Spatial Sequence Coding Differs during Slow and Fast Gamma Rhythms in the Hippocampus

Highlights

- Place cells code relatively long, temporally compressed paths during slow gamma.
- Place cells accurately follow ongoing trajectories during fast gamma.
- Slow gamma phases of spikes code spatial information.
- Fast gamma phases of spikes do not provide spatial information.

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In Brief

Zheng et al. show that place cells code sequences of locations differently during slow and fast gamma rhythms. Upcoming, relatively long paths are coded in a time-compressed manner during slow gamma, whereas representations closely follow current locations during fast gamma.
Spatial Sequence Coding Differs during Slow and Fast Gamma Rhythms in the Hippocampus

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SUMMARY

Spatiotemporal trajectories are coded by “theta sequences,” ordered series of hippocampal place cell spikes that reflect the order of behavioral experiences. Theta sequences are thought to be organized by co-occurring gamma rhythms (~25–100 Hz). However, sequences of locations are represented during distinct slow (~25–55 Hz) and fast (~60–100 Hz) gamma subtypes remains poorly understood. We found that slow gamma-associated theta sequences activated on a compressed timescale and represented relatively long paths extending ahead of the current location. Fast gamma-associated theta sequences more closely followed an animal’s actual location in real time. When slow gamma occurred, sequences of locations were represented across successive slow gamma phases. Conversely, fast gamma phase coding of spatial sequences was not observed. These findings suggest that slow gamma promotes activation of temporally compressed representations of upcoming trajectories, whereas fast gamma supports coding of ongoing trajectories in real time.

INTRODUCTION

The hippocampus is central to episodic memory and spatial navigation (O’Keefe and Nadel, 1978), both of which rely on sequences of spatiotemporal information. “Theta sequences” (Dragoi and Buzsáki, 2006; Foster and Wilson, 2007; Gupta et al., 2012; Skaggs et al., 1996) are precisely ordered series of spikes from place cells, hippocampal neurons that fire in specific spatial locations (O’Keefe, 1976; O’Keefe and Dostrovsky, 1971). Theta sequences provide temporally compressed representations of spatial paths, which activate within individual cycles of the extracellular theta oscillation (~6–10 Hz) and may be important for encoding or retrieval of spatial memories (Dragoi and Buzsáki, 2006; Foster and Wilson, 2007; Gupta et al., 2012; Wikenheiser and Redish, 2015). A second type of oscillation in the hippocampus, gamma (~25–100 Hz), is thought to interact with theta to temporally organize theta sequences (Dragoi and Buzsáki, 2006; Gupta et al., 2012; Lisman, 2005; Lisman and Jensen, 2013). The prevailing theory of how sequences of spatial locations are represented within theta-nested gamma rhythms posits that discrete spatial locations, not sequences of locations, are coded within individual gamma cycles (Dragoi and Buzsáki, 2006; Jensen and Lisman, 1996; Lisman and Jensen, 2013). A hypothesis that arises from this theory is that spatial paths represented during slow gamma are shorter than paths represented during fast gamma (Figure 1A), because fewer slow gamma cycles than fast gamma cycles occur within a theta cycle (Belluscio et al., 2012). Accumulating evidence indicates that slow and fast gamma rhythms represent distinct processing states in the hippocampal network. Slow and fast gamma occur on distinct theta phases (Colgin et al., 2009), and different types of hippocampal processing also occur on distinct theta phases (Hasselmo et al., 2002). Additionally, slow and fast gamma exhibit different relationships with behavior (Cabrál et al., 2014; Carr et al., 2012; Kemere et al., 2013; Takahashi et al., 2014; Yamamoto et al., 2014; Zheng et al., 2015). Also, place cells represent space differently during slow and fast gamma, coding locations ahead of the animal during slow gamma and behind the animal during fast gamma (Bieri et al., 2014). However, the question of whether longer sequences are represented during fast gamma and shorter sequences during slow gamma, as hypothesized above, has not yet been investigated. The current study addresses this gap in knowledge by comparing spatial trajectories coded by place cell ensembles during theta sequences associated with slow or fast gamma. The results support a novel model of spatial sequence coding during theta-nested slow and fast gamma rhythms (Figure 1B).

RESULTS

The local field potential (LFP) and spiking activity from 604 place cells were recorded in hippocampal area CA1 (Figure S1) of seven rats traversing a linear track. The spatial path represented by place cell ensemble activity during individual theta cycles was estimated using a Bayesian decoding approach (Bieri et al., 2014; Brown et al., 1998; Gupta et al., 2012; Jensen and Lisman, 2000; Zhang et al., 1998; see Experimental Procedures). A regression line was fit to the position estimates from each
decoded theta sequence of place cell spikes (Figure 2A). The regression line was subsequently used to measure the slope (cm/s), distance ("x span," cm), and duration ("t span," ms) of each sequence (Figure 2B). Each of these measures provides information about how spatial trajectories are represented by theta sequences. Slope measures the spatial distance represented by a sequence in a given amount of time, and thus can be used to quantify temporal compression of sequences. X span estimates the length of a represented trajectory, and t span reflects the time period of sequence activation. Sequences of ordered locations that were unlikely to arise by chance were classified as "significant sequences" (see Experimental Procedures). Theta sequences have previously been defined as sequences that occur in forward order (Foster and Wilson, 2007; Gupta et al., 2012), or in other words exhibit a "sweeping forward structure" (Feng et al., 2015). Thus, it is unclear how to interpret theta sequences with negative slopes (i.e., sequences occurring in reverse order). For this reason, theta sequences with negative slopes were excluded from the analyses presented in the main text (analyses of sequences with negative slopes are shown in Figure S2). Sequences with negative slopes (n = 17,193) constituted a minority of the total sequences (n = 48,098), and only a minority (36%) of sequences with negative slopes were defined as significant. In contrast, a majority (64%) of sequences exhibiting positive slopes were defined as significant.

Sequences of Place Cells Represent Longer and More Temporally Compressed Paths during Slow Gamma than during Fast Gamma

Theta sequences provide compressed representations of sequences of locations (Dragoi and Buzsáki, 2006; Foster and
sequences'' (n = 2,883); see Experimental Procedures; examples
(''slow gamma sequences'' (n = 2,958) and ''fast gamma se-
quences and fast gamma-associated theta sequences
compared slope values during slow gamma-associated theta
sequences (503.2 ± 7.5 versus 473.9 ± 7.4 cm/s; Z = 3.4,
p = 0.001, Mann-Whitney U test). The difference between slopes
increased when we compared slow and fast gamma sequences
ranked by ascending power; 47.7 ± 1.4 versus 43.6 ±
1.5 cm; Z = 2.4, p = 0.02). We next tested whether x span mea-
sures changed according to slow or fast gamma power. We
found that x spans were differentially related to slow and fast
gamma sequences. We found that x spans were significantly longer during slow gamma sequences (45.0 ± 0.6 versus 42.9 ±
0.6 cm; Z = 2.9, p = 0.004). The effect size increased when we
compared those slow and fast gamma sequences with particu-
larly strong gamma (i.e., the top 20% of slow or fast gamma se-
quencies increased as slow gamma rhythms increased. In
contrast, slopes decreased as fast gamma power increased (Figure 4A; b = −0.04, t(5,839) = −3.2, p = 0.001).

The finding that sequences represent more space per unit time during slow gamma may reflect an increase in the length of the represented path during slow gamma or representation of similar path lengths across shorter periods of time. To distinguish these possibilities, we separately analyzed the x span

Figure 3. Example Slow Gamma Sequences and Fast Gamma Sequences
Examples were selected from five different rats. (A) Examples of slow gamma sequences. The top row (left) shows rate maps for ensembles of simultaneously recorded place cells, ordered ac-
\n
cording to their place fields’ centers of mass (color coded according to the center of mass locations on the track). The black bar indicates the animal’s current location. The top row (right) shows color-
coded spatial probability distributions (resulting from Bayesian decoding analyses) for the example theta sequences. White dashed lines indicate the animal’s actual locations. The middle row shows raw CA1 recordings, and the bottom row shows corresponding slow gamma band-pass-filtered (25–55 Hz) versions. Vertical tick marks indicate spike times from the corresponding place cell ensembles. Each cell’s number identifier indicates its order within the theta sequence. (B) Same as (A) but for example fast gamma sequences.
The finding that shorter and longer paths are represented during fast gamma (Z = 3.25, p = 0.001) and slow gamma (Z = 5.4, p < 0.001) suggests that spatial representations more closely follow an animal’s current position during fast gamma but extend a greater distance away from the current position during slow gamma. To test this hypothesis, we devised a metric that combines slope and prediction error measures to assess the accuracy of decoded sequences. A sequence was considered accurate (i.e., the decoded path closely tracked the animal’s actual position) if it had a flat slope and a low prediction error (see Experimental Procedures). We found that different gamma types showed different relationships to accuracy (Figure 4D; multiple regression model interaction term; \( b = 0.2, t(11,678) = 8.1, p < 0.001 \)). High fast gamma power correlated with high accuracy (\( b = -0.09, t(5,839) = -5.4, p < 0.001 \)), whereas high slow gamma power correlated with low accuracy (\( b = 0.1, t(5,839) = 6.1, p < 0.001 \)). These findings suggest that sequences more accurately follow an animal’s current location during fast gamma than during slow gamma.

Prediction error was calculated by subtracting the actual position from the decoded position; thus, positive prediction errors indicate that decoded positions are ahead of the actual position, whereas negative prediction errors indicate that decoded positions are behind the actual position. Our previous results showed that place cells tend to code positions ahead of the animal during slow gamma and behind the animal during fast gamma (Bieri et al., 2014). We tested whether place cells were again more likely to code positions ahead of the animal during slow gamma and behind the animal during fast gamma in our present dataset (which includes 604 CA1 cells, 351 of which were also used in the Bieri et al. study; see Experimental Procedures). Consistent with our previous results, prediction errors were significantly higher during slow gamma than during fast gamma (\( Z = 5.0, p < 0.001 \), Mann-Whitney U test). Also, prediction errors were significantly positive during slow gamma (\( Z = -3.25, p = 0.001 \), Wilcoxon signed-rank test) and significantly negative during fast gamma (\( Z = -5.34, p < 0.001 \), Wilcoxon signed-rank test). The above collection of results suggests that place cells code relatively long paths extending ahead of the current position on a compressed timescale during slow gamma and code ongoing paths on a slower timescale during fast gamma.

**Slow Gamma Phase Coding of Spatial Information**

Place cell spikes occur at progressively earlier theta phases as an animal moves through a cell’s place field, and thus spikes’ theta phases provide spatial information (“theta phase precession”; O’Keefe and Recce, 1993; Skaggs et al., 1996). However, current hippocampal gamma theories assume that gamma phase variations do not code spatial information (Lisman and Jensen, 2013). Yet, the phase of ≈30-Hz gamma oscillations in the prefrontal cortex was shown to code the order of sequentially presented objects (Siegel et al., 2009), raising the possibility that slow gamma phase coding of sequences also occurs in the hippocampus. If sequences of locations, not individual locations, were represented at different phases within slow gamma cycles, it would help explain why relatively long paths are represented on a relatively compressed timescale during slow gamma (Figure 1B).

To address the question of whether slow or fast gamma phases code spatial information, we assessed whether slow and fast gamma phases of spikes changed systematically as
theta sequences progressed (Figure 5). We ordered individual gamma cycles during fast gamma sequences and slow gamma sequences (three and five gamma cycles per theta cycle for slow and fast gamma, respectively; Figures S5A–S5E), centered at the gamma cycle with the highest number of spikes (cycle “0”; Figures S5F and S5I). Cycles occurring before cycle 0 were labeled with descending integer values, and cycles occurring after cycle 0 were labeled with increasing integer values. The slow gamma phase of place cell spikes significantly shifted across successive slow gamma cycles (Figure 5A; W(4) = 125.2, p < 0.001; p < 0.001 for all pairwise comparisons; multisample Mardia-Watson-Wheeler test). In contrast, place cells did not shift their fast gamma firing phase preference across successive fast gamma cycles (Figure 5C; W(8) = 8.3, p = 0.4). Slow gamma phase variations across cycles were not due to differences in spike density (Figures S5G, S5H, S5J, and S5K).

Theta phase precession has previously been shown to be slightly disrupted during periods of slow gamma, with spikes tending to occur across a more limited range of theta phases during slow gamma than during fast gamma (Bieri et al., 2014). This finding was maintained in the present dataset. Theta phase distributions of spike times during slow and fast gamma sequences were significantly different (data were randomly downsampled such that each cell had equal numbers of phase estimates in slow and fast gamma sequences, as in Bieri et al., 2014; W(2) = 6.6, p = 0.04; Mardia-Watson-Wheeler test). For each cell, we also compared theta phase precession for slow and fast gamma sequences using circular-linear regression analysis (Kempter et al., 2012; Schlesiger et al., 2015). For cells exhibiting significant theta phase-position correlations (i.e., significant circular-linear correlations) during both slow and fast gamma sequences, we found significantly different theta phase-position correlations for slow and fast gamma sequences (chisquare goodness-of-fit test, $\chi^2(4) = 21.5$, p = 0.0003). Specifically, theta phase precession slopes for fast gamma sequences were significantly more negative than slopes for slow gamma sequences (paired t test, $t(78) = 2.1$, p = 0.04).

Still, theta phase precession was largely maintained during slow gamma. Thus, it is possible that the observed slow gamma phase precession was simply an epiphenomenon of theta phase precession. That is, slow gamma phase precession could occur as a result of segmentation of theta sequences. To test this possibility, we cut each slow gamma-associated theta cycle into contiguous segments using a “period” randomly selected from the range of slow gamma periods (i.e., $1/55-1/25$ s). The slow gamma phases for each of these mock slow gamma cycles were assigned by designating the first and last time points in the mock gamma cycle as $0/C14$ and $360/C14$, respectively, and linearly interpolating phase values for the intervening time points. Theta and mock gamma phases of spikes occurring within these mock slow gamma cycles were measured. For each slow gamma sequence, this procedure to produce mock slow gamma cycles and associated slow gamma spike phases was repeated 1,000 times and averaged to generate the probability distributions shown in Figure 5B. Although theta phases of spikes advanced in both real and mock datasets, slow gamma phase precession was not apparent across mock slow gamma cycles (Figure 5B). The mean phase shift across successive slow gamma cycles for

Figure 5. Slow Gamma Phase Precession
Gamma cycles within theta cycles were ordered, centered at cycle 0 (i.e., gamma cycle with maximal spiking).

(A) Probability distributions of slow gamma phases of spikes across slow gamma cycles within slow gamma sequences. Slow gamma phases of spikes shifted systematically across successive slow gamma cycles.

(B) Probability distributions of mock slow gamma phases of spikes across mock slow gamma cycles in slow gamma sequences. Mock slow gamma spike phases did not shift significantly across mock slow gamma cycles within slow gamma sequences. Preferred spike phases shifted significantly less across mock cycles than across real slow gamma cycles.

(C) Probability distributions of fast gamma phases of spikes across successive fast gamma cycles within fast gamma sequences. Spikes' preferred fast gamma phase did not significantly change across successive fast gamma cycles.

(D) Probability distributions of mock fast gamma phases of spikes across mock fast gamma cycles generated from fast gamma sequences. Preferred firing phases of spikes within mock fast gamma cycles did not change significantly across the sequence.

See also Figure S5.
Figure 6. Slow Gamma Phases Coded Spatial Information, but Fast Gamma Phases Did Not

Place cells were categorized by their place field location (early, middle, and late correspond to place fields near the beginning, middle, and end of the track, respectively).

(A) Cells fired on different phases of slow gamma according to their place field location, for slow gamma sequences that occurred across the whole track (left), on the first half of the track (middle), or on the second half of the track (right).

(B) Cells tended to fire on a similar fast gamma phase, regardless of where their place field was located or where their corresponding fast gamma sequence occurred on the track.

See also Figure S6.

the real data was −46.0°, which was significantly different from the phase shifts observed across the sets of successive mock slow gamma cycles (−0.01° ± 2.9°, mean ± angular deviation; 95% confidence intervals for mock data: [−5.7°, 5.7°], p < 0.0001). This finding supports the conclusion that slow gamma phase precession was not epiphenomenal to theta phase precession and segmentation of theta sequences. Analogous analyses were performed for fast gamma (Figure 5D), using contiguous segments with periods randomly selected from the range of fast gamma periods (i.e., 1/100–1/60 s). Not surprisingly, considering that spikes did not exhibit fast gamma phase precession in the real data, mock fast gamma phases of spikes did not systematically change across successive mock fast gamma cycles (Figure 5D). The mean phase shift across successive real fast gamma cycles was −6.0°, which was not significantly different from the phase shifts across mock fast gamma cycles (−6.2° ± 2.9°, mean ± angular deviation; 95% confidence intervals for mock data: [−11.9°, −0.4°], p = 0.95). These results were maintained when an alternative method was used to construct mock gamma cycles (Figure S5L).

The finding that slow gamma phases of groups of place cell spikes change systematically across theta sequences raises the hypothesis that slow gamma phases of spikes code spatial information. To test this hypothesis, we investigated whether place cells fired on different gamma phases depending on the location of their place fields. We grouped place cells that fired during each theta sequence into three categories based on the location of their peak firing rate along the trajectory of the track. “Early” place cells fired near the beginning of the trajectory, “middle” place cells fired in the middle, and “late” place cells fired near the end. We then estimated the mean phase of slow or fast gamma at which spikes occurred for place cells within each spatial category. Spike phases during slow gamma sequences were significantly different depending on spatial category (Figure 6A; W(4) = 81.0, p < 0.001; multisample Mar-dia-Watson-Wheeler test), with early and late slow gamma phases coding early and late place field locations, respectively. On the other hand, spike phases during fast gamma sequences did not change significantly across place field locations (Figure 6B; W(4) = 7.0, p = 0.1). The results were not due to a differential distribution of slow and fast gamma across different track locations (Bieri et al., 2014), because the effects persisted when sequences occurring on the first and second halves of the track were analyzed separately (Figures 6A and 6B; first half of the track, W(4) = 41.5, p < 0.001 for slow gamma phases and W(4) = 3.3, p = 0.5 for fast gamma phases; second half of the track, W(4) = 55.1, p < 0.001 for slow gamma phases and W(4) = 2.4, p = 0.7 for fast gamma phases). These findings suggest that slow gamma phases provide information about spatial location, whereas fast gamma phases do not code spatial information.

A previous study described two classes of place cells that fire at different phases of gamma (trough-firing pyramidal cells, termed “TroPyr,” and rising phase-firing pyramidal cells, termed “RisPyr”; Senior et al., 2008). This previous study did not distinguish between slow and fast gamma, leaving open the possibility that each place cell class is selectively driven by a gamma subtype. The gamma phase of TroPyr and RisPyr spikes varied differently across a theta cycle (Senior et al., 2008), which could be related to the different gamma phase variations exhibited by spikes during slow and fast gamma. This proposition was not supported by the present data, however. Although slow and fast gamma power were negatively correlated, as reported previously (Colgin et al., 2009), firing rates of individual cells were positively correlated during slow and fast gamma sequences (Figure S6). This finding suggests that place cells are equally active during either gamma type and not selectively activated during slow or fast gamma.

Number of Fast Gamma Cycles per Theta Cycle Increases with Path Length

A previous study showed that the number of gamma cycles within a theta sequence increases with increasing path length (Gupta et al., 2012). However, this study measured gamma across a broad range of frequencies (i.e., 40–100 Hz). Here, we investigated whether the number of gamma cycles within a theta sequence changed with path length separately for slow and fast gamma (Figure 7). We found a significant interaction effect between path length and gamma type on the number of gamma cycles within a theta cycle (multiple regression model interaction term; b = −0.002, t(5,571) = −2.1, p = 0.04). The number of fast gamma cycles within a theta sequence was positively correlated with path length (Figure 7A; b = 0.002, t(2,761) = 3.2, p = 0.001), but the number of slow gamma cycles per theta sequence did not significantly change with path length (Figure 7B; b = 4.8 × 10⁻⁶, t(2,810) = 0.8, p = 0.4). These results are consistent with the hypothesis shown in Figure 1B. That is, if sequences of locations are represented within slow gamma cycles, then the number of slow gamma cycles would not be expected to change much with path length. However, if different
believed to be stored in, and retrieved from, CA3 (Jensen and
guide an animal’s path (Cabral et al., 2014). Sequences are
shown to be enhanced when previously experienced sequences
 gamma (Bieri et al., 2014). In addition, slow gamma has been
 cells has also been associated with the occurrence of slow
2007; Wikenheiser and Redish, 2015). Predictive firing by place
locations (Muller and Kubie, 1989). Such predictive firing during
late locations, respectively. In contrast, place cell ensembles
with early and late slow gamma phases representing early and
studies have shown that fast gamma links CA1 to inputs from the
and Hasselmo, 1997), and CA3 transmits activity to CA1 during
slow gamma (Colgin et al., 2009; Kemere et al., 2013; Schomburg
et al., 2014). This collection of findings supports the hypoth-
thesis that slow gamma promotes the retrieval of place cell
sequences from CA3 as animals plan and envision future trajec-
tories. However, this kind of predictive function would require
temporal compression of spatial sequences to allow a rapid
“look ahead,” and the aforementioned studies did not investi-
gate how this would occur. The present results suggest a way
in which such temporal compression arises during theta-related
behaviors. Specifically, sequential locations may activate at the
slow gamma timescale during retrieval, thereby providing a
more compressed timescale than the theta timescale of
encoding.

The present results were limited to theta-related behaviors
(i.e., active movement). However, sequences also activate in a
temporally compressed manner during sharp wave-ripples
(SWRs) (Diba and Buzsáki, 2007; Lee and Wilson, 2002;
Nádasdy et al., 1999), and sequences replayed during awake
SWRs were recently shown to predict animals’ future trajectories
toward goal locations (Pfeiffer and Foster, 2013). Interestingly,
slow gamma increases during awake SWRs, with stronger slow
 gamma coinciding with higher-fidelity replay (Carr et al.,
2012). Considering these prior findings from SWR-associated
behaviors together with the current results during theta states
raises the possibility that slow gamma promotes temporally
compressed spatial memory representations during both active
exploration and rest. However, different mechanisms likely
regulate sequence compression during SWRs compared to
sequence compression during theta. Hippocampal neurons
receive high-frequency shunting inhibition during ripples,
preventing most cells from firing (English et al., 2014). This shunting
inhibition likely prevents spikes from occurring across a wide
range of slow gamma phases, as was seen in the present study.
In line with this viewpoint, trajectories coded within SWRs
jumped from one location to another across slow gamma cycles
(Pfeiffer and Foster, 2013), rather than activating within slow
 gamma cycles.

Regarding the functional significance of fast gamma, previous
studies have shown that fast gamma links CA1 to inputs from the
medial entorhinal cortex (Colgin et al., 2009; Kemere et al.,
2013; Schomburg et al., 2014), which convey information about
current location (Brun et al., 2002; Hafting et al., 2005; Zhang
et al., 2013). Additionally, place-based navigation is thought to rely
on fast gamma (Cabral et al., 2014), and place cells represent
recent locations, not upcoming locations, during fast gamma
(Bieri et al., 2014). Also, fast gamma frequencies increase with
increasing running speeds, which may enable faster transitions
across sequentially coded locations as running speeds increase
(Ahmed and Mehta, 2012; Zheng et al., 2015). These findings
support the hypothesis that fast gamma supports coding of cur-
rent spatial positions, rather than activating previously stored
memories. The present results provide additional support for
this hypothesis by showing that fast gamma sequences code
relatively short paths that closely follow an animal’s position in
real time.

In addition, our findings provide the first evidence of gamma
phase coding of spatial sequences and reveal that phase coding

Figure 7. Number of Gamma Cycles per Theta Cycle Increased as a
Function of Decoded Path Length for Fast Gamma, but Not Slow
Gamma

For fast gamma sequences (A), the number of fast gamma cycles per theta
cycle increased as a function of x span. For slow gamma sequences (B), the
number of slow gamma cycles per theta cycle did not significantly change as a
function of x span. The expected gamma cycle count versus path length re-
lationships (gray dashed lines) were estimated based on relationships of path
length and gamma cycle count to running speed (see Experimental Pro-
cedures), as in an earlier study (Gupta et al., 2012). Data are presented as
mean ± SEM.
occurs selectively during slow gamma. Specifically, sequences of locations were represented within individual slow gamma cycles, with increasing slow gamma phases coding successive positions within a spatial sequence (Figure 6). It is unlikely that this finding relates to theta phase shifts of slow gamma amplitude peaks that have been reported at increased running speeds in mice (Chen et al., 2011), first because our mock slow gamma results do not support this conclusion (Figure S5L), and second because we did not observe such shifts in rats (Zheng et al., 2015). The observed slow gamma phase precession contrasts with prevailing theories of spatial coding during theta-nested gamma, which assume that discrete locations are coded within individual gamma cycles and that gamma phase variations do not provide spatial information (Dragoi and Buzsáki, 2006; Jensen and Lisman, 1996; Lisman, 2005; Lisman and Jensen, 2013). However, there is a precedent for slow gamma phase coding of sequences. In the prefrontal cortex, different phases of ~30-Hz gamma have been shown to code series of items maintained in short-term memory (Siegel et al., 2009). In contrast to slow gamma, we found no evidence of fast gamma phase coding. Spikes preferentially occurred at the same fast gamma phase across successive fast gamma cycles within a theta sequence, in agreement with theories of theta-nested gamma in the hippocampus (Dragoi and Buzsáki, 2006; Jensen and Lisman, 1996; Lisman, 2005; Lisman and Jensen, 2013).

The ability of the hippocampus to handle sequences of spatiotemporal information is a dynamic process, involving both encoding of ongoing events and retrieval of previously stored memories. Earlier work indicated that encoding and retrieval optimally occur at different theta phases (Hasselmo et al., 2002). At the encoding phase, inputs from the entorhinal cortex dominate, and long-term potentiation in hippocampal synapses is enhanced. At the retrieval phase, inputs from CA3 are strong, whereas inputs from the entorhinal cortex and long-term potentiation mechanisms are attenuated. The hypothesis that fast and slow gamma play roles in encoding and retrieval, respectively, is consistent with this earlier work in that fast and slow gamma preferentially occur at different theta phases (Colgin et al., 2009). However, in the earlier study (Hasselmo et al., 2002), encoding and retrieval occurred within the same theta cycles, whereas the present results, and previous results (Colgin et al., 2009), have found that slow and fast gamma tend to occur on different theta cycles. This difference may be explained by the behavioral tasks. In the present study, and in Colgin et al. (2009), rats were merely running through familiar environments. In tasks explicitly requiring encoding and retrieval, perhaps slow and fast gamma would be observed more often on the same theta cycles.

The current study suggests a possible mechanism for achieving dual encoding and retrieval functions through different gamma subtypes. During sequence retrieval, an initial cue may trigger the first ensemble in a linked sequence of place ensembles. The relatively long slow gamma period may then allow successive items within the stored sequence to be retrieved rapidly, until gamma-modulated inhibition (Buzsáki et al., 1983; Penttonen et al., 1998; Soltesz and Deschênes, 1993) arrives and shuts down place cell firing. In contrast, the short period of fast gamma may ensure that only one cell assembly is activated per fast gamma cycle. This type of scheme may be well suited for coding sequences of discrete locations in ongoing behavior, preventing preemptive retrieval of locations that previously followed the current location but may or may not become part of the current trajectory. These dual slow and fast gamma mechanisms may also explain how spatial memories can be encoded in real time but later retrieved in a time-compressed manner.

**EXPERIMENTAL PROCEDURES**

**Subjects**

Seven male Long-Evans rats weighing ~350–500 g and ~4–10 months of age were used in the study. Data from four of these rats were included in a previous study (Bieri et al., 2014). Rats were maintained on a reverse light/dark cycle (lights off from 8 a.m. to 8 p.m.) and tested during the dark phase. After surgery, animals were singly housed in cages (40 cm × 40 cm × 40 cm) constructed from clear acrylic and containing enrichment materials (e.g., plastic balls, cardboard tubes, and wooden blocks). Rats recovered from surgery for at least 1 week prior to the start of behavioral testing. During the data collection period, rats were placed on a food-deprivation regimen that maintained them at ~90% of their free-feeding body weight. All experiments were conducted according to the guidelines of the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

**Recording Drive Implantation**

Recording drives were surgically implanted above the right hippocampus (anterior-posterior [AP] 3.8 mm, medial-lateral [ML] 3.0 mm, dorsal-ventral [DV] 1 mm in six rats; AP 5.0 mm, ML 5.0 mm, DV 1 mm in one rat). Bone screws were placed in the skull, and the screws and the base of the drive were covered with dental cement to affix the drive to the skull. Two screws in the skull were connected to the recording drive ground.

**Testing Procedures**

Following the postsurgery recovery period, rats were trained to run three 10-min sessions per day on a linear track. The linear track was 2 m long and 10 cm wide and was placed 64 cm above the floor. Rats were trained to run back and forth on the track, receiving small pieces of sweetened cereal or vanilla cookies at the ends. Every rat was trained on the linear track for at least 3 days prior to the start of recording to ensure familiarity with the environment. Between recording sessions, rats rested for ~10 min in a towel-lined, elevated ceramic container.

**Results, Statistics, and Data Analyses**

Data were analyzed using custom software written in MATLAB (MathWorks), unless indicated otherwise. Statistics were computed with MATLAB, SPSS 22 (IBM), R 3.1.1 (R Foundation for Statistical Computing), and Oriana 4.02 (Kovach Computing Services). Mann-Whitney U tests were used to compare sequence properties for slow gamma sequences and fast gamma sequences. Wilcoxon signed-rank tests were used to test whether mean prediction errors during slow gamma and fast gamma sequences were significantly different from zero. Multiple regression analyses were applied to test for gamma type × gamma power interaction effects on theta sequence properties. Then, linear regression analyses were performed separately for each gamma type to test for correlations between gamma power and theta sequence properties. Comparisons of gamma phase distributions across successive gamma cycles (Figure 5), or across different place field location categories (Figure 6), were performed using multisample Mardia-Watson-Wheeler tests. Data are presented as mean ± SEM, unless indicated otherwise. Additional analysis methods are described in detail below and in Supplemental Experimental Procedures.

**Detecting Individual Theta Cycles for Bayesian Decoding**

LFP signals were band-pass filtered for theta (6–10 Hz) and delta (2–4 Hz), and the time-varying power for each was determined as described in Oscillatory
Power Estimation in Supplemental Experimental Procedures. For theta cycle selection, a single LFP signal was chosen for each session by identifying the channel with the highest time-averaged theta power and at least one recorded CA1 cell. Periods of theta activity were defined using the theta:delta power ratio (Csicsvari et al., 1999). Specifically, theta power was required to be at least three times greater than delta power. Data with insufficient theta activity were not included in the analyses. Individual theta cycles were cut at the theta phase with the lowest number of spikes (typically at the peak or close to the peak) from all recorded CA1 cells during that session.

Bayesian Decoding Analyses

The most likely position represented by spiking activity from CA1 place cells was estimated using a Bayesian decoding approach (Bieri et al., 2014; Brown et al., 1998; Gupta et al., 2012; Jensen and Lisman, 2000; Zhang et al., 1998). Recording sessions with fewer than 20 cells were not analyzed, and only theta cycles containing at least three active place cells and a running speed greater than 5 cm/s were decoded. Place fields were constructed for each recording session on the linear track, as described in Place Fields in Supplemental Experimental Procedures. Decoding was performed for each theta cycle using a 40-ms sliding time bin shifted by 10 ms at each step. The probability of the animal being at position \( x \), given the number of spikes \( n \) from each cell collected in time bin \( t \), was estimated using Bayes’ rule:

\[
P(x|n) = \frac{P(n|x) \cdot P(x)}{P(n)}
\]

\( P(n|x) \) was estimated using the firing rates from the experimentally obtained place fields in the same 10-min linear track session. It was assumed that the firing rates of different place cells were statistically independent and that the number of spikes from each cell followed a Poisson distribution (Jensen and Lisman, 2000; Zhang et al., 1998). \( P(n) \), the normalizing constant, was set so that \( P(x|n) \) summed to 1. \( P(x) \) was set to 1.

Theta Sequence Analysis and Significance Determination

Theta sequences were characterized by first determining the longest contiguous set of time bins (length of each bin: 40 ms, 10-ms sliding window) that contained spikes. If spikes did not persist across an entire theta sequence, then contiguity was considered to be broken when there were two or more adjacent time bins (i.e., 50 ms or longer) without spikes. The temporal duration of the longest set of contiguous time bins was defined as the \( t \) span of the sequence. For each time bin within the \( t \) span, 1,000 spatial positions were randomly selected from a weighted distribution, namely the probability distribution \( P(x|n) \) of positions obtained from the Bayesian decoding analyses (see above), and fit with a regression line. The slope of the regression line defined the slope of the theta sequence. The path length \( (x \) span) of the theta sequence was defined as the difference between positions associated with the start and end of the regression line.

To determine whether a theta sequence was significant, we employed the following shuffling approach. For each sequence, we circularly shifted the probability distribution of estimated positions by a random distance at each time bin 1,000 times (the same as the “column-cycle” shuffle described in Davidson et al., 2009) for sharp wave–associated sequences. Two methods were used to detect significant sequences. First, we compared the \( R^2 \) value of the linear regression of each sequence to its corresponding shuffled versions to determine significance. Specifically, sequences with an \( R^2 \) value >95% of \( R^2 \) values for shuffled data were classified as significant sequences. However, this classification excluded sequences that accurately followed an animal’s actual position, and thus exhibited \( R^2 \) values that were close to 0 (see Figure 2). In order to allow such sequences to be defined as significant, we used a second definition of significance for those sequences with \( R^2 \) values close to 0 (i.e., the lowest 10% of \( R^2 \) values). For these sequences, the residual sum of squares (RSS) of the linear regression was compared between each sequence and corresponding shuffled versions. These sequences were then defined as significant if they exhibited an RSS value <95% of RSS values for shuffled data.

Because theta sequences have previously been defined as sequences occurring in a forward order (Foster and Wilson, 2007), sequences with negative slopes were excluded from all analyses, except in Figure S2, in which sequences with negative slopes were analyzed separately. Theta sequences with negative slopes may represent a different phenomenon with different underlying mechanisms from theta sequences with positive slopes, considering that reverse replay during awake sharp waves is thought to arise from different mechanisms and serve a different function from forward replay (Colgin and Moser, 2006; Diba and Buzsáki, 2007; Foster and Knierim, 2012; Foster and Wilson, 2006).

Categorization of Slow Gamma Sequences and Fast Gamma Sequences

To categorize slow and fast gamma sequences (Figures 3, 4, 5, 6, and 7; Figures S3–S6), significant sequences were first ranked according to their slow gamma power and fast gamma power (ranked separately for slow and fast gamma). A rank of 0 corresponded to lowest power, and a rank of 1 corresponded to highest power. Slow gamma sequences and fast gamma sequences were then defined as those significant sequences exhibiting power rank values for the gamma type of interest that were above 0.5 and power rank values for the other gamma type (i.e., not the gamma type of interest) that were below 0.5. The total number of theta sequences detected was 48,098. Of the 48,098 sequences, 18,059 were defined as significant; 13,537 of these significant sequences had positive slopes (i.e., activated in forward order). Of the 13,537 significant sequences with positive slopes, 2,958 were classified as slow gamma sequences and 2,883 were classified as fast gamma sequences.

Sequence Accuracy

To assess how accurately a theta sequence represented an animal’s current location (Figure 4D), we applied the following method to the combined set of slow and fast gamma sequences. First, two measures, slope and mean prediction error (i.e., the average difference between the actual position of the animal and the position of the regression line that was fit to the sequence), were separately ranked. Lower rank values corresponded to lower numerical values. The slope rank and mean prediction error rank for each sequence were then averaged and normalized between 0 and 1 to create an accuracy measure. Accuracy measures close to 0 corresponded to high accuracy, and accuracy values close to 1 corresponded to low accuracy (i.e., accuracy scores were high when slopes and prediction errors were low, and accuracy scores were low when slopes and prediction errors were high).

Phase Analyses

The time-varying phases of slow gamma, fast gamma, and theta were determined by Hilbert transformation of respective band-pass-filtered signals (25–55 Hz for slow gamma, 60–100 Hz for fast gamma, and 6–10 Hz for theta). Oscillatory peaks were defined as 0° for theta, and oscillatory troughs were defined as 0° for slow and fast gamma. Degrees were defined in this way to allow plotting from 0° to 360° for both theta and gamma, given that cycles were cut according to the phase associated with minimal spiking (peak to peak for theta and trough to trough for gamma; see Detecting Individual Theta Cycles for Bayesian Decoding above and Figure S5).

Estimation of Gamma Phase Shifts across Gamma Cycles

The gamma cycle with maximal spiking across all simultaneously recorded cells was defined as cycle 0 (Figure 5; Figures S3F and SSI). Cycles occurring before cycle 0 were numbered with decreasing integer values, and cycles occurring after cycle 0 were numbered with increasing integer values. Incomplete cycles at the beginning or end of the sequence were excluded from analyses. Gamma phases and theta phases of spike times were estimated for place cells that spiked within at least two slow gamma cycles within a theta cycle or at least three fast gamma cycles within a theta cycle. The number of cycles analyzed within each theta sequence was limited to three (cycles 1 to 1) for slow gamma and five (cycles 2 to 2) for fast gamma. These selection criteria correspond to the estimated average number of cycles encompassed within a gamma episode, based on the well-defined theta-gamma coupling bands seen in Figures S5A and S5C. Two-dimensional histograms of gamma phases and theta phases for spikes from each cycle number were plotted using 30° bins and smoothed across five bins.
Categorization of Place Cell Locations
Within each significant slow or fast gamma sequence, active place cells were ordered according to the spatial location of their peak firing rate using all spikes recorded within a given session (Figure 8). The peak firing positions of active cells in each sequence were ranked and then normalized between 0 and 1, in order to group cells into three categories. The cells representing relatively early locations (i.e., closest to the beginning of the track, 0 [start position] < peak firing position rank ≤ 1/3), middle locations (i.e., 1/3 < peak firing position rank ≤ 2/3), and relatively late locations (i.e., 2/3 < peak firing position rank < 1 [end position]) were placed in early, middle, and late categories, respectively. Histograms of spike phases for each spatial category were plotted using 20° bins and smoothed across seven phase bins.

Number of Gamma Cycles as a Function of Path Length
To assess the number of gamma cycles as a function of path length (Figure 7), individual gamma cycles were defined from trough to trough, using the time point closest to when the gamma phase was 0°. For each significant slow or fast gamma sequence, the total number of slow or fast gamma cycles per theta cycle was counted, and the corresponding x span (i.e., path length of decoded sequence) was found. The expected gamma cycle number versus x span relationship, based solely on the relationships of these variables to running speed, was estimated by multiplying the gamma cycle number versus running speed relationship by the running speed versus x span relationship (as in Gupta et al., 2012). The gamma cycle number versus running speed relationship was estimated by calculating the average number of gamma cycles per theta cycle for each set of sequences falling within a given running speed bin (bin width 5 cm/s). The running speed versus x span relationship was determined by creating an n x n matrix, where n is the running speed bin number and n is the x span bin number. For each element in the matrix, the number of sequences exhibiting that particular set of running speed and x span values was then counted. The number of sequences within each x span column of the matrix was then normalized across running speeds such that summation of the measures within each column equaled 1.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.12.005.

AUTHOR CONTRIBUTIONS

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Spatial Sequence Coding Differs during Slow and Fast Gamma Rhythms in the Hippocampus

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Figure S1, Related to Figure 2. Schematic showing location of all 56 recording sites in this study (red dots).
Figure S2, Related to Figure 4. No significant effects of slow and fast gamma on theta sequences with negative slopes. In all panels, gamma power measures (x-axes) were ranked separately for each gamma type. Slope (A), x-span (B), and t-span (C) as a function of slow (blue) and fast (red) gamma power for significant sequences exhibiting negative slopes. There was no gamma type × gamma power interaction effect on slope (A, multiple regression interaction term; \( b = -0.02, t(3958) = -0.7, p = 0.5 \), \( n = 1981 \) sequences), nor was there a main effect of gamma power (\( b = -0.008, t(3958) = -0.5, p = 0.6 \)). There was no gamma type × gamma power interaction effect on x-span (B, multiple regression interaction term; \( b = 0.01, t(3958) = 0.4, p = 0.7 \)), nor was there a main effect of gamma power (\( b = 0.01, t(3, 3958) = 0.6, p = 0.5 \)). No gamma power × gamma type interaction was found for t-span (C; multiple regression interaction term; \( b = -0.04, t(3958) = -1.4, p = 0.2 \)), nor was there a main effect of gamma power (\( b = 0.002, t(3958) = 0.1, p = 0.9 \)).
Figure S3, Related to Figure 4. Effects of fast and slow gamma power on properties of theta sequences for different theta sequence selection criteria. Slope (A), x-span (B), and t-span (C) as a function of slow (blue) and fast (red) gamma power for all significant sequences (i.e., including those with both positive and negative slopes, n = 7775 sequences). Absolute values of slopes were used to combine positive and negative slope values (A). We found a significant interaction effect of gamma power and gamma type (i.e., slow or fast) on both slope and x-span measures (multiple regression model interaction term; slope: $b = 0.07$, $t(15546) = 4.2$, $p < 0.001$; x-span: $b = 0.06$, $t(15546) = 3.4$, $p = 0.001$) but not t-span ($b = -0.02$, $t(15546) = -1.3$, $p = 0.2$). Also, gamma power did not significantly affect t-spans ($b = 0.002$, $t(15546) = 0.3$, $p = 0.8$). Slope and x-span measures increased as a function of slow gamma power (linear
regression; $b = 0.04$, $t(7773) = 3.2$, $p = 0.002$ for slope, and $b = 0.03$, $t(7773) = 2.8$, $p = 0.005$ for x-span) but decreased as a function of fast gamma power ($b = -0.03$, $t(7773) = -2.7$, $p = 0.006$ for slope, and $b = -0.02$, $t(7773) = -2.0$, $p = 0.04$ for x-span). (D) Slow and fast gamma power as a function of accuracy for all significant sequences (i.e., including those with both positive and negative slopes). We found that different gamma types showed different relationships to accuracy (multiple regression model interaction term; $b = 0.2$, $t(15546) = 9.8$, $p < 0.001$). High fast gamma power correlated with high accuracy ($b = -0.1$, $t(7773) = -6.6$, $p < 0.001$), and high slow gamma power correlated with low accuracy ($b = 0.1$, $t(7773) = 7.3$, $p < 0.001$).

In the main analyses, significant sequences were comprised of two groups based on two methods for determining significance of sequences (See “Theta sequence analysis and significance determination” section of Experimental Procedures). One group contained sequences with relatively steep slopes ($n = 4668$), and the other group contained sequences with relatively flat (i.e., near zero) slopes ($n = 1173$). The latter group contained a small proportion (~1/5) of all slow and fast gamma sequences (see Figure 2B). When we separated these two groups of sequences, we found a significant interaction of gamma type (i.e., slow or fast) and slope type (i.e., steep or flat) on gamma power (repeated measures ANOVA, $F(1,5839) = 7.1$, $p = 0.008$), indicating that slow and fast gamma power exhibited different relationships with different slope types. In the group of sequences with steep slopes, we found a significant interaction of gamma power and gamma type (i.e., slow or fast) on slope (multiple regression model interaction term; $b = 0.05$, $t(9332) = 2.8$, $p = 0.005$). For steep slope sequences, slow gamma power and slope were positively correlated ($b = 0.03$, $t(4666) = 2.9$, $p = 0.004$),
but fast gamma power and slope were not \( b = -0.01, \ t(4666) = -1.1, p = 0.3 \). The narrow range of slope values in the group of significant sequences with flat slopes (see Figure 2B) hindered analysis of changes in flat slopes as a function of gamma power. Perhaps not surprisingly then, the values of flat slopes did not change significantly with gamma power (multiple regression model: interaction term, \( b = 0.01, t(2342) = 1.5, p = 0.1 \); effect of gamma power, \( b = -0.002, \ t(2342) = -0.5, p = 0.6 \)). However, for sequences with flat slopes, fast gamma power ranks were significantly higher than slow gamma power ranks (paired t-test; \( t(1172) = 2.4, p = 0.02 \)).
Figure S4, Related to Figure 4. Effects of behavioral state of the animal, position on the track, and time within a session on theta sequences. (A) Histogram showing
distributions of running speed, acceleration, and jerk estimates for all significant sequences (green), significant fast gamma sequences (red), and significant slow gamma sequences (blue).  (B) Correlation between slope and fast (red) or slow (blue) gamma power as a function of normalized running speed, acceleration, and jerk. Note how the correlation between slope and gamma power does not change as a function of speed, acceleration, or jerk.  (C) Correlation between path length (x-span) and fast (red) or slow (blue) gamma power as a function of normalized running speed, acceleration, and jerk. Note how the correlation between x-span and gamma power does not change as a function of speed, acceleration, or jerk.  (D) Correlation between slope and fast (red) or slow (blue) gamma power as a function of normalized position on the track and time within a session.  (E) Correlation between path length (x-span) and fast (red) or slow (blue) gamma power as a function of normalized position on the track and time within a session. Note that correlations between gamma power and slope, and between gamma power and path length, do not change systematically with track position or time within a session.
Figure S5, Related to Figure 5. Gamma phase analyses. (A-D) Theta-gamma phase-phase plots of continuous local field potential recordings (from 56 tetrodes) (A,
and corresponding shuffled signals \((B, D)\), during slow gamma sequences \((A, B)\) and fast gamma sequences \((C, D)\). Theta-gamma phase-phase coupling was apparent for both slow and fast gamma, consistent with previously published reports (Belluscio et al., 2012). Shuffling procedures were based on those published by Belluscio and colleagues (Belluscio et al., 2012; see “Phase-phase coupling” section of Supplemental Experimental Procedures). (E) Mean radial distance \((R\) value\) from the distribution of the differences between theta phases and gamma phases calculated for different \(n:m\) (number of theta cycles: number of gamma cycles) relationships. Note that peaks can be seen around 1:4 for slow gamma and 1:9 for fast gamma, consistent with previous findings (Belluscio et al., 2012). (F, I) Spike counts at successive slow gamma cycles \((F)\) and fast gamma cycles \((I)\). Gamma cycles within theta sequences were temporally ordered, centered at cycle 0 (corresponding to the gamma cycle with maximal number of spikes). (G-H, J-K) Neither the preferred slow gamma phase of spiking during slow gamma sequences \((G-H)\) nor the preferred fast gamma phase of spiking during fast gamma sequences \((J-K)\) varied significantly according to the number of spikes within a theta sequence. Data represent angular mean ± angular deviation. (L) Probability distributions of gamma phases of spikes in slow gamma sequences (top) and fast gamma sequences (bottom) across mock gamma cycles that were created using an alternative method (see “Alternative method for creating mock gamma cycles” section of Supplemental Experimental Procedures). In this method, the real gamma phases were maintained within each gamma cycle, but gamma cycles were shuffled within each theta sequence. Spike times were not shuffled such that the original theta phases of spikes within each theta sequence were preserved. Slow gamma phases at spike times did
not shift significantly across mock slow gamma cycles within slow gamma sequences (top). The mean phase shift across successive slow gamma cycles for the real data was -46.0 degrees, which was significantly different than the phase shifts observed across the mock slow gamma cycles (-0.01 ± 2.9 degrees, mean ± angular deviation; 95% confidence intervals for mock data: [-4.0, 5.6] degrees, p < 0.0001). Fast gamma phases at spike times also did not shift significantly across mock fast gamma cycles within fast gamma sequences (bottom). The mean phase shift across real fast gamma cycles (-6.0 degrees) was not significantly different than the phase shifts across mock fast gamma cycles (-6.2 ± 2.9 degrees, mean ± angular deviation; 95% confidence intervals for mock data: [-9.8, -2.0] degrees, p = 1.0).
Figure S6, Related to Figure 6. Slow and fast gamma sequences did not co-occur, but individual place cells fired during both. (A) Average power spectra for fast gamma sequences (red) and slow gamma sequences (blue). (B) Cross-correlogram for slow gamma peaks vs. fast gamma peaks. The procedure used to detect slow and fast gamma peaks is described in the “Cross-correlation of gamma peaks” section of the Supplemental Experimental Procedures. Data represent mean ± SEM. (C) There was a significant positive correlation between the spike rate of each cell during fast gamma sequences and slow gamma sequences. Each open circle corresponds to measures from one cell. (D-E) Place cells were divided into low firing and high firing categories for each gamma type (see “Place cell spike rates during slow and fast gamma sequences” section of Supplemental Experimental Procedures). Place cells exhibited similar levels of activity during both gamma types. This finding is inconsistent with the hypothesis that distinct classes of place cells, corresponding to
‘TroPyr’ and ‘RisPyr’ cells (Senior et al., 2008), fire selectively during slow and fast gamma.
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Tetrode and recording drive preparation.
Recording drives contained 14 (6 rats) or 26 (1 rat) independently movable tetrodes constructed from 17 μm polyimide-coated platinum-iridium (90%-10%) wire (California Fine Wire). Electrode tips in tetrodes targeted toward cell body layers were platinum-plated to reduce single channel impedances to ~150-300 kΩ at 1 kHz.

Tetrode placement.
Over the course of a few weeks after drive implantation, tetrodes were slowly lowered toward their target locations. We targeted 6 of the 14 tetrodes toward the CA1 cell body layer and 6 toward the CA3 cell body layer in 4 rats (CA3 data was not used for this study), and 12 of the tetrodes toward the CA1 cell body layer in 2 rats, and 24 tetrodes toward the CA1 cell body layer in 1 rat. In all rats, 1 tetrode was targeted toward the apical dendritic layers of CA1 and was used for hippocampal depth estimation as the rest of the tetrodes were turned down. In all rats, 1 tetrode was used as a reference for differential recording. This reference tetrode was placed at the level of the corpus callosum or higher and was recorded against ground to make sure that it was placed in a quiet location. All recording locations were verified histologically after experiments were finished (see “Histology” section below). Figure S1 shows the recording locations for the 56 tetrodes that were included in this study (i.e., those that had place cells and were located in or near CA1 stratum pyramidale on the days of recording). In one rat, two tetrodes targeted toward CA1 appeared in histological sections to be on the border
of CA2-CA1. However, place cells and LFPs recorded from these tetrodes were indistinguishable from other CA1 recordings collected simultaneously. Therefore, place cells and LFPs from these tetrodes were included in this study.

**Data acquisition.**

Experiments began when spikes with amplitudes exceeding ~5 times the noise levels emerged around the estimated depth of CA1 (i.e., ~2 mm). Robust theta rhythms and sharp wave-ripple activity were used as on-line verification that tetrodes were in the CA1 cell body layer. Recording drives were connected to multichannel, unity gain headstages (Neuralynx, Bozeman, MT, USA). The headstage output was conducted via lightweight tether cables through a multichannel slip-ring commutator to a data acquisition system that processed the signals through individual 24 bit AD converters (Digital Lynx, Neuralynx, Bozeman, MT, USA). Unit activity was bandpass filtered from 600 to 6000 Hz. Spike waveforms above a threshold set by the experimenter (~55-75 μV) were time-stamped and recorded at 32 kHz for 1 ms. Light-emitting diodes (LEDs) on the headstages were used to track rats’ movements at a 30 Hz sampling rate. Additionally, video files were collected during every recording session. LFPs (1 per tetrode) were recorded continuously in the 0.1-500 Hz band at a sampling rate of 2000 Hz. Notch filters were not used. Continuously sampled LFPs were recorded differentially against a common reference electrode placed in an electrically quiet region (see “Tetrode placement” section above). The common reference signal was duplicated using a breakout board (MDR-50 breakout board, Neuralynx, Bozeman, MT, USA) and recorded continuously against ground.
Spike sorting and cell classification.

Spike sorting was performed offline using graphical cluster-cutting software (MClust v3.5; A.D. Redish, University of Minnesota, Minneapolis). Spikes were clustered manually in two-dimensional projections of the multidimensional parameter space (consisting of waveform amplitudes, energies, and peak-to-valley ratios). Autocorrelation and cross-correlation functions were additionally used to identify single units. Putative place cells were distinguished from putative interneurons on the basis of spike width, average firing rate, and bursting properties (Fox and Ranck, 1981; Frank et al., 2001; Harris et al., 2000; Henze et al., 2000). Firing rate maps across behavioral sessions (see “Place fields” section, below) were also used to identify place cells.

Place fields.

Rats’ trajectories were tracked using red and green LEDs on the headstage. Spatial firing rate distributions (“place fields”) for each well-isolated putative pyramidal cell were constructed by summing the total number of spikes that occurred in a given location bin (3 cm), dividing by the amount of time that the animal spent in that bin, and smoothing with a 15 cm (5 bins) SD Gaussian centered on each bin. A total of 604 CA1 place cells were used; 351 of these cells were included in a previous study (Bieri et al., 2014).

The two track directions were analyzed separately; this was also the case for data sets containing bidirectional place cells. A minority of place cells in this study (92 out of 604 cells) were defined as bidirectional. Bidirectional place cells were defined using the following criteria from (Battaglia et al., 2004): (1) exhibited a mean firing rate
> 0.3 Hz in each direction; (2) spatial information content > 0.25 bits/spike in each
direction; and (3) maximum rate overlap (R) between the rate maps in the two directions
> 0.35.

**Estimation of running speed.**
The running speed for each position ($x_n$) was estimated by taking the difference
between the preceding position ($x_{n-1}$) and the following position ($x_{n+1}$) and dividing by
the elapsed time ($2*1/position$ sampling frequency) (Bieri et al., 2014). The sampling
frequency for position data was 30 Hz, thus allowing a temporal resolution of 1/15
seconds for running speed. Acceleration and jerk (Figure S4) were calculated as the
first and second derivatives, respectively, of the running speed.

**Oscillatory power estimation.**
Time varying power for slow gamma (25–55 Hz), fast gamma (60–100 Hz), theta (6–10
Hz), and delta (2–4 Hz) were computed, using a wavelet transform method described
previously (Tallon-Baudry et al., 1997). Signals were prewhitened and then convolved
by a family of complex Morlet’s wavelets $w(t,f)$, one for each frequency, as a function
of time:

$$w(t,f) = Ae^{-t^2/2\sigma_t^2}e^{2i\pi f t}$$

With $\sigma_f = 1/2\pi\sigma_t$. The coefficient A was set at:

$$(\sigma_t\sqrt{\pi})^{-1/2}$$
in order to normalize the wavelets such that their total energy was equal to 1. The family
of wavelets was characterized by a constant ratio $f/\sigma_f$, which was set to 5. Slow and
fast gamma power estimates were then z-scored across the entire recording session and averaged across respective frequency ranges and across tetrodes with place cell recordings. The maximum value of the resulting time-varying power was used to obtain a single slow gamma value and a single fast gamma value for each theta cycle.

**Phase-phase coupling (Figure S5).**

Phase–phase coupling plots were constructed based on the methods presented in a previous paper (Belluscio et al., 2012). Theta phase-slow gamma phase coupling was calculated as a bivariate histogram of the instantaneous theta and slow gamma phases (see “Phase analyses” section of Experimental Procedures) across significant slow gamma sequences (Figure S5A). Shuffled data were generated for comparison purposes, and shuffling procedures were based on those used previously (Belluscio et al., 2012). In each slow gamma sequence, slow gamma phases were randomly shifted between 1 and 100 ms, whereas theta phases remained the same. A 2-D histogram for shuffled data was then generated using the shifted slow gamma phases and non-shifted theta phases, and this procedure was repeated 1000 times. The final shuffled theta phase–slow gamma phase coupling plot was constructed by averaging all of the histograms from shuffled data (Figure S5B). The same procedure was performed for theta-fast gamma phase-phase coupling, except that instantaneous theta and fast gamma phases from significant fast gamma sequences were used (Figure S5C-D).

**n:m phase-phase locking (Figure S5E)**
In order to estimate how many gamma cycles occurred in a theta cycle, the n:m phase-locking ratio was calculated between theta and slow gamma, and between theta and fast gamma (Belluscio et al., 2012; Tass et al., 1998). We measured the mean resultant length (R value) of the distribution of phase differences:

$$\Delta\text{phase}_{n:m}(t) = n \times \text{phase}_{\text{theta}}(t) - m \times \text{phase}_{\text{gamma}}(t)$$

on each theta sequence for different n:m ratios (i.e., 1:1, 1:2, ..., 1:12). $R = 1$ represents a perfect unimodal distribution of n:m phase differences (Rayleigh test for uniformity), whereas $R = 0$ indicates that the n:m phase difference comes from a uniform distribution. The R value curves plotted for theta-slow gamma phase-phase coupling and theta-fast gamma phase-phase coupling were averaged across all significant slow gamma sequences and all significant fast gamma sequences, respectively (Figure S5E).

**Alternative method for creating mock gamma cycles (Figure S5L)**

For each slow and fast gamma sequence, slow and fast gamma phases were estimated within gamma cycles as described in the “Phase analyses” section of the Experimental Procedures. Then, for each slow and fast gamma sequence, wholly intact gamma cycles (i.e., those cycles occurring entirely within the slow or fast gamma sequence) were identified and randomly shuffled within their associated sequence. Partial gamma cycles occurring at the ends of the sequence were swapped within their associated sequence. This method provided a way to create mock gamma cycles without requiring arbitrary assignment of gamma phases to time points (i.e., the actual estimated gamma phases were maintained within each mock gamma cycle). Spike times were not
shuffled such that the original theta phases associated with spike times were preserved. Thus, gamma phases were shuffled relative to spike times, but the sequence of theta phases associated with spike times remained intact. This procedure was repeated 1000 times for each slow and fast gamma sequence.

**Cross-correlation of gamma peaks (Figure S6B)**

For LFP recordings from the whole session, slow and fast gamma peaks were identified as time points corresponding to slow and fast gamma phases of ~180° (see “Phase analyses” section of the Experimental Procedures) and exhibiting peak power exceeding 2 SD above the mean slow and fast gamma power, respectively. Numbers of slow gamma peaks and fast gamma peaks were separately counted within 100 ms sliding time bins, shifted by 10 ms. The resulting time series were then used to calculate cross-correlations between time-varying slow gamma and fast gamma peak counts.

**Place cell spike rates during slow and fast gamma sequences (Figure S6C-E).**

For each cell, spike rates were calculated within slow gamma sequences and fast gamma sequences for each recording day. Cells were considered ‘high firing’ or ‘low firing’ for each gamma type if their spike rate was higher or lower, respectively, than the median spike rate from all cells for that particular gamma type.

**Histology (Figure S1).**
For verification of tetrode locations, brains were cut coronally into 30 μm sections and stained with cresyl violet. All tetrode tracks were identified, and the deepest location of each tetrode was determined by comparison across adjacent sections.

SUPPLEMENTAL REFERENCES


