Hippocampal Network Dynamics Constrain the Time Lag between Pyramidal Cells across Modified Environments

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The hippocampus provides a spatial map of the environment. Changes in the environment alter the firing patterns of hippocampal neurons, but are presumably constrained by elements of the network dynamics. We compared the neural activity in CA1 and CA3 regions of the hippocampus in rats running for water reward on a linear track, before and after the track length was shortened. A fraction of cells lost their place fields and new sets of cells with fields emerged, indicating distinct representation of the two tracks. Cells active in both environments shifted their place fields in a location-dependent manner, most notably at the beginning and the end of the track. Furthermore, peak firing rates and place-field sizes decreased, whereas place-field overlap and coactivity increased. Power in the theta-frequency band of the local field potentials also decreased in both CA1 and CA3, along with the coherence between the two structures. In contrast, the theta-scale (0–150 ms) time lags between cell pairs, representing distances on the tracks, were conserved, and the activity of the inhibitory neuron population was maintained across environments. We interpret these observations as reflecting the freedoms and constraints of the hippocampal network dynamics. The freedoms permit the necessary flexibility for the network to distinctly represent unique patterns, whereas the dynamics constrain the speed at which activity propagates between the cell assemblies representing the patterns.

Key words: theta rhythm; temporal coding; place cells; plasticity; phase shift; hippocampus; synaptic communication; synchrony; spatial memory; stability

Introduction

The propagation speed of neuronal information in the brain depends on numerous factors. Axonal conduction speeds, the synaptic strength of neuronal connections, the firing rate and synchrony of presynaptic assemblies, and the often oscillatory balance between global excitation and inhibition each affect the ability of leading cell assemblies to influence postsynaptic spiking patterns in trailing assemblies. Some of these variables, such as the state of inhibition, can change instantaneously, whereas others, such as synaptic strength, may require prolonged exposure of the network to the same conditions. Nevertheless, it is not well understood which parameters of neuronal activity change robustly in response to environmental perturbations and which ones remain relatively constant due to the constraints of network dynamics. We addressed these questions by simultaneously monitoring large numbers of neurons in the hippocampus after a modification of the testing conditions.

Hippocampal neurons in the rat fire based on the animal's location and direction of movement (O'Keefe and Dostrovsky, 1971; McNaughton et al., 1983). The hippocampal spatial code is robust and the network generates spike trains that can reliably

decode the rat's current position and trajectory in a given environment (Wilson and McNaughton, 1993; Brown et al., 1998; Zhang et al., 1998; Jensen and Lisman, 2000). In a single neuron, when the rat passes through its place field, spikes are fired at progressively earlier and earlier phases of the ongoing theta local field potential (O'Keefe and Recce, 1993). For cell pairs with overlapping place fields, the temporal structure of spike trains within a theta cycle reflects the distance between the place-field centers and their sequential activation during the run (Skaggs et al., 1996; Dragoi and Buzsáki, 2006); larger distances are associated with larger temporal offsets within the theta cycle, a phenomenon now called "sequence compression." Such precise temporal relationships are considered to play a fundamental role in hippocampal function during episodic memory formation and sequence learning (Jensen and Lisman, 1996; Redish and Touretzky, 1998; Wagatsuma and Yamaguchi, 2004). A large number of studies document that changing local and distant environmental cues or alterations of emotional or contextual cues strongly affect the firing of individual neurons (Muller and Kubie, 1987; O'Keefe and Speakman, 1987; Gothard et al., 1996a,b; O'Keefe and Burgess, 1996; Lever et al., 2002; Battaglia et al., 2004; Wills et al., 2005). However, it is less known how the ensuing firing pattern changes of single neurons affect their interactions and the state of the network in which they are embedded. To this end, we analyzed the place fields and neural activity recorded from the CA1 and CA3 regions of the hippocampus in rats running on a long elevated track, and compared these to data after the length of the track was shortened (Gothard et al., 1996b).

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Figure 1. Place fields on a linear track with modified lengths. CA1 (**a**) and CA3 (**b**) place fields are ordered for a rat running left to right on a linear track before and after the length is shortened. Many fields vanished and others shifted to new locations on the short track. Others fired only on the short track. **c**, Histograms indicate preferred firing locations for the long (top) and short (bottom) tracks. The white portions of the histograms represent cells that fired only in that environment. The dark (black for long, red for short) portions of the histograms represent cells that had place fields in both environments. **d**, The peak firing rates (left) were lower and field-sizes (right) were smaller on the short track than on the long track. Colors correspond to cells shown in **e**: the positions of place fields on the long and short tracks are suggestive of four general categories (shown in black, green, blue, and cyan; see Results). The diagonal, corresponding to a rescaling, is plotted in magenta, and the identity is plotted in orange. **f**, The amount of place-field shifting was related to the place-field location on the long track. Fields at the beginning (green) shifted out toward the center, whereas fields at the end (blue) shifted in toward the center. The orange line indicates no shift (identity). **g**, The population firing rates increased from the long to the short track for pyramidal cells (top) but were conserved for interneurons (middle). The cofiring among the pyramidal population (bottom) also increased from the long to the short track.

Materials and Methods

We trained three male Sprague Dawley rats (335–400 g) to run back and forth on a linear track (6.2 cm width) for water reward at both ends (end platforms $22 \times 22 \text{ cm}^2$). After learning the task, the rats were implanted with 32- and/or 64-site silicon probes in the left dorsal hippocampus under isoflurane anesthesia. The silicon probes, consisting of four or eight individual shanks (spaced $200 \mu\text{m}$ apart) each with eight staggered recording sites ($20 \mu\text{m}$ spacing) (Csicsvari et al., 2003), were lowered to CA1 and CA3 pyramidal cell layers (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). After recovery from surgery (~ 1 week) we tested the animals again on the track. Tracks were shortened by moving both platforms in full view of the rat, where it was resting or grooming on a platform after reward. All protocols were approved by the Institutional Animal Care and Use Committee of Rutgers University. We continuously recorded all channels at 32,552 Hz over the following 7–10 d with a 128-channel DigitalLynx recording system. After recording, we obtained the local field potential (LFP) from each channel by low-pass filtering up to 1252 Hz. We high-pass filtered the data (0.8–16 kHz), and thresholded for spike detection (Hazan et al., 2006). For each putative spike, we sampled 54 data points at each of the 8

recording sites on the shank, centered on the maximum spike amplitude (supplemental Fig. S2, available at www.jneurosci.org as supplemental material). Based on the resulting set of 54 dimensional vectors, we calculated three principal components for each recording site. We clustered these 3×8 principal components using previously described methods, based on the Mahalanobis distance and autocorrelations within the refractory period of clusters (Harris et al., 2000; Hazan et al., 2006). We separated pyramidal cells and interneurons on the basis of their autocorrelations, waveforms and mean firing rates (Csicsvari et al., 1999).

One-dimensional place fields were constructed from the two-dimensional place fields (in turn constructed from occupancy and spike maps for 1.44 cm^2 bins) by projecting onto the track axis. An eight point smoothing filter was subsequently applied. The position was tracked with an LED. Trials were marked by onset and end of motion in a trajectory that crossed the track. Spikes otherwise fired on the platform ends were discarded. We considered only one field per cell: the field with the maximum peak firing rate. The preferred firing location was the position of the peak rate. Consistent results were obtained by repeating all calculations using centers-of-mass for the preferred firing locations. Well isolated pyramidal cells with stable place fields of peak

≥ 2 Hz on the track (on either or both of the left to right, and the right to left trajectories) were used in subsequent analysis. This rate threshold was used so that place fields whose firing rates were dramatically reduced on the short track would not be excluded from the analysis. Higher thresholds, however, yielded consistent results. We obtained 292, 1207, and 583 fields from rats 1, 2 and 3 respectively, in 12, 33, and 9 sessions (from 7 to 58, median = 23, recorded place cells per session). The maze was rotated 90° between alternate sessions (from 1 to 4, median = 2 sessions per day; the position of the track remained the same for comparing runs on long and short tracks). Some cells may have contributed in more than one session, but electrodes were typically advanced between sessions in the same configuration. Session firing rates on the track were determined by dividing the total number of spikes by the total amount of time spent running. Cofiring rates were calculated from the average the number of cells active per second in 120 ms bins.

Spikes fired within a boundary defined by 10% of the peak firing rate, and when the running speed was > 2 cm/s, were used to calculate the cross-correlations in 1 and 5 ms time bins for time lags of up to 2.5 s (supplemental Fig. S3, available at www.jneurosci.org as supplemental material). These were subsequently bandpass-filtered between 2 Hz and 30 Hz. The time lags of the peaks were determined as follows: we first found the local maxima from the bandpass (2–30 Hz) filtered 1-ms-binned cross-correlogram (CCG). The time lag on the long track was determined by the shortest time lag in the direction of the largest peak (i.e., the behavioral time scale lag). To correct for a potential noise-thresholding effect around zero, if the time lag in the alternate direction was nearer the best-fit line of the sequence compression relationship, this value was used instead. The algorithm next identified the nearest CCG peak on the short track, unless a peak with a shorter time lag was present, in which case the latter peak was chosen. This method avoided missed time lag preservation across conditions, although the statistics were still robust with more error prone methods. To evaluate the size of the peak in the CCG, we used the number of counts in the 5-ms-binned CCG (which is less vulnerable to noise jitter) at the relevant time lag. For all analysis involving time lags, we used only data where the CCG peaks were > 5 counts, and there was a clear dip to at least half the CCG peak within 75 ms (i.e., cell pairs that demonstrated a degree of theta modulation). Of 16,587 possible cell pairs on the long track (1456, 7446, and 7685 from rats 1, 2, and 3), 3352 (302, 1540 and 1510) displayed enough overlap and theta-modulation to contribute to the analysis. Of 12,309 possible cell pairs on the short track, 3020 contributed, with 1218 cell pairs contributing in both long and short tracks.

Theta-band calculations were performed on the local field potential signal from the electrode judged nearest the pyramidal cell layer, in turn from the shank that yielded the most units. Signals were whitened and the spectrum was analyzed with multitaper techniques (Mitra and Pesaran, 1999) using 0.8 s windows with 50% overlap. Theta power was taken as the mean value in bins corresponding to frequencies 4–12 Hz.

The equidistant dataset was created to simulate cell pairs that shift physiological distances without altering their sequence compression. We shuffled cell identities in the long and short track independently among cell pairs that were the same distance apart, to within 1.5 cm. We repeated this shuffling procedure 5000 times to create a surrogate dataset.

Figure 2. The average profiles (across all sessions and animals) of different variables are calculated for the long (black) and short (red) tracks. *a*, Significant differences were observed for the population firing rates of pyramidal cells, which cannot be accounted for by the different running speed profiles (*d*, *e*). *b*, The interneuron firing rates were not systematically different. *c*, The spiking profiles were similar between the long and short track, with higher occupancy at the beginning of the track. *d*, The running speed profiles reflect a higher peak running speed in the middle of the short track. *e*, Firing rate is plotted against running speed for each position pixel on the track (see also supplemental Fig. S5, available at www.jneurosci.org as supplemental material). The best-fit lines are also shown. The higher firing rates on the short track cannot be explained by running speed. *f*, The theta-frequency increases by a small (0.6%) but significant (rank-sum, $p < 10^{-20}$) amount from the long to the short track. The local field potential theta power decreased in both CA1 (*g*) and CA3 (*h*) on the short track. *i*, Coherence in the theta-frequency band also decreased between the two structures (see also supplemental Fig. S7, available at www.jneurosci.org as supplemental material).

Results

Initially, rats ran back and forth on an elevated track (170 cm length) between two platforms (22 × 22 cm) for water reward at each platform. Each running trajectory (left to right vs right to left) was treated separately. CA1 and CA3 neurons fired with fields tuned to locations along the track (Fig. 1*a,b*). In all figures, fields were ordered from left to right corresponding to leaving one platform (at the left) and approaching the opposing platform (at the right). No intrahippocampal regional differences could be seen for the measures we calculated (supplemental Fig. S4, available at www.jneurosci.org as supplemental material), so all data are hereby pooled. After ~20 trials, we shortened the track (to 100 cm) by sliding in the platform ends from both sides. Across 54 sessions, the preferred firing locations, as measured by the peak firing rates (mean = 15.0 Hz), of 1750 (1062 CA1 and 688 CA3) recorded place fields covered the extent of the track (Fig. 1*c*). After shortening the track, 603 place fields vanished, whereas 1147 others maintained fields on the short track (Gothard et al., 1996a). The peak firing rates of the persisting subset of fields were significantly lower (Fig. 1*d*), from a mean peak of 16.4 Hz on the long track to 13.2 Hz on the short track (20% decrease; paired *t* test, $p < 10^{-3}$). Although running speed can affect the peak firing rate, mean in-field running speeds in this population were not significantly different on the two tracks (mean 53.1 cm/s long, 50.3 cm/s short, paired *t* test $p = 0.6$; but see also supplemental Fig. S5, available at www.jneurosci.org as supplemental material).

Figure 3. Sequence compression. **a**, The theta-scale time lag was correlated with the distance between the preferred place-field firing locations for both the long (black) and short (red) track lengths. All cell pairs on each track were included. The error bars denote the large SDs within each distance bin. These relationships are well described by sigmoids. **b**, Several examples from panel **a** are shown, with the reference (blue) and overlapping (green) place fields shown in the left columns, and theta-scale time lags shown in the right.

Figure 4. Theta-scale timing was preserved between long and short tracks. **a**, The timing on the short track was identically (gray line) related to the timing on the long track. **b**, Cell-pair distances were also correlated between the long and short tracks, with deviation from the identity most noticeable at large pair-distances on the long track. **c**, When the pairs were shuffled between equidistant pairs, the correlation in theta-scale timing was considerably weakened.

The sizes of the place fields, as measured by the portion of the track with >2 Hz firing (or by full-width at half-maximum in supplemental Fig. S5, available at www.jneurosci.org as supplemental material), also decreased significantly from a mean of 52 to 42 cm (19% decrease; rank-sum $p < 10^{-12}$) (Fig. 1*d*), again with great variability. An additional 332 (183 CA1 and 149 CA3) place fields fired only on the shortened track.

Among the cells that fired in both environments, there were some general patterns in the displacements of the preferred (peak) firing locations (Fig. 1*a,b,e,f*). We performed clustering on the positions and place-field displacements to delineate the following categories: place fields at the beginning of the track tended to shift out toward the center (green), those in the middle did not shift or barely shifted (black), and those at the end of the track shifted in toward the center (blue). A small fraction of fields, (3.4%; cyan) which might be an underestimate, appeared to deviate from this general behavior, likely because they completely remapped between the long and the short track. This view is further supported by a comparison of the population vectors in supplemental Figure S6, available at www.jneurosci.org as supplemental material (Gothard et al., 1996*a*).

In summary, between the long and the short environments, there was generalized representation as well as distinct representation. Place fields in the generalized representation persisted on both the long and short tracks: neurons moved their place fields in conjunction with the shifting reward platforms, consistent with previous results (Gothard et al., 1996*a*; Redish et al., 2000; Maurer et al., 2006). We simultaneously found place cells that distinguished between the two configurations of the track; some fired only on the long or the short track, whereas others rate and/or location remapped between the two configurations (Leutgeb et al., 2005*b*). In all, we recorded 1479 (903 CA1 and 576 CA3), persisting and new, place fields firing on the short track, with peak firing rates at a mean of 13.3 Hz.

Despite the 15% fewer place fields on the short track, the overall representation was in fact more spatially “focused,” with 10.3 recorded neurons/cm, compared with 8.2 recorded neurons/cm on the long track (26% increase; binomial $p < 10^{-14}$), summed across all sessions. As a result, the spatial overlap, expressed as a percentage of the place-field spatial tuning curves, increased as well from 48% to 56%, (rank-sum, $p < 10^{-150}$). Coactivity, defined as the number of cells active within 120 ms (~ 1 theta cycle), likewise increased (34%, t test, $p < 10^{-6}$) (Fig. 1*g*, bottom). The increased spatial overlap, and consequent increased temporal overlap of neuronal spikes, compensated for the decreasing peak firing rates, and resulted in a net increase in the global firing rate for the pyramidal cell population; the mean firing rates of the sum of all active cells on the track were 24% higher upon shortening the track (t test, $p < 10^{-5}$) (Fig. 1*g*, top). The interneuron population, however, maintained the same level of activity across the testing conditions and showed no significant change in global firing rate (t test, $p = 0.6$) (Fig. 1*g*, middle). To examine how these variables changed over the extent of a run, we calculated their firing rate profiles in spatial bins along the track

Figure 5. Theta time scale relationship of neuron pairs remained stable after track length change. Each panel (*a–d*) depicts a reference place field (blue) and overlapping place field (green) on the long (top, black) and short (bottom, red) track. Left to right, The first column depicts the firing rates versus position. The second column illustrates the phase and position of each spike in the place fields. The third column plots the unfiltered cross-correlogram between the reference and related cell, with the time lag of the peak from the bandpass-filtered CCG indicated by the arrow. Note that the time lag of the peak changed very slightly for the cell pairs, even though the firing rate maps changed substantially. The fourth column depicts the smoothed cross-correlograms on a 1 s long behavioral time scale.

(Fig. 2*a–c*). The pyramidal firing rate (Fig. 2*a*) was higher in all portions of the short track, independent of the speed profile (Fig. 2*d,e*), whereas the interneuronal firing rate was not systematically different. The environmental change resulted in a lower theta power in the local field potential measured in both CA1 and CA3 (Fig. 2*f–i*) and a lower theta-band coherence between the two structures on the short track, indicating that firing rate, running speed, and theta power may vary orthogonally. These results were also confirmed by within session comparisons (supplemental Fig. S7, available at www.jneurosci.org as supplemental material).

We investigated additional firing characteristics of the cells for systematic changes between the track length changes. The phase-precession slopes of single neurons were not systematically different for the tracks (data not shown). Because the phase-precession slope is potentially noisy and vulnerable to outliers, we also calculated the oscillation frequency of the autocorrelogram and, a related measure, the time-offset of the first peak in the autocorrelogram. Despite expectations from the field size-slope relationship (Huxter et al., 2003), the oscillation frequencies were in fact slightly lower on the short track (8.59 vs 8.73 Hz on long; *t*

Figure 6. The time lag between pairs after large distance changes. **a**, The top panel shows color-coded lines connecting the same pairs on the long and short track. The color code is based on the time slopes (histogram shown on the bottom panel). Most cell pairs showed very little change in the theta-scale time lag (as reflected by the horizontal lines), resulting in different sequence compression on the long versus short tracks for this subset (inset). Similar analysis performed on surrogate equidistant datasets (black) indicates an expected peak at a slope of 1.0 ms/cm (bottom). The two slope distributions were significantly different (Kolmogorov–Smirnov test, $p < 10^{-14}$). **b**, In a shuffled equidistant dataset, the timing preservation vanished. The histogram (bottom) follows the expected distribution.

test, $p < 10^{-4}$), consistent, instead, with the decreased peak firing rates of the neurons on the short track (Maurer et al., 2006; Geisler et al., 2007). In summary, virtually all examined firing properties of single pyramidal neurons, including firing rate, field size and preferred firing location, changed across the two environments, whereas inhibitory firing rates remained relatively stable.

We next examined the temporal dynamics of neuronal spikes in the two environments, by comparing the spike correlations between cell pairs. As in previous reports (Skaggs et al., 1996; Dragoi and Buzsáki, 2006; Geisler et al., 2007), the theta-scale time lag and the distance between the preferred locations of cell pairs were correlated on both the long and short tracks ($r = 0.75$ long; $r = 0.67$ short) (Fig. 3). However, we uncovered nonlinearity in the sequence compression for larger distances and found that a sigmoidal fit was a suitable descriptor. This relationship was unchanged if we considered CA1 or CA3 cell pairs alone (supplemental Fig. S4, available at www.jneurosci.org as supplemental material). Unexpectedly, we also found a strong correlation between the time lags of the same neuron pairs in the long and short track ($r = 0.81$) (Fig. 4a). This provided a direct indication that the theta-scale timing of neurons remains stable across environments. However, distances between cell pairs varied, with most cell pairs unchanged, yet an important fraction altered (Fig. 4b).

Therefore, we first considered the possibility that the lack of change in pair distances, along with sequence compression, would be enough to explain the preserved timing. We created an “equidistant” dataset by randomly shuffling the time lags for each pair, on the long and short tracks separately, with the time lag from all other pairs ≤ 1 distance bin away (see Materials and Methods). Although in this equidistant dataset the sequence compression correlations between time lag and distance were mostly unaffected ($r = 0.76$ long; $r = 0.63$ short), the correlation between the time lags across tracks was noticeably weakened ($r = 0.39$) (Fig. 4c), suggesting that the time lags are not solely determined by the distances between place fields or by a simple common phase mechanism, driven by hippocampal theta, that trans-

lates distance into time lag, but are instead controlled by neuronal interactions (see Discussion). We focused next on the subset of the cell pairs where the distance between the preferred firing locations changed most significantly (i.e., decreased by >25 cm) between the long and short representations (other thresholds yielded consistent results). Inspection confirmed that most of these pairs (84 of 142) were typically composed of one cell from the beginning or end of the track and another in the middle or opposite end, i.e., cells representing different reference frames (Gothard et al., 1996a; Redish et al., 2000). In many cases, the place fields changed considerably, yet timing within the theta cycle, as reflected in the cross-correlograms, was unaltered (Fig. 5). Overall, we observed a significant number of instances where the time lag was unchanged (near zero sequence compression slope) (Fig. 6a,b), compared with the equidistant distribution (Fig. 6a, bottom). Although the number of cell pairs with large distance shifts represented a small segment

of the dataset (142 of 1218 pairs), the preservation of the time lag apparently resulted in different sequence compression behavior among the relevant cells. To test the robustness of the time lag, we also compared time lags from the first half of trials to those from the second half, in sessions on the short track, and between trials with fast and slow running speeds (supplemental Fig. S8, available at www.jneurosci.org as supplemental material). We did not observe a difference in either instance (see also Geisler et al., 2007), indicating that the theta-scale time lag indeed represents a fundamental property of the circuitry.

Discussion

Our findings show that hippocampal network activity adjusts to task conditions. Changing the size and geometry of the testing environment has a profound effect on the firing patterns of hippocampal neurons (Muller and Kubie, 1987; Gothard et al., 1996a; O’Keefe and Burgess, 1996; Leutgeb et al., 2005a; Wills et al., 2005). In support of these previous observations, changing the length of the track altered many of the firing properties of neurons, including their preferred firing location, peak firing rate, field-size and field overlap. However, the theta-scale timing of neurons and the interneuron firing rate remained unaffected, indicating that these parameters set constraints on the mechanisms by which hippocampal networks can represent environments.

Relationship of theta-scale time lag and place-field distance

Within a single theta cycle, the relative timing of neuronal spikes reflects the upcoming sequence of locations in the path of the rat, with larger time lags representing larger distances (Skaggs et al., 1996; Dragoi and Buzsáki, 2006). We found that this relationship is best described by a sigmoid, resulting from the fact that the magnitude of sequence compression (i.e., the inverse slope of the sigmoid for a given distance) is smaller for short distances, but increases with distance. The sigmoidal relationship is likely a consequence of the theta-cycle dynamics of pyramidal neurons. Population firing rates are modulated by the ongoing theta oscillation.

tion, and are maximal at the trough of the CA1 pyramidal layer theta. Thus, the natural upper limit of ~ 150 ms for theta-cycle cofiring, results in a plateau in the sequence compression relationship. As a result, upcoming locations that are more proximal are given better representation within a given theta cycle, with poorer resolution of locations in the distant future. The behavioral consequence of this mechanism is that objects and locations far away are initially less distinguishable, but as the animal approaches, they are brought into the “fovea” of the theta-scale code and become better resolved. The large error bars in the sigmoidal relationship suggests that there is some variability in the strict nature of the sequence compression relationship; indeed a few cell pairs observed a slightly different relationship on the long versus the short track (Fig. 6a, inset).

If locations can be regarded as analogous to individual items in a memory buffer (Buzsáki, 2005; Jensen and Lisman, 2005), this suggests a context-dependent “register capacity” for the number of items that can be stored within a single theta cycle “memory buffer.” As further support, context (i.e., track length) also affected the degree of spatial and temporal overlap of place fields, resulting in increased cofiring among cells during each theta cycle and increased overall firing in the pyramidal population on the short track, relative to the long. Interestingly, this change in activity resulted in decreased power and coherence in the theta-band of local fields measured from CA1 and CA3 regions. Because the local field potential is considered to arise mainly from local processing of synaptic signals (Nadasdy et al., 1998; Logothetis, 2003), it displays independence from the firing rate, decreasing in instances where spiking is increasing, and may instead reflect different aspects of the task (see also Hirase et al., 1999; Huxter et al., 2003).

Implications and neuronal mechanisms of stable time lags

A robust finding in our study is that time lags between pairs are preserved despite changes to the tuning curves of individual neurons. What mechanism can be responsible for maintaining the time lags? In the framework of continuous attractor dynamics, environmental cues determine the effective topography of the manifold or “chart” (in which individual neurons represent nodes) on which the ongoing activity packet propagates (Amari, 1977; Samsonovich and McNaughton, 1997; Rolls, 2007), presumably according to input from motor and head-direction systems. The stable nature of the time lags at first approach appears consistent with the notion of a fixed synaptic matrix for the manifold (Samsonovich and McNaughton, 1997) or one in which no additional learning takes place on the shrunken track (Kali and Dayan, 2000). Indeed, whereas place-field firing rates changed instantaneously in the new environment, no evidence for plasticity was observed in spike timing within a single session. However, the emergence of new place cells on the short track suggests that the synaptic matrix on the short track is not a simple perturbation of that on the long (see also Knierim, 2002; Lenck-Santini et al., 2005). The proposed existence of multiple simultaneous activity packets may provide a phenomenological explanation (Sam-

Figure 7. Model for temporal lag stability. Pyramidal cell (1) excites a second pyramidal cell (2) and an interneuron (inh 2; other sources of depolarization are not shown). In this model, cells fire when excitation exceeds inhibition. The middle panel depicts the excitatory drives for the two interdependent place cells 1 (green) and 2 (blue) on the long track, with inhibition for each superimposed with a dashed line. The inhibition of cell 2 is delayed relative to cell 1, resulting in net time lag dt . When the track length is shortened (bottom), the rise in excitatory drives occur over a shorter duration (i.e., fewer theta cycles), and the place fields are shifted relative to each other. Inhibition in the place cells preserves timing with an appropriate shift, relative to that of the long track (superimposed in cyan), thus maintaining the time lag.

sonovich, 1998; Stringer et al., 2004). Nevertheless, the chart model posits that place cells are controlled by distal (room) and local (maze) cues, and the influence of these cues varies dynamically so that the brain’s representation shuttles back and forth between charts representing different reference frames: the start platform, distant room cues and the end platform (Gothard et al., 1996a; Redish et al., 2000; Maurer et al., 2006), consistent with the groups outlined in our study. Yet, the preservation of time lag took place not only for pairs representing the same reference frame, but even for pairs with place fields anchored to different segments of the environment (Gothard et al., 1996a; Redish et al., 2000), suggesting that transitions between independent charts cannot account for the present findings.

The mechanism responsible for maintaining the time lag must also dissociate the contribution of firing rate and timing: an important aspect of the present findings is that changes to the firing rate did not affect the timing across neuron pairs. Neither firing rate changes caused by environmental modification, nor firing rate changes caused by the animals running speed (Huxter et al., 2003) were sufficient to alter the time lag between cell pairs. We predict that under other manipulations, either environmental, or motivational, which alter the firing rates of individual neurons, time lags will remain preserved (Wood et al., 2000; Moita et al., 2003; Leutgeb et al., 2005b; Terrazas et al., 2005). How can firing rates vary without affecting timing? We conjecture that interneurons play a critical role: interneurons can provide a “window of opportunity” during which a postsynaptic neuron may spike. The timing of this window may be established by the combined effect of presynaptic excitatory activity and inhibition. A network variant of the single cell model proposed by Mehta et al. (2002), illustrates our hypothesis (Fig. 7): through recurrent and feedforward connections, changes in the drive from the leading assembly may modify the timing of interneurons inhibiting the trailing assembly, which in turn establish the time lag. Thus, the stability of time lags between neurons may arise from the network dynam-

ics responsible for maintaining the steady state of theta oscillations, rather than from the fixed synaptic matrix of a chart. Interestingly, in other states, such as hippocampal sharp waves associated with decreased inhibition and resulting relative hyperexcitability (Buzsáki et al., 1983; Csicsvari et al., 1999), the temporal lag between place cell pairs is shortened (Diba and Buzsáki, 2007). In extreme cases, such as during epileptic discharges or when networks are artificially driven by strong electrical stimulation, the time lag between neurons is further drastically decreased (Bragin et al., 1997).

In summary, numerous aspects of firing patterns can be altered by environmental and locomotion speed changes: the location and magnitude of the peak firing rates, the oscillation frequency of cells (Harris et al., 2002; Mehta et al., 2002; Huxter et al., 2003; Maurer et al., 2006), the shape and size of the place fields, the coactivity and the balance of pyramidal and interneuron populations, the synaptic processing evident in the theta power and coherence among regions. Considering that timing on the scale of tens of milliseconds, as studied here, is necessary for synaptic learning and memory formation, the spatial tuning curves of pyramidal cells may be modified based on the relevance and demands of different aspects of the task. Other measures vary in an interdependent manner across different environments and are of secondary consequence to the phase sequence generated in each theta cycle. From this perspective, the timing of spikes relative to that of other neurons in the assembly is a fundamental mechanism, which guides state-dependent operations in the hippocampal system.

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