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Author
Chen, Zhiping

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The Speed Dependence of Hippocampal Neural Oscillations

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Physics

by

Zhiping Chen

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ABSTRACT OF THE DISSERTATION

The Speed Dependence of Hippocampal Neural Oscillations

by

Zhiping Chen

Doctor of Philosophy in Physics

University of California, Los Angeles, 2013

Professor Mayank Mehta, Chair

Hippocampus plays important roles in episodic memory formation, learning and spatial navigation. In rodents, hippocampal theta and gamma rhythms become prominent during locomotion and disappear during immobility. Many studies have been done to understand their functional roles. However, how these rhythms transit between mobile and immobile states is largely unknown. The most intuitive way to address this problem is to look at their dynamics as a function of the running speed of the animal. This thesis is focused on the speed dependence of theta and gamma rhythm as well as their coupling. In chapter 2, I showed that hippocampal gamma rhythm can be further divided into two subbands whose amplitudes are differentially correlated with the running speed. This speed dependence of gamma amplitude was restricted to a narrow range of theta phases. The preferred theta phase for slow gamma showed a precession with speed while the preferred theta phase for fast gamma remains unchanged. These results demonstrate a novel influence of speed on the amplitude and timing of the hippocampal gamma
rhythm which could contribute to learning of temporal sequences and navigation. In chapter 3, I found that LFP-theta modulation of spikes (TMoS) is the largest during immobility when theta is weak and irregular. This can be explained by the fact that the hippocampal gamma oscillations become stronger with running speed and take over the control of spike-timing while reducing theta modulation through transient phase-phase coupling. This transition leads to improved precision of spike timing within theta cycle at high speeds which could facilitate precise spike-timing based neural computations and learning. Finally, in chapter 4, I am trying to explain these novel speed dependences of theta and gamma as well as theta-gamma coupling. The simulation results suggest that there could be just one parameter that speed modulates in order to generate all these results. This hypothesis, although needs to be confirmed by the experiments, provides a really profound possibility on how the running speed of the animal may influence the neural oscillations and thus control the neural activities.
The dissertation of Zhiping Chen is approved.

Alcino Silva

Robijn Bruinsma

Katsushi Arisaka

Mayank R Mehta, Committee Chair

University of California, Los Angeles

2013
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CURRICULUM VITAE

EDUCATION

UNIVERSITY OF CALIFORNIA, LOS ANGELES
• MS in Physics (2007-2009)
PEKING UNIVERSITY
• BS in Physics (2003-2007)

EXPERIENCE

UNIVERSITY OF CALIFORNIA, LOS ANGELES
Research assistant – Department of Physics & Astronomy
Jan 2010 – present
• Designed and automated various time series, statistical and spectrum analyses on large electrophysiology datasets using MATLAB
• Modeling hippocampal CA1 neural network and studying the mechanism of theta-gamma coupling

Research assistant – Department of Electrical and Engineering
Jan 2008 – Dec 2009
• Characterized the electrical and optical properties of GaAs nanowires
• Simulated and designed the active region of terahertz quantum cascade lasers

Teaching assistant – Department of Physics & Astronomy
Sep 2007 – Sep 2008
• Supervised physics labs and discussion sessions, graded homework and exams

MARINE BIOLOGY LABORATORY
Summer course – Neuroinformatics
Woods Hole, MA
August 15, 2010 – August 29, 2010
• Featured topics: stochastic process theory, spectra analysis, multivariate time series analysis, machine learning, fMRI, neuroimage processing

SKILLS

• Experiment: Fabrication of Si and III-V optoelectronic device, Cryogenic system, FTIR
• Computer: C/C++, R, MATLAB, LABVIEW, Microsoft Office, Solidworks

PRESENTATION/PUBLICATION

CHAPTER I

INTRODUCTION

1.1 The electrical signal of the brain

The brain consists of billions of neurons and their inter-connected networks. The basic functions of the brain originate from the coordinated activities of groups of neurons. Such activities can greatly perturb the electrical potential of the surrounding extracellular medium and therefore result in electrical signals that are large enough to be detected even on the scalp. The earliest records on these electrical signals which was later widely referred as EEG (encephalography) can be traced all the way back to more than a century ago when Caton observed feeble current passing through two electrodes placed on the skulls of rabbits and monkeys.

Decades later, equipped with improved galvanometer and the ability to record the signal on papers, people started to study the rhythmic patterns of the EEG and their response to external stimuli. The term ‘desynchronized EEG’ was used for the first time to describe the observation that the rhythmic patterns became weaker or even disappear after stimulation. The first study on human EEG began from Berger’s work, although the motivation was to study the possibility of telepathic communication between brains through electromagnetic waves. In his famous 1929 paper, he reported two types of waves. The larger amplitude waves had an average duration of 90 milliseconds and presented when the objects’ eyes were closed while the smaller amplitude waves were a little faster with an average duration of 35 milliseconds and presented mostly when the objects’ eyes were open. He then used ‘alpha’ and ‘beta’ to name them and thus
started the Greek alphabetic naming system on brain rhythms. It is worth pointing out though that the frequency order of these rhythms not necessarily follows their alphabetic order since they can be only named after being discovered and some slower oscillations were discovered later like delta and theta rhythm⁶. Obviously, these waves are no useful for telepathy because their amplitude are too small (~200 micro volts) to be able to transmit through air. Yet, it opened a window for the early pioneers to observe and probe the activities of the brain. Because EEG’s non-invasive nature, it has been used in various diagnostic applications including the detection of epilepsy, coma, and brain death⁷. It can also be used to study sleep and sleep disorders⁸. There are also many applications that use EEG to control electronic devices⁹.

One of the main limitations of EEG is its poor spatial resolution which is bond to its physical nature and is unlikely to be solved. The signal detected on the scalp is the result of a highly attenuated ensemble average activity of a large number of neurons which is far away from the probing electrode. As the signal of the local activity reaches the surface of the scalp, it is spatially smoothed out and the spatial pattern of EEG becomes a slow varying function around the scalp and therefore the spatial resolution will not be improved by increasing density of electrodes since the neighboring electrodes will looks more like each other at a smaller spacing. This will be like listening to a concert outside the concert hall. What you hear will be the same no matter you are close to the east entrance or the west entrance.

In early 1950s, Penfield and Jasper developed ECoG (electrocorticogram) as part of their Montreal procedure which is used to treat patients with severe epilepsy¹⁰. This technique placed electrodes directly on the surface of the cortex after the removal of skull. Since signals from neural activities attenuate dramatically when passing through the skull and there are lots of
artifacts involving muscle and eye movement when recording on the scalp. ECoG yields a magnitude larger signal and a much higher spatial resolution compared with EEG. It is therefore a powerful tool to localize the epileptogenic region before resection. Because of its invasive nature, ECoG is only suitable for patients with intractable epilepsy. However, it is widely used in many research laboratories on various kinds of mammalians.

Even though ECoG is one step closer to the brain than EEG, it shares many limitations in EEG. First, both methods cannot provide enough temporal resolution to resolve action potentials and therefore are unable to study single neuron activity. Second, the signals recorded in both methods are mostly coming from the activity in the superficial layers of the cortex. The contribution from the deeper layers is orders of magnitude smaller. It is also impossible to detect signals from the regions underneath the cortex. It is then very intuitive to think that the solution would be to place the electrode even deeper in the brain and closer to the neurons. Indeed, electrode placed in the extracellular medium of the brain will be able to record the action potentials from neurons near (<140 micrometers) its tip. The low frequency component recorded from these electrodes has the same physical origin as the EEG from the scalp except that its source is confined in a much smaller space than the classic EEG recorded on the scalp. Therefore, the name local field potential (LFP) is more suitable for this new type of recordings. The brain rhythms studied in this thesis are all coming from local field potentials.

Local field potential in the extracellular medium helps to reveal that different parts of the neuron have different electrical activities and therefore produce very different LFP signals around them. Neuron is not a point source that just fires action potentials. In the simplest version, the dendrite receives and integrates signals from upstream neurons and transmits them to
the soma which then decides whether or not to fire an action potential. This makes the LFP recorded near the dendrites very different from the one recorded near the soma. This case is especially true for pyramidal neurons since they often have very extended dendritic structures. Inputs from different brain regions are organized along the long axis of the dendrite and therefore these parts are likely to yield different LFP patterns when electrodes are placed near them. This different LFP pattern as a function of depth of the electrode not only helps to reveal the organization of the neural networks but also can be a valuable tool to determine the depth of the electrode\textsuperscript{13}. Since action potentials are very high frequency oscillation and attenuate quickly as a function of distance, it is usually ideal to place electrodes in the cell layer when picking up spikes is the priority. When it is more important to study the rhythm in the LFP, signals from near the dendrite often have larger amplitude oscillations.

Putting an electrode deep in the brain offers much informative recordings than classic EEG. However, there is one big difficulty. Since the amplitude of the spike extracted from LFP is a function of the distance between the soma and the tip of the electrode, neurons that have more or less equal spatial distance to the tip of the electrode become almost indistinguishable in the recordings. In order to overcome this difficulty, O’Keefe and McNaughton invented a new type of electrode (tetrode) by twisting 4 very thin (12-15 microns in diameter) electrical wires with insulating outer shell and gluing them together to create a single electrode with 4 independent channels\textsuperscript{14–16}. Since the four tips are spatially separated, each neuron will then have four distances instead of just one to present its spatial location relative to the tips of the electrode (tetrode). This remapping of neurons’ spatial locations onto a new 4 dimensional space based on the distance profile to the tips of the tetrode makes sure that each neuron will have a unique spot.
in this new 4 dimensional space with no overlap. Similarly, each spike will be recorded 4 times simultaneously by the 4 channels on a tetrode. The amplitudes of the spikes extracted from the 4 channels can then be mapped onto a new 4 dimensional space and spikes from the same neuron should aggregate around a unique spot to form a cluster. Since no one can work with 4 dimensional space, the most practical operation is to combine these 4 coordinates two at a time to form six unique 2D spaces (1-2, 1-3, 1-4, 2-3, 2-4 and 3-4). Spikes that are not separable in one space may become separated in other combinations. By scanning through all six combinations, all the separable clusters of spikes can be identified. In the most ideal conditions, a single tetrode can record up to 20+ single neurons although the total number of neurons electrically within the reach of the tetrode is much more (~1000)\(^{17}\). This could be the case that only a small fraction of the neurons are active during a recording session. It is also possible that some neurons are damaged by the blunt tip of the tetrode on its way to the target brain area. Simultaneously recording of hundreds of neurons can be achieved by using multiple tetrodes. Although the depth of the tip of the tetrode can be estimated by counting the number of turns of the driving screw, this estimated depth is usually not reliable due to the fact that tetrodes are flexible and therefore can be bent when advancing into the brain. The most reliable method is to use the unique LFP patterns associated with the target brain region to determine its depth. For example, the existence of ripples and the polarity of sharp wave can be used to determine whether the tetrode has reached the hippocampal cell layer\(^{13}\).

The advantages of tetrode compared to sharp-tip electrode are first it can record multiple neurons using triangulation. Second, it will cause much less damage to the neurons and brain tissue when advancing into the target area. Third, its flexible tip can move together with the
surrounding brain structures and therefore more suitable for chronic recordings. However, tetrode has its own limitations. First, each tetrode can be only made manually and therefore it is difficult to control the quality. Second, tetrode cannot be reused. It has to be made again every time before implantation. Third, it is hard to estimate the spatial location of the tip. As described above, because tetrode can be easily bent when facing relatively rigid brain structures, it is almost impossible to happen that the tip of each tetrode remains straight underneath its guide tube. This makes it hard to estimate the depth as well as the horizontal location of the tip.

Multisite recording probe was first introduced by Wise at University of Michigan and is often referred as Michigan probe\(^1\). The probe is made out of silicon. A thin layer of metal is patterned onto one or both sides of the probe to form all the recording sites and signal lines. This process is based on the mature MEMS (microelectromechanical system) technology. Therefore the probes can be prepared at a relatively low cost and high precision. Michigan probe can also record multiple single units like tetrode while offering more advantages over tetrode like its reusability and the ability to record LFP at different depth simultaneously. The latter is especially useful for CSD (current source density) analysis\(^1\).

The methods and technologies covered so far are specifically for in vivo electrophysiology. ‘In vivo’ stands in Latin as ‘within the living’ as oppose to ‘in vitro’ which stands for ‘within the glass’. Simply speaking, the in vivo methods use live objects while the in vitro methods use slices. In vitro electrophysiology contains a whole different category of methods and will not be covered in this thesis.
Electrical signal is not the only source that can be used to probe the activities of the brain. MEG (magnetoencephalography) is a method that uses SQUID (superconducting quantum interference device) to detect weak magnetic field on the surface of the scalp. fMRI (functional magnetic resonance imaging) use BOLD (blood-oxygenation-level-dependent) signal to locate the area where neurons have high level of activities. Optical method enjoys its booming development in recent years. Combined with powerful genetic tools and advanced microscopy, it offers unprecedented possibilities to probe and modulate activities of selected groups of neurons both in vivo and in vitro.

1.2 The rhythm of the brain

Rhythmic patterns are the most frequently observed phenomenon in nature. From the transition of seasons to the beating of the heart, everything living or non-living operates in a periodic fashion. This reoccurring process brings the sense of time which then gives rise to the past, the present and the future. It is worth pointing out though that the mechanism of oscillation in the physical world and in a living organ can be very different. In physics, the most common form of oscillation is harmonic oscillation. It can be produced just by hanging a weight on a spring. The up and down movement of the weight is harmonic oscillation. Theoretically, as long as there is a force that drives the object back to an equilibrium point and its magnitude is proportional to the displacement from the equilibrium point, a harmonic oscillation will be formed. Harmonic oscillation contains a single frequency and produces a continuous sinusoidal time series. It is not too surprise that the oscillating patterns recorded from the brain is very
different from these harmonic oscillations. Those in the brain are usually much more dynamic in terms of individual cycle length, amplitude and duration of existence. The shape of each individual cycle is also not necessarily sinusoidal like (this will be discussed more in detail in chapter III). The mechanism responsible for generating the rhythmic patterns in the brain is far more complicated than the one for harmonic oscillators and therefore is far from being fully understood. We do know that it is highly non-linear and different oscillating patterns require different mechanism and recruit different pool of neurons. For the above reasons, the neuroscience community usually prefers to use the term ‘rhythm’ instead of ‘oscillation’ in order not to confuse with the harmonic oscillation in physics.

Since Berger’s pioneering work in 1929, people began to fill up the 0.5Hz-500Hz frequency space with various bands named after Greek letters. Higher frequency is not practical since EEG and ECoG cannot provide higher temporal resolution while LFP recording will have large artifact from extracellular spike waveforms in frequency band beyond 500Hz. The bands reported so far in frequency order are delta (0.5-4Hz), theta (4-8Hz), alpha (8-12Hz), beta (12-30Hz), gamma (30-120Hz) and ripple (150-250Hz). These frequency bands have been found in various species, during different behavior stages and of course at different brain regions. Since the mechanism responsible for generating these bands is largely unknown. Therefore, the determination on the frequency boundary for each band is sort of arbitrary. Even for the same type of rhythm, its frequency boundary varies among different experimenters, test objects and the regions of the brain. For example, theta rhythm in human EEG is around 4-8Hz but becomes faster (4-12Hz) when measured in the hippocampus of rodents. Another example is gamma
rhythm, the hippocampal gamma band in rat is often reported at 30-120Hz while this entire band oscillates a little slower in mice\textsuperscript{24,25}.

As already stated above, brain rhythm is no way close to a simple harmonic oscillator. On the frequency domain, each band occupies a range of frequencies rather than a single frequency. The width of the band is determined by three factors: the duration of the rhythm, regularity of periods and the shape of the waveform. Shorter duration, irregular period and non-sinusoidal like waveforms will all contribute to widen the spectrum. Deviation from sinusoidal waveform will create higher order harmonic bands (see more in chapter III). The reason to talk about this is because people nowadays are so use to the spectrum analysis when study brain rhythms. They gradually neglect the fact that brain rhythms are not ideal harmonic oscillators. The frequency domain analysis may blind people from the more detailed information provided in the time domain analysis. This is not to say that the frequency domain analysis provides no useful information. A more appropriate way would be to use both methods and look at the signals from as many different aspects as possible in order to capture all the dynamics.

Since local field potential reflects the average neuronal activities around the electrode (so is the case for EEG and ECoG but on a much larger scale)\textsuperscript{26}, the rhythmic patterns can only exist if the majority of the contributing sources oscillate at the same frequency and at the same phase. This step is critical. Imagine that if all the sources oscillate at the same frequency but their phase relation is totally random, the average activities reflected in the local field potential will get smoothed out. This co-variation of activities among neuron assemblies is referred as ‘synchronization’ in neuroscience. To be more specific, the contributing sources mentioned here refer to the local synaptic activities. Spikes generated from the soma only contain high frequency
component that is above the upper bond of brain rhythms while synaptic activities (EPSP and IPSP) have relatively longer time constants which are more suitable for generating these low frequency field oscillations. The synchronization of synaptic activities is necessary since the contribution from a single postsynaptic site is usually not strong enough to activate the soma. Therefore, multiple postsynaptic sites need to receive inputs within a short time window in order to efficiently sum up the inputs. Here comes the problem. Since action potential travels along the axon at a much slower speed compared to the speed of electromagnetic waves, the variation on the distance between the soma of the upstream neuron to the postsynaptic site of the downstream will greatly change the time when the downstream neuron receives an EPSP/IPSP. Since fast rhythm (gamma) has a narrower time window to integrate all the synaptic inputs, it can only recruit neurons within a relatively small region. On the other hand, slower rhythm (theta) is more tolerant to this variation and can reach more neurons in a larger area. It is therefore believed that brain is organized by a series of different rhythms on various spatiotemporal scales.

1.3 Theta rhythm

Theta rhythm is perhaps the most extensively studied rhythm in the brain. The term ‘theta’ was first introduced by Walter to describe the 4-7c/sec oscillating pattern he observed in human EEG\(^6\). Only a year later, Green and Arduini reported their finding of a slow wave oscillation at 3-6c/sec during the ‘arousal state’ of hippocampus\(^28\). The term ‘arousal’ here refers to the state after peripheral stimuli was applied to the subject in the acute experiment. Since the two fell in the same frequency range, ‘theta’ was used again to name this hippocampal rhythm. However,
the two theta rhythms are found to be associated with different behaviors and are shown not to be coherent with each other when detected simultaneously\textsuperscript{29,30}. Another interesting finding in their paper is the inverse relationship between the simultaneously recorded activity in hippocampus and in cortex. While cortex becomes desynchronized after external stimulation, hippocampus exhibits strong and regular rhythm. This relation maintains during the rest state of the brain while cortex becomes more rhythmic and hippocampus begins to show irregular activities. The picture becomes more complicated and species dependent when people tried to correlate theta rhythm with behavior during in vivo experiments\textsuperscript{31,32}. The most robust correlation with behavior exists in rodents. While hippocampal theta rhythm arises during voluntary movement and paradoxical sleep, it is largely absent during immobility\textsuperscript{23,33}. This is one of the reason that rodent is preferred over many other species in studying hippocapal theta rhythm. However, robust theta can also occur during immobility in rabbits, cats and guinea pigs\textsuperscript{34,35}. It turns out though that these two types of theta have slightly different frequency with the voluntary movement associated theta (7-12Hz) oscillates a little faster than the immobility associated theta (4-9Hz). Since the fast theta mostly associated with the type I movement (walking, jumping, swimming, head movement), it was referred as type I theta while the slower theta was referred as type II theta in most of the later studies. More interestingly, type I and type II theta have been shown to have very different responses to certain pharmacological manipulations\textsuperscript{36}. Type I theta is resistant to atropine, a cholinergic antagonist, and is sensitive to anesthetic agents such as urethane, ether and alcohol. On the other hand, type II theta is resistant to most anesthetic agents while can be completely abolished by atropine. These results imply that there might be two independent mechanism responsible for generating type I and type II theta. I will cover this topic shortly in this section.
Theta rhythm exists in the entire hippocampal formation, including dentate gyrus, CA3 and CA1. The most prominent theta rhythm exists in the str. lacunosum-moleculare of CA1 region. Theta waveform has a depth profile in terms of both its amplitude and phase. The abrupt phase change begins at pyramidal cell layer and the phase difference grows all the way to 180 degrees from the most apical to the most basal part of the dendrite. Theta waveforms at the same depth are highly coherent across the entire hippocampus. The recent result even suggests that theta is a travelling wave which roughly propagates along the septotemporal axis of the hippocampus. The phase of theta modulates the discharge of neurons. The theta phase-locking on firing rate is not just limited to neurons in hippocampus but also found in other brain regions. These features make theta a powerful clock which orchestrates the entire hippocampus and other brain regions.

An important step in the search for the generators of hippocampal theta rhythm was made by Petsche when he reported the finding of a bursting cell type that fires at theta frequency in medial septum. Further evidences on this medial septum ‘pace maker’ emerged when studies show that the lesion of medial septum completely abolishes theta rhythm. The early version of this septum ‘pace maker’ hypothesis believes that the cholinergic neurons in medial septum sends rhythmic excitatory inputs to hippocampal neurons at theta frequency and thus are responsible for generating the hippocampal theta rhythm. This hypothesis was supported by the finding that hippocampal theta in the anesthetized animal can be completely blocked by the cholinergic antagonist atropine. However, the complication arises due to the fact we talked about earlier that type I and type II theta respond differently with atropine and anesthetic agents. The current hypothesis can only explain the results on type II theta. Actually, the selective block of
cholinergic neurons in medial septum will not completely eliminate type I theta. Even though its amplitude suffers a great reduction, the frequency remains unchanged and theta power is still significant in power spectrum analysis\textsuperscript{46}.

It turns out that cholinergic neurons are not the only cell type that project into hippocampus in medial septum. There is a group of GABAergic neurons that send inhibitory projections to hippocampus\textsuperscript{47}. In the expanded version of the classic theta mode, both cholinergic and GABAergic neurons in medial septum send rhythmic inputs to hippocampal basket interneurons which then lead to a current source around the soma in CA1 pyramidal neurons. On the other hand, entorhinal cortex sends rhythmic and excitatory input to CA1 and creates a current sink around the distal dendrite of CA1 pyramidal neurons. This theta dipole from the soma to the distal dendrite of CA1 pyramidal neuron can explain the depth profile of theta phase and amplitude\textsuperscript{42}. The classic theta mode is no way near a complete story of theta generation in hippocampus\textsuperscript{48}. The recent study even suggests that intact hippocampus can have self-sustained theta rhythm\textsuperscript{49}.

Since the discovery of this prominent rhythm in hippocampus, theta rhythm has been suggested to play an important role in various hippocampal functions. One of the most important tasks for rodents is spatial navigation. O’Keefe has shown in a series of his pioneering works that hippocampus is crucial for the formation of spatial map and the lesion of hippocampus impairs the animal’s ability for spatial navigation\textsuperscript{50–52}. In 1993, he reported a novel relationship between the theta phase and the location where the hippocampal place cell fires spikes\textsuperscript{53}. He found that place cell fires progressively earlier in each subsequent theta cycle as the rat moves throughout the place field. This relation of reduced theta phase as a function of position (not as a function of
time) is the well known phase precession. The author proposes that the theta phase coding of position can help to improve the accuracy of place coding. Another and more interesting potential application of phase precession is the compression of temporal sequence for the place cells that have overlapping place fields\textsuperscript{54}. This mechanism could facilitate the induction of long term potentiation (LTP) which is crucial for the formation of episodic memories. Phase precession was also found in prefrontal cortex\textsuperscript{55} and in MEC II grid cells\textsuperscript{56}. The latter persists even during the inactivation of hippocampus, suggesting that phase precession might represent a more general mechanism that is utilized in multiple brain regions.

The jewel on the crown is the mechanism that could explain phase precession. So far the models still survive the scrutiny are the two oscillator model\textsuperscript{54,57} and the ramp model\textsuperscript{58}. Since the validation of both models requires the in-field membrane potential of place cell, virtual reality system have been developed to allow in vivo whole cell recording of hippocampal place cell while the mice is navigating in a virtual maze\textsuperscript{59}. However, the results seem to partially match both predictions from the two models. While the membrane potential theta indeed oscillates fast than the field theta and lead to phase precession, asymmetric ramp have been confirmed to exist in the averaged membrane potential of place cell as a function of position. Two-oscillator model is very successful in explaining the formation of grid cells\textsuperscript{60}. However, the recent finding of grid cells in bats implies that theta is not necessary for generating grid patterns\textsuperscript{61} and therefore poses new challenge for the two-oscillator model.

1.4 Gamma rhythm
The fact that gamma comes right after beta in Greek alphabetic order implies that gamma rhythm has long been observed in EEG recording\textsuperscript{62}. However, its highly transient and irregular nature plus its smaller amplitude following the $1/f$ law makes it easily overwhelmed by the alpha and beta rhythms which on the other hand are usually much stronger, more regular and persistent. Another reason that the importance of gamma rhythm has been neglected for so many years after its first discovery could be the fact that cortical gamma rhythm exists predominantly during the waking and the arousal stage of the brain. The very well established observation of desynchronized EEG during these stages may easily lead people to conclude that these small and transient oscillations are just some high frequency irregular activity in the desynchronized EEG.

The most fundamental problem in visual pattern recognition is the ‘binding problem’, namely how spatial distributed neuron assemblies that response to different attributes of a feature in an object coordinate their activities and achieve an integrated response to that feature. The classic theory proposes that the neuronal organization in visual cortex follows a hierarchical order such that neurons respond to lower order features integrate their output and send to the downstream neurons which can then respond to higher order features. This theory was supported by the discovery of ‘simple cells’ which have restricted receptive fields to oriented stimulus and ‘complex cells’ which have bigger and more spatially invariant receptive field\textsuperscript{63}. The easiest way for complex cell to achieve this is to integrate the inputs from several ‘simple cells’. Higher orders of integration will lead to more complicated and specialized receptive that can even respond to a face, hand or an particular item\textsuperscript{64}.

The beauty of the hierarchical organization theory is that it is straight forward and intuitive. However, it has its own limitations and therefore cannot be the whole story. First, this
purely feed forward network does not include any feedback loops which may account for up to half of the circuitries in the neural systems. Second, this purely wiring dependent feature recognition system does not have any flexibility. The recognition of certain type of feature is predetermined by its specific wiring in the brain. As we know that the recognition process of the brain is highly dynamic, the inclusion of the recognition of new features would require different wiring which may be difficult for the adult brain. Third, different brains recognize the same feature very differently based on the prior experience and memories. A purely feed forward network would not allow the addition of internal input.

The breakthrough was initiated by a series of Gray and Singer’s work. In 1987, they showed that neurons in cat visual cortex have oscillatory response to moving stimulus in gamma frequency range (40-60Hz) and this oscillatory firing pattern is tightly phase locked to the oscillatory patterns in local field potential. In 1989, they further found that the gamma rhythm in the firing sequence detected in spatially separated columns can become synchronized with each other when the stimulus they respond to share some common features. A temporal binding theory was then proposed that neurons in visual cortex are dynamically organized by the synchrony of brain oscillation. In the temporal binding framework, neurons that have distinct receptive fields can fire synchronously at gamma frequency for several cycles, this time window allows for efficient integration of EPSP for the downstream neurons. The advantage of this mechanism is that first it has infinite possibilities of recruiting different neuron assemblies to achieve very complicated pattern recognition. Second, it is highly dynamic and therefore can quickly switch between binding patterns just by a few gamma cycles. Third, Interneurons can play an important role under this framework since the interneuron network has been shown to be
critical for maintaining gamma oscillation\textsuperscript{67,68}. Finally, the length of a single gamma cycle (15-30ms) falls right in the range of spike timing dependent plasticity (STDP) and therefore provides a powerful tool to modulate the synaptic weights of the network which is believed to be essential for memory formation and consolidation.

Gamma band activities also exist in hippocampus. Unlike cortical gamma that is induced by sensory input, hippocampal formation is believed to be able to have self-sustained gamma oscillation. This feature makes hippocampus an ideal structure to study the cellular-synaptic basis of gamma oscillations\textsuperscript{19,69,70}. Various network models have been proposed to generate gamma oscillations. The commonly agreed understanding is that gamma frequency is largely determined by the synaptic time constant of excitatory and inhibitory input and interneuron plays a critical role in synchronizing the local networks.

The functional role of hippocampal gamma oscillation is still not clearly understood. Colgin’s paper reported that gamma oscillation in CA1 contains two distinct subbands, namely slow gamma (25-50Hz) and fast gamma (65-140Hz)\textsuperscript{24}. While slow gamma couples most strongly between CA3 and CA1, fast gamma shows strongest coupling between MEC layer III and CA1. Since both CA3 and MEC send input into CA1, the differential coupling of slow and fast gamma with the different source of input implied their different origins. A more subtle hypothesis has been made regarding the functional role of slow and fast gamma. Slow gamma is proposed to be in charge of memory retrieval while fast gamma can facilitate memory encoding. The cycle length for slow gamma is not optimal for induce STDP and slow gamma power is concentrated around the theta phase where pyramidal neurons fire less spikes, these features can help to prevent the recoding of previously stored memories and not to interfere with the on-going
encoding process. On the other hand, fast gamma has a smaller cycle length which makes it more sensitive and more effective to modify the synaptic weights of events occurred within the same cycle. Its preferred theta phase is also consistent with the theta phase that is believed to most easily induce LTP. The fact that the slow and fast gamma prefer different theta phase makes it possible to quickly switch between the two functions with minimum interference.

1.5 Cross frequency coupling

Different brain rhythms can exist simultaneously during cognitive process. However, their interactions are not very well understood. In theory, there are four types of coupling that could possibly exist between two oscillators at different frequencies. They are amplitude-amplitude, phase-phase, phase-frequency and phase-amplitude couplings. Except the third phase-frequency coupling, experimental support for all other three types of couplings has been found. The most popular form of coupling is phase-amplitude coupling, namely the phase of a slow frequency oscillation modulates the amplitude of a fast frequency oscillation. This type of coupling has been observed in EEG as well as in hippocampal local field potential over different frequency combinations. So far, the most studied case is theta-gamma coupling in hippocampus.

The exact functional role of hippocampal theta-gamma coupling is still unknown. Experimental results showed that theta-gamma coupling is related with various cognitive tasks. For example, it can be dynamically modulated by different behavior stages. The magnitude of the coupling is strongly related with the performance during item-context association task. It could also help to separate different subbands of gamma and avoid interference of their distinct functions. Theta-gamma coupling has also been observed in human with its coupling strength
and spatial distribution closely related with the task performed\textsuperscript{75}. It is proposed that theta oscillation can synchronize spatially distributed gamma oscillation and therefore integrate inputs over different neuronal assemblies. Chapter V provides a theory that is consistent with this hypothesis. Another idea is that gamma oscillation divides a single theta cycle into up to seven ‘time slot’ which could be used to store working memories\textsuperscript{76}.
CHAPTER II
SPEED CONTROLS THE AMPLITUDE AND TIMING OF HIPPOCAMPAL GAMMA OSCILLATION

2.1 Abstract

Cortical and hippocampal gamma oscillations have been implicated in many behavioral tasks. The hippocampus is required for spatial navigation where animals run at varying speeds. Hence we tested the hypothesis that the gamma rhythm could encode the running speed of mice. We found that the amplitude of slow (20–45 Hz) and fast (45–120 Hz) gamma rhythms in the hippocampal local field potential (LFP) increased with running speed. The speed-dependence of gamma amplitude was restricted to a narrow range of theta phases where gamma amplitude was maximal, called the preferred theta phase of gamma. The preferred phase of slow gamma precessed to lower values with increasing running speed. While maximal fast and slow gamma occurred at coincident phases of theta at low speeds, they became progressively more theta-phase separated with increasing speed. These results demonstrate a novel influence of speed on the amplitude and timing of the hippocampal gamma rhythm which could contribute to learning of temporal sequences and navigation.

2.2 Introduction

Gamma rhythmic (~20–120 Hz) modulation of neural activity has been demonstrated in the cortex^{65,75,77–83} and hippocampus^{19,24,73,74,84,85}. Gamma oscillations are thought to increase synchronization of neural activity to mediate a variety of cognitive functions including
attention\textsuperscript{79}, learning\textsuperscript{74}, temporal binding and awareness\textsuperscript{83,86}. Gamma oscillations in the hippocampal LFP of rats are also modulated by theta oscillations, and they occur in two distinct bands, the lower frequency slow gamma (30–55 Hz) and the higher frequency fast gamma (55–120 Hz). The slow and fast gamma oscillations in CA1 are synchronous with slow gamma in CA3 and fast gamma in the entorhinal cortex respectively\textsuperscript{24}. Gamma rhythm also separates into the slow and fast bands in the human sensorymotor cortex\textsuperscript{78} and the rodent olfactory bulb\textsuperscript{82}.

Gamma oscillations in CA1 are larger in mice than in rats\textsuperscript{84}. Thus, mouse hippocampal gamma provides a reliable way of studying its modulation by behavior. In behaving mice, the hippocampal gamma rhythm and its coupling to theta are influenced by parvalbumin containing interneurons\textsuperscript{87}, interneuron-interneuron gap junctions\textsuperscript{84,88}, and acetylcholine\textsuperscript{89}, thereby implicating complex interactions between cellular properties, the excitatory and inhibitory neuronal networks and neuromodulators. The contribution of gamma oscillations to navigation is unknown.

During spatial navigation hippocampal pyramidal neurons or place cells fire at elevated rates in restricted regions of space\textsuperscript{50}, thereby providing information about the subject’s position through a rate code\textsuperscript{15}. The pyramidal neurons’ activity also provides information about a rat’s position through a temporal code known as theta phase precession such that the phase of the LFP theta rhythm where a pyramidal neuron spikes systematically precesses to lower values as a function of the position of the rat\textsuperscript{53,54,56,58,59,90}.

In order to navigate, it is not only necessary to know the current position but also to predict the future position. A necessary condition to achieve this is to know the current speed, in
addition to knowing position and head direction information. Firing rates of place cells contain information about position and head direction\(^{14}\). In addition, place cells’ firing rates\(^{14,91}\), and hippocampal interneuron firing rates\(^{92}\) also increase with speed. However, since position and speed are orthogonal variables, using firing rates to encode both position and speed could be confounding. We hypothesize that hippocampal high frequency oscillations could provide an independent and fast code for running speed.

Here we show that the amplitude of the gamma rhythm is strongly modulated by running speed. Unlike previously reported abrupt changes in gamma with task variables, we report a gradual increase in gamma amplitude with running speed that differentially influences slow and fast gamma rhythms. Further, we demonstrate a theta-phase precession like phenomenon where the preferred theta-phase of the slow gamma rhythm precesses to lower values with increasing running speed of the mouse.

2.3 Results

We measured 214 LFPs along with spiking activity, from the dorsal hippocampal area CA1 in 63 sessions from twelve mice using tetrodes. The mice ran on a 1.5 m long linear track to obtain rewards at the two ends of the track (see Methods and figure 2.5). The only selection criteria for using the data were that the tetrode was in the hippocampus while the mouse ran on the linear track. For the analysis of spiking activity, only those (160) tetrodes with at least 500 spikes on the track were used to ensure reliable quantification. As is
Figure 2.1: Hippocampal gamma rhythm splits into two bands, fast and slow, whose magnitude increases during locomotion. A) Power spectrum of a dorsal hippocampal LFP during locomotion (red) and immobility (black) in one example session. Shaded areas indicate 95% confidence intervals. B) Change in spectral power during run compared to stop as a function of
frequency in the gamma band. Data are averaged across the ensemble of 214 LFP traces. Shaded regions correspond to s.e.m. here and in subsequent figures. Inset shows the change in power for the example data set from fig. 1a. The increase in power is significantly lower at 45 Hz than in the surrounding frequency band, thereby demarcating a clear border between slow (20–45 Hz) and fast (45–120 Hz) gamma bands. C) Running speed of a mouse as a function of time (black), and corresponding amplitude of slow (red) and fast (blue) gamma rhythms. Insets show slow and fast gamma amplitudes at higher temporal resolution for high (<1>) and low (<2>) speeds. Both fast and slow gamma amplitudes are larger during run than stop. D) Slow (red) and fast (blue) gamma amplitudes were 15±1.5% (p = 5.3e-24) (mean±s.e.m., Wilcoxon Ranksum test here and in subsequent figures), and 31±1.0% (p = 1.2e-65) larger during run than stop, with fast gamma amplitude showing a greater increase than slow gamma (p = 1.5e-13).

common, it was difficult to detect a clear boundary between the fast and slow gamma bands based on the spectral power of the LFP in the gamma range alone (figure 2.1a, the same data were used for all subsequent example figures). This could result from noise masking a potential boundary between the gamma sub-bands. To overcome this difficulty, we assumed that the noise would remain relatively unchanged between the stop and run epochs, and computed the percentage change in spectral power at each frequency during run relative to the corresponding power during immobility. This procedure not only showed a clear increase in power in the gamma range during locomotion, it also revealed the change in gamma power was bimodal, clearly separating in two sub-bands within the gamma range (figure 2.1b, see methods): slow gamma (20–45 Hz) and fast gamma (45–120 Hz). The boundary between the two gamma bands
was defined as the frequency (45 Hz) at which there was the smallest change in gamma power between run and stop. This split in the gamma band is similar to studies in rats\textsuperscript{19,24,73}, but the entire gamma band is \(~10\) Hz lower in our data in mice.

Having detected the boundary between the two gamma bands, all subsequent analyses were done using the raw LFP, without any subtraction. The instantaneous amplitudes of the LFP were calculated separately for the slow (20–45 Hz) and fast (45–120 Hz) gamma bands, in order to obtain greater temporal precision in our estimates of gamma dynamics (figure 2.1c and figure 2.5). The amplitude of both slow and fast gamma rhythms increased by 15±1.5%, \(p = 5.3\times e^{-24}\) and 31±1.0% \(p = 1.2\times e^{-65}\) (median±s.e.m., Wilcoxon rank sum test, here and in all data) during run compared to immobility respectively (figure 2.1d and figure 2.5). Thus, there was a significant increase in the amplitude of slow and fast gamma rhythms during locomotion compared to immobility.

To compare data obtained from different electrodes and mice, the gamma band amplitudes were measured in z-scored units (see methods). Not only did the gamma amplitude increase during locomotion, this increase was proportional to running speed (figure 2.2). While the slow gamma amplitude increased linearly with running speed (figure 2.2a, c), fast gamma amplitude depended on the logarithm of running speed (figure 2.2b, d and figure 2.6). We denote the slow and fast gamma amplitudes by \(A_S\) and \(A_F\) respectively, and running speed by \(S\). The dependence of slow and fast gamma on speed can be modeled by:

\[
\frac{dA_S}{dS} = \alpha \quad \text{and} \quad \frac{dA_F}{dS} = \frac{\beta}{S}
\]
with $\alpha = 0.017 \pm 0.0012$ s/cm and $\beta = 0.17 \pm 0.0057$. In other words, since slow gamma amplitude is a linear function of speed, the derivative of slow gamma amplitude with respect to speed is independent of speed. Similarly, since the fast gamma amplitude increases as the logarithm of running speed, the derivative of fast gamma amplitude with respect to speed is inversely proportional to speed. The small magnitude of standard errors in $\alpha$ and $\beta$ compared to their mean is indicative of a remarkably consistent fit across datasets. These functions were also very good fits to the data, as confirmed by the analysis of residual errors (less than 5%) of linear and logarithmic fits (figure 2.7). Thus, the amplitudes of fast and slow gamma rhythms were differentially, gradually, stochastically but reliably modulated by running speed.

The amplitude of the gamma rhythm is modulated by the phase of a lower frequency LFP theta rhythm in rats\textsuperscript{19,24,73,74}, mice\textsuperscript{84,93} and humans\textsuperscript{75}. Hence, we investigated the speed dependence of cross-frequency coupling between the phase of low frequency rhythms and the amplitude of higher frequency rhythms (figure 2.2e and figure 2.5). Briefly, cross-frequency coupling was computed using a modulation index based on Shannon information such that a uniform phase distribution would yield a modulation index of zero. Consistent with theta-gamma coupling reported in rats we found significant theta-gamma coupling in mice. In fact, of all the low (2–20 Hz) and high (15–300 Hz) frequencies tested, the strongest cross-frequency phase-amplitude coupling was found between the phase of the theta rhythm (6–12 Hz) and the amplitudes of the slow and fast gamma rhythms. In addition, theta-gamma coupling was significantly larger for fast gamma compared to slow gamma (figure 2.2e and figure 2.8). The cross-frequency coupling increased linearly with speed for slow gamma but logarithmically for fast gamma (figure 2.9), as with the amplitudes.
Figure 2.2: Joint influence of running speed and theta phase on gamma amplitude. A) Each red dot depicts the amplitude of slow gamma in a window of 250 ms around each LFP theta peak as a function of running speed. The value of the slow gamma amplitude was averaged within a given speed bin (~7 cm/s wide, with 80% overlap between neighboring bins) (red squares). Black line shows the best linear fit. B) Same as A for fast gamma (blue dots and squares) with logarithmic fit. (See Methods S1 for methods and Supplement S1, Supplement S2 for details). C) Ensemble averaged data showing linear increases in slow gamma amplitude with speed. D) Same as C with logarithmic speed-dependence for fast gamma. E) Each vertical panel shows the cross-frequency coupling between the amplitude of a fast (15–300 Hz) signal (y-axis) and the phase of
a slow (2–20 Hz) signal, whose frequency is shown on the x-axis). Separate panels show coupling at different running speeds (top) for the example data in figures 2A,B. Colorbar to the right indicates modulation index (see Methods S1). Significant cross-frequency coupling is found only between the phase of the theta (6–12 Hz) oscillation and the gamma amplitude (20–120 Hz). Fast-gamma-theta coupling is greater than slow-gamma-theta coupling (bottom panel) at all speeds. The coupling increases logarithmically and linearly with speed for fast and slow gamma respectively (see Supplement S2). F) Slow gamma amplitude changes with running speed and theta phase for the example data set in figure 2A, B. G) Similarly for fast gamma. H) Slow gamma amplitude at the preferred phase (at 236±2.2°) of theta, averaged across all data, is linearly correlated with running speed (solid line, R² = 0.90±0.018, median±s.e.m.), but slow gamma amplitude around the theta trough changed minimally (dotted line). I) Similarly, fast gamma amplitude around the peak (260±1.8°) of theta increased logarithmically with speed (solid line, R² = 0.94±0.016), but fast gamma amplitude around the theta trough changed minimally (dotted line). J) Distribution of the slope of slow gamma amplitude around the theta peak as a function of running speed (solid line) across the ensemble of data, showing that it was significantly positive (0.017±0.0012, p = 1.9e-40) and far greater than the slope around the theta-trough (dotted line, 0.00067±0.00032, p = 4.3e-4). K) Similar results were true for the slope of fast gamma amplitude as a function of the logarithm of running speed around the theta peak (solid line, 0.17±0.0057, p = 4.1e-68) and the theta trough (0.013±0.0040, p = 0.0054).

To understand the fine temporal structure of theta-gamma coupling, we computed the joint influence of theta phase and speed on slow (figure 2.2f) and fast (figure 2.2g) gamma
amplitudes. The trough of theta was designated as phase 0° or 360°. The multiunit firing was maximal around the trough of theta (358°±3.7°, see methods). The phase of theta where gamma amplitude was maximal was called its preferred theta phase (see methods). Both slow and fast gamma had their preferred theta phases in the descending part of theta (figure 2.2f, g and figure 2.10). Further, only the gamma amplitude around the preferred theta phase showed modulation with speed (figure 2.2h, i). The slow gamma amplitude around its preferred theta phase showed a 44±2.9% linear increase with speed, whereas slow gamma amplitude 180° away from the preferred phase, corresponding to the anti-preferred phase, showed little speed-dependent increase (2.6±1.1%). Similarly, fast gamma amplitude around its preferred phase showed a 32±1.0%, logarithmic increase with speed, whereas fast gamma amplitude 180° away from its preferred phase increased by only 6.5±1.0%. These results were confirmed independently by comparing the slopes of the dependence of gamma amplitude on speed at their preferred theta phase or 180° away from their preferred theta phase for slow (figure 2.2j) and fast (figure 2.2k) gamma respectively.

These findings show that the influence of speed on gamma amplitude was restricted to a narrow range of preferred theta phases. In addition, the preferred theta phase of slow gamma seemed to change with running speed (figure 2.2f). To demonstrate this visually, it is necessary to suppress the speed-dependent change in gamma amplitude. Dividing the gamma amplitude at all theta phases for a given speed by the gamma amplitude averaged across all the phases in that speed bin achieved this suppression and revealed not only that the depth of modulation of slow gamma amplitude by theta phase was 158% larger at higher speeds, but also that the preferred theta phase of slow gamma was phase advanced by 63° at high compared to low speeds (figure
This is similar to precession of spike phase as a function of position\textsuperscript{53,54,56,58,59,90}, that is accompanied by increasing firing rate\textsuperscript{58,94} and membrane potential depolarization\textsuperscript{59}, that is characterized by the hippocampal spatio-temporal receptive field\textsuperscript{56,58}.

The gradual precession of slow gamma preferred theta phase with speed can be analogously characterized by a so-called velo-temporal receptive field (VTRF figure 2.3b and figure 2.9). The VTRF averaged across the ensemble of data showed robust precession of slow gamma preferred theta phase with speed (figure 2.3c). Although slow gamma amplitude increased linearly with speed, slow gamma preferred theta phase precessed approximately logarithmically with speed. Unlike slow gamma, fast gamma preferred phase showed only a small amount of precession for the example data set (figure 2.3e), as well as when averaged across the ensemble of data (figure 2.3f and figure 2.10).

Across the ensemble of data, the preferred theta phase of slow gamma precessed by $61^\circ\pm2.7^\circ$ between low and high speeds, whereas the preferred theta phase of fast gamma precessed by only $16^\circ\pm1.8^\circ$ (figure 2.4a and figure 2.11). Thus, while slow and fast gamma rhythms preferred similar phases of theta at low speeds, the two rhythms became increasingly phase-separated with increasing running speed (figure 2.4a–c). This was further demonstrated by computing the slopes of the slow (and fast) gamma preferred theta phase versus the logarithm of speed. Slopes of gamma preferred theta phase versus speed were negative for both slow (-23±1.4) and fast (-3.0±0.78) gamma, with slow gamma
Figure 2.3: Theta-phase precession of gamma amplitude as a function of running speed.

A) Slow gamma amplitude as a function of theta phase for one example dataset (figure 2F) was averaged across all the theta cycles at low (dashed) and high (solid) speeds. Further, unlike figure 2F, the amplitude of gamma at each phase of theta was divided by the sum of gamma amplitudes across all phases of theta at that speed. This enabled a comparison of the depth of modulation of gamma amplitude with running speed and theta phase, independent of changes in gamma amplitude with running speed. The theta phase modulation of gamma amplitude was 158% greater and the preferred phase of theta was 63° lower at high speeds compared to low speeds. B) Same data as A, but as a function of (the logarithm of) a range of running speeds. The hippocampal velo-temporal receptive field for speed (VTRF) for this example dataset shows a progressive precession of slow gamma preferred phase of theta with speed (white dotted line). C) VTRF averaged across all the data show a robust increase in the depth of modulation as well as
precession (white dotted line) of slow gamma preferred phase of theta with increasing running speed. D) Same as in A, but for fast gamma showing only a small change in the depth of modulation of fast gamma amplitude (75%) and preferred theta phase (10.7°) with speed. E) Same as B showing only small changes in fast gamma VTRF with speed. F) Same as C showing minimal changes in the ensemble averaged fast gamma VTRF with speed.

showing significantly greater change with speed than fast gamma (p= 9.14e-34, figure 2.4b). As a result, the slow and fast gamma preferred theta phases were similar at low speeds, but at the highest speed the slow gamma preferred theta phase occurred 37°±2.1° ahead of the fast gamma preferred phase (figure 2.4c and figure 2.12). Under laboratory conditions, mice did not attain speeds beyond 50 cm/s. In the wild, mice can attain much higher speeds. Extrapolation of the traces in figure 4a suggests that at these naturally attainable higher speeds, slow and fast gamma can achieve a greater degree of theta-phase separation.

Consistent with previous studies\textsuperscript{24,69,84}, the probability of spiking of the hippocampal neural ensemble, as measured by multi-unit activity, was significantly influenced by the phase of both fast and slow gamma rhythms recorded on the same tetrode (figure 2.4d and figure 2.5). Additionally, the phase of the large amplitude slow gamma rhythm had a significantly (78.01%, p = 1.9e-14) greater influence on spiking probability than the phase of fast gamma (figure 4d, e, see Methods S1). Further, spikes preferred nearly the opposite phases of slow and fast gamma rhythms\textsuperscript{24} (figure 2.4d, f) with maximal spiking probability occurring at 240°±5.6° of slow gamma (p= 1.7e-7, Rayleigh test) and 80°±5.7° of fast gamma (p= 2.9e-6, Rayleigh test).
Figure 2.4: Speed dependent separation of slow and fast gamma preferred theta phase and modulation of spiking by fast and slow gamma.

A) Averaged across the ensemble of data, slow and fast gamma preferred theta phases were nearly coincident (−6.0±1.2°) at low speeds, but the slow gamma preferred phase precessed by 61±2.7° with increasing speed whereas fast gamma preferred phase precessed by only 16±1.8°.

B) The slope of slow (fast) gamma preferred phase as a function of running speed is shown in red (blue). Maximal speed in each session was normalized to unity to allow comparison across data. The vast majority (95%) of slow gamma LFP showed speed-dependent phase advancement, but only 67% of fast gamma LFP showed phase advancement. C) There was only a small difference in the preferred phases of slow and fast gamma at low speeds (dashed line,−6.0±1.2°,
p = 5.0e-7), but the two rhythms were separated by 37±2.1° (p = 1.3e-25) at high speeds (solid line). D) Multi-unit spike probability as a function of fast (blue) and slow (red) gamma phase was computed separately for 141 data sets and averaged across the entire ensemble (see Methods S1). E) Scatter plot of fast gamma phase vs. slow gamma modulation index of spiking for 141 data sets. Spike probability was more strongly modulated by the phase of slow gamma than fast gamma (p = 1.9e-14) F) Scatter plot of the preferred fast (81±5.7 degrees) and slow gamma phases (242±5.6 degrees) of spikes.

2.4 Discussion

These results demonstrate a gradual, large, differential and significant modulation of the amplitude and timing of hippocampal slow and fast gamma oscillations with running speed. Virtually all of our data show that slow gamma amplitude increases linearly with speed whereas fast gamma amplitude shows a logarithmic dependence on speed (figure 2.2c, d, figure 2.6 and figure 2.7). This differential modulation could arise due to mechanisms within MEC and CA3, which are hypothesized to generate the fast and slow gamma respectively19,24, or due to the differential nature of excitatory-inhibitory networks and dendritic properties within the distal versus the proximal parts of CA1 where these inputs terminate respectively. Further, cholinergic levels may increase with speed which could differentially enhance gamma oscillations. Speed-dependent changes in gamma rhythm could not be an artifact of speed-dependent change in spiking probability because the multi-unit activity showed far greater phase locking to slow gamma than fast gamma, even though the fast gamma band has more similar frequency content
to spikes, thereby increasing the chances of spike bleed over. Further, spikes prefer nearly the opposite phases of slow and fast gamma.

Speed modulation of gamma was restricted to a narrow range of preferred theta phases. The preferred phase of theta, where slow gamma amplitude was maximal, precessed to lower values with increasing speed. This precession of the gamma preferred theta phase was larger for slow than fast gamma. This provides the first demonstration of a phase-precession like phenomenon within the hippocampus that is independent of position and depends on speed. As a result, at low speeds, the highest amplitudes of slow and fast gamma occurred at similar phases of theta, but with increasing speed, they became increasingly more phase-separated such that slow gamma occurred increasingly earlier than fast gamma within each theta cycle.

Further studies are required to determine the biophysical mechanisms underlying these results. One possibility is that with increasing speed, stimuli occur at a greater pace, which increases the firing rates of hippocampal excitatory\textsuperscript{14,91} and inhibitory\textsuperscript{92} neurons. This increased spiking activity would facilitate the generation of gamma oscillations in the recurrent excitatory-inhibitory circuits\textsuperscript{95} which could explain our finding of gradually increasing gamma amplitude with speed. Since the firing rates of hippocampal neurons are modulated by the phase of the theta rhythm, such a mechanism could also explain the comodulation of gamma amplitude by theta phase and running speed.

Precession of slow gamma preferred theta phase with running speed could be explained by a mechanism similar to the rate-to-phase transformation mechanism proposed to explain theta-phase precession of spikes as a function of position\textsuperscript{58,94,96,97} as follows. The firing rate\textsuperscript{94} and
excitability\textsuperscript{59} of place cells increases as a function of the rat’s position within the place field. The interaction between this ramping excitation and the theta rhythm could result in theta phase precession of place cell spikes. Similarly, slow gamma oscillations could require a specific balance of excitation and inhibition which occurs at a specific phase (\( \sim 270^\circ \)) of theta at low speeds. The excitation-inhibition balance would be influenced by both theta rhythm and running speed. Higher running speeds could result in increased excitability of neurons which could result in the optimal balance occurring at earlier phases of theta, resulting in slow gamma oscillations appearing at earlier phases of theta, i.e. precession of slow gamma preferred theta phase with speed.

Similar mechanisms could apply to the fast gamma rhythm. However, fast gamma amplitude showed a weaker dependence on speed than slow gamma (figure 2.2). This could arise due to a smaller speed-dependent increase in firing rates in the entorhinal cortex\textsuperscript{98} than the hippocampus\textsuperscript{14,91}, or due to local mechanisms within distal regions of CA1 where the entorhinal inputs terminate.

Unlike the theta phase precession of spikes as a function of position, where the spike-phase progressively decreases with increasing position, the preferred theta-phase of slow gamma can both increase and decrease with corresponding change in running speed. These observations are consistent with the rate-phase transformation model\textsuperscript{58,94,96,97} because position increases only monotonically in the highly directional place cells on linear tracks, whereas speed can change bidirectionally. Consistent with this model, bidirectional changes in preferred gamma phase of spikes have been recently observed\textsuperscript{99}.
These results can have significant functional consequences. In order to navigate, it is not only necessary to know the current location but also the running speed. Position and speed are orthogonal variables that should be represented by independent parameters. The firing rates of hippocampal neurons are modulated by both spatial location and by the running speed of the animal. Hence, the firing rate provides an ambiguous code for position and speed. Speed-dependent changes in gamma rhythm can provide an independent parameter to encode speed. Just as gamma oscillations can arise through excitatory-inhibitory networks, gamma-rhythmic modulation of spikes can be readily decoded by downstream excitatory-inhibitory networks to extract unambiguous information about speed.

Several studies have shown increased gamma power occurs with attention or task demands, and it is associated with improvement on a number of behavioral measures\textsuperscript{74,79,83,86}. Increased gamma power with speed could similarly improve synchrony of hippocampal spikes that could facilitate induction of synaptic plasticity and learning of navigational routes and temporal sequences. It has been suggested that hippocampal activity predicts or anticipates the upcoming events in a temporal sequence\textsuperscript{97}. Increasing speed would require faster prediction of future events. If slow and fast gamma arise in CA3 and MEC respectively\textsuperscript{19,24}, our results of a speed-dependent increase in the theta phase separation of slow and fast gamma would suggest that CA3 activity increasingly anticipates MEC activity by about 40u of theta phase, or at least 15 ms at high speeds.

Several studies have shown that coincident activation of the entorhinal and CA3 inputs to CA1 results in enhanced activation of CA1 and induction of long-term potentiation of synapses\textsuperscript{100–104}. Thus, at low speeds, coincident activation of CA3 and entorhinal inputs at
similar theta phases would increase their efficacy in activating CA1, and facilitate associative synaptic plasticity between these inputs resulting in improved place learning. Increasing theta-phase separation between the slow and fast gamma rhythms at higher speeds would result in anticipatory learning of sequences of neural events between CA3 and MEC inputs within CA1. Such predictive coding is consistent with the observation that stimulation of CA3 before the entorhinal cortex increases the transmission of entorhinal inputs to CA1 neuron’s soma\textsuperscript{102} by overcoming fast inhibition. Indeed, synchronous activation of entorhinal activity during up-down states results in fast activation of the R-LM interneurons\textsuperscript{105} which reduces the level of depolarization of CA1 pyramidal neurons\textsuperscript{106}. Thus, the speed-dependent increase in the thetaphase separation of slow and fast-gamma amplitudes could facilitate a speed-dependent enhancement of the efficacy of entorhinal activity, or the sensory inputs, in driving CA1 neurons.

In sum, just as the spiking rates of pyramidal neurons and their preferred theta phases are modulated by spatial position, we show that the magnitude of slow and fast gamma rhythms and their preferred theta phases are modulated by running speed. This suggests that while the firing rate and theta phase of pyramidal neurons encode position, the amplitude and theta phase of gamma oscillations can provide an independent estimate of running speed that could be useful in navigation and learning.

2.5 Methods and supplements

2.5.1 Experimental Methods

All experiments were carried out in 12 wild-type (C57/BL6) male mice. Mice were housed individually, maintained on a 12-h light/dark cycle and had ad libitum access to food and
water unless otherwise specified. All experiments were conducted in accordance with the animal welfare guidelines of the Max Planck Society.

**Electrophysiology:** The mice were implanted with hyperdrives above the right dorsal CA1 region of the hippocampus (AP-2.0 mm, L1.5 mm with respect to bregma). Each hyperdrive contained up to 4 independently movable tetrodes and a reference electrode. Tetrodes were made of four 15-µm, Teflon coated Nichrome wires (Kanthal, Palm Coast FL), which were twisted and heat-fused together. Two stainless steel screws were implanted over the opposite frontal cortex (for anchoring) and the cerebellum (for ground).

**Behavioral procedures:** One week after surgery, the mice were trained to run back and forth on a linear track for food reward (sweetened milk) located at the opposite ends of the track. The track (length 140 cm, width 3 cm) was made of wood and painted black. Throughout the entire training and further recording the mice were food-restricted and maintained at ~85% of their postoperative *ad libitum* body weights.

**Recording methods:** Throughout the training period, the tetrodes were advanced gradually over the course of many days to place them in the CA1 pyramidal cell layer. The arrival of each tetrode into the hippocampus was recognized by several criteria, including the presence of 100-300 Hz “ripples” in the local field potential (LFP), the polarity of “sharp waves” in the LFP, and the appearance of multiple cells with complex spikes. During recording, the mice were attached to a unitary gain head-stage preamplifier (HS-16; Neuralynx, Tucson, AZ) via a cable suspended on the supporting metal string to minimize an additional load to the animal’s head. Signals were filtered and differentially amplified against the reference
electrode by Lynx-8 programmable amplifiers (Neuralynx). Whenever the amplitude of the spike signal exceeded a predetermined threshold, each tetrode channel acquired a 1-ms sample of data at a rate of 32 kHz. These spike samples were time-stamped, amplified by a factor of 5,000-10,000 and stored on a personal computer running Cheetah data acquisition software (Neuralynx). Local field potentials (LFP) were recorded from one (of the four) electrodes of each tetrode. The LFP was sampled at 2 kHz, amplified 2000 times, and filtered between 0.5-900Hz.

The animal’s position and head direction was measured using overhead CCD camera (Cohu iDome, USA) that tracked two light-emitting diodes (red and blue) attached to the headstage. Video recording was made with spatial resolution of 0.25 cm/pixel and a sampling rate of 50Hz. Daily recording sessions consisted of 8-40 laps on the linear track surrounded by rest/sleep sessions (20 min) on an elevated platform.

**Histology**: Following completion of the experiments the mice were deeply anesthetized and electrolesion (200 µA for 4 sec via one channel of each tetrode with respect to the ground screw) was performed to confirm that the tetrodes were in CA1.

**Multi-unit extraction**: All the spikes with peak amplitude greater than 100 µV and peak to valley duration ranging between 0.125ms to 0.375ms were extracted from each tetrode. Only data from tetrodes with more than 1000 such spikes in one session were used. These criteria yielded 141 data sets.

**2.5.2 Data analysis**
**Computation of running speed:** Position data were smoothed using a Gaussian kernel of width 200ms to remove noise from the video tracking data. Speed was computed as the first temporal derivative of position (Figure 2.5.1a)

![Figure 2.5.1: Speed and spectral analysis. A) Speed of one mouse as a function of time. B) Broad band (0.5-900Hz) LFP (black) and theta band (6-12Hz) LFP (pink). Each theta event (250ms) was assigned a number (t, t+1, …). C) Hilbert transform of the theta band LFP from B.](image)

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**Figure 2.5.1:** Speed and spectral analysis. A) Speed of one mouse as a function of time. B) Broad band (0.5-900Hz) LFP (black) and theta band (6-12Hz) LFP (pink). Each theta event (250ms) was assigned a number (t, t+1, …). C) Hilbert transform of the theta band LFP from B.
Troughs of theta in B were assigned a phase 0° or 360°. D) Slow gamma (20-45Hz) LFP (black) and the envelope of the filtered LFP obtained using Hilbert transform (red).

**Power spectral analysis:** LFP were filtered in appropriate bands using a two-way least squares FIR filter. LFP spectrum was computed using the multi-taper method from the Chronux open source MATLAB toolbox (http://www.chronux.org/). For figure 1A, the spectrogram was analyzed for one LFP trace from one example session. The spectrum was calculated in 2s epochs, and was averaged separately for data when the animal was running or immobile. Mice alternated between immobility and run in each trial. Spectral power was computed in a 2s wide window placed at the center of each run or immobile epoch. There were 29 run and stop epochs in the calculation. Confidence interval was estimated using jackknife method.

For figure 1B, the change of spectral power was estimated between the run and stop spectra calculated in figure 1A using:

\[
\text{Percentage change} = 100 \times \frac{S_{\text{run}} - S_{\text{imm}}}{S_{\text{imm}}}
\]

Where \( S_{\text{run}} \) is the run spectrum and \( S_{\text{imm}} \) is the stop spectrum. The result was averaged across 214 LFP traces obtained in 63 sessions from 12 mice. The shaded region indicates the standard error of the mean.

**Theta event based analysis of gamma amplitude:** Each LFP was first filtered in theta band (6-12Hz) and then Hilbert transformed to locate the peak for each individual theta cycle. Theta peak was assigned a phase of 180 degrees and the relative phase of theta computed within each theta cycle. The entire data set was then labeled with a series of theta events centered on
theta peaks with a 250ms window length (~50% overlap with both adjacent theta events, Figure 2.5.1b). Slow (fast) gamma amplitudes were obtained by filtering the LFP in 20-45Hz (45-120Hz) bands and computed from the absolute value of the analytic signal. After this labeling was done, the average speed, and fast and slow gamma amplitudes were calculated for each theta event. Figures 2.2a-b were obtained by plotting speed vs. slow or fast gamma amplitude for all the theta events from one data set.

**Speed dependent cross frequency coupling (CFC):** For ease of depiction, only six speed bins were used in this analysis (figure 2.2e). The first bin was for immobility with speeds between 0 - 0.5cm/s. The remaining five equally spaced bins were generated between 2cm/s to the top 99% speed for all the theta events. There was no overlap between neighboring speed bins. The range of frequencies used for amplitude-frequency sweep was from 15-300Hz, step size 2Hz, bandwidth 4Hz, while the range used for phase-frequency sweep was from 2-20Hz, step size 1Hz, bandwidth 2Hz. To accommodate these lower frequencies, CFC and speed were computed in coarser, 500ms wide windows. The results were qualitatively insensitive to these choices of parameters.

For each amplitude and phase frequency pair in a given speed bin, the data were grouped into 60 equally spaced phase bins spanning 0-360° based on their phase value, and the mean amplitude of the high frequency signal $f_{amp}$ was calculated for each low frequency phase bin $f_{phase}$ for a given speed bin $S$. Using these, the shannon entropy of cross-frequency coupling and its modulation index (MI) were calculated as:

$$H(f_{amp}, f_{phase}, S) = -\sum_{j=1}^{N} P(j) \log[P(j)]$$
\[ MI(f_{\text{amp}}, f_{\text{phase}}, S) = \frac{\log(N) - H}{\log(N)} \]

Where \( H \) is the Shannon entropy, \( MI \) is the modulation index, \( P(j) \) is the normalized amplitude of the high frequency signal in each low frequency phase bin \( j \), and \( N \) are the number of phase bins (60). Figure 2.2e and figure 2.9 were obtained using this procedure.

**Computation of joint influence of speed and theta phase on gamma amplitude and hippocampal velo-temporal receptive fields for speed (VTRF):** Since cross-frequency coupling was mostly restricted to theta-gamma coupling (see above), subsequent analysis focused on the fine temporal structure of theta gamma CFC as a function of speed. LFP was filtered in theta (6-12Hz) band and theta phase was computed as described above. The amplitude of LFP in the slow and fast gamma bands was also computed as detailed above. To obtain finer temporal resolution, theta phase was separated into 120 phase bins at each speed. To obtain finer resolution of speed, thirty speed bins with 80% overlap between adjacent speed bins were used. Both theta and gamma data were sorted into one of the thirty speed bins as described above (Figure 2.5.2a, b). The reason to use a fixed number of speed bins rather than a fixed speed bin size was to allow ensemble average across different data sets where mice spanned different ranges of speed. The mean gamma amplitude within each speed-phase bin was calculated separately for slow and fast gamma. This procedure yielded a 2D picture (Figure 2.5.2c) that showed the joint modulation of slow (fast) gamma amplitude by running speed and theta phase, called the velo-temporal receptive field for speed (VTRF) Figure 2.2f and g.
Figure 2.5.2: Velo-temporal receptive fields for speed (VTRF). A) Speed S, theta phase and slow/fast gamma amplitude were calculated within each theta event $t$. B) Slow/fast gamma amplitude envelope for each theta event was rescaled to the same length spanning 0-360° of the theta cycle. C) The rescaled gamma amplitude envelopes were grouped into different speed bins and the average gamma amplitude value at each theta phase was obtained for each speed bin (right panel). Slow gamma amplitude (colorbar, z-scored units) as a function of speed and theta phase (left).
Computation of speed-dependent shift of preferred theta phase for slow and fast gamma: This was obtained by first calculating the preferred phase of slow (or fast) gamma amplitude in each speed bin from the previously calculated VTRF. Circular analysis was used to calculate this preferred phase:

\[
\theta^{\gamma_{S/F}}(S) = \arg\left(\sum_{j=1}^{N} P^{\gamma_{S/F}}(S) \times e^{i\theta_j}\right)
\]

Where \(N\) is the number of phase bins, \(S\) is the speed, \(\gamma_S\) and \(\gamma_F\) is the slow and fast gamma band. \(P^{\gamma_{S/F}}\) is the mean gamma amplitude in each phase bin at a certain speed for slow or fast gamma, \(\theta_j\) is the value of phase at the center of each phase bin \(j\).

Normalization of gamma amplitude across data: To calculate the dependence of gamma amplitude on speed and theta phase, averaged across data sets from different tetrodes, sessions and animals, gamma amplitude has to be normalized across data sets. Since different animals ran at different speeds, and gamma amplitude varied from across electrodes and sessions, simple averaging of gamma amplitude across all speeds would generate variable results. Hence, the filtered slow (or fast) gamma traces were divided by the standard deviation of the data points when the animal is immobile. This would make the mean amplitude envelope of slow (or fast) gamma during immobility close to one for all the data sets. This resetting of gamma amplitude was done separately for fast and slow gamma and for each LFP. As a result, all the speed dependent gamma amplitude traces (figures 2.2-4, figure 2.7-10) start close to unity at the lowest speed, which allows unbiased comparison and averaging across diverse data.

Realignment of gamma phase precession across data: Similar procedure was followed to compute the ensemble averaged velo-temporal receptive fields for speed (VTRF) (figure 2.3c,
The preferred theta-phase of slow (or fast) gamma varied across electrodes (figure 2.11). To remove the variability introduced by this, a procedure similar to that used to compute the ensemble averaged phase precession of spikes as a function of position was used. Specifically, since the preferred phase of slow and fast gamma was around 270° (figure 2.11), preferred theta-phase of slow gamma was reset to 270° for all LFP. Speed-dependent change in the slow gamma’s preferred theta-phase was then averaged across all the data to obtain figures 2.3c, 2.4a and figure 2.10.

Notably, the exact amount of constant phase realignment done for slow gamma for one LFP trace was also carried out for fast gamma phase for that LFP. This ensured that the phase difference between slow and fast gamma theta-phases was unchanged in each electrode.

**Gamma phase locking of spike probability:** Hilbert transform was applied to LFP in slow and fast gamma bands to obtain their respective phases. The slow and fast gamma phase for each spike at the time of occurrence was computed. The instantaneous slow and fast gamma cycles where each spike occurred were identified and the slow and fast gamma amplitudes at the peak and the trough were assigned to each spike. The gamma amplitude was normalized with the same method described above to facilitate the comparison between different data sets. Only the spikes with slow or fast gamma amplitude greater than 1.5z both at the peak and trough were used in the slow or fast gamma phase locking analysis.

Preferred phase and modulation index of spike phase locking were computed separately and similarly for slow and fast gamma as follows: Spikes were sorted into N phase bins according to their slow (or fast) gamma phase. The number of spikes in each bin was divided by
the total number of spikes to yield spike probability (whose sum is unity). The preferred gamma phase \( \varphi \) and modulation index \( MI \) were defined as:

\[
MI = \text{abs}\left(\sum_{j=1}^{N} p_j e^{i\theta_j}\right)
\]

\[
\varphi = \text{arg}\left(\sum_{j=1}^{N} p_j e^{i\theta_j}\right)
\]

Where \( N \) is the number of bins, and \( p_j \) is the probability of having spikes in a phase bin \( \theta_j \). These definitions have the advantage that the modulation index is independent on the mean firing rate and the number of phase bins.

2.5.3 Supplement figures
Figure 2.6: More examples for figure 2.1c from six different mice (mouse label to the left) demonstrating increased slow and fast gamma amplitudes during run compared to stop. Each panel is identical to figure 1C. See figure 2.1C legend for details.
Figure 2.7: More examples for figure 2.2a-b from six different mice, showing speed-dependent, gradual increase in slow and fast gamma amplitude with speed. Each panel is identical to the corresponding panels in figure 2.2a and 2.2b. See figure 2.2a, b legend for details.
Figure 2.8: Slow gamma amplitude increases linearly with speed whereas fast gamma amplitude increases logarithmically with speed. The average amplitude of slow gamma in each speed bin was computed for 30 speed bins (figure 2.2a and figure 2.7). A linear fit was made to the average amplitude of slow gamma as a function of speed. The absolute value of residual error was averaged across all speed bins and divided by the average slow gamma amplitude at all speeds to yield the percentage residual (Solid red line, figure 2.8a). $R^2$ values of the best fit (solid red line, figure 2.8b) were also computed. Small values of percentage residual error (1.9%) and the large value of $R^2$ (median=0.90) indicate that linear model is a good fit. However, when the slow gamma amplitude as a function of speed was fitted with a logarithmic curve, the percentage residual error was significantly larger (2.9%, dashed line, figure 2.8a), and the $R^2$ value was significantly lower (median=0.78, dashed red line, figure 2.8b).

Similar procedure was followed to compute the goodness of fit of a logarithmic relationship between fast gamma amplitude and speed. In contrast to slow gamma, the percentage residual error was significantly smaller when using a logarithmic fit to fast gamma
(1.2%, dashed blue line, figure 2.8c), compared to a linear fit (1.9%, solid blue line, figure 2.8c). Further, $R^2$ values (median=0.94, dashed blue line, figure 2.8d) were significantly higher with a logarithmic fit for fast gamma than with a linear fit (median=0.82, solid line, figure 2.8d).

Additionally, in panel E the x axis is the percentage error for a linear fit while the y axis is for logarithmic fit. Red dots represent slow gamma and blue dots represent fast gamma for each data sets (214 data sets). Most of the red dots were distributed the diagonal line indicating a better linear fit for slow gamma. On the other hand, most of the blue dots were distributed below the diagonal line indicating a better logarithmic fit for fast gamma. Similar scatter plot was done for $R^2$ values in panel F showing the same results.
Figure 2.9: Ensemble averaged speed dependent cross-frequency coupling (CFC) and differential increase of slow and fast gamma modulation indices. A) This figure is similar to figure 2E. Speed-dependent CFC similar to figure 2E was computed for each LFP and averaged across the ensemble of 214 LFPs to obtain this figure. In order to reduce noise, the panel with lowest speed was subtracted from the subsequent panels, leaving only the speed dependent component. Each vertical panel, for a given speed, shows the cross-frequency coupling between the amplitude of
fast (15-300Hz) signal as a function of the \textit{phase} of slow (2-20Hz) signal. Significant cross-frequency coupling is visible between the phase of theta (6-12Hz) and amplitude of gamma (20-120Hz). Consistent with figure 1B, cross-frequency coupling is distinct in slow and fast gamma band (bottom panel shows inset for slow gamma). B) Modulation index averaged over slow (red line) and fast (blue line) gamma bands and plotted as a function of speed, indicating their differential dependence on speed.
Figure 2.10: Theta-phase precession of preferred gamma phase as a function of running speed. Same as in figure 2.3 but from another six different mice (mouse number is to the left of each panel). A) Normalized slow gamma amplitude as a function of theta phase at the highest (solid) and lowest (dotted) speeds. B) Similar data as a) with slow gamma amplitude as a function of theta phase and logarithm of running speed. C) Same as in a) for fast gamma. D) Same as in b) for fast gamma.

Figure 2.11: Distribution of preferred theta phase of slow (red) and fast (blue) gamma at low speeds before realignment. At low speeds the mean phase of low gamma was (281± 4.1°) and that of high gamma was (269±4.0°).
Figure 2.12: Relationship between the magnitude of speed-dependent precession of slow and fast gamma preferred phase of theta. For each LFP the difference in theta-preferred phase of slow gamma at highest minus the lowest speed was computed. Similar difference in theta-preferred phase was computed for fast gamma. The speed-dependent change in theta-preferred phase of slow and fast gamma were correlated ($r=0.48$) with slow gamma showing a significantly greater degree of phase precession than fast gamma.
Figure 2.13: A summary of changes in slow and fast gamma amplitude and timing with running speed and theta rhythm. The multiunit activity (green dots) is maximal at the trough of theta (black trace, top and bottom). At low speed (top) the fast gamma amplitude is maximal (blue) just before the slow gamma reaches maximal value (red). At high speed (bottom) the amplitude of both slow and fast gamma, as well as the multiunit firing rate, increase. Further, maximal slow gamma amplitude appears about 15 ms before maximum fast gamma amplitude.
CHAPTER III
PERSISTENT THETA MODULATION AND ITS SPEED DEPENDENT MICROSTRUCTURE

3.1 Abstract

Hippocampal theta rhythm is critical for synaptic plasticity\textsuperscript{112–115} learning\textsuperscript{116} and neural coding. However, theta rhythm in the rodents while awake is thought to appear only during locomotion\textsuperscript{27,28,53,58} and mostly disappear during periods of immobility in the absence of any task. Here we show that although the amplitude of hippocampal local field potential (LFP) theta rhythm in mice is weak during immobility, significant LFP-theta modulation of spikes (TMoS) is present at all times, indicating persistent theta modulation during immobility and locomotion. Surprisingly, TMoS is even greater during immobility than during locomotion. This is because the hippocampal gamma oscillations become stronger with running speed and take over the control of spike-timing while reducing theta modulation through a novel mechanism. Thus, theta modulation of spikes is highest during immobility whereas the precision of spike timing within theta cycle is highest at high speeds which could facilitate precise spike-timing based neural computations and learning\textsuperscript{53,54,58}.

3.2 Introduction

The rodent hippocampal activity is strongly modulated by a 4-12Hz theta rhythm during locomotion\textsuperscript{27,28,53,58} whose disruption impairs learning\textsuperscript{116} and neural code\textsuperscript{117,118}. Hence, theta-burst stimulation is widely used for inducing synaptic plasticity in vitro\textsuperscript{112–115} which is thought to modulate neural spiking and facilitate learning\textsuperscript{53,54,58}. However, the rodent hippocampal local
field potential (LFP) shows clear theta rhythm only during locomotion, but not during periods of immobility\textsuperscript{23,69}, which poses a question: whether theta rhythm plays a role in learning during vast periods of immobility? In order to answer this question, the first thing needs to be tested: whether theta modulate neural activities during immobility. We therefore analyzed the data of LFP, multi-unit activity (MUA) and single unit activity using tetrodes implanted chronically in the dorsal CA1 of C57BL6 mice as they ran on linear tracks for liquid rewards at the two ends (see methods). The mice were largely immobile at the reward locations. Data from 154 tetrodes, obtained from 78 sessions in 11 mice were used.

3.3 Results

Consistent with previous observations, the LFP amplitude in theta range was indeed smaller during immobility than during locomotion (figure 3.1a). However, the LFP theta modulation of spikes (TMoS, supplement method) was not only present during immobility, it was much larger (146±13\%, p=9.74e-28) than during high-speed locomotion (figure 3.1a). This was true for almost all (85\%) the data sets (figure 3.1b).

We developed a technique to determine the significance level of TMoS (see method and figure 3.5) taking into account of the different number of spikes used in the calculation during immobility and locomotion. We found that TMoS was significant in the vast
Figure 3.1: Persistent Theta Modulation of Spikes (TMoS). A) Running speed (black), theta amplitude (blue) and TMoS (red) as a function of time in one recording session. TMoS is higher during immobility than locomotion. The data between the two dotted red lines are shown at greater magnification to depict
increased phase locking during immobility (bottom left) than high speed (bottom right). Each small red bar in these panels represents one multiunit spike and the black oscillating traces are LFP filtered in theta range (4-12Hz). B) TMoS during immobility vs. TMoS during run. Each dot represents the result obtained from one electrode in one session. C) Same as B but showing the degree of significance of TMoS. Dotted red line corresponds to three standard deviations. Data above this line could occur by chance with p< 0.01 (supplement method). D) TMoS during immobility for the LFP theta cycles with large amplitude (top 25%) was larger than the ones with the lowest amplitude (bottom 25%). E) Same as D but for the significance levels showing that a majority of even the lowest amplitude theta cycles showed significant (p<0.01) TMoS.

The majority of data sets during both immobility and locomotion (96% and 94% respectively), while the significance level of TMoS was (61±11% p=8.43e-16) greater during immobility than locomotion (figure 3.1c). Similar results were also seen for isolated pyramidal neurons and interneurons (figure 3.6). To confirm the persistent nature of theta modulation at all times during immobility, we split all theta cycles during immobility into four quartiles based on their instantaneous theta amplitude and calculated TMoS for each group separately. While TMoS was (57±8%, p=1.97e-17) larger during large theta amplitude epochs (figure 3.1d), a majority of data sets (89%) showed significant TMoS even during epochs with lowest theta amplitude (figure 3.1e). Thus, significant TMoS was present at all times during immobility, indicative of persistent theta modulation in the hippocampal spiking during all epochs of a spatial task.

Our finding that significant theta modulation of spikes was present during immobility and was even larger than during running is in seeming contradiction with commonly held belief that
theta modulation should diminish or disappear during immobility. This is because instead of theta modulation is usually quantified by computing the auto-correlation of the LFP or the spike trains instead of using TMoS. Indeed, the autocorrelations of the LFP, place cells, interneurons or multiunit show far weaker or insignificant theta rhythm at low speeds (figure 3.7). How can the discrepancy between TMoS and the autocorrelation be reconciled? We note that while TMoS is influenced only by the depth of theta modulation of spikes by the LFP within a given theta cycle (figure 3.8), the autocorrelation is influenced by the rhythmicity or the variability of theta waveform from cycle to cycle. We demonstrated using a simulation that not only the variability of theta period but the variability of theta amplitude and shape reduce the autocorrelation (figure 3.9) such that, it is possible to have strong TMoS in every theta cycle but have weak or insignificant rhythmic modulation in the autocorrelation.

In order to test this hypothesis, we examined the microstructure of individual theta cycles as a function of running speed (figures 3.2). Indeed, at low speeds theta period varied considerably from cycle to cycle (Figure 3.2a) and this variability in theta period reduced with increasing speed (figure 3.2b), showing a 39.4±1.4% reduction with speed averaged across all the data sets. In addition, theta amplitude increased by 25.7±2.3% with speed while its variability reduce by 25.6±1.6% at higher speed. Consistent with previous observations in rats\textsuperscript{22,119,120}, theta cycles were also asymmetric in mice, showing rapid rise from trough to peak and a slow decay from peak to trough (figure 3.2a). Additionally, the
Figure 3.2: Change in theta shape and variability with running speed. A) Two example LFP (raw LFP in pink, theta band filtered LFP in black) traces at low speed (left) and high speed (right). The dotted vertical lines are located at the true theta peaks and troughs. Red bars represent theta rise time from true-trough to true-peak. Blue bars represent theta-decay time from true peaks to true trough. B) Scatter plot of theta period vs. running speed in one data set. Each dot is one theta cycle. Black line is the average over each speed bin. Theta period does not change significantly with speed but the variability of theta period does reduce substantially. C) Ensemble average of the standard deviation of theta period as a function of speed. Shaped area indicates the standard error of the mean. There was a 39.4±1.4% reduction of theta-period variability with speed. D) Same as B but for theta amplitude. E) Same as C but for the standard deviation of theta amplitude which reduced by 25.6±1.6% with speed. F) Same as B but for theta shape. G) Same as C but for the mean value of theta shape (solid line) and its standard deviation (dashed line).
Theta shape became 23.8±0.3% more asymmetric and the standard deviation of theta-asymmetry reduced by 23.8±1.2% with speed.

theta cycle asymmetry was speed dependent (figure 3.2d, e). While theta cycles were symmetric (Theta Shape Index=−0.013±0.001) at low speeds there was a large increase in their asymmetry (Theta Shape Index=0.119±0.003) with increasing running speed. Further, the variability in theta shape was reduced by 23.8±1.2% (figure 3.2d, e) with increasing speed. Thus, the observed variability in theta period, amplitude and shape can diminish theta rhythm in the autocorrelation during immobility, despite the presence of large and significant theta modulation (TMoS).

In order to explain the reduction of TMoS at high speeds, we note that in addition to theta, hippocampal activity is also modulated by slow (20-45Hz) and fast (45-90Hz) gamma, that are phase locked to theta rhythm and whose amplitudes grow with running speed. We hypothesize that the gamma oscillations could interfere with theta rhythm, especially at high speeds, thereby reducing TMoS. To test this hypothesis we computed the modulation of the amplitude and phase of slow and fast gamma by theta phase and running speed. Consistent with recent studies, both slow and fast gamma’s envelope amplitudes grew with running speed, but only around the peak of theta (figure 3.3a, b for slow gamma in single dataset and ensemble average). This shows speed-dependent theta-gamma amplitude-phase coupling. Surprisingly, we also detected a transient, phase-phase coupling between slow-gamma and theta such that 2-3 cycles of slow-gamma oscillation occurred around the peak of theta (Figure 3.3c). This transient
Figure 3.3: Speed-dependent amplitude and phase coupling between slow gamma and theta phase. A) Amplitude of slow-gamma envelope as a function of theta-phase and running speed, i.e. the slow-gamma velo-temporal receptive field. Left and right panels show several example traces used for generating A at the speed indicated by the white boxes. B) Same as B but for slow gamma waveform, suggestive of
transient phase-phase locking. C) The standard deviation of slow gamma phase as a function of theta-phase and running speed showing significant amount of phase-phase locking only at high speeds and only near theta peak.

Phase-phase coupling between slow-gamma and theta was only present around theta peak, and only at high speeds. This was quantified using the standard deviation of slow gamma phase as a function of theta phase and running speed, which was small (15.5±0.6%) only for slow gamma, only around theta peak and only at high speeds (figure 3.3c, f). Such transient phase-phase coupling was not found between fast-gamma and theta (figure 3.10).

To determine the joint effect of theta, theta-locked gamma and running speed on TMoS, we calculated the multiunit spiking probability as a function of theta phase and running speed (figure 3.4b). At low speeds, the multiunit spiking probability was unimodal (figure 3.4c, red line), showing strong theta modulation and no sign of gamma modulation. This is consistent with no significant theta-locked slow-gamma in the LFP at low speeds (figure 3.4c, blue line).

In contrast, at high speeds the multiunit spiking probability as a function of theta phase had a multi-modal structure (figure 3.4d, red line). The multiple peaks in spike-probability within a theta cycle corresponded to a few cycles of slow-gamma, centered around theta peak, and reflected a similar structure seen in the theta-locked slow-gamma LFP (figure 3.4d, blue line). This slow-gamma induced multimodal nature of spiking interfered with theta modulation and thus reduced TMoS with increasing running speed (figure 3.4j), whereas the slow-gamma phase modulation of spikes increased with running
Figure 3.4: Multi-modal theta phase modulation on spike timing. A) VTRF on slow gamma waveform in one data set. Two sections of the data at low speed (blue 1) and high speed (blue 2) as indicated by the white boxes will be used to calculated traces in C-D. B) VTRF on multiunit firing rate in the same data set. Two sections of the data (red 1 and 2) in the same speed bins as in A will also be used in C-D. C) Averaged slow gamma waveform (blue line 1) and averaged multiunit firing rate (red line 1) as a function of theta phase at low speed. D) Same as C but at high speed, notice the similarity between the two traces.
E) Ensemble average on the vector based MI at theta and slow gamma band as a function of speed. F) Joint effect of theta and slow gamma on spike timing measured by the entropy based method. J) Schematic plot shows the transition from theta to slow gamma dominated spike timing control with increasing speed. The thickness of the arrows indicates the strength of the modulation.

speed (figure 3.4j). To estimate this joint influence of theta and gamma on precise spike timing, we computed the entropy of spike timing (see methods) as a function of theta phase. Greater the entropy, more precise is spike-timing, irrespective of whether it occurred due to theta or gamma. This showed that spike timing was indeed about twice more precise(183.5±32.5%) at high speeds than at low speeds, due to the elevated but transient slow gamma-theta phase-phase coupling (figure 3.4k).

Thus, at low speeds including immobility, theta amplitude is small but gamma amplitude is even smaller, hence theta exerts a greater control over spiking (figure 3.4e, f). On the other hand, theta rhythm is more variable at low speeds resulting in poor theta rhythmicity. With increasing running speeds theta amplitude grows to some extent but theta-peak locked gamma amplitude increases even more 21, which takes over the control of spiking resulting in reduced theta modulation of spikes but increased precision of spike-timing on sub-theta scale (figure 3.4e).

3.4 Discussion
These results have several significant implications. They demonstrate that significant theta modulation of hippocampal spikes is present at all times, even during periods of immobility. Thus, theta modulation is a persistent hippocampal state, present throughout a task session. Therefore, the well-established role of theta burst spiking in inducing long-term plasticity\textsuperscript{112–115} could also contribute to learning during the vast periods of immobility in a navigational task. In fact, we find that theta modulation of spikes is even greater during immobility than during running at high speeds.

These findings contradict commonly held belief that theta modulation is weak or absent during immobility and strong during locomotion. This belief is based on the autocorrelation (or the power spectrum, which is just a Fourier transform of the autocorrelation). We show that the autocorrelation is influenced not only by theta modulation at a given time but the variability of theta waveform across time. Even though large and significant theta modulation of spikes occurs during immobility, large variability of theta waveform across time masks this modulation. However, most theories of neural information processing rely on synchronous activation of neurons at a given time and do not depend on the degree of rhythmicity across long periods of time, thus theta modulation of spikes based inferences are appropriate. Further studies are needed to understand the mechanisms responsible for speed-dependent reduction in the variability of theta waveform, and their role in neural information processing\textsuperscript{122}.

Differentiation of theta modulation of spikes from the autocorrelation has important implications beyond our findings. For example, recent findings\textsuperscript{61} and the ensuing debate about theta modulation in bats, was entirely based on autocorrelation analysis, showing no significant theta rhythmicity. This suggests, paradoxically, that theta-burst based mechanisms of plasticity
may not be relevant for learning in bats. This debate and paradox could be removed if significant theta modulation of spikes was found in those data, which remains to be tested.

We further found that at high speeds, when the amplitude of the LFP theta rhythm is larger than that during immobility, theta modulation of spikes is paradoxically reduced. We show that this occurs because of the appearance of a few cycles of theta-locked gamma oscillations which takes over the control of spiking. As a result, while theta modulation of spikes reduces, the temporal information or precision of spikes is increased, narrowed down to the finer temporal scale of slow-gamma compared to theta. This increased temporal precision of spikes may facilitate learning of temporal sequences\textsuperscript{54} by the mechanisms of spike timing-dependent plasticity\textsuperscript{113–115}. The speed-dependent increase in the asymmetry of theta cycles could also facilitate this process\textsuperscript{58,94}.

3.5 Methods and supplements

3.5.1 Experimental Methods

Same as in 2.5.1

3.5.2 Data analysis

\textbf{Redefine theta cycle and quantify theta shape}: The peaks and troughs from 4-12Hz filtered LFP was first located using Hilbert transform. The nearest maximum (minimum) on raw LFP to each previously located peak (trough) was then defined as the true peak (trough) for
theta cycle was redefined between two neighboring troughs. The shape was defined as the difference from the center of the cycle to the true peaks divided by half of the cycle length. This definition yielded a shape index ranging from -0.5 to 0.5 which has the same polarity if the shape was measured in terms of skewness.

**VTRF on gamma amplitude envelope, gamma waveform, gamma phase and multi-unit firing rate:** We used the similar method described in the previous paper to calculate the VTRF for various time series including gamma amplitude envelope (figure 3a), gamma waveform (figure 3.3c) and gamma phase (figure 3.3e). Gamma waveform was filtered in slow (20-45Hz) or fast gamma (45-120Hz) band. Gamma amplitude envelope and phase is the absolute value and angle of the Hilbert transform of gamma waveform. The VTRF on multi-unit firing rate (figure 3.4b) was calculated by dividing the number of multiunit spikes in each speed and theta phase bin by the total time that animal spent in the same bin. 30 non-overlapping phase bins and 30 speed bins with 80% overlapping window were used in the above calculation. The sample points with the top 1% speed were discarded in the calculation to reduce motion artifact.

**The measurement on LFP phase modulation of spiking probability:** The LFP phase modulation of a given spike train can be measured by quantifying its phase distribution. Two methods are typically used as described below. TMoS in the main text refers to MIvec in theta band.

\[
MI_{vec} = \text{abs}(\sum_{j=1}^{N} p_j e^{i\theta_j})
\]

\[
H = -\sum_{j=1}^{N} p_j \log(p_j)
\]
\[
MI_{ent} = \frac{\log(N) - H}{\log(N)}
\]

Where \( N \) is the number of bins, \( p_j \) is the probability of having spikes in phase bin \( j \) which is centered at \( \theta_j \) and \( H \) is the Shannon entropy\(^{111}\). While the two methods were qualitatively the same in the single modal distribution, the entropy based method was more sensitive and accurate in the case of multi-modal distribution.

**Significance test of phase locking:** The significance of phase locking in real data set is determined by comparing it with the degree of phase locking calculated from randomly generated (uniform distribution) spike phases (surrogated data set) which has the same number of spikes as in the real data set. This is necessary since the number of spikes affects the degree of phase locking. In order to do this, we first estimated the mean and standard deviation of \( MI_{vec} \) in surrogated data set at a given spike count. The result was averaged over at least 500 trials. This process was repeated at a variety of spike numbers from \( 10^2 \) to \( 10^5 \) (figure 3.5). The significance level of phase locking of the real data set is then simply estimated by the likelihood that the \( MI_{vec} \) in the real data can purely arises from surrogated data set.

\[
Sig = \frac{MI_{real} - MI_{sur}}{std(MI_{sur})}
\]

3.5.3 Supplement figures
Figure 3.5: Mean and standard deviation of TMoS in surrogated data sets as a function of number of spikes.

Figure 3.6: TMoS and its significance level of pyramidal neurons and interneurons at low speed and high speed. A) Histogram of TMoS of pyramidal neurons during immobility (blue line) and
during locomotion (red line). B) Significance of TMoS of pyramidal neurons during immobility (blue line) and during locomotion (red line). C) Histogram of TMoS of interneurons during immobility (blue line) and during locomotion (red line). D) Significance of TMoS of interneurons during immobility (blue line) and during locomotion (red line).

Figure 3.7: Spike train autocorrelation of pyramidal neuron (PN), interneuron (IN) and multi-unit (MUA) and also LFP autocorrelation during immobility (blue lines) and during locomotion (red lines). The panels in the first row are from single session and the panels from the second row are ensemble average.
Figure 3.8: TMoS of MUA calculated by using broadly (dashed lines) and narrowly (solid lines) filtered theta during immobility (blue lines) and during locomotion (red lines). TMoS measures the degree of covariance between spiking probability and theta phase regardless of the regularity of theta cycles. In order test it, we filtered theta in a very narrow band (band width =1Hz) so that its cycles are forced to become regular. Since theta cycles are highly irregular during immobility and low speed and become more and more regular with increasing speed, the difference between TMoS calculated using narrowly filtered theta (TMoS narrow) and the TMoS calculated using broadly filtered theta (TMoS broad, this is how TMoS is normally calculated) should be much bigger during immobility than during locomotion, especially at high speed. To be more specific, TMoS narrow should be smaller than TMoS broad during immobility while both TMoSs should remain the same at high speed. A) Single session result. TMoS broad (dashed blue line) is much larger than TMoS narrow (solid blue line) during immobility across the entire theta band. While TMoS broad (dashed red line) is roughly the same as the peak value of TMoS narrow (solid red line) during locomotion. B) Same as A, but is averaged over the entire 154 data sets. C) Scatter plot of the difference of TMoS narrow and TMoS broad during immobility (x axis) and locomotion (y axis). Most of the differences at low speed are negative while distributed around zero at high speed.
Figure 3.9: Simulation on how the regularity among theta cycles influences its autocorrelation. The variations are applied to period, amplitude, and shape. A) Same variation in amplitude and shape while the variation in period doubles. B) Same as in A, but for amplitude C) Same as in A, but for shape.

Figure 3.10: Same as in figure 3.3 but for fast gamma. There is no phase-phase coupling between fast gamma and theta. Raw LFP traces are not shown in this figure.
CHAPTER IV

SPEED DEPENDENT SYNCHRONY OF THETA OSCILLATORS AND THETA TRIGGERED TRANSIENT THETA-GAMMA PHASE-PHASE COUPLING

4.1 Overview

The findings described in the previous two chapters showed that the running speed can profoundly influence the amplitude of theta and gamma rhythms as well as their interaction. For example, in chapter 2, I showed that the amplitude of slow gamma (20-45Hz) is linearly correlated with the running speed while its preferred theta phase becomes progressively earlier. In chapter 3, the shape of theta waveform and its variability is strongly dependent on the running speed such that the average theta shape becomes more asymmetric and more regular at high speed. The question is what the roles that speed plays in these novel findings. Are they independent events or is there a unified theory that could explain all of them? Two models will be presented and discussed in this chapter which may shed some light on this question.

4.2 Speed dependent synchrony of theta oscillators

In the classic theta generator model, hippocampal theta is entrained by medial septum. Medial septum neurons fire at theta frequency and send long range cholinergic and GABAergic projections onto the hippocampal interneurons\(^{36,43,46,48}\). It is believed that these coherent septum inputs generate the hippocampal theta rhythm. However, no work has been done to study the dynamics of the synchrony among these theta sources associated with animal’s behavior. It is
possible that the degree of synchrony among these theta sources is behavior dependent. Since the level of synchrony would greatly affect the sum of the input, it therefore can be used to explain the speed dependence of the shape of the theta waveform.

In order to test this, I used an asymmetric triangular wave at theta frequency (8Hz) to simulate the single medial septum input. The asymmetric triangular wave has a fast rise time and slow decay time in order to match the general shape of EPSP/IPSP. Ten traces have been used as the contributing theta sources whose sum creates hippocampal theta. For simplicity, the ten traces have identical period, amplitude and shape which also does not vary among cycles. A spread of the onset time was created to represent the imperfect synchrony among these theta sources. The level of the synchrony can therefore be controlled by changing the range of the spread.

Figure 4.1 shows two cases of synchrony. The top panel shows the case when the theta oscillators have a low degree of synchrony among them while in the bottom panel they are highly synchronized. The averaged waveform is used to show the ensemble effect. In the case of weak synchrony, the averaged waveform has a very sinusoidal like shape. The reason for is because the peaks of these theta oscillators arrive within a relatively large window and spread out. The higher order harmonics which contribute to the asymmetry of the waveform will have a larger phase spread than the first order harmonic and therefore get averaged out. When the synchrony is strong, the spread becomes narrower. The higher order harmonics will be less attenuated and maintain its asymmetry in the averaged waveform.
Figure 4.1: Synchrony among theta inputs affects the shape of their ensemble average (simulation). Top panel shows a case with weak synchrony while the bottom panel shows a case with strong synchrony. Colored lines represent 10 different theta inputs from medial septum. The black line is the ensemble average.

If we combine this result with the experimental observation, one can make a hypothesis that increasing running speed will improve the synchrony among different theta inputs from medial septum. This should address the part that the average theta shape becomes more asymmetric with increasing speed. But how the theta shape also becomes more regular? In order to explain this, I calculated the shape of the averaged theta waveform as a function of the width of the spread (figure 4.2a). Since there are only ten sample points within a given spread, they will have a slightly different distribution for each simulation and therefore yield a different averaged theta shape. This is particular true when the spread is large and is less significant as the spread becomes narrower. The fact that figure 4.2a resembles figure 3.2f indicates that the underlying mechanism for this speed
Figure 4.2: Simulation results on the A) Theta shape index as a function of the time jitter (reflect the degree of synchrony) among different theta oscillators. B) Same as A, but for theta amplitude.

dependence on the theta shape is due to the improved synchrony among different theta inputs from medial septum with increasing speed.

This hypothesis predicts that the theta amplitude should also increase as a function of speed while its standard deviation reduces at the same time (figure 3.2d and 3.2e). This is confirmed by the simulation result (figure 4.2b). Thus, the hypothesis that the running speed improves the synchrony of external theta inputs provides a very straight forward way to explain the speed dependence on the LFP theta shape.

4.3 Theta triggered theta-gamma coupling
Theta-gamma coupling is the most frequently studied form of cross frequency coupling. It is suggested to play an important role in many cognitive processes. However, its mechanism is still not clearly understood. In this section I will present a model that could explain most of the theta-slow gamma coupling results that I covered previously in chapter 2 and chapter 3.

There are two ways that theta and gamma rhythms can couple with each other. The first one is that the phase of theta modulates the amplitude of gamma. It is observed by many studies\textsuperscript{19,24,25,73–75,93}. The second one is that the phase of theta and the phase of gamma follow a 1: \textit{m} relation such that every theta cycle contains \textit{m} gamma cycles. A recent study claims that they found phase-phase coupling between theta and gamma rhythms\textsuperscript{22}. However, 1: \textit{m} phase relationship requires that there should always be \textit{m} cycles of the fast oscillation ride in a single cycle of the slow oscillation regardless of the variation of the cycle length. One prediction of this relationship is that the two should linearly correlate with each other with a slope close to \textit{m}. This is not the case in their paper and their slope is 10 times smaller than the theoretical value\textsuperscript{22}. Their paper has another weak point in the way they calculated the theta phase. Like what I did in chapter 3, they quantified the shape of theta waveform by finding the true theta peak and trough. However, they went further to redefine the theta phase using these true peaks and troughs such that theta phase is always 0° or 360° at the trough and 180° at the peak. This redefinition of theta phase suffers greatly from artifact since the local LFP maximum (true theta peak) will be very likely also the peak of a gamma cycle while the local LFP minimum (true theta trough) will most of time be also a gamma cycle trough. This redefinition of theta phase will artificially boost the 1: \textit{m} phase correlation between theta and gamma and therefore lead to incorrect conclusions.
Another way to think of this 1: m phase relationship is that it requires that each gamma cycle appears at exactly the same theta phase. In practice, this will lead to the prediction that the standard deviation of gamma phase at a given theta phase should be close to a constant and does not change with theta phase. We showed that this is not the case in chapter 3 figure 4.3c. While the phase of fast gamma is not related with theta phase, slow gamma phase shows a strong correlation with theta phase only at high speed and only around the theta peak. This observation that the phase-phase relation between theta and slow gamma only at certain theta phase implies that theta phase may not necessarily influence slow gamma phase throughout the entire theta cycle but rather trigger slow gamma at certain theta phase, most likely around the theta peak. This mechanism could explain the result in figure 4.3c that the standard deviation of slow gamma phase is minimal around the theta peak and increase at both earlier and later theta phase.

In order to simulate this process, I first use Wilson-Cowan model to simulate slow gamma oscillation. The equations in time derivative form are as follows:

\[
\tau_e \frac{dE}{dt} = -E + \Phi(g_{ee}E + g_{ei}I + S_e(t) + a_e) + N_e(t)
\]

\[
\tau_i \frac{dI}{dt} = -I + \Phi(g_{ie}E + g_{ii}I + S_i(t) + a_i) + N_i(t)
\]

where E and I represent the overall activities of excitatory and inhibitory populations, g is the synaptic matrix of the E-I network. S_e and S_i are the simulations applied to the two populations:
Figure 4.3: Wilson-Cowan model of hippocampal gamma oscillation.

\[
\begin{align*}
S_e(t) &= S_i(t) = 0 & t < t_{\text{stim}} \\
S_e(t) &= S_0 \cos(\theta)e^{-(t-t_{\text{stim}})/\tau_{\text{stim}}} & t > t_{\text{stim}} \\
S_i(t) &= S_0 \sin(\theta)e^{-(t-t_{\text{stim}})/\tau_{\text{stim}}} & t > t_{\text{stim}}
\end{align*}
\]

\(\Phi\) is the sigmoidal activation function which has the form:
\[ \Phi(a) = \frac{1}{1 + e^{-a}} \]

And $N_c$ and $N_i$ are the Gaussian noise. The parameters are chosen based on a previous work with the aim to match the slow gamma oscillation in the real data set as much as possible with an average frequency around $\sim$35Hz. The result is shown in figure 4.3.

Figure 4.4: Theta entrained synchrony among slow gamma oscillators
Following the multiple theta source idea in the previous section, local field gamma oscillation could also be the ensemble result of several gamma sources around the electrode. Therefore, I simulated 10 slow gamma sources using the parameters from the above example but with slightly different synaptic matrix for each source so that they are not fully synchronized. Gaussian noise was also added in the simulation. Another important step is to incorporate theta-gamma coupling. From the classic theta generator model, we know that hippocampal interneurons are entrained by medial septum at theta frequency. Therefore, we can add an external theta frequency stimulation on the inhibitory group activity. This strong inhibition will greatly perturb the phase of all the gamma oscillators and reset their phase to a fix value right after the turn on of each pulse (figure 4.4). Their synchrony will quickly relax after this strong reset during the rest of the theta cycle and get reset again in the next one.

This theta entrained synchrony among multiple slow gamma sources gives arise to the theta-gamma phase-amplitude coupling (figure 4.5a) as well as the theta-gamma transient phase-phase coupling (figure 4.5b) observed in chapter 3.
Figure 4.5: Data used to calculate this figure is from the part of the data used in figure 4.4 when theta is turned on. 0° degree theta phase was sign to each onset of theta stimulation and theta phase was then interpolated between the two onset times. Slow gamma trace was E(t) filter between 20-45Hz. This makes sense since LFP is mainly contributed by the synaptic activities from excitatory neurons.

4.4 Speed dependent slow gamma amplitude

From the above two models, my hypothesis is that the speed controls the synchrony of theta oscillators and therefore modulates the amplitude and shape of theta. On the other hand, theta entrains the synchrony of slow gamma oscillators and lead to the phase-amplitude and transient phase-phase coupling. However, there is one more major result that is not yet explained under this framework. That is the linear relationship between speed and slow gamma amplitude. I will show that it can be easily solved to combine the two models together.

In the previous section, we assume that there is one theta source that stimulates all the slow gamma oscillators. This is not necessarily the case because we already propose in chapter 4.2 that there might be multiple theta sources whose synchrony is controlled by the running speed. Therefore, I used 10 identical theta inputs other than one as the external input to the 10 slow gamma oscillators on a one by one basis. The synchrony of these theta inputs varies slowly following a sinusoidal shaped curve which has a period of 20s and ranges from 5s to 50s. The results show that the synchrony of theta inputs can greatly influence the ensemble slow gamma power. Therefore, if speed really controls this theta synchrony such that when the running speed becomes higher the synchrony is also better, the positive correlation between running speed and slow gamma amplitude can be explained by combining these two models.
Figure 4.6: Simulation on each slow gamma oscillator is entrained by a different theta input. A) The slow varying profile of theta synchrony matches the speed profile of the mice in a linear track task from the real data. B) The averaged slow gamma amplitude as a result of this changing theta synchrony.

This combined model predicts a novel way that the running speed of the animal modulates the hippocampal neuronal activities. The running speed improves the synchrony of medial septum neurons which provide the theta clock to the hippocampus. As the speed increases, the hippocampal theta becomes stronger, more asymmetric. Theta triggered slow gamma oscillation also become stronger and strongly couples with theta under both phase-amplitude and transient phase-phase coupling regime. Theta controlled spike timing therefore gradually switch to slow gamma controlled spike timing at high speed. The narrower window for grouping spikes may facilitate the induction of LFP through STDP which will lead to improved learning. Further
experiments, especially the dual recording between medial septum and hippocampus needs to be done to test this hypothesis. Large array electrode recording will also help to confirm this improved theta synchrony with increasing speed.
REFERENCE


