Dynamic Gamma Modulation of Hippocampal Place Cells Predominates Development of Theta Sequences

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ABSTRACT

The experience-dependent spatial cognitive process requires sequential organization of hippocampal neural activities by theta rhythm, which develops to represent highly compressed information for rapid learning. However, how the theta sequences were developed in a finer time scale within theta cycles remains unclear. In this study, we found that sweep-ahead structure of theta sequences developing with exploration was predominantly dependent on a relatively large proportion of FG-cells, i.e. a subset of place cells dominantly phase-locked to fast gamma rhythms. These ensembles integrated compressed spatial information entrained in a theta sequence by cells consistently firing at precessing slow gamma phases within the theta cycle. Accordingly, the sweep-ahead structure of FG-cell sequences was positively correlated with the intensity of slow gamma phase precession, in particular during early sequence development. These findings highlight the dynamic network-modulation by fast and slow gamma in the development of theta sequences which may further facilitate memory encoding and retrieval.

Keywords: Hippocampus, Place cells, Theta sequences, Gamma rhythms, Sequence development

INTRODUCTION

The hippocampus is known as its central role in encoding and storing experience and thus is essential for forming episodic memories (Buzsaki, McKenzie, & Davachi, 2022; Cohen & Eichenbaum, 1993). In the context of active navigation in an environment, hippocampal place cells are organized to fire sequentially within individual cycles of theta oscillations (4-12Hz) and form time-compressed representations of behavioral experiences, referred to as theta sequences (Colgin, 2020; Comrie, Frank, & Kay, 2022; Dragoi & Buzsaki, 2006; Skaggs, McNaughton, Wilson, & Barnes, 1996; Wallis, 2018). By sweeping ahead from the past to the upcoming locations, theta sequences represent information about potential future paths that could guide goal-directed behaviors (Gupta, van der Meer, Touretzky, & Redish, 2012; Joshi et al., 2023; Zheng, Hwaun, Loza, & Colgin, 2021). These predictive theta sequences exhibited experience-dependent development when rats were exploring a novel environment or learning a novel goal location (Feng, Silva, & Foster, 2015; Gobbo et al., 2022; Wikenheiser & Redish, 2015; Zheng et al., 2021). However, how the hippocampal network modulated the development of theta sequences' temporospatial-compressed organization remains incompletely understood.

A common assumption was established by previous studies that theta sequences were manifestations of theta phase precession, in which an individual place cell fired at progressively earlier theta phase as the animal traversed the place field (Aoki et al., 2023; Foster & Wilson, 2007; O'Keefe & Recce, 1993; Qasim, Fried, & Jacobs, 2021; Skaggs et al., 1996). However, when examined the theta sequences with experience at single trial level, their sequential structures were not developed on the first experience of a novel environment even if the phase

precession of individual place cells has been observed (Colgin, 2020; Feng et al., 2015). This suggests that the dynamic process of theta sequences development could be modulated by another network-level mechanism with neuronal firing organized at a finer timescale within theta cycles. Gamma (25-100Hz) could be a candidate that is thought to interact with theta to temporally organize theta sequences (Dragoi & Buzsaki, 2006; Fernandez-Ruiz, Sirota, Lopes-Dos-Santos, & Dupret, 2023; Lisman & Jensen, 2013). As two subtypes of gamma, fast and slow gamma rhythms (Colgin et al., 2009; Schomburg et al., 2014; Zhang, Lee, Rozell, & Singer, 2019) occur at distinct theta phases and coordinate theta sequences in different manners (Zheng, Bieri, Hsiao, & Colgin, 2016). This indicates that fast and slow gamma may act as different tuners but collaborate together in modulating theta sequence development.

Fast gamma (~65-100Hz), associated with the input from the medial entorhinal cortex, is thought to rapidly encode ongoing novel information in the context (Fernandez-Ruiz et al., 2021; Kemere, Carr, Karlsson, & Frank, 2013; Zheng et al., 2016). During fast gamma rhythms, place cell spikes occurred across all positions within a place field and displayed theta phase precession (Bieri, Bobbitt, & Colgin, 2014). This type of cells was identified recently as "phase-precessing" cells, in which the phase-precession pattern emerges with strong fast gamma (Guardamagna, Stella, & Battaglia, 2023). Their discrete distribution of theta phases is likely to contribute to the sequential structure formation when the sequences have been developed (Wang, Foster, & Pfeiffer, 2020). However, a question is raised about how these fast gamma-modulated cells be coordinated for the developmental process of theta sequences.

Slow gamma (~25-45Hz) in the hippocampal CA1 reflects information received

from CA3, which is responsible for the integration processing of learned information

and experience (Colgin et al., 2009; Zhu et al., 2023). In contrast to fast gamma,

spiking during slow gamma exhibited dominant theta phase locking and attenuated

theta phase precession (Guardamagna et al., 2023; Zhang et al., 2019). This

concentrated distribution of theta phase could be interpreted as they are coordinated

within slow gamma cycles, as a finer time-scale than a theta cycle. Place cells

spiking exhibited slow gamma phase precession, to generate mini-sequences in an

individual theta sequence, for highly time-compressed representation of ongoing and

future information (Zheng et al., 2016; Zheng et al., 2021). Thus, how fast and slow

gamma rhythms coordinate place cells spiking to modulate the dynamic process of

theta sequence development remains unclear.

Here, we proposed a scheme that fast gamma rhythms coordinate a subgroup

of place cells for rapidly encoding and processing sensory information since from the

early phase of theta sequence development (Fig.1). The developing process would

be then dependent on the slow gamma modulation, that the spikes are fired at slow

gamma phase-precessed pattern for spatial information compression within theta

cycles. To test this hypothesis, we investigated how fast and slow gamma

separately modulated place cells activities during the whole process of theta

sequence development. We found that the fast gamma phase-locked place cells

(FG-cells) were crucial for the development of theta sequences, without which the

temporospatially compressed structure of theta sequences could not be developed.

Furthermore, these cells exhibited slow gamma phase precession within theta cycles

associated with the sweep-ahead structure of theta sequences, which occurred

earlier than those cells not phase locked to fast gamma (NFG-cells). Our findings

highlight the dynamic network-modulation by neural oscillations fast and slow

gamma, at finer timescales within theta, in development of theta sequences which

may further facilitate memory encoding and retrieval.

RESULTS

A subgroup of hippocampal place cells phase locked to fast gamma rhythms during active behaviors

To investigate the temporal organization of place cells' firing associated with the

gamma rhythms during the development of theta sequences, we recorded neuronal

spiking of place cells and local field potentials simultaneously in hippocampal CA1

from 4 feely behaving rats (Fig.2-S1). As shown in a previous study, 32% of

hippocampal pyramidal cells were phase-locked to gamma rhythms during waking

theta states (Senior, Huxter, Allen, O'Neill, & Csicsvari, 2008). We further tested how

the hippocampal place cells were modulated by slow and fast gamma rhythms

respectively. The slow and gamma episodes were detected in time periods when the

power of each gamma type (Fig.2A-B, fast gamma: 65-100 Hz, slow gamma: 25-45

Hz) was above a preset threshold (see Materials and Methods). We then detected

the slow or fast gamma phase-locked place cells by quantifying the gamma phase

distributions of all spikes across all trials. In this study, 488 place cells were included

with place field distributed on the circular track but not located at the resting box. We

found that 120 (24.6%) out of these cells were significantly phase-locked to gamma

rhythms, a majority of which (113 cells, 23.2%) exhibited significant phase-locking to

fast gamma rhythms (Fig.2C). Their preferred fast gamma phases (202.38±2.01

deg) were concentrated around the peak of fast gamma cycle, which was consistent

with the findings in Schomburg et al. 2014 (Schomburg et al., 2014). However, a

very small number (3.7%) of cells were found to phase-locked to slow gamma

rhythms. Thus, these place cells were subdivided according to whether they were

significantly phase-locked to fast gamma (FG-cells) or not (NFG-cells) during active

behaviors in the subsequent analyses. Furthermore, the fast gamma phase-locking

of FG-cells was stable across trials, with a significantly higher mean vector length of

fast gamma phase-locking than those of NFG-cells (Fig.2D, repeated measure

ANOVA, main effect of cell type, $F(1,22)=33.9$, $p=7.5×10^{-6}$, partial η^2 =0.6; main

effect of trials, $F(4,88)=1.3$, $p=0.3$, partial η^2 =0.06, no cell type x trials interaction,

$F(4,88)=0.5$, $p=0.8$, partial η^2 =0.02). Meanwhile, we also found that a larger

proportion of neurons in FG-cells had significant theta phase precession than NFG-

cell (Fig.2-S2, chi-squared test, ratio of theta phase precession cell among the FG-

cell vs. NFG-cell, n=457cell，$\chi^2=13.7$, $p=2.1×10^{-4}$, Cramer V=0.2). These results

suggested that the FG-cells were stably modulated by fast gamma rhythms during

active behaviors.

The FG-cells were crucial for theta sequence development

As theta sequences were developed dependent on experience, we next

investigated whether FG-cells and NFG-cells played different roles in the

development of theta sequences. We then used a Bayesian decoding approach

(see Methods) to identify trajectories represented as rats running on a circular track,

and detected significant theta sequences in each single lap (Fig.3A). Another two

decoders were built by excluding either FG-cells spikes or down-sampled NFG-cells

spikes respectively, in order to compare the structures of theta sequences without

each type of cells. It can be observed that posterior probability distribution of theta

sequences was changed by excluding spikes from FG-cells (Fig.3B). The weighted

correlation of each sequence (Feng et al., 2015) was then measured to quantify the

temporospatial correlation of a theta sequence structure. We found that the

weighted correlation of theta sequences decoded by excluding FG-cells spikes

(exFG-sequences) was much lower than that of theta sequences decoded by

excluding down-sampled NFG-cells spikes (exNFG-sequences) in the same theta

cycles (Fig.3C, repeated measure ANOVA: main effect of cell type, $F_{(2,4844)}=128.7$,

$p=3.5\times10^{-48}$, partial η^2 =0.05; post-hoc test, raw sequences vs. exFG-sequences,

$p=8.0\times10^{-56}$, raw sequences vs. exNFG-sequences, $p=1.6\times10^{-26}$, exFG-sequences

vs. exNFG-sequences, $p=4.1\times10^{-9}$). This suggests that the sequential structure

could be dominantly interrupted by excluding FG-cells rather than NFG-cells.

Consistent with the previous studies (Feng et al., 2015; Wang et al., 2020), we

also observed the development of theta sequences along with exploration

experience (Fig.3D-E, repeated measure ANOVA, main effect of trials, $F_{(4,44)}=7.0$,

$p=2.0\times10^{-4}$, partial $\eta^2=0.4$). This could not be accounted for by running speed, as

the rats were feely moving at stable running speed across trials (Fig.3F, repeated

measure ANOVA, main effect of trials: $F_{(4,44)}=0.9$, $p=0.5$ partial η^2 =0.07). We

further investigated whether the FG-cells or NFG-cells were required for the

development of theta sequence structure. Interestingly, the temporospatial structure

of the theta sequences cannot be developed with experience when the FG-cell

spikes were excluded, with significantly lower weighted correlation than theta

sequences constructed by excluding NFG-cell spikes (Fig.3D,G, repeated measure

ANOVA, trials x cell type interaction, $F_{(4,44)}=3.0$, $p=0.03$, partial $\eta^2=0.2$, main effect

of cell type, $F_{(1,11)}=7.8$, $p=0.02$, partial $\eta^2=0.4$; main effect of trials, $F_{(4,44)}=4.0$,

$p=0.008$). Since the theta sequences have been developed after Lap2, we defined

the early development phase of sequences as the first 2 laps and late development

phase of sequences as the last 2 laps of exploration experience, respectively. We

found that the weighted correlation of both exFG-sequences and exNFG-sequences

was low during early development (Fig.3H, paired t-test, t(24)=0.2, p=0.9, Cohen's

d=0.04). During late development, however, the weighted correlation of exNFG-

sequences was higher than that of exFG-sequences (Fig.3H, paired t-test, t(24)=-

3.7, p=0.001, Cohen's d=0.8). These results suggest that fast gamma modulation of

place cells may contribute to the development of theta sequence with exploration

experience.

In order to estimate to what extent, the FG cells contributed to theta sequence

development, we investigated the relationship between the percentage of FG-cells

and the formation of sequential structure in different developments. In our dataset,

the percentage of FG-cells out of all place cells varied across days, thus we detected

theta sequences from trials with relatively low percentage (~10%) and high

percentage (~35%) of FG-cells. The number of excluded NFG-cells for decoding

was matched up with the number of excluded FG-cells by a pseudo-random method

in each day. During early development, the temporospatial structure of theta

sequences has not been developed by excluding either low or high percentage of

each type of cells (Fig.4A). During late development, however, the sweeping-ahead

structure of the theta sequence was significantly disrupted when excluding a high

percentage (~35%) of FG-cells, which was not observed when excluding a low

percentage (~10%) of FG-cells (Fig.4B). The sequential structure could be

maintained by excluding NFG-cells at either low or high percentage. To quantify the

relationship between sequence structure and the number of excluded neurons during

early and late development, we then measured the weighted correlation difference

between exFG-sequences and exNFG-sequences as a function of the fraction of

excluded cells. The results showed that the weighted correlation difference was

close to 0 regardless of the fraction of excluded cells during early development

(Fig.4C, Pearson's correlation, Corr=0.1, p=0.7). During late development, however,

we found that the weighted correlation difference was positively correlated with the

fraction of excluded cells (Fig.4D, Pearson's correlation, Corr=0.6, p=0.03). These

results suggest different roles of FG-cells and NFG-cells in the development of the

sweeping-ahead structure of theta sequence with experience, that the more

temporospatially compressed structure of theta sequences may require a larger

percentage of FG-cells.

In addition, we tested whether these results could be accounted for by the

different firing characteristics between FG-cells and NFG-cells. We found that

although these two types of cells took different proportion of all recorded place cells,

the place fields of both types of cells could cover the entire track uniformly (Fig.2C,

Fig.5A). We measured the distribution of center of mess (COM) of main place field

of FG-cells and number-matched NFG-cells (Fig.5B), and found similar distribution of

place field COM between cell types (Fig.5C, two-sample Kolmogorov-Smirnov test,

Z=0.1, p=0.8). Furthermore, we found that the theta sequences in the above

analyses were decoded by excluding similar numbers of spikes from FG-cells and

NFG-cells (Fig.5D-E, Student's t test, spike number: $t(22)=1.8$, p=0.09, Cohen's

D=0.7; mean firing rate in field: $t(22)=2.0$, p=0.06, Cohen's D=0.8), indicating that the

deferent development patterns between exFG-sequences and exNFG-sequences

were not due to quality difference between two decoders. The two type of cells

exhibited similar place fields size (Fig.5F, $t(22)=0.7$, p=0.5, Cohen's D=0.3) and

spatial information (Fig.5G, $t(22)=1.0$, p=0.3, Cohen's D=0.4). These findings

support the hypothesis that FG-cells, but not NFG-cells, may contribute to the

development of theta sequences with experience.

FG-cells exhibited slow gamma phase precession for information compression required in theta sequence development

In our previous study, we found that the place cell spikes occurred at progressively earlier slow gamma phases across slow gamma cycles in theta sequences, so-called slow gamma phase precession (Zheng et al., 2016). As sequences of locations were represented at different slow gamma phases, theta sequences exhibited temporospatially compressed structure representing long paths. We next investigated if the development of theta sequence structure was related to the slow gamma phase modulation of place cells. By detecting the slow gamma phases of spikes within all theta cycles, we observed that the slow gamma phases significantly shifted across successful slow gamma cycles (Fig.6A, multi-sample Mardia-Watson-Wheeler test, $W(4)=369.4$, $p<2.2\times10^{-16}$; post-hoc test, Cycle -1 vs. Cycle 0, $W(2)=97.5$, $p<2.2\times10^{-16}$, Cycle 0 vs. Cycle 1, $W(2)=95.4$, $p<2.2\times10^{-16}$), which was consistent with the previous finding (Zheng et al., 2016). Then we separated out the spike activity from the early and late development, and tested if the slow gamma phase precession still existed during different developments. Interestingly, we observed slow gamma phase shifting, but not phase precession, across successive gamma cycles during early development. The distribution of slow gamma phases in Cycle0 was similar as that in Cycle-1, then was dispersed in Cycle1 (Fig.6B, multi-sample Mardia-Watson-Wheeler test, $W(4)=18.5$, $p=9.8\times10^{-4}$; post-hoc test, Cycle -1 vs. Cycle 0, $W(2)=0.8$, $p=0.7$; Cycle 0 vs. Cycle 1, $W(2)=15.5$, $p=4.4\times10^{-4}$). However, the slow gamma phase precession existed during late development (Fig.6C, multi-sample Mardia-Watson-Wheeler test, $W(4)=21.2$, $p=2.8\times10^{-4}$; post-hoc test, Cycle -1 vs Cycle 0, $W(2) = 8.8$, $p = 0.01$; Cycle 0 vs.

Cycle 1, W(2)=8.2, p=0.02).   This finding suggests that slow gamma phase

precession of place cells may occur with exploring experience.

Furthermore, we investigated how the slow gamma phase precession occurred

in theta sequences developed with experience.   We detected theta sequences

during slow gamma episodes and measured the distribution of phase shifts across

gamma cycles within single unites.   In the significant theta sequences during slow

gamma episodes, we observed the slow gamma phase precession from single unit,

that the place cell spikes occurred at earlier slow gamma phases in later slow

gamma cycles (Fig.7A).   To quantify the slow gamma phase precession at single unit

level, we calculated the phase shift as the phase difference within spike pairs from

adjacent active slow gamma cycles (See Methods).   We found that slow gamma

phase difference was significantly lower than 0 between 2 active slow gamma cycles

(Fig.7B, one sample t-test, $t(485)=-11.9$, $p=6.3×10^{-29}$, Cohen's D=0.5), as well as

among 3 or ≥4 active slow gamma cycles for strict limitation of theta sequences

modulated by slow gamma (3 active cycles, $t(140)=-6.8$, $p=3.0×10^{-10}$, Cohen's

D=0.6; ≥4 active cycles, $t(27)=-3.2$, $p=0.003$, Cohen's D=0.6).   Furthermore, we

averaged the slow gamma phase differences across different cells within a theta

sequence and compare their distribution along with learning trials.   In the first lap, we

found that the slow gamma phase differences were not significantly different from

their surrogate data (Fig.7C, median of phase difference from lap1 was in 95%

confidence intervals of those from shuffled data, p=0.3).   However, the distribution of

slow gamma phase difference was negatively biased compared with their surrogate

data (Fig.7C, median of phase difference from lap2-5 was beyond the 95%

confidence intervals of those from shuffled data; lap2 p=0.001, lap3 p=0.001, lap4

p=0.002, lap5 p=0.002).   These results can also be replicated by averaging the slow

gamma phase differences from a single unit across successive theta sequences

(Fig.7D, lap1 p=0.1, lap2 p=0.001, lap3 p=0.001, lap4 p=0.002, lap5 p=0.01).

These findings raised a question of whether the development of slow gamma

phase presession existed for both FG-cells and NFG-cells. To address this question,

we quantified the distribution of slow gamma phase difference in FG-cells and NFG-

cells respectively. We found that the slow gamma phase difference of FG-cells

consistently exhibited a stable phase precession across trials, independent with

learning experience (Fig.7E, lap1 p=0.007, lap2 p=0.006, lap3 p=0.007, lap4

p=0.002, lap5 p=0.02). In contrast, the slow gamma phase precession of NFG-cells

was experience-dependent, with negative biased distribution of phase difference

occurring from Lap2 (Fig.7E, lap1 p=0.9, lap2 p=0.01, lap3 p=0.001, lap4 p=0.009,

lap5 p=0.1). These results suggest that FG-cells and NFG-cells have different

dynamic modulated by slow gamma rhythms during exploration experience. The

slow gamma modulation of FG-cells may occur earlier than that of NFG-cells,

thereby driving the location information to be coded within theta cycles in a highly

temporospatially compressed way.

FG-cells and NFG-cells exhibited different dynamics of slow gamma

modulation along with theta sequence development

Finally, we wondered if there was correlation between slow gamma phase

precession and the development of theta sequence structure, and how the FG-cells

and NFG-cells coordinated during this process. Within each theta sequence, we

measured its weighted correlation of the sequence structure, and plot it as a function

of slow gamma phase difference across slow gamma cycles. Interestingly, we found

most data were distributed in the Quadrant1 (Q1) compared to the shuffled data

distributed at the center (Fig.8A-B). The theta sequences with negative slow gamma

phase difference and positive weighted correlation were significantly dominant

(Fig.8C, the probability of observed data from lap1 exceeded 95% confidence

intervals of those from shuffled data, p=0.001). This finding supports our hypothesis

that the theta sequence development could be associated with the slow gamma

phase precession.

We further investigated the contribution of FG-cells and NFG-cells to the slow

gamma modulated theta sequence development during different exploring phases.

Importantly, the distribution of FG-cell-dominant theta sequences was significantly

biased to Q1 than that of the shuffled data, not only in late development when the

theta sequences have been developed, but also in early development (Fig.8D-F,

early development, p=0.001; late development, p=0.004). However, for NFG-cell-

dominant theta sequences, their distribution was significantly biased to Q1 than that

of the shuffled data only in late development (Fig.8G-I bottom, p=0.046), but not in

early development (Fig.8G-I top, p=0.08). Taken together, these results support the

hypothesis that FG-cells and NFG-cells may exhibit different dynamical

characteristics in gamma modulation, thus their coordination may contribute to the

development of theta sequences. FG-cells were consistently modulated by slow

gamma phase precession, which would be precondition for theta sequence

development.

DISCUSSION

In current study, we employed a circular track exploration task to investigate the

different roles of fast and slow gamma rhythms in the coordination of neurons during

the experience-dependent development of theta sequences. We first found that a

subgroup of place cells (FG-cells) that were phase-locked to fast gamma played a

crucial role in the development of theta sequences. By excluding a sufficiently high

proportion of FG-cells, the sweep-ahead structure of theta sequences was

significantly disturbed compared to that of excluding equivalent number of NFG-cells.

These FG-cells exhibited slow gamma phase precession within single theta cycles

throughout the developing process of theta sequences. Finally, we showed positive

correlation between the intensity of slow gamma phase precession and development

of the sweep-ahead structure of theta sequences.

Fast gamma episodes were detected with dominantly high probability as initial

exploration of each day and decreased probability as being familiar to the

environment (Bieri et al., 2014). This could be related to the feedforward

communication from upstream to downstream regions by using fast gamma

frequencies (Vinck et al., 2023). Although the information propagation of visual

stimulation was unlikely synchronized between visual cortex and hippocampal CA1

(Schneider, Tzanou, Uran, & Vinck, 2023), the MEC inputs could also be entrained

by fast gamma to process ongoing sensory information to the hippocampus (Buzsaki

& Schomburg, 2015; Colgin et al., 2009). Thus, the fast gamma phase-locked

ensemble may act as a receiver of novel information that organized its intrinsic

individual neurons in a time-compressed and spatially-ordered sequence to integrate

the information under the mesoscopic cellular substrate modulated by fast gamma

rhythms (Fernandez-Ruiz et al., 2023). These neurons exhibited similar firing

characteristics as those of fast gamma non-phase-locked neurons, however, they

showed higher probability presenting theta phase precession. Therefore, this fast

gamma phase-locked ensemble likely has overlapping with "bimodal cells" involved

in the formation of theta sequence structure including reverse and forward windows

(Chu et al., 2023; Wang et al., 2020). In this case, regardless of these neurons, the

sweep-ahead structure of theta sequences could not be developed with learning,

with the extent of structure impairment positively correlated with the proportion of

excluded fast gamma phase-locked neurons.

The important role of fast gamma in modulating theta sequence development

was also supported by a specific perturbation experiment. Liu and colleagues

optogenetically entrained the MEC GABAergic cells at an artificial gamma frequency

(53Hz) and observed the loss of phase precession patterns in CA1 place cells, and

thereby the disrupted development of theta sequences (Liu, Todorova, Tang, Oliva, &

Fernandez-Ruiz, 2023). The failure of sequential firing organization during behaviors

impaired the formation of a predictive map related to the highly time-compressed

awake replay sequences executing information storage. Fast gamma was also

found diminished between MEC and CA1 in transgenic model mice of Alzheimer's

Disease, linked with spatial memory impairment in AD as a circuit mechanism(Jun et

al., 2020). On the other hand, our previous study showed that fast gamma-

dominated theta sequences represented shorter trajectories with relative less spatial

information within a theta cycle (Zheng et al., 2016). This is indeed not contradictory

with the current results, as we found that slow gamma, related to information

compression, was also required to modulate fast gamma phase-locked cells during

sequence development. We replicated the results of slow gamma phase precession

at ensemble level (Zheng et al., 2016), and furthermore observed it at late

development, but not early development, of theta sequences. Thus, a scenario

could be drawn out that the network modulator was transitioned from fast gamma to

slow gamma throughout the sequence development with learning.

At single neuron level, we also observed that spikes of an individual place cell
occurred at precessed slow gamma phases across consecutive slow gamma cycles
under the time-scale of a theta window. At a finer scale within a theta cycle, slow
gamma cycles coordinated place cells in a "mini-sequence" way (Zheng et al., 2016),
in order to integrate information of long spatial trajectory related to the memory
retrieval and predictive behaviors. This negatively-biased phase difference
distribution appeared from lap2, when the sweep-ahead structures of theta
sequences haven't been fully developed, suggesting that slow gamma phase
precession of place cells contributed to the information compression required to the
theta sequence development. More interestingly, we found that slow gamma phase
precession of a subset of neurons was absent at initial exploration when the theta
phase precession has already displayed (Feng et al., 2015). This finding was in line
with a previous study showing that silencing of CA3 output didn't affect theta phase
precession of a single cell, but impaired theta sequence structures (Middleton &
McHugh, 2016). A possibility was that recruitment of PV-interneurons during the
CA3-driven descending phase of the theta cycle, associated with the induction of
slow gamma oscillations in CA1, was found reduced in the absence of CA3 input
(Fernandez-Ruiz et al., 2023; Topolnik & Tamboli, 2022). We also provided
additional evidence about the relationship between slow gamma phase precession
and theta sequence formation, that the more negative slow gamma phase difference
across cycles, the more sweep-ahead structure represented at late development of
theta sequences.

It is noteworthy that the subset of neurons (FG-cells) was modulated by both
fast gamma and slow gamma rhythms during the theta sequence development

(Fig.1). These place cells, associated with both memory encoding and retrieval,

seem to function like memory engram-like representations (Goode, Tanaka, Sahay, &

McHugh, 2020; Josselyn & Tonegawa, 2020; Lamothe-Molina et al., 2022; Pettit,

Yap, Greenberg, & Harvey, 2022). Indeed, the proportion of c-Fos positive place

cells (putative engram cells) had higher proportion being phase-locked to fast

gamma than the c-Fos negative place cells (Tanaka et al., 2018). In contrast, the

NFG-cells exhibited experience-dependent phase coding, that they likely contributed

to the path representation at the late learning process. Thus, further studies would

be needed to investigate how memory engram cells contribute to theta sequence

development and facilitate spatial memory and navigation.

MATERALS AND METHODS

Subjects

Four male Long-Evans rats weighing 500 to 700g were used in this study. Rats

were housed in custom-made acrylic cages (40cm×40cm×40cm) and exposed to a

light cycle opposite to natural light (light exposure time: 8:00pm-8:00am). All waking

activity recordings were performed in the dark (recording time: 8:00am-8:00pm).

Rats were handled at the time of electrode implantation surgery and underwent at

least 7 days of behavioral pretraining. Behavioral training was initiated after at least

one week of recovery from implantation surgery. Rats were physically deprived

during experimental data collection so that their body weight was maintained at

~90% of their free-feeding body weight. All experiments were conducted according

to the guidelines of the Animal Care and Use Committee of Tianjin University.

Behavioral paradigm

We designed a circular track apparatus for this study. The circular track with an

inner diameter of 90 cm, height of 50 cm, and width of 10 cm, was placing an acrylic

resting box (height of 31 cm, length of 28 cm, and width of 21cm) as beginning and

ending. Each rat underwent training to navigate on a circular track. A total of

eighteen small black silicone dots were utilized as context cues along the circular

track, covering approximately two-thirds of its length. Each dot was at a distance of

12 cm (or equivalently 0.28 radians) away from its neighbors. Before surgery, all rats

were pre-trained on the same circular track for 1 week and recovered from surgery

for at least one week before behavioral training resumed. For every recording

session, rats ran 5 trials (or laps) on the circular track. For each running lap, the rats

started from the rest box, navigating clockwise to the rest box, and then returned to

the rest box counter-clockwise without receiving any food reward. A tone (1kHz sine

wave with a duration of 0.25s) was delivered at the beginning and end of the trial.

According to the recorded position, the 2D movement speed of the rat was

calculated by 2D coordinates and elapsed times of consecutive two points.

Surgery and tetrode implanting

Before surgery, tetrodes were built from 17μm polyimide-coated platinum-iridium

(10/90%) wire (California Fine Wire, Grover Beach, California). The tetrode tip was

platinum-plated using a NanoZ system (White Matter, Seattle, Washington), ensuring

that the impedance of each recording channel was below 300 kOhms to facilitate

single-neuron activity recording. Eighteen independently mobile tetrodes hyperdrive

were surgically implanted into the hippocampal dorsal CA1 region. The rat brain

stereotaxic coordinates of the center of the electrode bundle were anterior-posterior

[AP] -3.5 mm, medial-lateral [ML] 3.0mm and on day of implantation the tetrode tips

moved 1mm downward (dorsal-ventral [DV] 1mm). Screws were placed on the

surface of the skull. The hyperdrive bundle were attached to the screws and skull

with dental cement. Two screws placed in the skull above the cerebellum served as

a panel ground for recording. After surgery, tetrodes were gradually downward to

dCA1 stratum pyramidale. One tetrode was designed as recording reference.

Recording reference was used to differentiate from other recording tetrode to monitor

signal During other recording tetrode downwards, the recording reference was

moved up and down to a quiet position in the cortex above the hippocampus and

remained quiet throughout signal recording session.

Data acquisition

Behavioral and electrophysiological data were acquired using a Digital Lynx

system and Cheetah recording software (Neuralynx, Bozeman, Montana). LFPs

recorded continuously from a single channel in each tetrode, within the frequency

range of 0.1-500 Hz from raw signal at 2,000 Hz sampling rate. Before each

recording session starting, the input amplitude ranges were carefully adjusted to

optimize resolution and prevent signal saturation. Typically, the input ranges for

LFPs were within the range of ±2,000 µV to 3000µV. To detect unit activity, signals

from each channel in a tetrode were bandpass filtered from 600 to 6,000 Hz. The

spike detected threshold was 55 µV. All the recorded signals were obtained by

differentiating with the reference tetrode (see "surgery and tetrode planting"),

eliminating a large amount of background noise. The position of the rat during

navigation behavior was recorded by the video tracking function within Neuralynx

system. The video resolution of 720 × 480 pixels and a frame rate of 25 frames per

second. The actual position was tracked with a red light-emitting-diode (LED) on

one of the three HS-27-Mini-LED headstage (Neuralynx, Bozeman, Montana)

attached to the hyperdrive. The actual 2D coordinates were obtained according to

the pixel value where the red is brightest

Spike sorting

Detected spikes were manually sorted using graphical cluster-cutting software
(MClust; A.D. Redish, University of Minnesota, Minneapolis). Spikes were sorted
using two-dimensional projections of three different features of spike waveforms (i.e.,
energies, peaks, and peak-to-valley differences) from four channels. Clusters were
judged based on waveform shape, isolation distance, and lack of inter-spike-interval
violation. Units with mean firing rates of more than 15 Hz were identified as putative
fast-spiking interneurons and were excluded from further analysis. The units with
peak firing rates more than 1 Hz but mean firing rates of less than 15 Hz was
putative pyramidal cell for further analysis.

Spatial firing maps and place cell identification

The animal position on the circular track was linearized to increasing radian
values and divided into 90 bins (approximately 0.035 rad per bin and that is 3.5cm).
Spatial firing maps were calculated only during locomotor periods (> 5cm/s). To
calculate directional spatial firing rate-maps, the number of directional spikes was
divided by the time of occupancy at each position bin on the circular track with the
same direction, followed by smoothing with a Gaussian kernel (standard deviation of
2 bins). For identifying the place cell, we used all trial spikes to calculate rate-maps.
A continuous span of at least three consecutive bins in the rate-map with a peak
firing rate exceeding 1 Hz was calculated as a place field. A pyramidal cell contained
at least one place field was defined as a place cell. The place field size was defined
by 0.1Hz firing rate boundary. The firing rate in each place field is divided by the
number of bins in the field to calculate the mean firing rate in the field, the peak firing
rate. The primary place field used for analysis was defined as the field with the

maximal peak firing rate among all fields. Finally we accepted 488 place cells with

place field distributed on the circular track (region from dot 1 to 18) but not located at

the resting box.

Spatial specificity was quantified by computing the spatial information score

(Langston, Ainge et al. 2010). For each cell, the spatial information score in bits per

spike was calculated from the recording in each period as:

$$\text{Spatial information} = \sum_i P_i \frac{\lambda_i}{\lambda} \log_2 \frac{\lambda_i}{\lambda}$$

Where λ_i is the mean firing rate of a unit the ith bin, λ is the overall mean firing rate,

and p_i is the probability of the animal being in the ith bin (occupancy in the ith

bin/total occupancies bin).

The single-lap(or trial) rate-maps was calculated using the same method and

used to Bayesian decoding analysis.

Local field potential analysis

Theta, slow gamma, and fast gamma rhythms were extracted from the LFP by

applying band-pass filters with frequency ranges of 4-12 Hz, 25-45 Hz, and 65-100

Hz, respectively. The time-varying phases of the theta, slow gamma, and fast

gamma rhythms were computed using the Hilbert transform. As the previous study

showed, the gamma power spectra were measured across different running speeds.

The power spectra across running speeds and absolute power spectrum (both result

were not shown) was estimated through the multitaper spectral analysis (Mitra and

Bokil, 2008) in the Chronux toolbox (http://chronux.org/). We defined the gamma

episode as the 160ms window with gamma power higher than 3 standard deviations

to observe the firing activity of place cells.

Quantification of theta phase precession

Theta phase precessions were examined in the primary place field calculated by

all trial spikes. The phase-position correlation of spikes emitted by a place cell during

all lap of its place field was determined by circular-linear regression between the

theta phase of spikes and the actual position of animal. The significant negative

correlation was determined theta phase precession. Theta phase precession slope

was calculated by least-square fit of spike locations of the animal and shifted spike

phases.

Estimation of gamma phase-locking

Phase-lock analysis was implemented using custom scripts based on the

circ_stats function from Matlab CircStat toolbox. The slow gamma, and fast gamma

spike phase distributions for each place cell were then determined by the time-

varying phases of slow gamma, and fast gamma (see "Local field potential

analysis"), respectively, at the time point closest to each spike time. And each spike

phase was evaluated with the LFP recorded in the tetrode where the neuron was

located. Phase-locking was quantified by calculating the mean vector length of the

resulting phase distributions. Significant phase-locking at fast gamma is identified

only for place cells meeting the criteria of a Rayleigh test $p<0.05$ and firing at least

10 spikes on each lap. For easy presentation, these cells are named FG-cells. The

remaining place cell are not phase-locking at fast gamma, called NFG-cells.

According to our results, nearly a quarter of the neurons are significantly phase-

locked at fast gamma. Only a very small number of neurons are slow gamma phase-

locked.

Single-trial Bayesian decoding analysis

To translate ensemble spiking activity into radian positions on the circular track,

a memory-less Bayesian decoding algorithm was implemented. Decoding was

performed using a 20ms sliding time window that shifted 5ms at each step. For each

decoding window, the number of spikes occurring is denoted as n_i, so that the vector

of all spiking activity simultaneously recorded for the cell is $\boldsymbol{n} = \{n_1, n_2, \cdots, n_i\}$. The

probability of a rat being at position x, given the number of spikes $\boldsymbol{n}$ from each unit

recorded in a time window, was estimated by Bayes' rule:

$$P(x|\boldsymbol{n}) = \frac{P(\boldsymbol{n}|x) \times P(x)}{P(n)}$$

where $P(\boldsymbol{n}|x)$ was approximated from the single directional firing turning curve of

each unit. The approximation assumed that the turning curve of individual units was

statistically independent and the number of spikes from each unit followed a Poisson

distribution. Prior knowledge of position $P(x)$ was set to 1 to avoid decoding bias to

any particular location on the track. The normalizing constant $P(n)$ was set to

ensure $P(x|\boldsymbol{n})$, or posterior probability, summed to 1. The decoding position was is

the set of x that maximizes $P(x|\boldsymbol{n})$. Decoding error was defined as the distance

between the decoded position with maximum-likelihood and the rat's true position.

For excluded cell decoding, position reconstruction was performed by removing the

FG-cells or NFG-cells from $\boldsymbol{n}$, respectively. Bayesian decoding analyses were

performed using custom routines in MATLAB 2020b.

Theta cycle detection

A single tetrode located in the stratum pyramidale and physical center all

recording tetrodes was selected and the theta signal (including phase and power

etc.) from this tetrode was utilized for further analysis. According to previous studies,

almost all theta sequences fall completely into a theta cycle. Therefore, the global 0

phase (the beginning of the theta cycle) of theta rhythm was determined as the

phase when the theta sequence quantization indicators (see "Theta sequence

quantification") were maximal. Briefly, the global 0 phase was moved from the peaks

in steps of 10°, and in every step, the sequence of place cells was quantified in each

theta cycle. The peak of the histogram of the distribution of the quantization

indicators over the step size was used as the global 0 theta phase for further

analysis.

Theta sequence quantification

Theta sequences were quantified only when the following conditions also hold:

(1) the animal was in the middle of the track (two-thirds of the circular track length),

(2) the current running direction is consistent with the expected direction with running

faster than 5 cm/s, (3) the duration of the corresponding theta cycles ranged from

100 to 200 ms, (4) more than 3 place cells and emitted at least 5 spikes during the

corresponding theta cycle.

Averaged sequence was constructed over the laps by aligning the probabilities

over the theta cycle mid-time point and position. Each and averaged sequence were

quantified within an area that decoded probabilities ±35 cm around the position of

animal, and ±1⁄4 theta cycle around the mid-time point of the theta sequence.

Probability difference

The area used to quantify were divided equally into four quadrants. The

Quadrant 2 represents the region both physically and temporally ahead of the rat,

whereas as the Quadrant 3 represents the region physically and temporally behind

the rat. Quadrant 1 and 4 represents the region that opposite with the animal's

current running direction. The summed decoded probabilities in Quadrant 1 and 4

were subtracted from the summed decoded probabilities in Quadrant 2 and 3 then

divided by the summed decoded probabilities in all four quadrants. This value

represents the difference between the decoding probability of the normalized current

path and the opposite path in the theta sequence. Positive differences would imply

theta sequences sweeping ahead in current moving direction of the rat, whereas

differences close to zero would indicate a lack of sequential structure.

Weighted correlation

Weighted correlation of each or averaged theta sequence was calculated from

the same area mentioned above. With the decoding probability (*prob*) as the weight

of the position estimation, the correlation coefficient between the time (*t*) and the

decoding position (*p*) is as follows:

$$corr_w(t, p; prob) = \frac{cov(t, p; prob)}{\sqrt{cov(t, t; prob)cov(p, p; prob)}}$$

609   where weighted covariance between time and decoded position is as

610   follows:

$$cov(t, p; prob) = \frac{\sum_i prob_i(t_i - m(t; prob))(p_i - m(p; prob))}{\sum_i prob_i}$$

611   and weighted means of time and decoded position are as follows:

$$m(t; prob) = \frac{\sum_i prob_i t_i}{\sum_i prob_i}$$

612   and

$$m(p; prob) = \frac{\sum_i prob_i p_i}{\sum_i prob_i}$$

The apparent sweep-ahead structure in the current running direction of animal

produced large positive correlations (close to one), whereas the lack of sequence

structure produced close to zero correlations. For further analysis, we use weighted

correlation to quantify the theta sequence from different decoding result.

Flip correlation difference

We defined an Indicator to measure the front-sweep structure, named Flip

correlation difference, which of each or averaged theta sequence was calculated

also from the same area mentioned above. In the temporal dimension, the

correlation coefficient between the decoding probability and the decoding probability

after performing both temporal and spatial flips was calculated, from which the

correlation coefficient between the decoding probability and the decoding probability

after performing only temporal flips was subtracted. Positive difference represents

intact sequential structure in the positive, whereas differences close to zero or

negative would indicate a lack of sequential structure or defective sequential

structure.

Gamma phase shift analysis of cell ensemble

The gamma cycle with maximal spiking across all simultaneously recorded cells

was defined as cycle 0. Gamma cycles occurring before cycle 0 are represented by

decreasing integer values, after by increasing integer values. Incomplete cycles at

the beginning or end of the sequence or during immobile (speed<5cm/s) were

excluded from analyses. We estimated the gamma phases and theta phases of

spike times for place cells that exhibited spikes within at least two slow gamma

cycles or at least three fast gamma cycles within a single 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 (data not shown). Two-

dimensional histograms of gamma phases and theta phases for spikes from each

cycle number were binned into 40 bins and smoothed across nine bins.

Slow gamma phase shift analysis of individual cell

We employed Morlet's wavelet method to calculate the time-varying power of

slow gamma. The slow gamma energy in each theta cycle was estimated,

considering only cycles with energy higher than 1.5 times the standard deviation as

slow gamma events. The global 0 phase of slow gamma, set at the trough,

determined the phase shift. For each cell, spikes in different slow gamma cycles

within each theta cycle were identified. Spike pairs, representing adjacent spikes

from different slow gamma cycles, were used to calculate the average phase

difference as the slow gamma phase difference. In cases of multiple spikes in the

same slow gamma cycle, their average phase was taken. Negative values indicated

phase precession, while positive values indicated procession. To maintain firing

location constancy, spikes used for phase difference calculation were assigned a

random slow gamma phase within their cycle, repeated 1000 times for a distribution.

In the analysis, we calculated one-sided, corrected empirical p-values $[(r+1)/(n+1)]$

by comparing real data to its corresponding shuffle data. Where r is the number

values from the shuffled distribution that are either smaller or larger (depending on

the hypothesis) than the observed value and n=1000 is the number of shuffling.

Correlation between the slow gamma phase precession and weighted

correlation

The theta cycle satisfying the criteria (see Slow gamma phase shift analysis) for

calculating the phase difference was chosen to estimate the relationship between

slow gamma phase precession and weighted correlation. On the one hand, the

Pearson correlation coefficient was used to estimate the linear correlation between

them. On the other hand, the correlation between slow gamma phase precession

and theta sequence structure was analyzed by two-dimensional histogram. We

binned slow gamma phase difference and weighted correlation into ten bins to

construct a two-dimensional histogram, and smoothed it across three bins for

visualize. The Two-dimensional histogram was divided into four quadrants and the

sum of probability in Quadrant 1 (Q1) was calculated. The statistical significance

was determined by calculating the one-sided, corrected empirical p-value. The

shuffle data was same as slow gamma phase shuffle. The true data was considered

to be significantly concentrated in Q1. The null hypothesis was the probability in Q1

of shuffled data was larger than the true data. If the p value was less than 0.05, the

null hypothesis was rejected.

Histology

At the end of the experiment, histological sections were prepared as follows to

verify the tetrodes position. Rats were intraperitoneally injected with a lethal dose of

urethane solution. The heart was subsequently perfused with phosphate buffer and

formalin to fix the brain tissue. Rat brains were then cut into 30-µm coronal sections

and Nissl Staining was performed to determine the location of tetrode in CA1.

Quantification and statistical analysis

Data analyses were performed using SPSS (IBM) and custom Matlab (the Math

Work) and R scripts. Paired t-test was performed to test between exFG-sequences

and exNFG-sequences during early and late development (Fig.3H). Student's t test

was performed to test the spike number, mean firing rate in field, place fields size

and spatial information between the FG-cell and NFG-cell (Fig.5D-G). One-sample t

test was conducted to assess whether the slow gamma phase shift was significantly

lower than 0 (Fig.7B). Repeated measures ANOVA was performed to test the

weighted correlation of theta sequences (Fig.2D, Fig.3C,E,G) and running speed

(Fig.3F) as a function of laps. Kolmogorov-Smirnov test was performed to test the

distribution of place field COM (Fig.5C) on the track using the Matlab function

'kstest2'. Multi-sample Mardia-Watson-Wheeler test was performed to test the slow

gamma phase shift of place cells across successive slow gamma cycles (Fig.6)

using the R 'circular-package'. Pearson's correlation was performed to test the

correlation between weighted correlation difference and the proportion of excluded

cells (Fig.4C-D).

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COMPETING INTERSETS

714 The authors declare that they have no competing interests.

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 FIGURE LEGENDS

Fig.1 | The schematic of a model for theta sequence development

Fast gamma rhythms coordinate a subgroup of place cells which dominantly fires

phase-locked to fast gamma (FG-cells). The development process of theta

sequences is disrupted by excluding these FG-cells. A possible model would be that

development of sweep-ahead structure of theta sequences is dependent on the slow

gamma modulation, i.e. slow gamma phase precession, whereby spatial information

could be highly compressed within theta cycles. FG-cells are those cells exhibiting

slow gamma phase precession within theta cycles since from the early stage of

sequence development, which occurs earlier than the cells not phase locked to fast

gamma (NFG-cells).

Fig.2 | A subset of hippocampal place cells was modulated by fast gamma

rhythms during exploration on a circular track.

(A) An example of simultaneously recorded LFPs (raw LFP, 65-100 Hz 25-45 Hz,

bandpass filtered gamma rhythm) and spikes of place cells. (B) Firing

characteristics of two examples of FG-cell and NFG-cell, including firing rate map,

and phase modulation by fast and slow gamma during detected episodes. Spikes

were represented by red lines on top of the gamma waveforms. MVL is short for

mean vector length. (C) The normalized turning curve of place cells sorted by the

center of mass of their main place field. The proportion of 4 types of cells identified

according to different modulation by fast and slow gamma was shown on the right.

(D) Mean vector length of FG-cells and NFG-cells across successive running laps

(n=12 sessions from 4 rats). Data are presented as mean ± SEM. ***$p<0.001$.

Fig.3 | Theta sequences development was disrupted without FG-cells.

(A) Top, Example color-coded posterior probability distribution across time from a

single lap. The running trajectory of the animal was indicated as white dashed line.

Bottom, 4–12 Hz bandpass filtered theta rhythm. The beginning and end of theta

sequences were indicated as red bars. (B) Examples of detected theta sequences

decoded by 3 different decoders, all spikes from all cells (top, i.e. raw sequences), all

spikes excluding those from FG-cells (middle, i.e. exFG-sequences) and all spikes

excluding those from NFG-cells (bottom, i.e. exNFG-sequences). The white triangle

indicates the animal's current position. (C) Weighted correlations between time and

decoded positions among three types of sequences. (D) Averaged decoded

probabilities by 3 types of decoders over 63 cm centered by the animal's current

running position and 170 ms centered by the mid-time point of theta sequence for

each lap, in a single recording session. (E) Weighted correlation of raw sequences

was significantly increased with running laps (n=12 sessions). (F) The running

speed did not change across laps. (G) The sweep-ahead structure of the exFG-

sequences (red) was disrupted compared to that of exNFG-sequences (yellow). (H)

The effect of excluding either FG-cells or NFG-cells on early (left) and late (right)

development of theta sequences. Data are presented as mean ± SEM. *p<0.05,

**p<0.01, ***p<0.001.

Fig.4 | Temporospatially compressed structure of theta sequences required a

relatively large proportion of FG-cells.

(A) Examples of detected theta sequences in early development. Each column is a

pair of theta sequences decoded by 2 different decoders, exFG-decoder (top, all

spikes excluding those from FG-cells) and exNFG-decoder (bottom, all spikes

excluding those from NFG-cells), in a same theta cycle. The left 2 columns of

sequences were detected from laps with a relatively small proportion (~10%) of FG-

cells. The right 2 columns of sequences were detected from laps with a relatively

large proportion (~35%) of FG-cells. W.Corr was short for weighted correlation of

each sequence. (B) Same as (A), but for late development of theta sequences. (C)

The difference of weighted correlation of sequences between exNFG-decoder and

exFG-decoder in early development, as a function of the proportion of excluded

cells. Each scatter represents data from each recording session. The dashed line is

the linear regression line of all scatters. (D) Same as (C), but for late development of

theta sequences. The difference of weighted correlation of sequences between

exNFG-decoder and exFG-decoder in late development, was positively correlated

with the proportion of excluded cells.

Fig.5 | FG-cells and NFG-cells exhibited similar firing characteristics.

(A) Spatial tuning curves of representative FG- and NFG-cells during exploration on

the circular track. Each column of cells exhibited similar place field positions on the

track. (B) Distribution of the place field centers of mass (COM) of FG-cells and NFG-

cells as a function of position on the track. (C) Cumulative distribution of FG-cells

and NFG-cells' place field COM. (D) Spike counts, (E) Mean firing rate in the place

field, (F) Place field size, and (G) Spatial information FG-cells and NFG-cells. Data

are presented as mean ± SEM.

Fig.6 | Slow gamma phase precession across successive gamma cycles

occurred during late development of theta sequences.

(A) Probability distributions of slow gamma phases of spikes across successive slow
gamma cycles within theta sequences. Gamma cycles within theta cycles were
ordered, centered at cycle 0 (i.e., gamma cycle with maximal spiking). Slow gamma
phases of spikes shifted systematically across successive slow gamma cycles. (B)
Spikes' slow gamma phase did not significantly backward-shifted across successive
slow gamma cycles during early development of theta sequences. (C) Same as (A),
spikes' slow gamma phase significantly backward-shifted across successive slow
gamma cycles during late development of theta sequences. The white dots denote
the peak probability of the histogram.

Fig.7 | FG-cells constantly exhibited slow gamma phase precession across
laps.

(A) Examples of slow gamma phase precession within theta cycles on single cell
level. The top row shows posterior probability for theta sequences. The middle row
shows the LFPs waveform in the same theta cycle of the theta sequence, with each
slow gamma cycle divided by black lines. The bottom row shows the slow gamma
phases of spikes from a representative cell activated in this theta cycle. The spikes
occurred at earlier slow gamma phase in the late slow gamma cycles compared with
those in the early slow gamma cycles. (B) The averaged slow gamma phase shift
across N active cycles were significantly negative (N=2, 3 or $\geq$4). The red lines
indicate the median and the black lines indicate the 25% and 75% quantile of group
data. (C) Histogram of mean slow gamma phase shift averaged within each theta
cycle of each lap. The histograms of the real data are shown in blue with blue
triangle indicating median. The histograms of the mock data are shown in grey with
black lines indicating the 95th confident interval of the their median. (D) The same

as (C) but for histogram of mean slow gamma phase shift averaged within each

place cell of each lap. (E) Histogram of mean slow gamma phase shift averaged

within each FG-cell (above x-axis) or NFG-cell (below x-axis) of each lap. The red

and yellow triangles are the median of phase shift in FG-cells and NFG-cells

respectively. *p<0.05, **p<0.01, ***p<0.001.

Fig.8 | The sweep-ahead structure of FG-cell sequences was positively

correlated with the intensity of slow gamma phase precession during both

early and late sequence development.

(A) Scatter plot of averaged slow gamma phase shift across slow gamma cycles

within a theta cycle as a function of weighted correlation of all sequences during all

laps. Left panel shows real data (blue) and right panel shows mock data by shuffling

(gray). (B) Probability distributions of sequences falling in 4 quadrants (Q1-Q4) with

slow gamma phase shift (<0 or >0) and weighted correlation (<0 or >0). (C) The

count of sequences falling in Q1 (blue triangle) was significantly higher than 95%

quantile of the shuffled data (black line). The gray histogram indicates the relative

probability of mock data falling in Q1 by 1000 times shuffling. (D) Scatter plot of

averaged slow gamma phase shift across slow gamma cycles within a theta cycle as

a function of weighted correlation of FG-cell sequences during early development

(top) and late development (bottom) of sequences. (E) Probability distributions of

FG-cell sequences falling in 4 quadrants during early development (top) and late

development (bottom) of sequences. (F) The count of FG-cell sequences falling in

Q1 (red triangle) was significantly higher than 95% quantile of the shuffled data

(black line) during both early development (top) and late development (bottom) of

sequences. (G-H) Same as (D-E), but for NFG-cell sequences. (I) The count of

NFG-cell sequences falling in Q1 (red triangle) was significantly higher than 95%

quantile of the shuffled data (black line) only during late (bottom) but not early (top)

development of sequences. *p<0.05, ***p<0.001.

Figure 1

Figure 2

A

Fast gamma episode   Sow gamma episode   Slow gamma episode   Fast gamma episode

Raw LFPs

Fast gamma

Slow gamma

Spikes

100μV

100ms

B

FG-cell

Fast gamma episode   Slow gamma episode

Amp(μV)

Time(ms)

MVL = 0.31   MVL = 0.07

Spike count

Fast gamma phase   Slow gamma phase

NFG-cell

Fast gamma episode   Slow gamma episode

Amp(μV)

Time(ms)

MVL = 0.05   MVL = 0.08

Spike count

Fast gamma phase   Slow gamma phase

C

Normalized firing rate
0   1

Cell ID

0   Position on the track (cm)   314

Cells not phase-locked to either slow or fast gamma
75.4%

Cells phase locked to slow gamma only
1.4%

Cells phase locked to fast and slow gamma
2.3%

Cells phase locked to fast gamma only
20.9%

D

Mean vector length

0.3

0.2

0.1

FG-cell
NFG-cell

1   2   3   4   5
Laps

***

Figure 3

Figure 4

Figure 5

A

FG-cell #1

FG-cell #2

NFG-cell #1

NFG-cell #2

Firing rate (Hz)

Position on the track (cm)

B

Cell count

FG-cells NFG-cells

Position on the track (cm)

C

cumulative distribution

FG-cells
NFG-cells

n.s.

Place field COM (cm)

D

×10³

Spike count

n.s.

FG   NFG
cells

E

Mean firing rate in field (Hz)

n.s.

FG   NFG
cells

F

Place field size (cm)

n.s.

FG   NFG
cells

G

Spatial information (bit/spike)

n.s.

FG   NFG
cells

Figure 6

Figure 7

Figure 8