 2002; Hirschl et al., 2003; Andermann et al., 2004; Andermann and Moore, 2006).

Early studies of behavioral correlates have emphasized striking differences in the pattern of activation of putative excitatory and inhibitory cells, in the CA1 region of the hippocampus (Ranck, 1973; O'Keefe, 1976). When an animal is running through an environment, pyramidal cells display highly localized firing for places in the environment, hence the term “place cells.” The firing rate of a neuron drops to zero outside of the place field. In contrast, interneurons fire at very high rates (~20 spikes per second or more) through most if not all of the behavior of the animal. During exploration, the activity of interneurons increases with speed and is clearly modulated by the oscillatory theta rhythm, a phenomenon which has earned them the name “theta cells” (Ranck, 1973). Reports on the spatial firing properties of interneurons have concluded either that there was no demonstrable selectivity (Christian and Deadwyler, 1986), that it was marginal at best (McNaughton et al., 1983; Mizumori et al., 1990), or spatially inhomogeneous (Kubie et al., 1990).

The relation between hippocampal firing and the theta rhythm has been the subject of many investigations, exemplified in 1993 by the first report of phase precession (O’Keefe and Recce, 1993). As the rat traverses the firing field of a place cell, spikes display progressively earlier phases relative to the theta oscillation. It has been proposed that additional information about space might thus be encoded in the spike timing of a neuron relative to the population theta rhythm, beyond the information available from the place cells consid-
Tetrode Implantation and Recording

Three male Long–Evans rats (3–7-months old) were implanted under deep surgical anesthesia (isoflurane 0.5–5%) with two arrays of independently movable recording tetrodes (for detailed methods, see Jones and Wilson, 2005b). One array of 11 tetrodes was aimed to the CA1 pyramidal cell layer. The tetrodes were slowly advanced to their target position over the course of several weeks. One additional tetrode in the array was left in the white matter above CA1 as a reference for differential recordings, and a second one was lowered 300 μm below the CA1 pyramidal layer, in the stratum radiatum, for local field potential recording of the theta rhythm. This location was chosen because the theta rhythm recorded near the hippocampal fissure has a fixed phase relationship with the hippocampal oscillatory activity, whereas the phase of the signals recorded in or near the pyramidal cell layer varies a lot with precise location and thus cannot be used for comparisons across days or animals. An array of seven additional tetrodes was targeted to the ventral hippocampal commissure for purposes not included in this study. All electrode placements were confirmed with histology after the end of the recording sessions.

Both thresholded extracellular action potentials (31 kHz sampling, 600–6,000 Hz filtering) and continuous local field potentials (2 kHz sampling, 1–475 Hz filtering) were recorded from each tetrode. Action potentials were assigned offline to putative individual units using the XClust software (M. Wilson). Since the emphasis for this study was placed on analyzing interneuron activity, only very well isolated pyramidal cells were clustered from each tetrode. Furthermore, we verified that only interneuron action potentials were included in putative interneuron clusters by several methods. First, we examined the clustered clouds in the spike width per spike amplitude space for cells recorded together on a given tetrode. Because interneuron waveforms have a smaller spike width, they appeared distinct in that projection space from the pyramidal cells waveforms. Second, we verified that the putative interneurons did not show a tendency to fire complex spikes (short bursts of spikes with decreasing amplitude), a characteristic specific to the pyramidal cells. Finally, we systematically inspected all waveforms of each epoch visually, confirming that the same cell was recorded over time without contamination.

Behavioral tracking was achieved by two sets of three infrared light-emitting diodes mounted on the headstage and blinking with a sampling rate of 30 Hz each.

Behavioral Training

Before surgery, animals were food-deprived to 85% of their free-feeding weights. Training on the maze began either a few days before or after the surgery. Two circular tracks of different lengths were used (290 cm for the first two rats, and 360 cm for the third; width 10 cm). A fixed wall was placed at one location with food reward wells on both sides. Recordings were performed once the rats ran repeatedly back and forth from one end to the other in order to collect chocolate sprinkles.

Data from only one recording session (several hours of contiguous recording on one day) were selected for each of the two first animals. For the third animal, as the quality of recording on some tetrodes was better later in the experiment, data from three different sessions were used for three, four, and four tetrodes respectively. In all cases, data from only one session was analyzed for each tetrode to ensure that no cell was counted twice.

Recording sessions consisted of multiple alternating run and sleep epochs. Only data from run periods are presented here; in particular, cells that fired in sleep but not during run epochs are not included. For the population analysis, only the data from the last run epoch of each session was kept.

Data Analysis

Spatial firing map

For each cell and each recorded epoch, a linearized spatial map of firing rate was calculated. The recording was first divided into laps of alternating directions (clockwise/counterclockwise) and segments for which the velocity exceeded 30 cm/s were selected. This threshold was chosen because all traversals of the track were done at much higher velocities (up to 100 cm/s), so that it eliminated firing only near the reward sites and preserved all firing on the running portion. Also, this threshold corresponds to the velocity at which the cell firing rates reach their asymptotic value (Mizumori et al., 1990). The track was then divided in 5-cm bins along its length (ignoring the width), and for each bin, the number of spikes fired by the cell was divided by the total time spent by the animal in the bin. Clockwise (respectively counterclockwise) laps are represented as left-to-right (respectively right-to-left) movement on all figures where the x-axis contains the location on the track.

The firing rate profile for each direction of movement was then scanned for two consecutive bins exceeding a preset threshold of 10 spikes per second. Only spatial firing maps fulfilling this requirement were kept in the database. Cells could thus con-
Spatial information measure

A spatial information measure was obtained by calculating the mutual information between position (the “stimulus”) and firing rate (the “response”) (Cover and Thomas, 1991; Dayan and Abbott, 2001). The mutual information can be computed as the difference between two terms: the total response entropy, and the average response entropy on trials that involve repetitive presentation of the same stimulus. For our purpose, we considered position bins $S_i$ of width $\Delta S$ along the track as the set of stimuli, and the firing rates $R$ calculated on each position bin and on each individual trial as the set of responses. The total response entropy is then given by: $H = -\sum (P_i \times \log (P_i))$, where $P_i$ is the probability that $S_i$ was presented and $H_i$ is the entropy for the subset of responses $R$ to $S_i$. It can be determined that the mutual information has a maximum value of $\log (R_{max}/\Delta R)$, where $R_{max}$ is the maximum firing rate; in particular, one way in which the maximum is achieved is when the firing rate profile has a constant slope from 0 to $R_{max}$ over the whole track and there is no variability from lap to lap.

Note that in practice, this calculation depends both on the firing rate resolution $\Delta R$ and on the spatial resolution $\Delta S$. We used the same $\Delta R = 5$ spikes per second and $\Delta S = 5$ cm for both pyramidal cells and interneurons, because they seem in the range of what could be biologically relevant to the encoding of spatial information. However, we checked that our results still hold qualitatively for different values of these parameters.

This mutual information measure captures the information carried by the rate on the location. By contrast, the information per spike derived by Skaggs and collaborators (1993) expresses the information conveyed when one spike is emitted and thus is specifically aimed at quantifying spatial selectivity of individual spikes. Indeed, it is well suited to describe the highly localized place fields of pyramidal cells. However, in the case of the distributed firing of interneurons, spatial information could be conveyed by different levels of firing rate (encompassing several spikes) and/or by the absence of spikes, two characteristics of firing which are not captured by the information per spike. When the information per spike is multiplied by the mean firing rate of the cell, an information rate is obtained, measured in bits per second (Skaggs et al., 1993). It might seem at first that the information rate allows us to integrate over several spikes, and thus overcomes the limitations of the information per spike. However, the information rate is only meaningful for very small integration window and the interpretation over long intervals such as those required for estimating firing rates is difficult. By contrast, the mutual information measure directly derives from firing rates and not individual spikes, and preserves symmetry in the firing rate levels, so that localized decreases in firing rates encode as much information as increases.

Phase relative to the theta rhythm

The local field potential from stratum radiatum was filtered offline in the theta band (5–12 Hz). Peaks and troughs of the filtered signal were assigned to 0° and 180°, respectively, and were used to calculate a phase relative to the theta rhythm for each recorded spike. More precisely, the phase assigned to a spike was interpolated linearly from the spike time relative to the times of the preceding and following theta peak and trough (or trough and peak). Phase distribution histograms and the mean circular phase were calculated for each epoch (Zar, 1999). A minimum phase, that is, the theta phase at which the last firing occurred, was derived from fitting the distribution to a model circular distribution of the Von Mises family (Swindale, 1998). This same parameter was also calculated on a 15 cm sliding window (overlap 10 cm) along the track for each direction. This yielded a minimum phase curve as a function of position. Each time interval between two consecutive crossing of this curve was defined as a theta cycle for the cell. This allowed us to determine the first and last spike of each cycle, as well as all intermediate spikes in the cycle, along with their theta phase.

Correlation between phase and position was computed using modified linear statistics as developed by O’Keefe and Recce (1993). We searched for the best fit of the data when plotted as the phase of each spike against the position of the animal at the time of the spike. For a given putative regression line, we first added or subtracted 360° from the phase value of each data point as needed individually, so that the point was closest to the line. The distance between the line and the data points was then computed as the residual sum of squares as in standard linear statistics. The best fit was obtained by a least-squares minimization with respect to slope and intercept of the line, using a standard optimization function in Matlab. This yielded the Pearson’s correlation coefficient $r$ for the data set.

Because of the high and distributed firing of interneurons, this procedure could not be applied successfully to all the spikes together. Indeed, it is readily apparent in the phase/position raster plots or density plots that relations between phase and position hold only on a fraction of the track, and that in fact different relations might coexist on different sections of the track. We thus sought to extract the most salient position/phase relations in each data set. We first calculated the circular mean phase for each position bin (5 cm). We then computed the correlation coefficient between mean phase and position for each subsection of the track that contained contiguous firing and that was at least 30 cm wide. The best correlation was defined as the one that maximized the coefficient of determination $r^2$; it also had to be significant ($P < 0.05$). This procedure could be applied to both interneurons and pyramidal cells. In this latter population, it yielded results qualitatively very similar to the simple correlation of phase and position using all spikes in the place field. Quantitatively, it is expected that because of the maximizing procedure on $r^2$ across all subsections of the track, the regression lines obtained have higher absolute correlation coefficient values, smaller spatial extent and phase range, and higher slopes than when using the correlation for the whole track (this should be kept in mind when comparing to other results from the literature).
Still, with this method, the observed correlations could arise as a consequence of lumping the activity from all laps together, rather than reflecting a systematic phase shift on individual laps. To ensure that our procedure extracts regions of consistent phase changes in time, we calculated the phase difference between spike patterns in consecutive theta cycles inside each lap. Regression lines obtained as described earlier were kept only if the mean value of this phase shift was consistent with the regression slope (negative for precession and positive in the inverse case, Student’s t-test, \( P < 0.05 \)). In other words, we ensured that in the precession cases, there was a consistent phase advance, theta cycle after theta cycle, during individual laps.

We selected the best regression line per field for the population analysis in order to limit the contribution of each cell to one phase/position correlation per direction. Note however that additional nonoverlapping regression lines for the same field could be obtained by repeating the same procedure. We emphasize that the calculation described earlier does not introduce any bias for precession versus a phase increase with position; thus the presence of precession of firing could be tested in interneurons and confirmed in pyramidal cells by this procedure.

All data analysis was conducted using Matlab (MathWorks). No smoothing was applied unless explicitly stated.

## RESULTS

A total of 113 cells were recorded in three rats. Only very-well isolated cells firing during spatial exploration epochs were selected for analysis. Cells were classified as either pyramidal cells \((n = 89)\) or interneurons \((n = 23)\) on the basis of their average spike width, overall firing rate, and examination of the waveform (Fig. 1C). One cell could not be unambiguously classified and was excluded. All cells were located in the CA1 pyramidal cell layer. The recording of each interneuron was accompanied by the simultaneous recording of pyramidal cells on the same tetrode. The laminar location of the tetrodes was also confirmed with postmortem histology.

Two interneurons and 30 pyramidal cells did not reach the activity threshold on the firing rate profile (see Methods) and thus were not further analyzed. Those two interneurons were atypical, in that their firing rate was rather low, but their spike width and the absence of complex spike bursts matched interneuron characteristics. Also, the firing rate of these interneurons dropped as soon as the rat started running (which correlated with a prominent theta rhythm) and thus we believe these belonged to the rare class of theta-off cells previously described (Mizumori et al., 1990).

### Spatial Profile of Activity

We first examined the cells firing as a function of position. Unlike pyramidal cells, which showed typical localized place fields, the firing of all interneurons was distributed over most of the track and peaked at very high rates (50–100 spikes per second). The level of activity was not constant, however, but varied up or down along the track. Changes in firing rate with position were reliably reproduced lap after lap, and stayed identical between epochs. Figure 1A shows the activity lap-by-lap of an interneuron during two run epochs, one composed of 12 laps and the second of 24 laps, separated by a rest period. Only the activity corresponding to the clockwise direction of movement on the track is represented. For this cell in particular, there was a large drop in activity for one specific location on the track, as well as additional modulation of firing rate at other locations, that all could be matched from one epoch to the other. This specific profile of firing did not reflect differences in occupancy of the track or changes in the speed of the animal. For the interneuron of Figure 1B recorded during a different session, the spatial profile of activity was again robustly maintained lap after lap and across epochs.

### Spatial Information

We hypothesized that the reliable modulation of firing rate with position that we observed in interneurons could potentially encode spatial information. We calculated the mutual information between position and firing rate, an indicator of how much information about location can be retrieved from knowledge of the activity level (Cover and Thomas, 1991; Dayan and Abbott, 2001). Using this measure, the spatial information for interneurons was consistently higher than for pyramidal cells (Fig. 1D). Indeed, pyramidal cells typically fire only in one restricted location, so that on most of the track their firing rate is null and does not convey any information other than “not being in the place field.” By contrast, a slowly varying firing rate (such as in Fig. 1B; spatial information = 1.3 bits) always transmits some information about location. We conclude that spatial selectivity is present and reliable in interneurons and that this information may be used by other components of the network.

### Phase-Locking to the Theta Rhythm

We recorded the local field potential in the stratum radiatum of CA1. Peaks in the filtered local field potential were detected and assigned phase 0° (see Methods). Figure 2A shows the average unfiltered local field potential after aligning all peaks for one run epoch, for both a cell layer recording and a simultaneous stratum radiatum recording. As expected, there is a large difference in absolute phase, amplitude, and shape between the two signals, and this difference depends on the animal. Also, the theta rhythm shape in the stratum radiatum was consistently skewed and exhibited an irregular rising slope.

We examined the distribution of spikes along the theta cycle. Interneurons as well as pyramidal cells showed strong phase-locking to the theta rhythm. The firing probability as a function of phase is depicted in Figure 2B for two interneurons as well as one pyramidal cell. All three showed a deep modulation that was highly reproducible from one run epoch to another, and that peaked at different phases. The distributions of mean theta phase in the two populations of cells were similar (Fig. 2C). However, in the interneuron population, there were clearly two distinct groups of cells, corresponding to two modes of the mean phase histogram (Figs. 2C,D, illustrated by the two examples in Fig. 2B). Twelve cells had a mean circular phase under –30° and nine cells over –30°. These two groups of cells did not differ in intrin-
sic firing properties (spike width, mean firing rate). Note that we cannot rule out the possibility that the bimodal shape of the histogram arose by chance from a single continuous distribution. However, given that it was reported in the literature that different types of anatomically identified interneurons found in or near the pyramidal cell layer show different phase-locking to the theta rhythm (Klausberger et al., 2003, 2004, 2005; Somogyi and Klausberger, 2005), we investigated in the rest of this study whether the two groups of interneurons that we isolated by mean phase alone exhibited differences in firing field characteristics as well.

FIGURE 1. Spatial selectivity and reproducibility of interneuron firing. A: Raster plots and linearized spatial firing rate maps for an interneuron recorded during two different run epochs separated by a rest period. Only time segments for which the animal velocity was above the threshold (see Methods) and in the clockwise direction (represented left-to-right after the linearization; black arrow) were used for these graphs. The gray histogram below the firing rate map shows occupancy, that is, the total time spent by the animal in each of the position bins (width = 5 cm) after velocity filtering. Firing rates were obtained by dividing the number of spikes in each bin by the occupancy. The black dotted curve is the instantaneous speed of the animal averaged across laps. B: Raster plot, linearized spatial firing rate map (black curve), occupancy (gray histogram), and speed of the animal (black dotted curve) for an interneuron recorded during 20 laps in the counterclockwise direction. The gray curve shows the rate map obtained for a previous 19-lap run epoch completed 1 h before. C: Left, average firing rate during run epochs as a function of spike width for interneurons (n = 21) and pyramidal cells (n = 59). For this plot, the firing rate was averaged for the entire run epochs, regardless of the velocity of the animal. The spike width was measured as the average time between the peak and the trough of the action potential, calculated on the largest waveform across the four tetrode wires. Right, example of the average waveforms recorded on the four wires of a tetrode for an interneuron (top) and a pyramidal cell (bottom). The dotted lines indicate the times of peak and trough for each cell. Only cells that reached the activity threshold on the firing rate spatial maps are included in this graph. D: Distribution of the spatial information measure for the two populations of cells. For each cell, only data from the last run epoch recorded in the session (which also was the epoch containing the largest number of laps) was used for this plot. Each cell may contribute up to two fields, one for each direction on the track. The spatial information measure was computed using ΔR = 5 spikes per second and ΔS = 5 cm. Interneurons: 42 fields from 21 cells, black histogram; Pyramidal cells: 72 fields from 59 cells, white histogram.
Phase Precession: Correlation of Position and Phase

The phase locking to the theta rhythm was further examined as a function of the location of the animal on the track. In Figure 3A, we superimposed on the local field potential the spike firing patterns for one interneuron (red ticks) and one pyramidal cell (blue ticks) recorded simultaneously. The phase precession of the pyramidal cell can be seen as a progressive advancement of short bursts of spikes or single spikes in each cycle, relative to the peaks of the theta rhythm (dotted lines). For the interneuron, the spikes are more numerous and spread out in long bursts, which makes phase precession less obvious on the raw display. We have indicated for each burst the first spike, last spike and mean spike above the spike train. Two periods of phase precession can be observed. In the first half of the display, the phase of the first spike stayed constant while the mean phase advanced. In the second half, the whole burst shifted while there were changes in firing rate as well.

We plotted the theta phase of all spikes versus position for all counterclockwise laps for the same interneuron and pyramidal cell on Figure 3B. After normalization by occupancy, the density of spikes varies both with the firing rate and the specific phase distribution for each location (third row). We analyzed the phase distribution along the track and extracted several features as a function of position: minimum phase, average phase of first and last spike in a theta cycle, and mean overall phase (fourth row of Fig. 3B; see Methods). In the case of the interneuron, the dynamics of these four parameters is complex and involves slow variations across tens of centimeters as well as sharp transitions. In particular, we frequently noticed sections of slow shifts downwards of the theta phase while the rat advanced on the track. These appear as upper-right to lower-left trends in the spike plots.
and density maps for a right-to-left direction of movement, similarly to the signature of phase precession in pyramidal cells. To detect and quantify the phase dynamics, we applied a modified procedure to evaluate correlation between theta phase and position in the interneuron data (see Methods). Specifically, we determined a set of nonoverlapping regression lines for the correlation between phase and position by considering all possible subsections of the track. Four segments were isolated for the interneuron of Figure 3B. The black line indicates the highest correlation and was the one kept for the population analysis. Note that in this example, there were three segments of phase precession and one segment of phase increase with position. Although we mainly observed phase precession, the inverse dynamics was occasionally encountered in the interneuron population and seemed to coincide with relative drops in firing rate. This inverse pattern was not specific to separate cells: for each interneuron of Figure 3B. The black line indicates the highest correlation and was the one kept for the population analysis. Note that in this example, there were three segments of phase precession and one segment of phase increase with position. Although we mainly observed phase precession, the inverse dynamics was occasionally encountered in the interneuron population and seemed to coincide with relative drops in firing rate. This inverse pattern was not specific to separate cells: for each
case of inverse dynamics, we observed periods of phase advances on other parts of the track for the same cell.

The same procedure was applied to pyramidal cells as well. An example is given on the right of Figure 3B where the best regression fit (black line) can be seen to closely follow the mean phase as a function of position.

The population results are summarized in Figure 4, where the best regression line for each "field" (activity of one cell for one direction of movement) was plotted. Pyramidal cells show a very homogeneous behavior, all lines spanning the same theta range around the peak of theta at 0° and with similar slopes. In the interneuron population, regression lines were more heterogeneous and included three cases of phase increase with position. Nonetheless, there was a significant bias for precession of theta phase with advancing position rather than for the opposite phenomenon (36 fields out of 39; \( \chi^2, P < 1.10^{-10} \)). Some regression lines spread over only a small fraction of the theta cycle and others over more than 360°, up to 410° (mean = 189° ± 132° vs. 229° ± 67° for pyramidal cells; see the 10 occurrences in eight cells of large phase advances on Fig. 4A). Also, their slopes were more distributed than for pyramidal cells (−3.6 ± 2.4 deg/cm vs. −4.9 ± 1.4 deg/cm). The distribution of the coefficient of determination \( r^2 \) shows that the correlations tended to be weaker than in the pyramidal cells (Fig. 4B). Note that in both populations, phase precession periods did not occur on any specific subsection of the track but were scattered along its length, suggesting that this phenomenon did not depend on changes in behavior. In particular, we verified that there was no systematic change in the speed of the animal during phase precession. We conclude from this analysis that interneurons do exhibit phase precession, albeit with a more variable pattern than pyramidal cells.

We reasoned that the heterogeneity of interneuron phase precession dynamics might originate from the diversity of inhibitory cells. In particular, we examined whether the mean circular phase, for which we had separated two groups of cells, was correlated with characteristics of phase dynamics. We did not find a significant correlation between the preferred phase and the slope, the \( P \)-value, or the theta range of the regression line \( (P > 0.2) \). This result suggests that phase precession is not preferentially expressed by a particular subgroup of the interneurons that we recorded from.

**Correlation of Rate and Phase**

Next, we investigated the correlation between the firing rate and the theta phase. The distribution of correlation coefficients for interneuron fields was tight and centered close to 0, with a bias towards negative values (Fig. 5A). In pyramidal cells, there was a strong negative correlation between phase and rate in the majority of the cases. Thus, although there exists a bias in the interneuron population towards a negative relationship between theta phase and rate, this bias is weaker than for pyramidal cells.

**FIGURE 4.** Correlation between phase and position. A: Regression lines of mean circular phase with position, for all fields for which a significant regression could be calculated (Top, 39 in 21 interneurons; Bottom, 57 in 49 pyramidal cells). Gray lines indicate data from the two first animals, which ran on a smaller track (Track 1) than the third animal (Track 2, black lines). B: Distribution of the coefficient of determination \( r^2 \) for the correlation of phase with position for interneurons (top) and pyramidal cells (bottom), separated in precession cases \( (r < 0) \) and inverse relationships \( (r > 0) \).
FIGURE 5. Relationships between phase, position and rate and possible mechanisms of phase dynamics generation in interneurons. A: Distribution of the correlation coefficient $r$ of phase with rate for interneurons (top, $n = 42$ fields) and pyramidal cells (bottom, $n = 72$). B: Normalized firing rate profile for the track segment where the best regression of phase with position was found. For each regression line (see Fig. 4A), the firing rate profile for the corresponding segment of the track was normalized to fit in a $[0 1] \times [0 1]$ plot, and all profiles were averaged together. We separated the segments showing precession on the left from the segments having an inverse relationship between phase and position on the right. Gray shadings: ± standard error of mean (SEM). On the right, there were not enough cases in the interneuron population to calculate averages; thus we plotted the three individual curves. There were no cases at all for the pyramidal cells population. C: Interspike interval (ISI) as a function of spike rank during a theta cycle. For each field, the times of spikes in each theta cycle were recorded (see Methods) and ISIs were averaged with respect to spike rank in the theta cycle (one gray curve per field; top, $n = 42$; bottom, $n = 72$). Each individual curve only goes as far as the largest number of spikes in one cycle for that field. The black curves indicate the mean values across the populations, truncated when the number of fields dropped below 2/3 of the total numbers. D: Influence of the intrinsic bursting characteristics of a cell on the rate-to-temporal code model of Mehta and collaborators (2002). In this model, firing occurs when the incoming excitatory input overcomes the oscillatory inhibition. The potential window for firing in a theta cycle thus widens as the excitatory input increases. For cells firing short bursts followed by refractory periods (bottom), the phase of firing advances as excitation increases (precession model for pyramidal cells). However, for cells firing long bursts such as interneurons (top), firing spreads over a larger range of theta phase as the excitation increases, thus breaking the excitation/phase correlation. E: Influence of the shape of the oscillatory inhibition on the model. The inhibitory input to a cell could assume a temporal shape different from a sinusoid, in particular, an asymmetric shape. The exact profile and skewness of the incoming inhibition is responsible for the correlation between phase and activity, and could even switch the relation from precession (top) to an inverse relation (bottom).
To investigate further the relation between phase, position, and rate, we examined the profiles of firing rate versus position found during the segments of phase precession determined as above. For the interneuron of Figure 3B, phase precession periods were accompanied by local peaks in firing rate. This is similar to pyramidal cells for which phase precession occurs mostly when the firing rate increases while entering the place field (see the pyramidal cell example of Fig. 3B, right). The activity profiles were averaged for all phase/position regression lines showing a negative (left) and a positive (right) slope (Fig. 5B, top). If there were no particular relationship between precession and rate, we would expect to find flat curves. However, the mean firing rate profiles showed a pronounced peak both for clockwise and counterclockwise laps. These bell-shaped profiles had a similar shape than the profiles averaged for pyramidal cells (Fig. 5B, bottom). The smaller amplitude reflects the fact that phase precession segments were less homogeneous in the interneuron population than in the pyramidal cells population. Note that in the three cases of phase increase with position, the firing rate profile showed a local drop in activity.

**Modified Models for the Generation of Phase Precession in Interneurons**

We reasoned that interneurons might fundamentally follow the same simple model of phase precession as pyramidal cells, but show different precession characteristics (strength, correlation with rate) because of the details of their own set of excitatory and inhibitory inputs and of their intrinsic properties (input–output relationship of the cell, bursting features).

In particular, we calculated the average interspike intervals (ISIs) as a function of spike rank inside theta cycles (Fig. 5C). Interneurons exhibited long bursts with increasingly shorter ISIs, that is, spikes were closer and closer in time towards the end of the burst compared to the beginning, and the firing was spread over a long fraction of the theta cycle (see also Fig. 3A). By contrast, there were fewer spikes in each cycle for pyramidal cells; ISIs were short and tended to get slightly longer along the burst.

We examined how these different firing patterns could influence the generation of phase precession. In Figure 5D, we represented the model of Mehta and collaborators (2002), in which a cell emits spikes only when the incoming excitatory input exceeds the theta-locked oscillatory inhibition. For cells which fire short bursts such as pyramidal cells (bottom), this translates into phase precession in the input/phase domain. For cells which fire long patterns throughout the theta cycle (top), the model predicts that the phase range increases with increasing input, but without showing an asymmetry around a fixed mean phase. More precisely, the exact profile of the burst will bias the plot towards slightly increasing or decreasing phases, but the resulting correlation will be weaker than in the pyramidal cells case. In view of our results on the burst patterns (Fig. 5C), we conclude that the model predicts a looser relationship between phase and excitatory input for interneurons than for pyramidal cells.

Another parameter that can influence the dynamics of the spikes phases is the precise shape of the incoming oscillatory inhibition. The simplest model assumes a sinusoidal waveform for the strength of the inhibitory inputs arriving on any cell (as in Fig. 5D). However, there is evidence from the shape of the local field potential, and from the interneurons phase-locking histograms (Fig. 2), that the level of inhibition does not follow a purely sinusoidal time course. It is difficult to predict for a given cell what this pattern might be and it is probably different from one cell to another (for example, for the different types of interneurons observed in Fig. 2). Figure 5E shows that, depending on the skewness of the oscillatory inhibition, the phase/input relationship can switch from a precession pattern (top) to an inverse relationship (bottom). Note that when applied to the short burst pattern of pyramidal cells, these different inhibitory input shapes would only affect the slope of precession but not eliminate precession altogether.

It should be kept in mind that the model only predicts precession of phase as a function of the level of input. Our extracellular measurements do not provide direct access to the excitatory input to the cell but only to the spike output. If we assume a monotonic relationship between the excitatory input and the firing rate, we infer that the correlation between phase and firing rate should follow qualitatively the correlation between phase and input.

Even in the framework of a simple phase precession model, these considerations indicate that the precise features of the inputs and of the intrinsic neuronal mechanisms are likely to have a large influence on phase precession. They suggest that the phase dynamics observed in interneurons result from a combination of intrinsic properties and basic integration mechanisms, in the same way that phase precession in pyramidal cells is generated.

**Discussion**

**Spatial Selectivity of CA1 Interneurons**

The first finding of this study is that the firing rate of CA1 interneurons is modulated by location in a reliable and stable manner. Previous report of spatial selectivity in interneurons suffered from the difficulty of assessing location-specific firing independently of the particularities of behavior at different locations (Kubie et al., 1990). Indeed, the authors concluded that they could not reject the idea that some unidentified behavioral state creates the observable firing pattern. To restrain the behavioral repertoire expressed during the experiment, we used a linear circular track on which the rat ran back and forth repeatedly for food. Examination of the position and velocity as a function of time, as well as the occupancy histograms on the track, revealed a highly stereotyped behavior that could probably never be matched in an open-field enclosure (Kubie et al., 1990; Wilson and McNaughton, 1993). Thus, the linear maze running task might be optimally designed to assess spatial selectivity. Interneurons recorded simultaneously displayed firing rate profiles that varied over the whole track and with no distinct relation to each other or to the occupancy histogram. Not surprisingly,
The use of the mutual information measure implicitly assumes that a downstream cell can estimate firing rates, in other words, integrate spikes over a certain amount of time. In our calculation, we used a precision of 5 spikes per second for the firing rates, which would likely be integrating the activity from many neurons so that the required integration window could be greatly reduced and even may be compatible with fast postsynaptic potentials. Note that a precision of 5 spikes per second is also within the range of the variability in firing rate observed either on different trials or on different run epochs. Moreover, it corresponds to integration over roughly two cycles of the theta rhythm, that is, a period long enough so that the firing rate can be estimated independently of the temporal grouping of spikes due to phase-locking to the theta rhythm.

Phase Locking to the Theta Rhythm and Heterogeneity of Interneurons

In agreement with previous studies, we describe a tight phase-locking of interneurons to the theta rhythm (Csicsvari et al., 1999; Klausberger et al., 2003, 2004, 2005; Somogyi and Klausberger, 2005). One subpopulation of interneurons fired on average at the peak of the extracellularly recorded local field potential, in synchrony with the pyramidal cells. They could correspond to the bistratified cells of Klausberger et al. (2004). The second subpopulation fired a few tens of milliseconds earlier, and is probably mainly composed of parvalbumin-positive basket cells (Klausberger et al., 2003; Somogyi and Klausberger, 2005). We looked at the activity of both groups during sharp wave-ripple events in sleep epochs and found that all cells except one showed an increase in activity at the peak of the ripple (data not shown). This observation fits with the finding of an increased firing rate during ripples of basket and bistratified cells reported by these authors, providing more evidence that we recorded indeed from these particular cell types.

As suggested by previous studies, the basket cell population could synchronize pyramidal cells by resetting their activity through the rebound following an induced IPSP (Cobb et al., 1995; Somogyi and Klausberger, 2005). The reported delay is such that this rebound should occur at the peak of the theta wave, when pyramidal cells fire most. At the same time, the bistratified cells, terminating on more distal dendrites, would decouple the pyramidal cell soma from the entorhinal input and thus favor the influence of the Schaffer collaterals for that phase. Interestingly, it has been suggested that the alternating dominance of entorhinal and CA3 inputs on the CA1 pyramidal cells in the theta rhythm functions as a switch between encoding and retrieval states of the hippocampal network (Hasselmo et al., 2002). Phase locking to the theta rhythm could thus be at the core of the mechanisms of learning and memory. In this framework, the different types of interneurons would have a critical role in orchestrating the integration of information inside each hippocampal theta cycle.

We investigated whether our two subpopulations of interneurons exhibited different forms of spatial selectivity. The distributions of the spatial information measure were similar in the two groups, and neurons of both types had smooth deeply modulated spatial firing profiles. This observation suggests that despite the anatomical and physiological heterogeneity of inhibitory cells, all subclasses may be involved in the processing of spatial informa-
...tion. However, we have only indirect evidence through the mean circular phase distribution that we were recording from different types of interneurons. It would be interesting in future studies to determine anatomically the cell type in order to establish exactly how spatial selectivity is distributed in the interneuron population.

Phase Precession of CA1 Interneurons

We extend these findings by demonstrating that the phase locking of interneurons to the theta rhythm is not fixed but dynamically modulated by spatial location. The mean circular phase showed periods of precession as the rat ran on a linear track, particularly on subsections of the track where the firing rate of the interneuron displayed a transient increase with position.

The characteristics of phase precession in interneurons were clearly more heterogeneous than for the pyramidal cells population. Notably, the phase advance in interneurons spread over intervals of variable width and centered at different phases of the theta cycle. In 10 cases, we observed phase advances exceeding 360°. This finding could be interpreted in two ways. One possibility is that, contrary to pyramidal cells, for which the phase precession interval seems to be always restricted to one cycle, interneuron firing can effectively precess continuously over more than 360°. This is however difficult to reconcile with simple models of phase precession generation (Kamondi et al., 1998; Magee, 2001; Harris et al., 2002; Mehta et al., 2002). These models predict that even if the excitatory drive increased dramatically, the phase should not advance by more than 360°, but rather spikes should spread over the whole theta cycle.

Alternatively, large phase advances could arise as a result of the juxtaposition of two periods of phase precession, each restricted to 360°. Indeed, such a phenomenon has recently been described in the CA1 pyramidal cells, whereby close or overlapping subfields of one cell give rise to a compound field exhibiting multiple cycles of phase precession (Maurer et al., 2006). Analogously, the interneuron firing field can be thought of as a series of close or overlapping subfields, each corresponding to a transient increase in firing above a baseline. In this scheme, the large phase advances would coincide with the superposition of two or more close peaks in activity, which could add to many different profiles depending on the degree of overlap of the subfields. Indeed, the firing rate curves observed for the cases of large phase advances were highly variable, whereas those of the small phase advances often exhibited a local increase of activity. Interestingly, this multifield model also provides an explanation for the few cases of phase decrease with position, in which the spikes from the end of one subfield were presumably associated with the spikes from the beginning of the next subfield. The apparent drop in firing rate seen is those cases is compatible with the hypothesis of a transition period between subfields. Thus, it might be interesting to develop a cluster cutting technique in the phase/position space to describe interneuronal activity, such as that used by Maurer and collaborators on the pyramidal cells (Maurer et al., 2006).

Generation of Spatial Selectivity and Phase Dynamics in Interneurons

The modulated spatial profiles and phase dynamics of interneurons may originate from different sources. The simplest explanation is that they may reflect directly the summation of incoming inputs. Interestingly, some cells in the entorhinal cortex (EC) are now known to express both a spatial firing pattern in the form of a two-dimensional grid (Hafting et al., 2005), and phase precession (Hafting et al., 2006). If the EC indeed directly drives the spatial and temporal characteristics of CA1 cells, including via a possible CA3 relay, several situations can be considered.

First, the firing properties of hippocampal cells could arise initially in the pyramidal cells and then be reflected in the interneurons activity downstream. For example, a typical basket cell may receive input from a few hundred of pyramidal cells (Schwartzkroin and Kunkel, 1985; Halasy et al., 1996). Given the large variance in firing of each individual excitatory cell, some spatial selectivity could be retained when adding all inputs together, even for a random wiring. Also, if we suppose that the subset of presynaptic pyramidal cells active at each location fire around the same phase relative to the theta rhythm, phase-locking and phase precession in the interneuron might simply follow from their presence in the intrahippocampal excitatory input. At present, too little information is known on the inputs to a given interneuron to evaluate what their superposition would really look like. Further, the integration time course in the recipient interneuron might smooth out most of the fine temporal tuning. In this perspective, intracellular recordings during spatial exploration such as developed by Brecht and colleagues will undoubtedly yield fascinating data on the incoming inputs and ongoing computation in each cell type (Lee et al., 2006).

The second possibility, mirroring the first one, postulates that the input from the EC entirely shapes the properties of the interneurons, and that the place fields of the pyramidal cells derive from the properties of their inhibitory inputs. In favor of this idea, basket cells do receive profuse innervation from extra-hippocampal inputs, and their divergent projection to ~1,000 pyramidal cells has been described in detail (Schwartzkroin and Kunkel, 1985; Halasy et al., 1996). Each pyramidal cell is thus contacted by a few tens of basket cells (Halasy et al., 1996). A place field could originate in the disinhibition of a pyramidal cell at a particular location where the ~20 presynaptic basket cells happen to be less active.

Finally, firing properties could develop jointly in both populations, involving recurrent loops between them. For example, the entorhinal projection from grid cells could interact with the hippocampal local inhibition, so that starting from the periodic entorhinal input, a single peak of excitation wins over in a given pyramidal cell and yields a place field.

However, an alternative hypothesis to this class of models has to be considered, in which the firing properties of cells emerge, or re-emerge, in CA1 through the interaction between excitatory and inhibitory inputs and the cells intrinsic properties. In this scheme, the spatial and temporal structure present in the inputs
would be smoothed out by the summation of many differently tuned afferents in a postsynaptic cell, especially given the long time course of some postsynaptic potentials (Connors et al., 1988; Forsythe and Westbrook, 1988). This is especially relevant to phase-locking and phase precession. Although the excitatory inputs to CA1 exhibit fine temporal precision, it is unclear to what degree this could be preserved in a downstream cell. More concretely, unless a basket cell receives inputs exclusively from pyramidal cells that have highly colocalized place fields, it is likely that for a given location, the phase of spikes coming from different place cells will be scattered over the theta cycle. Phase-locking and phase precession might be lost altogether when integrating all the excitatory postsynaptic potentials.

Thus, in this second hypothesis, it is interesting to consider how phase precession could arise in interneurons from poorly tuned inputs. In particular, we wish to evaluate whether simple mechanisms put forward for pyramidal cells could also account for the generation of phase precession in interneurons. If this were the case, it would provide strong support to these mechanisms and would also suggest that phase precession might in fact be expressed in all cell types, as long as an interaction between an oscillatory activity and a changing excitatory input is taking place.

We re-examined a simple model of the generation of phase precession (Mehta et al., 2002). Briefly, the model predicts that phase precession occurs when the activity of the cell increases in the presence of the theta rhythm. Indeed, as in pyramidal cells, phase precession in interneurons was coupled with simultaneous dynamics of the firing rate profile. We explored how the particular characteristics of interneurons could explain the differences in precession that we observed. We found that the long sustained bursts of spikes of the inhibitory cells during theta cycles could explain the wider scatter and weaker phase precession than in the pyramidal cells case. Also, the specific oscillatory shape of the inhibitory input to a cell may be responsible for the direction and slope of the mean phase dynamics. Given the diversity of inhibitory cells and the complexity of the connectivity between them, it should not be surprising that phase precession in these cells displays heterogeneous profiles. Rather, the fact that we found both spatial selectivity and phase precession in our two subpopulations of interneurons argues for a general mechanism underlying this phenomenon regardless of cell type.

Other characteristics of this model are that it depends on the asymmetry in the firing field, which has been shown to develop gradually for place cells during exploration (Mehta et al., 1997, 2000). It would be interesting to determine whether in interneurons, phase precession is present from the beginning of exploration or if it forms progressively over several laps, as for pyramidal cells (Mehta et al., 2002), and whether one can detect in parallel the development of an asymmetry in the firing field.

Interestingly, theta phase precession has recently been described outside of the hippocampus, in prefrontal cortex (Jones and Wilson, 2005a). Although it has not been reported yet, some form of precession may also exist in structures where phase locking to the hippocampal theta rhythm has been observed, such as amygdala (Pare and Gaudreau, 1996), striatum (Berke et al., 2004) and visual cortex (Lee et al., 2005). In those studies, the lack of a position correlate makes it necessary to look for phase advances in time by other means, which might prove more difficult than in a simple linear spatial exploration task. Coupling of preferred phase changes to rate changes, if it was demonstrated in those instances, would further validate a generalized model of how the integration of excitatory and inhibitory inputs may lead to the synchronization of firing within the oscillatory theta rhythm.

### References


