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Hippocampal information processing and transmission via slow wave synchrony

THESIS

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MASTER OF SCIENCE

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by

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Thesis Committee:
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DEDICATION

To my family and friends for their unconditional support,

To Dr. Gregory Brewer for his guidance that has taught me to research and write independently with confidence,

To Assistant Professor R. Nithya for her unconditional support and encouragement in helping me pursue my interests,

To the late Manoj Niranjan for helping me find my interests in Bio-medical Signal and Image processing.

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ABSTRACT OF THE THESIS

Hippocampal Information processing and transmission via slow wave synchrony

By

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The hippocampus has long been known to play a central role in various behavioral and cognitive functions. Despite extensive behavioral, anatomical studies postulating roles of the hippocampus and its sub-regions, the underlying dynamics of the neural circuits responsible for region-specific roles in the hippocampus remains poorly understood. In addition, the communication between the hippocampal sub-regions also plays a major role in learning and memory but has not been explored as much due to the lack of access to the individual axons. This paper seeks to understand the role of slow waves (4-100 Hz) known as Local Field Potentials (LFP) in the hippocampal sub-regions, their dynamics and role in information transmission to bind cell assemblies through synchrony by timed-bursts and individual spikes, by space (EC, DG, CA3, CA1) in the hippocampal sub-regions and by frequency (4-11 Hz theta and 30-100 Hz gamma bands). We used a four-chambered polydimethysiloxane (PDMS) micro-tunnel device over a multi-electrode array (MEA) with the engineered living reconstruction of the hippocampal sub-regions. The 5 x 10 μm micro-tunnels between each chamber allow axonal growth and inhibit the migration of 15 μm diameter cell bodies or traversal by dendrites providing insight into the role of axons in inter-regional

communication. A significant advantage of our system is the ability to separate activity in the axons in the tunnels from the computations of the neural cells in the wells of the 4-chambered device. Functional connectivity within hippocampal sub-regions and the axonal tunnels helped in ascertaining the role of LFP in information processing and transmission. A computationally simple and linear method, Cross-Correlation of the theta power and the cross correlation of the gamma power was used to estimate synchrony of LFP. Comparison between the theta-theta and gamma-gamma LPF's from the different sub-regions indicates that the CA3 has the strongest correlation. CA3 sub-region is characterized by recurrent network wiring pattern and such strong correlation can be associated with this kind of network pattern. The spatial distribution of the theta and gamma correlation between electrodes was estimated by computing the correlation as a function of the inter-electrode distance within each sub-region. A common relationship of LFP power with the inverse square of distance during bursting events suggested LFP transmission through synapses of branching dendritic trees in the plane of the network. These first measures of LFP's in axons suggests axonal transmission of slow waves, independent of spikes and synapses. Understanding these network properties within the hippocampus could provide important information for steps in memory formation, neuromorphic computing and artificial intelligence systems in addition to better detection of hippocampal diseases such as epilepsy and Alzheimer's disease.

Chapter 1

INTRODUCTION

This chapter mainly focusses on the basics to understand the work done in this project beginning by understanding the hippocampal structure and its role in cognition and memory (section 1.1). The underlying neural dynamics that supports behavior of the subregions can be interpreted by understanding the Local field potential oscillations in each subregion of the hippocampus (section 1.2). Statistical dependence LFP between sub-regions and tunnels can be addressed by comprehending connectivity and its measures (section 1.3). Here, we propose that LFP synchrony indicates a potential interaction between the recorded sites and a possible mechanism to facilitate synaptic transmission and network-to network communication. We wanted to draw a comparison of the LFP synchrony in the different sub-regions of the hippocampus and how well each sub-region was correlated to the axonal information in the tunnels. This would allow us to more accurately determine the relationships of network communication to LFP synchrony in inter-network communication, which is achieved by axon isolation in the tunnels. To provide more information on the spatial dependence of LFP synchrony, a distance cross-correlation based approach was used to determine the relationship between the correlation of the LFP oscillations with increase in inter-electrode distance. In this paper, we report electrophysiological, exploratory data analysis for LFP synchrony and visual evidence to show the association of the LFP oscillations to bursts of spikes in the hippocampal sub-regions and the axonal tunnels thereby suggesting a possible role of slow waves in binding of cell assemblies for information processing and transmission.

1.1 Hippocampus and its role, an overview

Located deep within the medial temporal lobe of the brain is a sea horse shaped structure namely the hippocampus. The hippocampus has been a leading area of research among the researchers since the 1957 case of H.M. who lost his memory after surgical removal of the hippocampus. Anatomical studies clearly delineate at least 3 subregions of the hippocampus that receive inputs from the entorhinal cortex (EC): Dentate Gyrus (DG), CA3 and CA1[1]. The Hippocampal region which includes the CA and DG sub-regions along with adjacent EC is essential for declarative memory[2] as illustrated in Fig 1.1(a). This system of the Hippocampus and EC are principally concerned with memory and its consolidation, in addition to operating maintaining and establishing long term memories. The hippocampus may have a special role in tasks that depend on relating or combining information from multiple sources, such as tasks that ask about specific events (episodic memory) or associative memory tasks that require different elements to be remembered as a pair (e.g., a name and a face) or what in the context of when and where[2].Behavioral studies attempt to decipher the various functions of the hippocampus as a whole. A number of studies including functional imaging and studies on animals characterizing the information encoded by single neurons have revealed the roles of hippocampus and para-hippocampal regions in memory[3], [4].

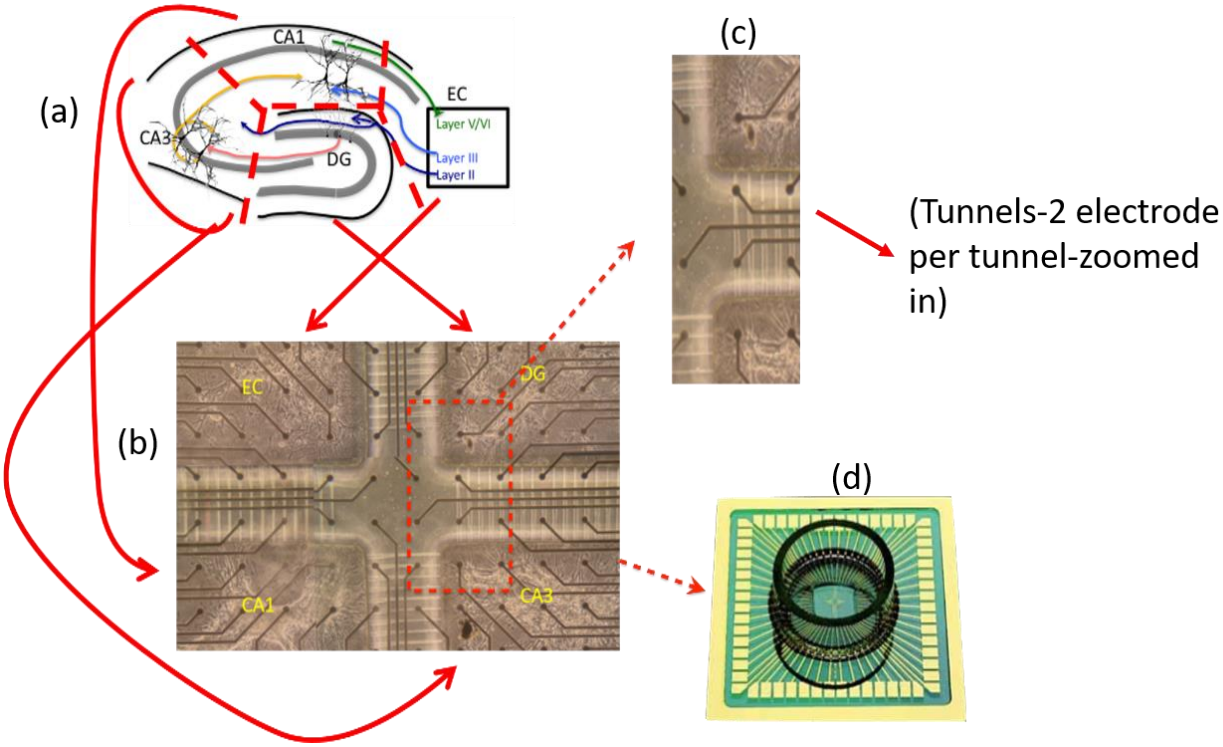

Figure 1.1: Reverse engineered sub-regions of the rat hippocampal network reconstructed in a four chambered device with axonal connectivity via micro-tunnels over a micro-electrode array (200 µm electrode spacing). (a) Entorhinal cortex (EC) and Hippocampal subregions (DG, CA3, CA1) that were co cultured on the 4 chambered PDMS device ; (b) Layout (Zoom in) of the four chambered reconstruction of the hippocampal sub-regions on a Multi-electrode array (MEA) showing micro-tunnel connectivity between the different sub-regions;(c) Micro-Tunnels between the sub-regions highlighted-there are 2 electrodes per tunnel; (d)The 4 chambered PDMS device on multielectrode array to measure the properties of the network.

Do all the sub-regions of the hippocampus process the same kind of information? How are the structural differences between the subregions associated with the tasks performed by them? In order to be able to answer such questions electrophysiological, functional imaging studies are carried out. Originally many memory tests were performed to assess the hippocampal function of the brain in contrast to tests that were sensitive to the other brain regions, however now these studies have differentiated the roles played by each individual sub-region. Studies have shown that the hippocampal regions differentially associate with standardized memory tests[5].Recent findings supports the idea that each individual subregion is associated with distinct cognitive and computational behavior[6]–[8].The roles of the different regions range from pattern separation, pattern completion to memory recall, sparse coding and information storage. Episodic memories are the memory of experience or events that took place at time, location with other information like associated emotions and circumstantial knowledge. An important property of such episodic memory is the retrieval of information. The ability to recall a whole memory from a partial cue is an important property of episodic memory and is referred to as completion[9]. Proposed roles of CA3 include the formation and storage of these episodic memory as well as their recall in pattern completion. The auto-association nature of the CA3 networks enables the retrieval of the whole information in response to a small cue[6], [9]. CA1 is considered as the end sub-region of the hippocampus, most of the output to the neocortex is from CA1, thus it can also be called the output sub-region of the hippocampus. In addition, there are a number of roles associated with the CA1 region of the hippocampus namely parallel processing of information, novelty detection, enhancement and distribution of information to the other regions of the cortex, retrieval of remote episodic memory and are also considered important for autonoetic(self-knowing)- which gives rise to remembering in the sense of self-recollection of previous events at which on was present[10]–

[14].DG being the first site to receive information form the EC can be considered to a play a major role in production of episodic memories[15]. Another most important property of episodic memories is its ability of pattern separation which allows for discrimination between similar inputs thereby enabling to avoid overwriting information similar to existing information and preventing catastrophic interference[16]. Electrophysiological studies have shown the role of DG in pattern separation[8].In spite of such extensive behavioral, anatomical studies postulating the roles of hippocampus and its sub-regions, the underlying dynamics of the neural circuits responsible for these roles of the different regions and the hippocampus remains poorly understood. Understanding these algorithms could provide important information for neuromorphic computing and artificial intelligence systems in addition to better detection of hippocampal diseases such as epilepsy and Alzheimer's disease, for example.

1.2 Network patterns and underlying dynamics

Neural oscillations are pervasive across several regions of the cortex and the hippocampus. A clear understanding of the oscillations is thus important for the understanding of the complex behaviors and functions of the different sub-regions of the hippocampus as discussed in (section 1.1). The oscillations are postulated to regulate the encoding and retrieval of information in the hippocampus. The neural oscillations within the hippocampus can either be the information itself or assist in the flow of spiking information within the neural circuits of the hippocampus, or an artifact of the spiking[17]. Cognitive functions requires dynamic coordination of different groups of the neurons[18]. Synchronization of the neural oscillations-a well-known mechanism play a major role in the aforementioned function of dynamic coordination. The hippocampal local field potential (LFP) exhibits three major types of rhythms, theta (~4-11Hz), sharp wave-ripples

(~110-250 Hz ripples superimposed on ~0.01-3 Hz sharp waves) and gamma(~25-100Hz)[19]. These rhythms defined by their frequencies, have behavioral correlates in several species including rats and humans, and have been proposed to perform distinct functions in hippocampal memory processing. We know limited about the origins of these oscillations, the mechanism and the role of these oscillations in connection with the overall behavior of the different sub-regions of the hippocampus.

Gamma Oscillations

The high frequency of the gamma oscillations (30-300 Hz) supports its function during behaviors which require high level of coordination on a time scale beyond perception. Such fast coordination is required for various activities such a selection of inputs, grouping of neurons and retrieving memories[18], [20]. Gamma oscillations occur in bursts at particular times within the theta cycle[21], [22] and have been proposed to select particular cell assemblies for processing at those times.[18], [23]. Current source density analyses have supported the evidence of the presence of two independent hippocampal gamma generators in EC and CA3. Experiments on guinea-pigs conducted by S.Charpak et al. [24] also supports the presence of individual gamma generators in the entorhinal cortex. In addition, studies on animals with lesion in EC have shown the more apparent presence of slower gamma oscillations (30-100 Hz) suggesting multiple origins of gamma oscillations. Gamma generators were independent of each other but also coupled at certain times suggested by recent study[25]. Routing of information was thus a hypothesized function of the gamma oscillations, it provides the temporal segregation of information from different sources[25]. Pascal Fries[27] suggested the hypothesis that synchronized gamma oscillations facilitate information transmission between the sending and the receiving neurons.

Colgin et al. [18] were able to illustrate the presence of distinct fast (~ 65-140 Hz) and slow (~ 25-50Hz) gamma oscillations in the CA1 region and how the 2 components of gamma oscillations differentially coupled to inputs from CA3 and EC. In addition to information transmission and routing, memory encoding is also an important function of the gamma oscillations. Intracranial studies on epilepsy patients demonstrated that the gamma oscillations were able to predict encoding of the new verbal memories[26]. Another study conducted in the macaque hippocampal formation suggested an increase in neural synchronization in the gamma band reflecting enhanced coordination among the neurons, facilitating memory encoding[27]. Memory retrieval typically involving the CA3 and CA1 regions of the hippocampus. Gamma oscillations proposed to serve as the underlying physiological mechanism in retrieval of information [28]. The increase in gamma power and coherence at the CA3-CA1 interface exhibit dynamic coupling of the hippocampal networks depending on the behavioral demands. Thus, these studies discussed, demonstrate a range of critical functions of the gamma oscillations, however the exact source of these coupling and synchronization functions is unknown. A two-dimensional neuronal network may reduce complexity to be better able to decipher specific roles for gamma oscillations in synchronizing spiking for stronger inputs or directing those inputs to specific subregions.

Theta Oscillations

Theta oscillations are most regular in hippocampal CA1 regions, but also found in DG, CA3, Entorhinal cortex(EC) and several other cortical structures[28], [29].These structures are thus considered the main current generators of theta rhythm[30].Theta waves in all cortical targets were abolished on inactivation of medial septum-diagonal band of Broca (MS-DBB) has suggested MS-DBB to be the an ultimate rhythm generator of theta oscillations[31]. However the exact

mechanism of these oscillatory patterns remains poorly understood. The Hippocampal theta rhythm is associated with numerous functions ranging from memory and spatial behavior, rapid eye movement sleep, anxiety[32]. EEG data analyzed showed changes in theta power band supporting the hypothesis that the episodic memory was indeed primarily reflected in the theta band[33]. Animals studies also showed strong evidence supporting presence of theta oscillations in hippocampus[34]. Hippocampal theta rhythm correlates with learning and memory functions in rats[35]. Notably, theta oscillations play a major role in hippocampal information processing in both humans and animals including timing of neuronal spiking thereby suggesting an association or intervention of LFP in causing spike occurrence and other neuronal computations [36]. Stark et al.,[37] showed Firing rates and rate modulations of individual neurons, and multineuronal sequences of pyramidal cell and interneuron spiking, were correlated during theta oscillations, spontaneous ripples, and synthetic ripples. However there exists disconnect between research in the rodents versus other species where there is no evidence of prominent theta oscillations[38]. Theta oscillations also play a major in sensory input processing[34]. Several investigations also linked hippocampal theta oscillations to spatial navigation which is also considered as type of sensory information processing[35], [39]. Stronger than these associations, Caplan et al [40] correlated theta amplitude to the level of difficulty of a task. Thus these signals are of interest due to its relation to various aspects of cognition and behavior. Due to the existence of diverse range of functions of theta oscillations, it is important to understand role of LFPs to understand the functional and behavioral computations of the hippocampus as whole and its sub-regions. An important aspect is to understand the role of theta oscillations in inter-regional communication and how it establishes connections across the different neuronal networks. Future studies will also focus on revealing association of LFP rhythm and the output of active single cells-action potentials.

Future studies employing hippocampal-dependent tests and observing the flow of information will be essential for dissociating the contributions of gamma/theta oscillations to various types of memory processing. Results of future studies are likely to impact theories of memory operations such encoding, retrieval and storage in the hippocampus and may lead to novel and precise development of therapies to diseases due to aberrant development of connections between the networks of neurons.

1.3 Connectivity and its measures

Brain, a complex organ is made of numerous functional units that connect and communicate with each other. These units make anatomical, functional or effective connections with the other units within the nervous system. The units can be individual neurons, populations or different brain regions. Understanding the connectivity thus helps to understand the role of individual units in physiology, cognition, pathology, behavior and various other conditions. What are the different levels and modes of brain connectivity? What are the different methods in existence to analyze this connectivity?

1.3.1 Types of connectivity:

Neural connectivity gives us the knowledge of how the neuronal units of the brain are connected and understanding the neural connectivity also helps determine the functional properties of the neurons and the connections they form within the brain. The different levels of connectivity and the measurement scales are discussed in section 3.2. This section focuses on the fundamental distinction between connectivity and the different measures/metrics to quantify connectivity

1. Anatomical Connectivity: It refers to physical or structural connections between the neurons and dependent on the morphology of the neuronal elements.
2. Functional Connectivity: It describes the patterns of statistical dependence among neuronal elements.
3. Effective Connectivity: It refers to a combination of anatomical and functional connectivity. Combined understanding of the physical connection and the level of connection between the neuronal networks is important to understand the overall effective relationship between them.

Brain connectivity can be studied and analyzed using a broad range of network analysis. The functional and effective connectivity estimation can be achieved through a number of methods including pattern recognition[41], [42], entropy estimation, model fitting[43], [44] and data mining[45]. Structural connectivity on the other hand are more likely to be clearly elucidated using invasive tracing studies. Functional connectivity being a more statistical concept unlike the other 2 types of connectivity, explains the type of interactions between any 2 or more types of neuronal elements or population at any given time thereby explaining role of the neuronal elements in computational and behavioral interactions between the different networks of the brain. Understanding the role of neural oscillations and their interaction between the population of neurons will help determine the functional role of neurons and networks in information processing and communication between the different regions of the brain. Several methods, such as coherency [46], mutual information[47], partial-directed coherence[48] and phase synchronization [49], [50] have been used to measure the interactions among different areas of the brain to establish a network.

1.3.2 Measures of connectivity

Functional connectivity can be analyzed by linear statistical analysis using Pearson's correlation coefficient and cross-correlation analysis**.**

Cross-Correlation is a relatively simple and useful technique to measure the statistical dependence between two time series. It not only provides measures of similarity but also the time delay in similarity between the two signals. The time delay provides information on the lag between the two signals when there is maximum similarity. Cross Correlation as a function of delay τ can be found from equation 1, where f and g represent the time series signals.

$$\rho_{fg}(\tau) = \int_{-\infty}^{\infty} f(t)g(t+\tau) \quad (1)$$

In MATLAB, the cross correlation between two time series sequences can be obtained using the in-built function 'XCORR'

$$[r,lags]=xcorr(x,y,maxlag, scaleopt) \quad (2)$$

The xcorr function from equation 2 returns the cross correlation (r) of the time series signals x, y and also the lag (lags) at which the cross-correlations are computed. Maximum lag(maxlag), specified as an integer scalar to set the range over which delays are determined from -maxlag to maxlag. If you do not specify maxlag, the lag range equals 2N – 1, where N is the greater of the lengths of x and y. Finally, setting the scaleopt parameter to 'coeff' in the normalize property returns the correlation coefficient values(r) at each lag. Cross-correlation can help in identifying the directionality of functional connectivity and estimating lags between sites. The directionality of information transmission along with the lag between the sub-regions of the can be estimated using cross-correlation.

Pearson's correlation coefficient is another linear measure of correlation between 2 signals. Unlike the Cross-correlation measures, Pearson's correlation does not consider the lag between the two signals. It is mathematically defined as the covariance between the signals multiplied by the inverse product of their standard deviations.

$$\rho_{X,Y} = \frac{COV(X,Y)}{\sigma_X \sigma_Y} \tag{3}$$

In equation 3, X and Y are two time series signals of interest. $\rho_{X,Y}$ is the Pearson's correlation coefficient. COV(X,Y) refers to the Covariance between X and Y. σ_X refers to the standard deviation of X and σ_Y refers to the standard deviation of Y. The value of the correlation coefficient ranges between -1 to +1 with -1 indicating a negative linear correlation between X and Y, +1 indicates a positive linear relationship between X and Y, 0 indicates no linear correlation between X and Y.

In addition to the aforementioned methods, functional connectivity can also be described using measures such as coherence and phase synchronization which are the frequency domain equivalents of the former methods.

Fisher weighted average correlation coefficient

Because the value of the correlation coefficient is not a linear function of the magnitude of the relation between the variables, correlation coefficients cannot simply be averaged. In such cases, it must be first converted into additive measures. I use the method of Fisher Weight to compute the average correlation coefficient throughout the analysis. The r to Z fisher transformations can be defined by (4)

$$Z = \frac{1}{2}[ln(1+r) - ln(1-r)] = \frac{1}{2}[\frac{ln(1+r)}{ln(1-r)}] \tag{4}$$

According to Faller[75], an arithmetic mean can be computed after Z transformation. Fisher Weighted mean average is then obtained by re-arranging equation (5)

$$\overline{r} = \frac{e^{\overline{2z}} - 1}{e^{\overline{2z}} + 1} = \frac{e^{\overline{z}} - e^{\overline{-z}}}{e^{\overline{z}} + e^{\overline{-z}}} \tag{5}$$

Coherence is a widely used mathematical method that quantifies the phase synchrony between a pair of measured signals. Measures of coherence includes both phase and amplitude synchronization. The magnitude of the squared coherence between two channel waveforms can be calculated as follows:

$$C_{xy} = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)} \tag{6}$$

Cxy corresponds to the magnitude of the squared coherence of the signals. The magnitude of the squared coherence estimate is a function of frequency with values between 0 and 1 and indicates how well x corresponds to y at each frequency. Equation 6 indicates that the coherence is measure of Power spectral density (PSD) of x (P_{xx}) and y (P_{yy}), cross-power spectral density (P_{xy}). Coherence is an estimate of the consistency of relative amplitude and phase between signals detected in coils or electrodes within a set frequency band. Coherence is one mathematical method that can be used to determine if two or more brain regions, have similar neuronal oscillatory activity with each other.

In this work, linear analysis methods discussed will be predominately used to understand the functional connectivity of LFP oscillations in the hippocampal sub-regions and the role of LFP synchrony in neuronal information processing and transmission.

Chapter 2

MATERIALS AND METHODS

2.1 Engineered neural circuits in vitro

Information processing is dependent on the network's ability to generate action potentials. Although investigations on individual neurons has continued for decades, quantification of inter-regional communication between populations of neurons simultaneously at multiple locations has been limited recording methods. The 3-dimensional distribution of neurons in the brain makes access difficult for simultaneous multisite recording. Studying the neuronal structures, the factors affecting the communication and the behavior of the networks can be done using brain slices, animal behaviors as well as in vivo[52], [53]. However, engineered neural circuits could provide improvements in access to the individual components of multiple neurons. Many methods have been used to develop neuronal cultures with defined connectivity patterns. In order to control the connectivity and to develop stable patterns, methods like caging neurons using physical barriers[54]and patterning rough surface coatings[55] continue to be investigated. Our lab has developed engineered neuronal circuit with defined connectivity patterns [56]. With the notion that re-engineering neural circuits will help in uncovering the various mechanisms of the underlying neuronal circuit, there has been a recent increase in the use of engineered neuronal networks to study the brain in vitro. Arseniy Gladkov et al. [57] developed a microfluidic devices with microchannels that couple 2 chambers with cultures neuron networks to analyze and compare neurite growth within the mirco-channels. Edwards D et al. [58] were able to demonstrate directed formation of electrically active and synaptically connected small circuits of neurons of hippocampus of adult rats through chemically engineered culture surfaces that gives polarity to neuronal processes. Daniele Poli et al. [59] used a 2 chambered device with axonal connectivity

via tunnels to understand the process of encoding and decoding between the DG and CA3 regions of rat hippocampus. In this work, for the first time we use an engineered four chambered network to reverse engineer the hippocampal formation. This living neuron network of 4 hippocampal sub-regions EC, DG, CA3, CA1 isolated from rat, cultured within a 4 chambered device with axonal connectivity via 5 x 10 x 400 μm micro-tunnels (Fig 1.1). Our model of the four sub-regions of the hippocampal formation helps evaluate specific information transmission in axons and circuitry within and between these sub-regions, allowing insight into the roles of slow waves in facilitating information transmission. The 5 x 10 μm micro-tunnels allow axonal growth and inhibit the migration of 15 μm diameter cell bodies or traversal by dendrites[60], [61] allowing insight into the role of axons in inter-regional communication. The procedure for a 2 chambered device has been described in Brewer et al. [62]. The same protocol was used for the development of the 4 chambered devices used in this work. The hippocampus was removed from the neocortex of each hemisphere of postnatal day 3 rats before dissection of the sub-regions as described in short by Poli et al. [59]. Each hippocampal sub-region tissues were subjected to brief digestion in papain followed by trituration into a suspension of single cells. Tissues were plated in proportion to the anatomical density.

2.2 Micro Electrode Arrays (MEAs) and Recordings

Electrophysiology plays a vital role in understanding the varied behaviors of the different regions of the brain[64]. There are a wide range of recording techniques to capture these electrophysiological properties of the brain. However, it is important to know the advantages and the pitfalls of such systems to choose the most appropriate choice for analysis. What are MEAs and why are we interested in them? What is our spatial scale of interest and why?

The recording techniques are also classified according to spatial resolution into microscale, mesoscale and macroscale recordings. Different kinds of neurophysiological recordings contain comprehensive information pertaining to the connectivity between the brain regions at different scales. The microscale is focused towards the study of the individual neurons and their firing patterns. These allow the characterization of single cell behavior to stimuli. At the macroscale, fMRI, measures large-scale communication and the association of the different cortical regions of the brain. However, it is necessary to understand how the neurons behave as population in order to understand their role in communication between the different regions of the brain and as well the computational and behavioral of a group or a population of neuronal assemblies. Thus, recording of the local field potential at the mesoscopic scale bridges the gap between the microscale single neuron recording and macroscale whole cortical subregion analysis to study the behavior of a population of neuronal assemblies.

MEAs (multi-electrode arrays) have made simultaneous recording from multiple neuronal assemblies possible, an important feature since hippocampal coding likely proceeds through cell assemblies rather than between single neurons [57]. The electrical interface of the MEA with the cultured neurons has made it useful to study neuronal cultures. Cell patterning methods along with effective MEAs provides non-invasive and long-term recordings (5 mins in this work) simultaneously from multiple neurons[59]. They increase the yield of neurons per recording session and simultaneous recording from a population of neurons makes pairwise correlations easier[65]. MEAs measure the extracellular field potentials generated by the membrane currents including fast 1 ms action potentials and slower 10-1000 ms slow wave potentials.

To develop the 4 chambered compartment, poly-dimethyl-siloxane device was aligned over a multielectrode array MEA120 (Multichannel Systems, Germany; ALA Instruments, USA) consisting of a 12 X 12 layout grid sparing 6 electrodes in each corner, 120 recording electrodes with 4 internal reference electrodes was used to record the signals from the in-vitro hippocampal cell culture for this work. The spacing between the electrodes is 200 μm with an electrode diameter of 30 μm. This array thus made it possible to record simultaneously across the 4 chambers in 2D reducing the complexity of the brain network recordings (Fig 1.1). The unique design of the device also offers the prospects of measuring signals from both the somata and the axons. Activity from each multielectrode array was recorded at 25 kHz sampling frequency with 1100x amplification and a hardware filter of 1-3000 Hz. The recordings were made at 37^{o}C under continuous flow of hydrated sterile 5% CO_2, 9% O_2 and balance N_2 (Airgas custom, Santa Ana, CA, USA) over a Teflon film cover (ALA scientific) as suggested by Brewer et al. We waited 3-4 weeks for the robust development of the axons, dendrites and the synapses[67]. Five minutes of spontaneous activity was recorded using Multichannel System's MCRack software and analyzed offline using MATLAB Scripts.

2.3 Signal Processing

2.3.1 Local Field Potentials:

For analysis of band-limited LFP oscillations, the signals were down sampled to 625 Hz from 25 kHz, lowpass filtered < 300 Hz. To extract specific LFP frequencies, signals were filtered using band-pass filters with cutoff frequencies 4-11 Hz (Theta) and 30-100 Hz (Gamma). As with connectivity measures computed over time, the main parameter to select is the length of the time segment used to compute the correlation coefficient. If the time segment is too long, transient changes in connectivity might not be detected, but if the time segment is too short, you may have too little data for robust correlation coefficient estimates. The time segment was selected so that at least four cycles of theta oscillations were included (1 s), and this number decreases with higher frequencies. To analyze the LFP, we segmented them into 1 sec intervals for theta oscillations and 40 ms time segments for gamma oscillations after extraction of bursts and non-bursts segments obtained from burst detection algorithm discussed in section 2.3.2. LFP instantaneous phase and amplitude can be computed from the respective analytic signal via Hilbert transform.

Filters and filtering data before applying Hilbert transform

Filters generally serve two purposes (1) separation of signals that have been combined, and (2) restoration of signals that have been distorted in some way. Different types of filters are used in multiple applications ranging from online to offline, from analog to digital and from hardware to software in their implementation. Filters as such can also introduce undesired components into the signal. In particular, filters can induce changes in phase of different frequencies in addition to affecting the magnitude of the signal [65]. Such distortion in the signal can result in

misinterpretation of results and erroneous conclusions. The distortions that arise by online filtering is exacerbated by offline processing. Filters need to be designed to reduce the adverse effects of distortion of the signal of interest. One other way of correcting can be done if we know the exact properties of the filter and the original timing of the signals. In the offline correction process, a filter similar in properties to that of the online filter can be designed and the signal can be filtered in the reverse direction to nullify the effects of distortion thereby leading to phase shifts back to zero [65], [66]. This filtering is applied to signals using the "filtfilt" function in MATLAB, which is a zero-phase filtering technique. It processes the input data, x, in both the forward and reverse directions. After filtering the data in the forward direction, "filtfilt" reverses the filtered sequence and runs it back through the filter. The result has the following characteristics of zero phase distortion, a filter function equal to the squared magnitude of the original filter transfer function and filter order double the order specified by the transfer function coefficients b and a (7). It also minimizes the start-up and ending transients by matching initial conditions.

$$y = filtfilt(b, a, x) \tag{7}$$

The analytic signal obtained from the Hilbert transform can be difficult to interpret if all frequencies are present in the signal leading to biased interpretations of the data. Therefore, it is necessary to filter the data into separate frequency bands of interest before applying Hilbert transform for further computations of power or phase of a signal. This method of filtering and then transforming the data is considered as an advantage over wavelet transform where the control over the Morlet wavelet characteristics are lesser compared to a filter characteristic. However, each of the three methods of computation Fourier, Hilbert transform and Morlet wavelets are well-suited and produce similar results[67]. This particular work used Hilbert transform for signal processing.

Computation of the analytic signal with the Hilbert transform:

To estimate the power of a narrowly bandpass-filtered signal without using the wavelet transform the Hilbert transform was used to calculate an analytical signal. The analytical signal X(t) is complex and can be used to calculate the phase/power. The Hilbert transform X_h(t) is equal to the signal phase shifted by 90°. The real part of the analytic signal equals the raw signal, X(t) and the complex part is the Hilbert transformed signal (8)

$$H(t) = X(t) + iX_h(\mathrm{t}) \tag{8}$$

Given a complex signal as a function of time H (t), the following equation were used to estimate the instantaneous power R(t) (9). H(t) is the result of a Hilbert transform (8). with R{H} and I{H} the real and imaginary part of H, respectively.

$$R(t) = (R\{H\})^2 + (I\{H\})^2 \tag{9}$$

2.3.2 Spike/Burst detection:

The digitized signal from each electrode was filtered using a 300 Hz high pass filter followed by spike detection as discussed by Bhattacharya et al. [68] in detail. Briefly, spikes were identified as peak-to-peak voltages exceeding 11 × the minimum root-mean-square value of 200 ms contiguous windows (Spycode v3.9) [69], [70]. We imposed a 1 ms dead-time or refractory period after a spike before another could be detected. As mentioned by Brewer et al. [62], bursts were defined as four or more spikes with an inter-spike interval (ISI) of 1–50 ms, on a per electrode basis, not summed over multiple electrodes. The start and stop time of the bursts were extracted

into an array on a per electrode basis and used for segmentation of the LFP-theta and gamma oscillations into burst and non-bursts segments for connectivity analysis.

2.3.3 Cross-correlation

A common idea to understand the connectivity between brain regions is to use multi-site electrode studies to sample LFP simultaneously from multiple regions, to determine if one regions leads/lags with respect to the other and to estimate the time lag between the connected regions. There are number of methods to detect directionality of functional connectivity using LFP signals such as Granger causality [71], partial directed coherence [72] in addition to the non-linear methods discussed in the introduction section . However, these methods are mathematically complex, are also sensitive to noise. In this work, I use Cross correlation - a relatively simple and mathematically straightforward method to estimate connectivity between the hippocampal sub-regions. The mathematics of the method was already discussed in section 1.3.1. In brief, the LFP oscillations was divided into bursting and non-bursting segments. The cross-correlation within theta oscillations during the bursts and non-bursts duration between the electrodes was computed respectively. Similarly, the gamma cross correlation during the burst and non-bursts durations was computed between the electrodes respectively. A natural starting point for estimating spatial falloff of correlation between electrodes within a sub-region and between a sub-region electrode and an axonal electrode was also computed and plotted as function of the square of the inter-electrode distance in order to analyze the communication between electrodes. The cross correlation between electrodes were computed between all possible inter-electrode distances and the average cross correlation computed at unique inter-electrode distances to quantify the fall off in spatial correlation. The cross correlation between electrodes during the bursts and non-bursts durations in

the regions and the axonal tunnels were computed between all possible bursting electrode combinations. After computation of the cross-correlations of the filtered power vectors, the distribution of the lags at which the cross-correlation peaks occur was obtained. Wilcoxon's non-parametric rank sum test was performed on the sample of lags to verify whether the mean of the distribution was significantly different from zero.

Cross-correlation is designed to make inferences about synaptic connectivity between neurons. One can observe narrow peaks (a few ms) in the latency of the LFP of one neuron with respect to another in investigating synaptic connectivity. If such a peak is observed, then one can potentially make inferences about the number of synapses between each neuron [73]. Cross-correlation can also be applied to make inferences about local circuits [74]

2.4 Significance of functional interaction

In any analysis of functional interactions, one must establish clear criteria for statistical significance to determine if interactions observed are due to chance. The best way to establish empirical significance is to reapply the same statistical tests to the same data in which the dimension of interest is randomly permuted. This dimension is time in my analysis. Using shuffling, I tested the significance of a functional interaction by (1) Shuffling the LFP segments in time or in trials and (2) Applying functional interaction analysis of interest which is Cross Correlation to shuffled data [73]. This process can be easily achieved in MATLAB using the 'randperm' function. It returns a row vector containing a random permutation of integers. This type of shuffling is called random shuffling or Independent and Identically distributed (IID) shuffling These integers were used as row indices to shuffle our segmented LFP data and cross

correlation analysis was performed on the shuffled data to determine the if the observed interactions are due to chance.

2.5 Statistics

All statistical analyses were performed using the standard statistical toolbox in MATLAB. No specific analysis to estimate minimal population sample or group size was used. Mainly two sample t-test was used to compare between the bursts and non-bursts related LFP functional connectivity data, which returns a test decision for the null hypothesis that the data in vectors `x` and `y` comes from independent random samples from normal distributions with equal means and equal but unknown variances, using the two-sample *t*-test. The alternative hypothesis is that the data in `x` and `y` comes from populations with unequal means. The result `h` is `1` if the test rejects the null hypothesis at the 5% significance level, and `0` otherwise. Multiple comparison tests using 'multcompare' in MATLAB along with ANOVA from the statistics and machine learning toolbox in MATLAB. The purpose of one-way ANOVA is to determine whether data from several groups (levels) of a factor have a common mean.

Chapter 3

RESULTS

The goal of this work is to quantitatively determine the functional connectivity among the sub-regions of the hippocampus. In addition, the living engineered reconstruction of the hippocampal subregion (Fig 1.1) will enable ascertaining the role of the slow waves, their dynamics in the hippocampal subregion and in information transmission into the neighboring regions.

3.1 Recorded spontaneous activity from microelectrode arrays

The primary purpose of using the micro-electrode arrays over the engineered neuronal networks chambers is to measure the activity of single neurons in small populations from the dissected hippocampal sub-regions. The spontaneous spiking activity from each of the electrodes in each subregion and the tunnels over 5 arrays was computed in order to determine reproducible dynamic features while eliminating inactive electrodes from the analysis. Electrodes were considered inactive if there were less the 5 spikes per 5 minutes of recording. The spike rate across all the electrodes were computed and the spatial distribution of the log of spike rate on a standard MEA120 layout for all the 5 arrays is shown in Fig 3.1. All the electrodes showed activity greater than 5 spikes per 5 minutes and none were eliminated from analysis.

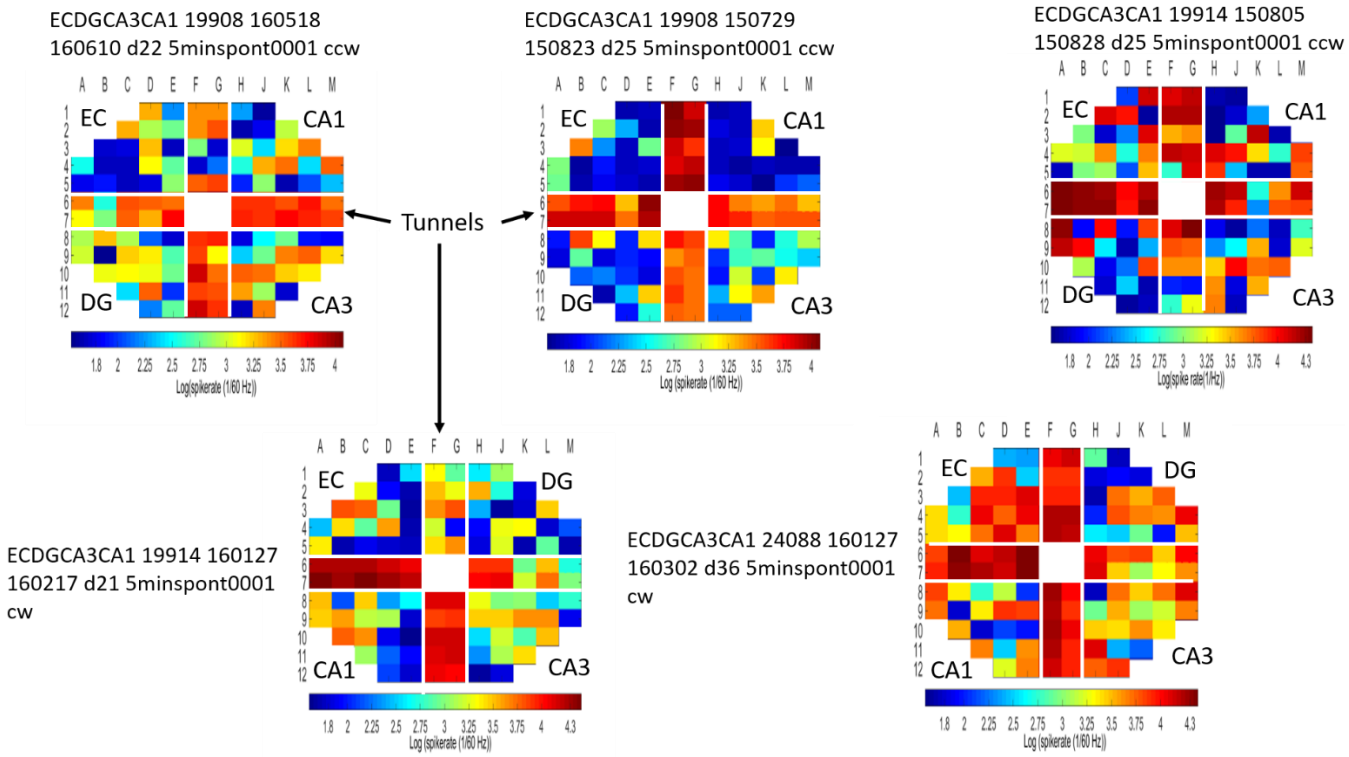

Figure 3.1: Spontaneous spike activity from electrodes in each sub-network of 5 arrays. Note higher spike rates in tunnels and variability from one array to another. File names of each recording are indicated for data rigor. Figure File name: spike_activity.fig

3.2 Directionality from relative timing of oscillatory peaks

Multi-site recordings are often used in identifying the directionality of functional connectivity and estimating velocities between sites. Cross-correlation is a simple, method to estimate directionality and velocity based on distance divided by lag from local field potentials (LFPs) generated by axons in the tunnels. This work is aimed at deciphering the lags from the sub-regions to the axons in the tunnels which is used to make inference about the type of connectivity between the regions and tunnels. However, a causal relationship cannot be established using cross-correlation. More complex mathematical methods like Granger Causality are used to establish causal relationship or better yet an intervention to promote or interfere with the LFP [73]. The distribution of lags between LFP oscillations is depicted in Fig 3.2 for theta oscillations and Fig 3.3 for gamma oscillations. The information about the lag is used to make inference about the synaptic connectivity in the neurons. Using the cross-correlation method, we demonstrate a circular path of theta waves from EC to the EC-DG tunnels into DG to the DG-CA3 tunnels, into CA3 to the CA3CA1 tunnels. However, in 3.15 D, the EC leads the EC-CA1 tunnels for feedback in the theta and gamma frequency range. Lag times of 5.1-8.2 ms for theta and 5.2-6.9 ms for gamma are consistent with transmission through an average of one synaptic delay on either side of the axons in the tunnels. The lags between the sub-regions and the tunnel found in the theta and gamma frequency ranges suggests that theta and gamma oscillations in the axons could drive functional connectivity between the hippocampal sub-regions. The next section thus shows few individual examples of correlation and transmission between the sub-region and tunnels.

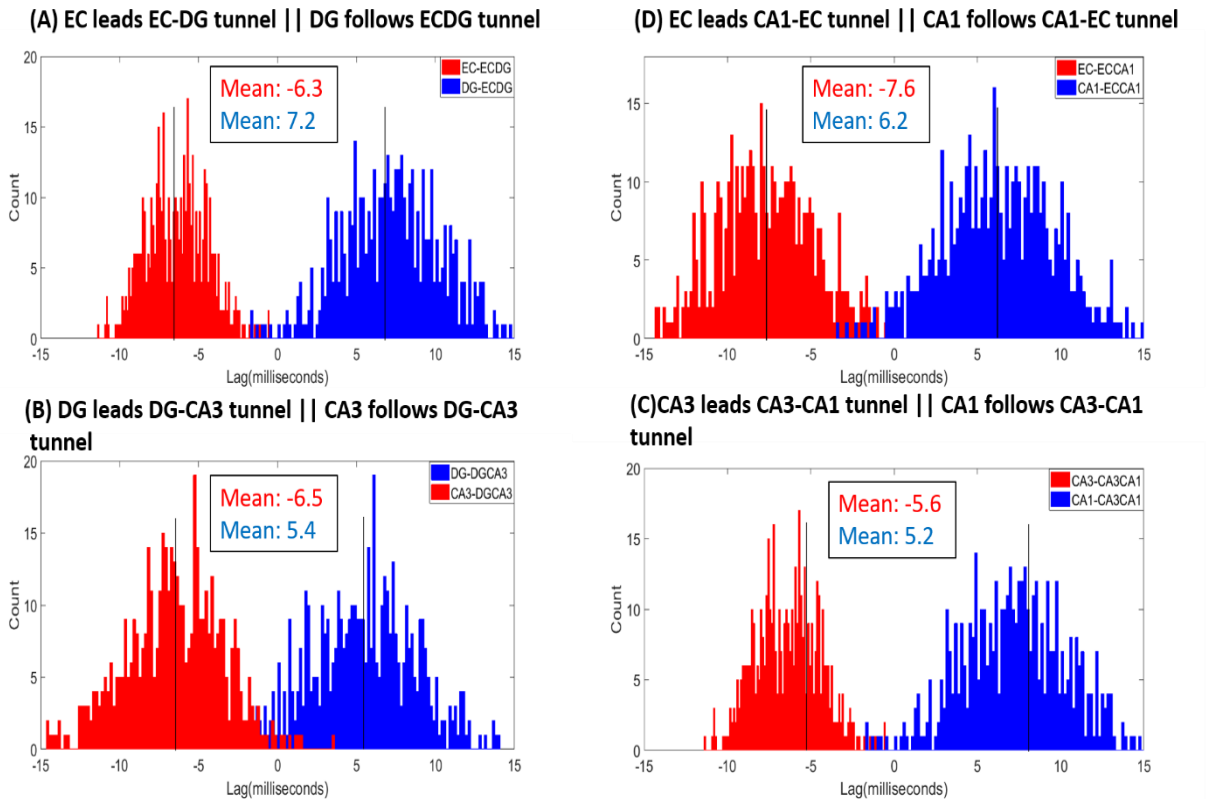

Figure 3.2: Distribution of lags of theta correlation between the sub-regions and the tunnels. The negative mean lag indicates that the sub-region leads the tunnel and a positive mean lag occurs when the sub-region follows the tunnels. The lags suggest at least one synaptic connection exists between the sub-region and tunnels. Wilcoxon's non-parametric rank sum test was performed on the sample of lags to test whether the mean of the distribution was significantly different from zero (p=0.01 in all cases; n=5 arrays). File name of this figure: theta_lag.fig

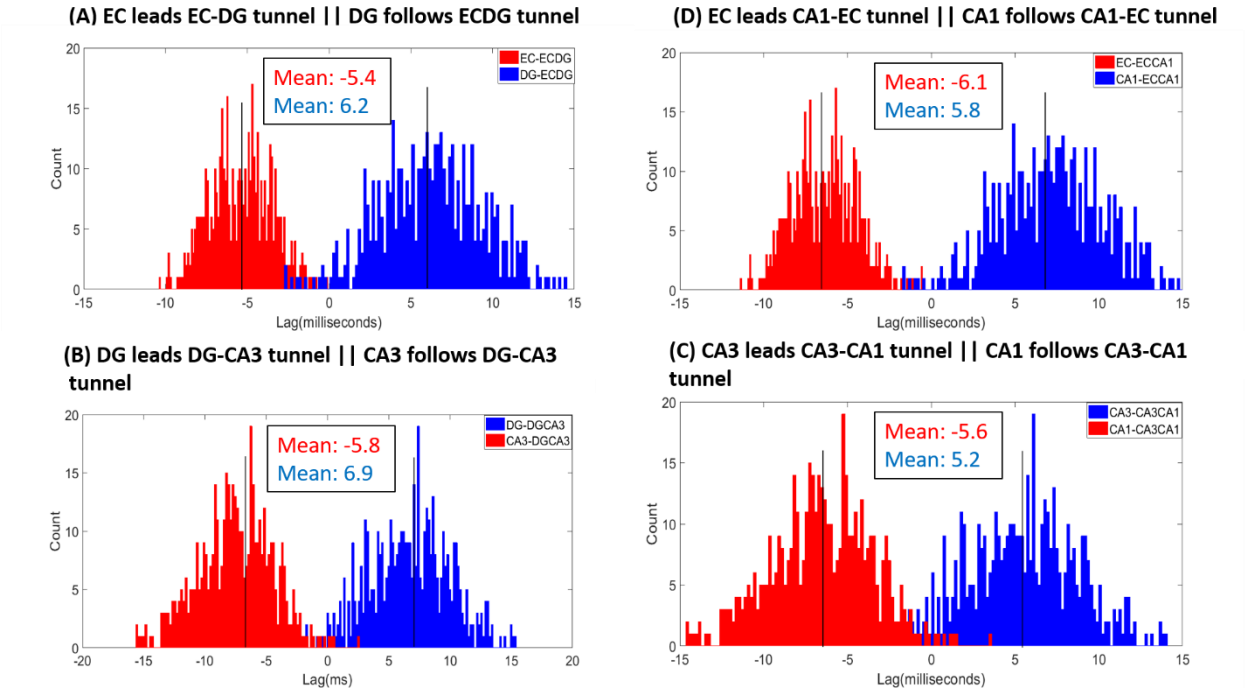

Figure 3.3: Distribution of lags of gamma correlations between the sub-regions and the tunnels. Negative lags indicate that the sub-region leads the tunnels and a positive lag indicates the sub-region follows the tunnels. These lag times can indicate an average of a single synaptic connection exist between each sub-region and tunnels. Wilcoxon's non-parametric rank sum test indicates the mean of the distribution was significantly different from zero (p=0.01 in all cases; n=5 arrays). File name of this figure: gamma_lag.fig

3.3 Relationship of LFP to bursts and their synchrony

Inspection of the neural signal in the time-domain is necessary to characterize how changes in neural LFP oscillations contribute to information processing and transmissions. It is important to account for their spatio-temporal characteristics and their association with burst of spikes. For this, we determined the theta (4-11Hz), gamma(30-100Hz) wave power by bandpass filtering with the Hilbert transform. The spikes were obtained by high pass filtering of the raw data. Fig 3.4 shows the association of robust theta oscillations with a burst of spikes in the subregion (CA3). Fig 3.5 shows the raw and filtered theta waveforms of adjacent axonal tunnel electrodes (CA3-CA1). Fig 3.6 illustrates the association of theta oscillation and bursts in the sub-region (CA3) considered as a source where the computation occurs and the axonal tunnel between CA3 and CA1 (CA3-CA1) that transmits the product of computation into the neighboring regions. In addition, Fig 3.4, 3.5, 3.6 also suggests certain level of correlation of LFP between neighboring electrodes suggesting the binding of cell assemblies for network wide communication through synchrony of LFP oscillations. The red boxes in the figure not only shows the association of theta oscillations with the bursts but also highlights the synchrony in the occurrence of both between electrodes. These results provide compelling evidence that time-domain features of both hippocampal LFP waveform and bursts, reflects local circuit properties. These results point to the possibility of inferring circuit states from local field potential features in the hippocampus and perhaps other brain regions with other rhythms. This analysis also serves as a starting point for analysis to infer the causal relationship between the LFP and spikes which will be analyzed in the future works.

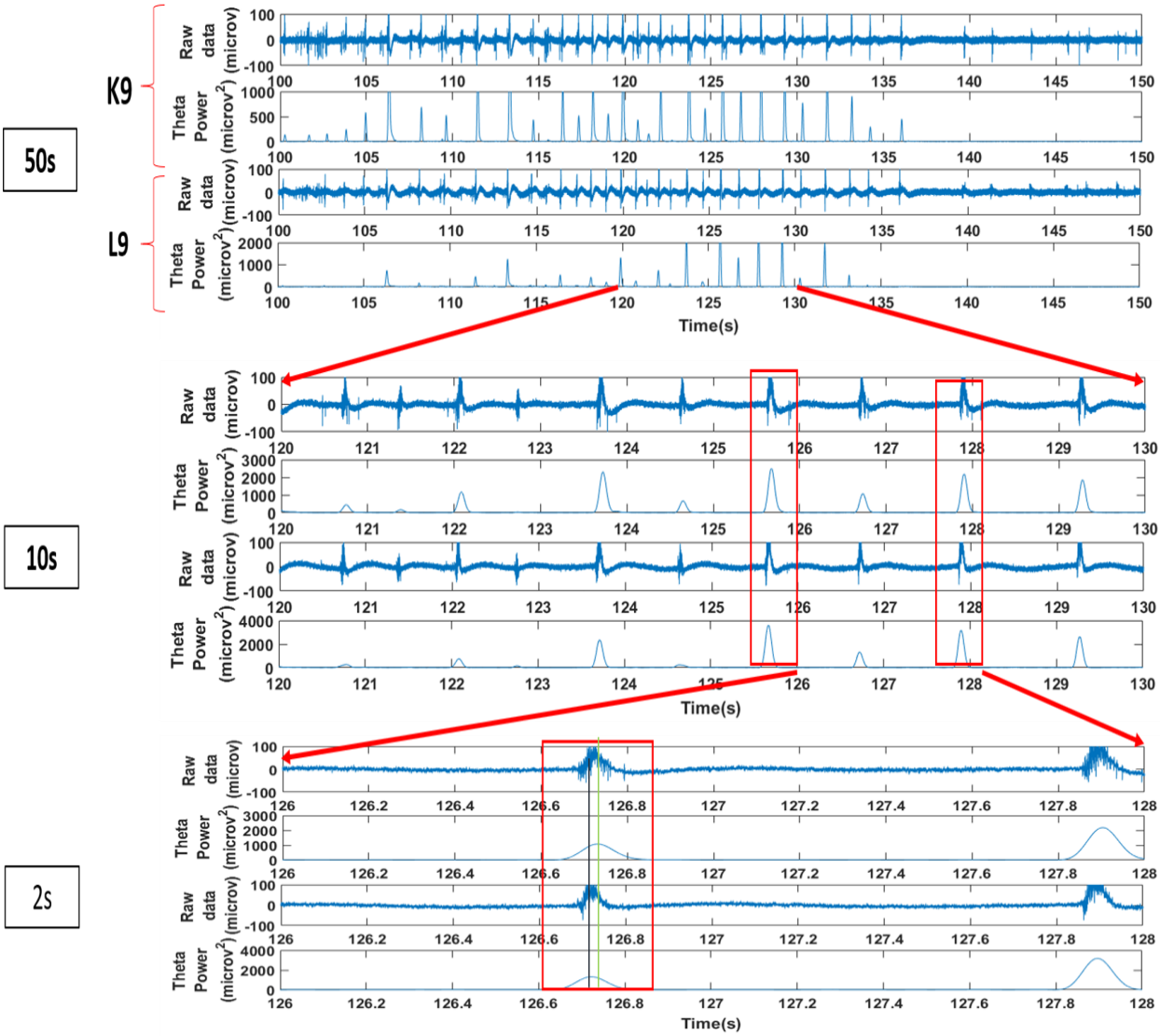

Figure 3.4: Raw signal and filtered theta waveforms of adjacent electrodes (K9, L9) in CA3 sub-region suggests theta synchrony between nearby electrodes and transmission of theta oscillations between neurons is associated with a burst of spikes. The peak of theta oscillation (black line) of L9 electrode at 126.69s and the peak of the theta oscillation (green line) of K9 at 126.73s suggest the propagation of theta oscillations. Magnification of a time interval to show burst and corresponding theta cycle. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001. Figure File name: Theta_region.fig

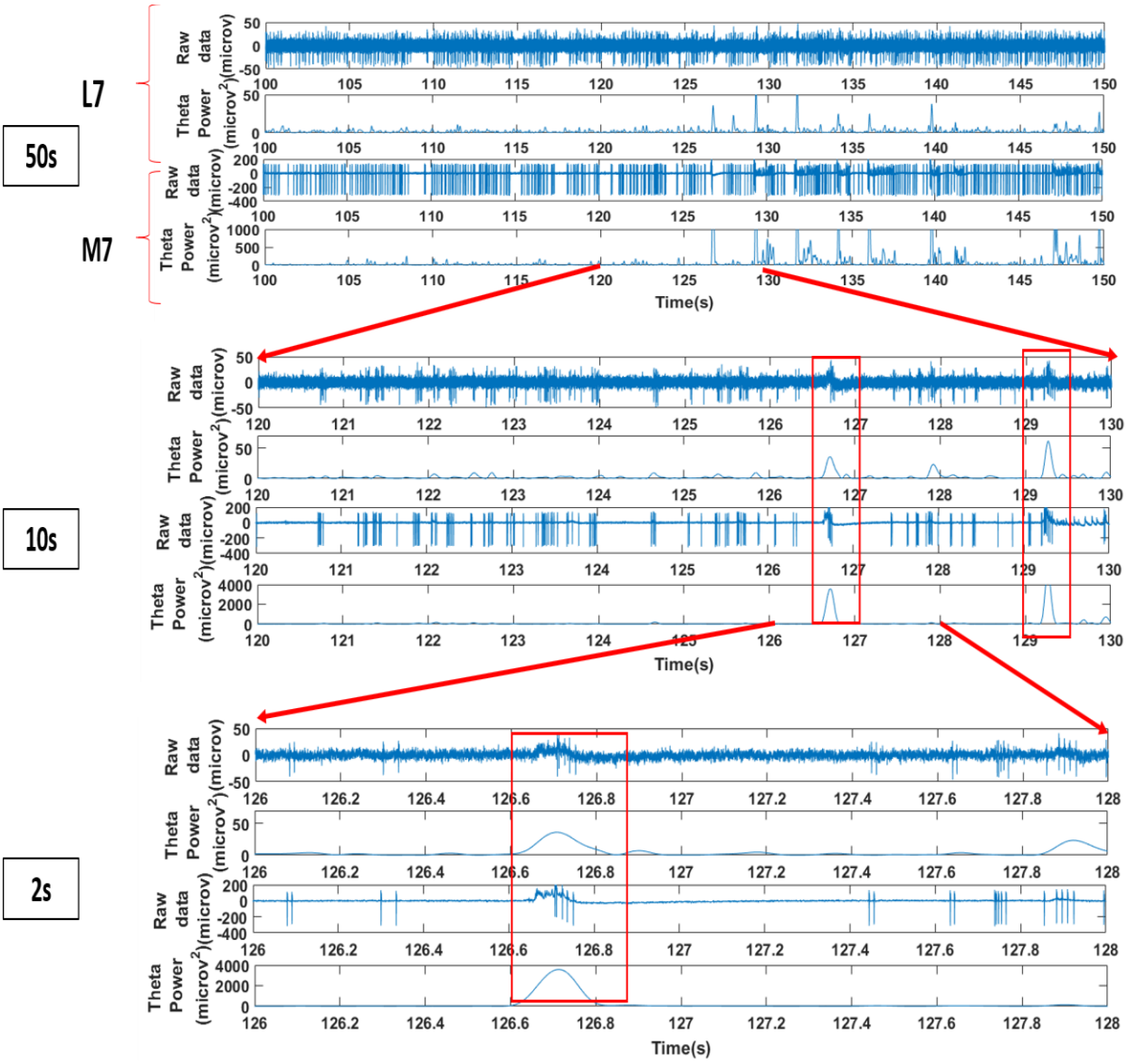

Figure 3.5: Raw signal and filtered theta waveforms of adjacent tunnel electrodes (L7, M7) in CA3 –CA1 tunnel suggests theta synchrony between electrodes and transmission of theta oscillations via the axon tunnels is associated with a burst of spikes. Magnification of a time interval to show burst and corresponding theta cycle. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: Theta_tunnel.fig

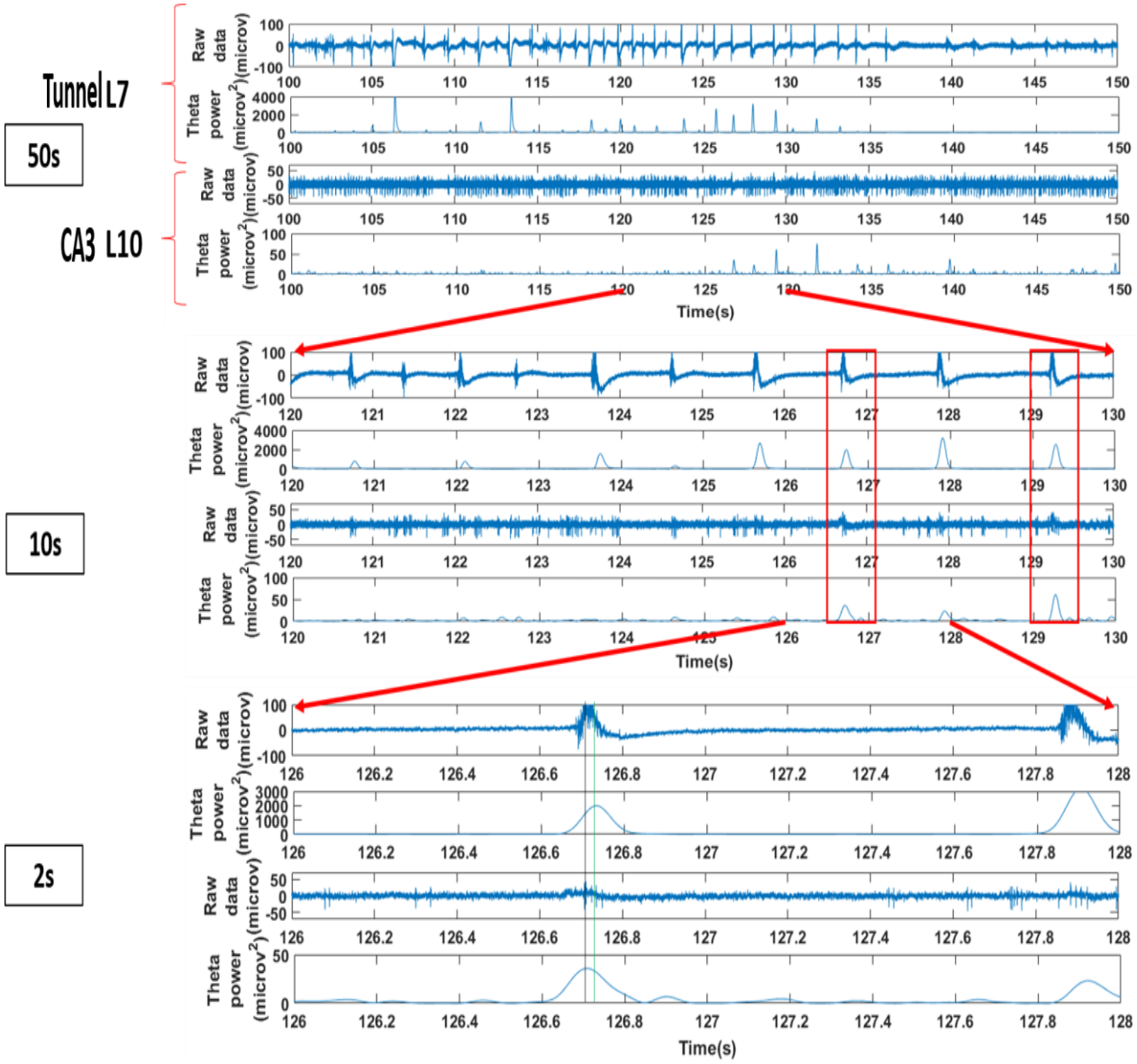

Figure 3.6: Electrode L10 in CA3 serves as source for axonal electrode L7 in a CA3 –CA1 tunnel suggests theta propagation from regional electrodes into the tunnel and their association with a burst of spikes at 126.7 s. Note opposite apparent direction for events at 127.9 s in which the axon appears to transmit a theta oscillation into the neighboring CA3 region. Note larger signals in the tunnels because of higher resistance there. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: Theta_source_tunnel.fig

We now shift focus to how the hippocampal gamma rhythms, relate to the network activity of neurons in hippocampal sub-regions and the axonal tunnels. The gamma oscillations also show similar behavior like the theta oscillations as illustrated in Fig 3.7, 3.8, 3.9. Such analysis approach allows us to analyze if the theta/gamma cycles contain information about the underlying neuronal sequences.

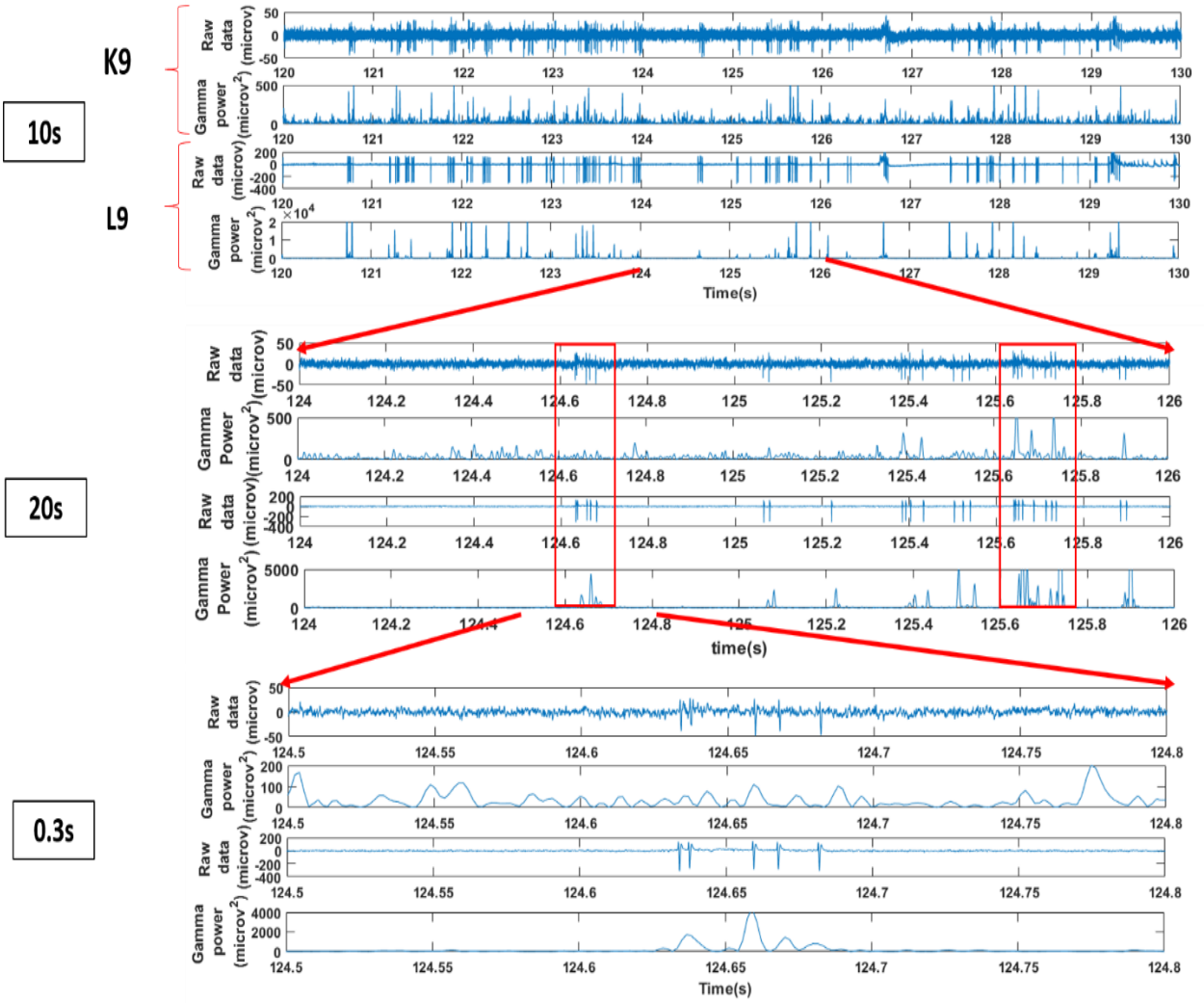

Figure 3.7: Adjacent electrodes (K9, L9) in CA3 sub-region suggests some gamma synchrony between nearby electrodes, transmission of gamma oscillations between neurons is associated with a burst of spikes. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: gamma_region.fig

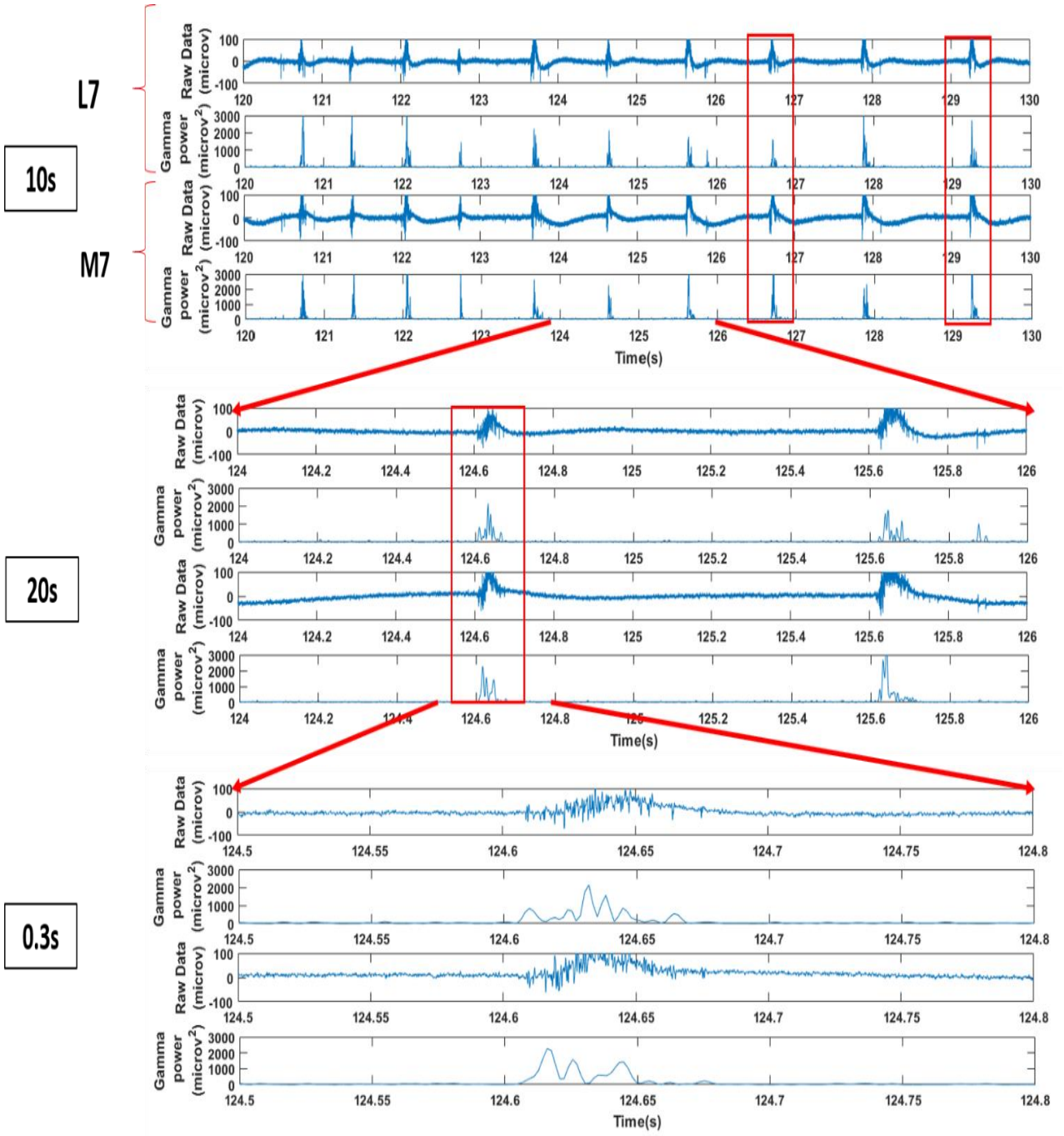

Figure 3.8: Raw signal and filtered gamma waveforms of adjacent tunnels on electrodes L7 and M7 between CA3 and CA1 regions suggests a type of gamma synchrony associated with bursts of spikes with unique gamma oscillations via the axon tunnels. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: gamma_tunnel.fig

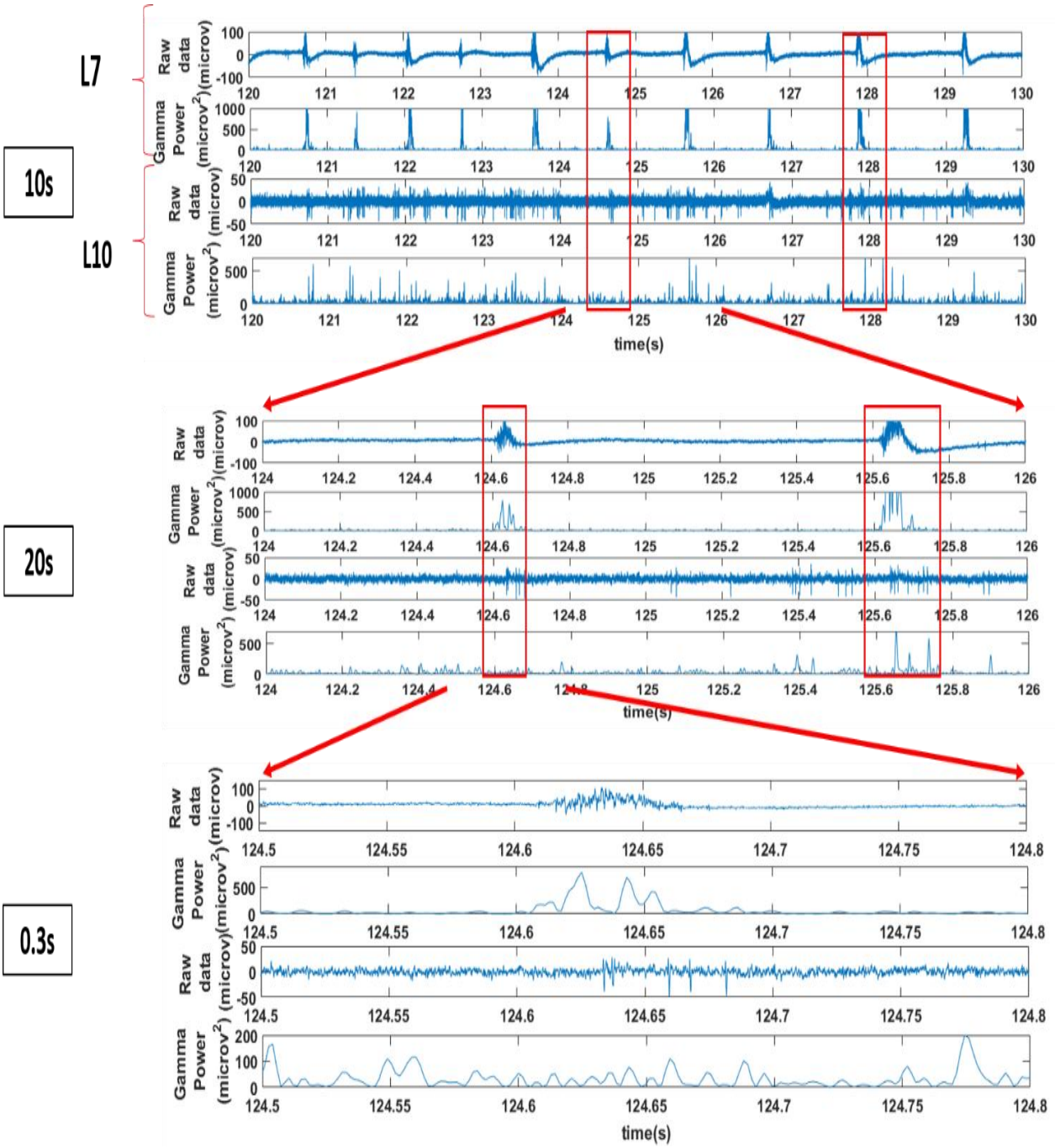

Figure 3.9: Electrode L10 in CA3 and electrode L7 in a CA3 –CA1 tunnel suggests gamma oscillations in regional electrodes, the tunnel and their association with a burst of spikes; Suggests gamma synchrony between electrodes from the region and tunnel separating computation within sub-region, the output of the computation in the axons and hence transmission of gamma oscillations into a neighboring region. Magnification of a time interval to show burst and corresponding gamma cycle. Also note gamma power in absence of spiking. Recording file: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001. Figure File name: gamma_source_tunnel.fig.

3.4 Quantification of hippocampal connectivity in theta

The above examples were from single pairs of electrodes. To statistically evaluate consistent behavior, we assessed connectivity between all pairs in each sub-region of the hippocampus and in the axonal tunnels, between pairs of electrodes, I adopted by a common linear synchronization approach of Cross-Correlation, which was described in detail in methods. Each sub-region contains a 19×19 connectivity matrix and a tunnel matrix of 5×5 elements with the connectivity weights being the cross-correlation of the theta oscillations from 4–11 Hz (Fig 3.10). Only 1 electrode from each tunnel was chosen since electrodes from the same tunnel are expected to carry the same information since both measure the LFP from the same axons. Node indices are organized by the electrode names per the indicators on the axes. Blue areas represent weaker connections and red being the stronger connectivity indicator. The white line on the diagonal represent the auto correlation. Fig 3.10 suggests strongest theta correlation in the CA3 sub-region, which could be an attribute of their recurrent neuronal networks [61]. Also, connectivity to CA3 from both sides, DG-CA3 and CA3-CA1 tunnels also seem to be the highly theta correlated in this network. Averages for 5 arrays in Fig 3.11 also confirms the strong correlation in the CA3 region.

These connectivity matrices also suggest the theta correlations are spatially concentrated i.e, stronger correlation between adjacent electrodes compared to electrodes far away which could be indicative of the fall in correlation by the inverse square law with distance from an electrode. Signal from a strong dipole source that propagates in the extracellular medium would be expected to fall off as the inverse-cube of the distance, while a monopole would dissipate as the inverse-square and a cable as 1/r.

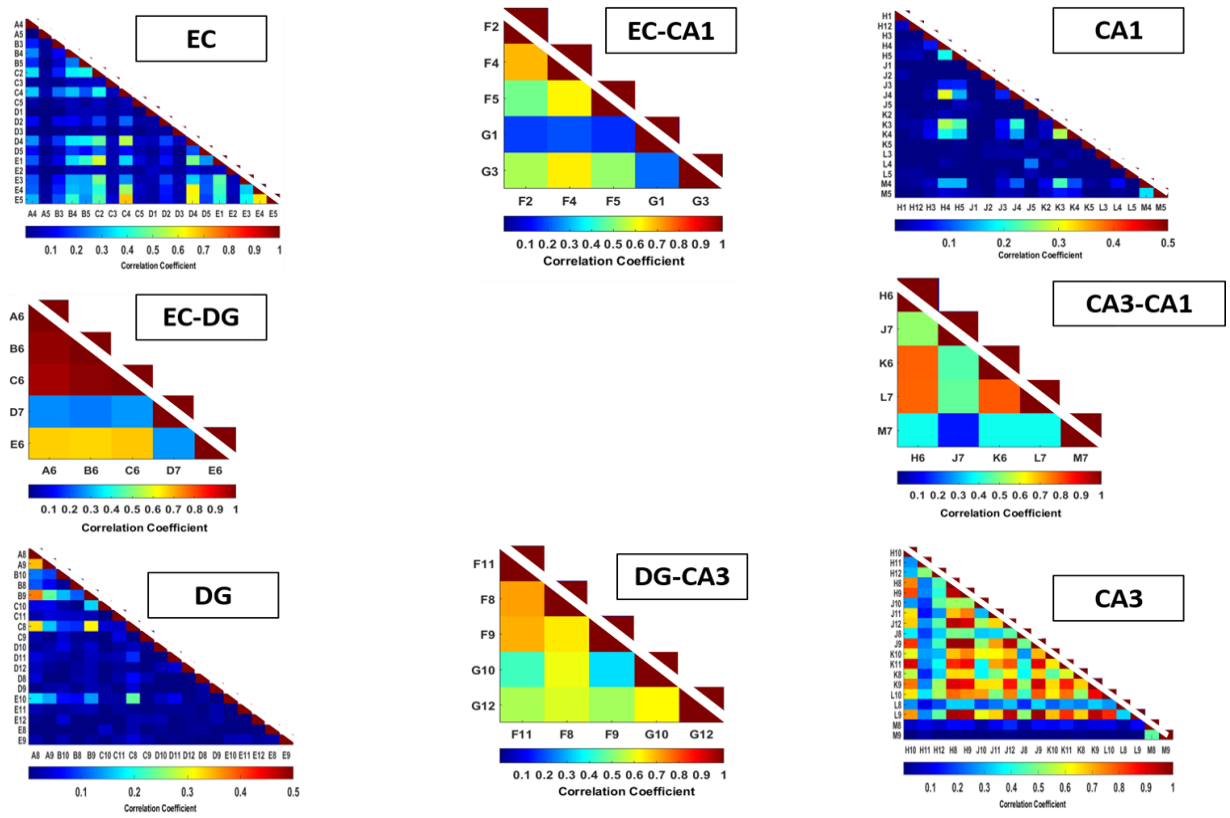

Figure 3.10: Theta band power correlations for the 19 by 19 electrode combinations for the sub-regions and 5 by 5 electrode combinations for the tunnels suggest strongest functional connectivity within CA3 and among the tunnels between CA3 and either CA1 or DG. In the correlation matrix, each row and each column represent the cross-correlation coefficient obtained from the XCORR between pairs of electrodes in each sub-region and tunnels. Recording file: Single array ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: theta_connectivity (EC, DG, CA3, CA1). fig

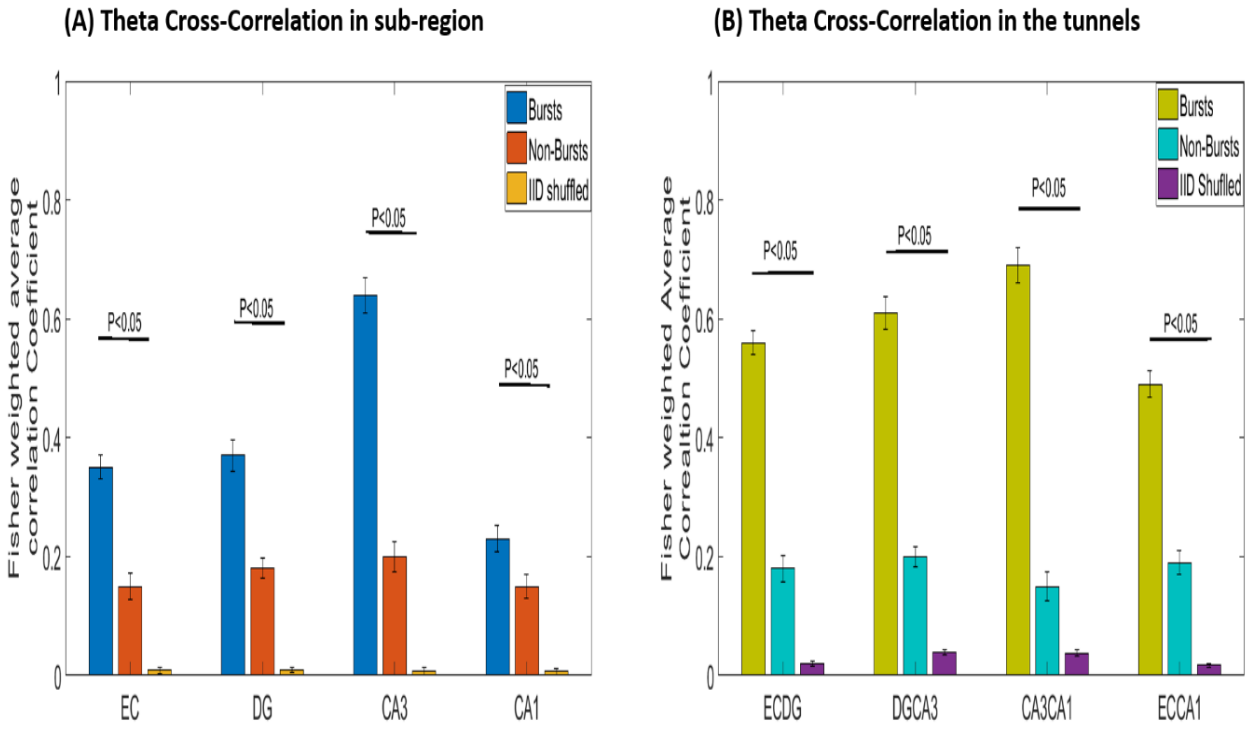

Figure 3.11: Sub-regional and axonal theta correlations suggest coordinated flow of theta oscillations during bursts. Significance test for theta correlations in subregions and tunnels (a) Synchronization of theta oscillations during bursts within a region were significantly greater than the non-burst segments with especially high burst correlation in CA3 compared to other sub-regions (N=5 Arrays); These measures were together above levels for IID randomly shuffled data; (P<0.05,ANOVA;N=5 Arrays) **(b)** Synchronization of theta oscillations in axonal tunnels during bursts and non-bursts is higher in tunnels (P<0.05,ANOVA) indicating the transmission of information from the same source or parallel transmission of the same output. Theta correlations within tunnels are significantly greater than shuffled data (P<0.05, ANOVA; N=5 Arrays). File name for this figure: theta_region_BNB.fig, theta_tunnel_BNB.fig

Differences in synchrony in the sub-regions and the tunnels could suggest differences in how each region processes information via LFP oscillations. Fig 3.11 (a) shows the correlation within the subregions during bursts and non-bursts segments of the theta oscillations. It suggests that overall the correlation of burst theta is significantly greater that than non-burst theta (2-sample t-test, P=0.015). However, the lower correlation of the non-burst theta is still significantly above shuffled data suggesting that it is not because of a random background fluctuation. In addition, comparison between the burst theta from the different sub-regions suggests that the CA3 has the strongest correlation with significant difference among all the other sub-regions (P=0.02, ANOVA multiple comparisons). CA3 sub-region is known to be a recurrent network wiring pattern and such strong correlation can be associated with this kind of network pattern [61]. EC sub-region was significantly different from the other sub-regions (P=0.015, ANOVA multiple comparison) except DG (P=0.67, ANOVA). Fig 3.11(b) shows results of similar analysis performed in the axonal tunnels. Measuring from individual axons, we were able to observe LFP in the axons suggesting that non-axonal membrane currents transmit LFP information. Tunnel correlations are also like that in the sub-regions, in that burst correlations are significantly greater than the non-bursts theta correlations (P~0.011). Note that the two-fold higher values of correlations in the tunnels between EC and DG and between EC and CA1 suggests coordinate axonal LFP information transmission to and from these regions. It is necessary to investigate if observations are due to random chance occurrence as opposed to a defined connectivity pattern. By random shuffling of the segments of LFP oscillations the relationships between neuronal population activity and random background fluctuations. The observed interactions are significant and are not due to chance (P~0.002 ANOVA multiple comparisons).

3.5 Quantification of hippocampal connectivity in low gamma

The low gamma network is similarly constructed from frequencies between 30–100 Hz. Fig 3.12 suggests strongest gamma correlation in the CA3 sub-region, but correlations were about 50% lower than those for theta. Again, DG-CA3 or CA3-CA1 tunnels seem to be the more highly correlated tunnels. These type of results for a single array were averaged for 5 arrays for statistical comparison (Fig 3.13). Differences in synchrony in the sub-regions could suggest differences in how each region processes information via LFP oscillations. Fig 3.13 (a) shows the correlation within the subregions during bursts and non-bursts segments of the gamma oscillations. It suggests that overall the correlation of burst theta is significantly greater that than non-burst gamma (2-sample t-test, P=0.02). However, the lower correlation of the non-burst gamma is still significantly above shuffled data. In addition, comparison between the burst gamma from the different sub-regions suggests that the CA3 has the strongest correlation (P= 0.03, ANOVA multiple comparison). Fig 3.13(b) shows results of similar analysis performed in the axonal tunnels. Measuring from individual axons, we were able to observe LFP in the axons suggesting that non-axonal membrane currents transmit LFP information. Tunnel correlations are also like that in the sub-regions, in that burst correlations are significantly greater than the non-bursts theta correlations (P=0.0015, ANOVA multiple comparisons). Note that the two-fold higher values of correlations in the tunnels between EC and DG and between EC and CA1 suggests coordinate axonal LFP information transmission to and from these regions. It is necessary to investigate if observations are due to random chance occurrence as opposed to a defined connectivity pattern. By random shuffling of the segments of LFP oscillations the relationships between neuronal population activity and random background fluctuations. The observed interactions are significant and are not due to chance (P=0.006, ANOVA multiple comparison).

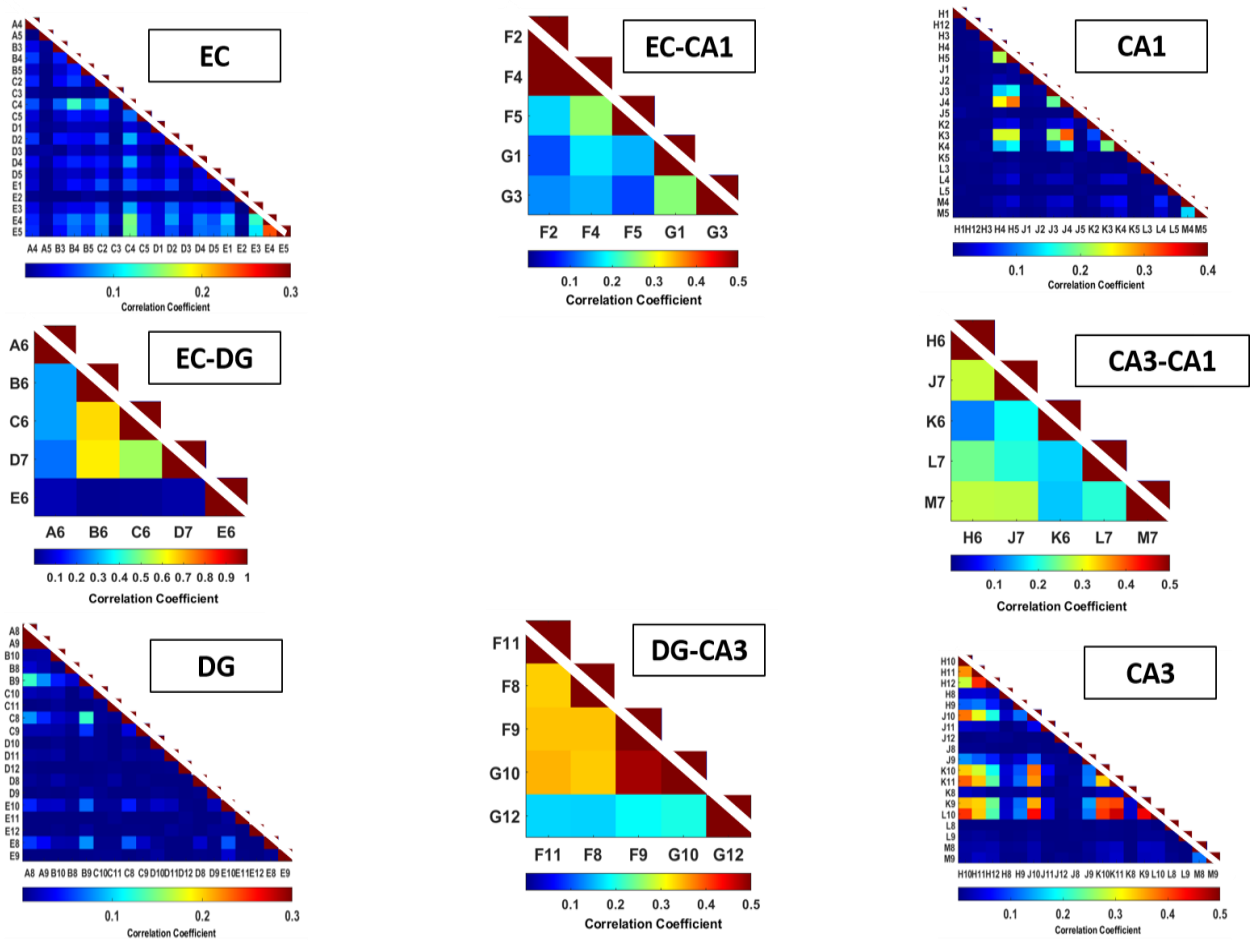

Figure 3.12: Gamma band power correlations for the 19 by 19 electrode combinations for the sub-regions and 5 by 5 electrode combinations for the tunnels suggest strongest functional connectivity within CA3 and among the tunnels between CA3-CA1 or DG-CA3. In the adjacency matrix, each row and each column represent the cross-correlation coefficient obtained from the XCORR between pairs of electrodes in each sub-region and tunnels. Recording file: Single array: ECDGCA3CA1 19914 150805 150828 d25 5minspont0001.Figure File name: gamma_connectivity (EC, DG, CA3, CA1). fig

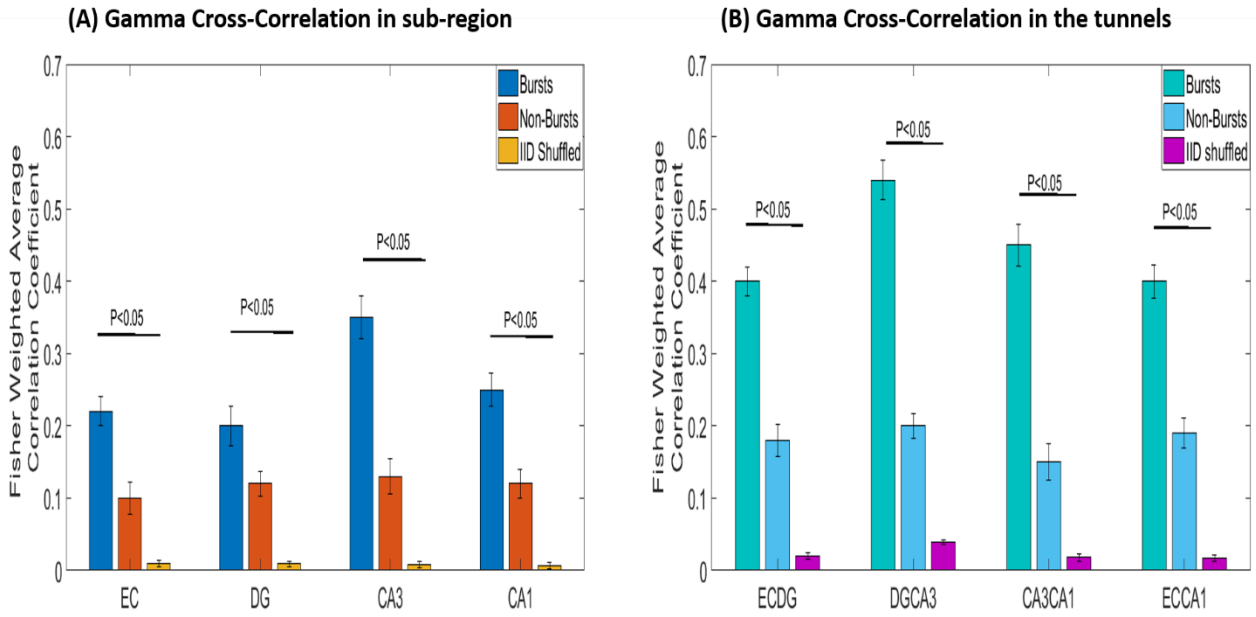

Figure 3.13. Sub-regional and axonal gamma correlations suggest coordinated flow of gamma oscillations during bursts. Significance test for gamma correlations in subregions and tunnels (a) Synchronization of gamma oscillations during bursts within a region were significantly greater than the non-burst segments with especially high burst correlation in CA3 compared to other sub-regions (N=5 Arrays); is. These measures were together above levels for IID randomly shuffled data; (P<0.05, ANOVA; N=5 Arrays) **(b)** Synchronization of gamma oscillations in axonal tunnels during bursts and non-bursts is higher in tunnels (P<0.05, ANOVA) indicating the transmission of information from the same source or parallel transmission of the same output. Gamma correlations within tunnels are significantly greater than shuffled data (P<0.05, ANOVA; N=5 Arrays). File name for this figure: gamma_region_BNB.fig, gamma_tunnel_BNB.fig

3.6 Slow wave correlations decrease as the inverse-square of the distance

The theta and gamma correlations between adjacent electrodes are spatially concentrated from the connectivity matrices (Fig 3.10, 3.12). Three types of relations might be expected for a decrease in correlated LFP power with distance from the origin. 1) For direct connections from a slow wave of varying membrane potential that propagates down an axon or dendrite, power would decrease according to the cable equation as 1/distance 2) For LFP's originating from synapses that fan out from a strong source in two dimensions, correlated power would follow an inverse-square law ($1/r^2$). 3) For LFP's originating from neuronal dipoles that source and sink currents, correlated propagation into the medium would follow an inverse cube law ($1/r^3$). To define the spatial relationship of the theta and gamma correlation, we determined correlations of LFP power vs distance between the electrodes as shown in Fig. 3.14, Fig 3.15 respectively. These functions were fit separately for both bursts, non-bursts theta and gamma correlations, allowing maximum goodness of fit and equal opportunity for different functions to describe the different frequencies. The best-fit is the one with the highest R-squared value using a least-squares method. For each component, the best-fit was chosen to represent the average falloff of correlation versus distance. To adequately capture the falloff, we used only pairwise correlations. The inverse square falloff pattern was a good fit for both theta and gamma band correlations, but the goodness of fit R^2 values for theta in CA3 appeared higher and gamma correlations were about half those of theta. The correlation analysis was repeated for all the sub-regions of the hippocampus for theta and gamma bands, again suggesting the same effect in the fall-off of correlation with increase in the inter-electrode distance for the theta and gamma bursts duration.

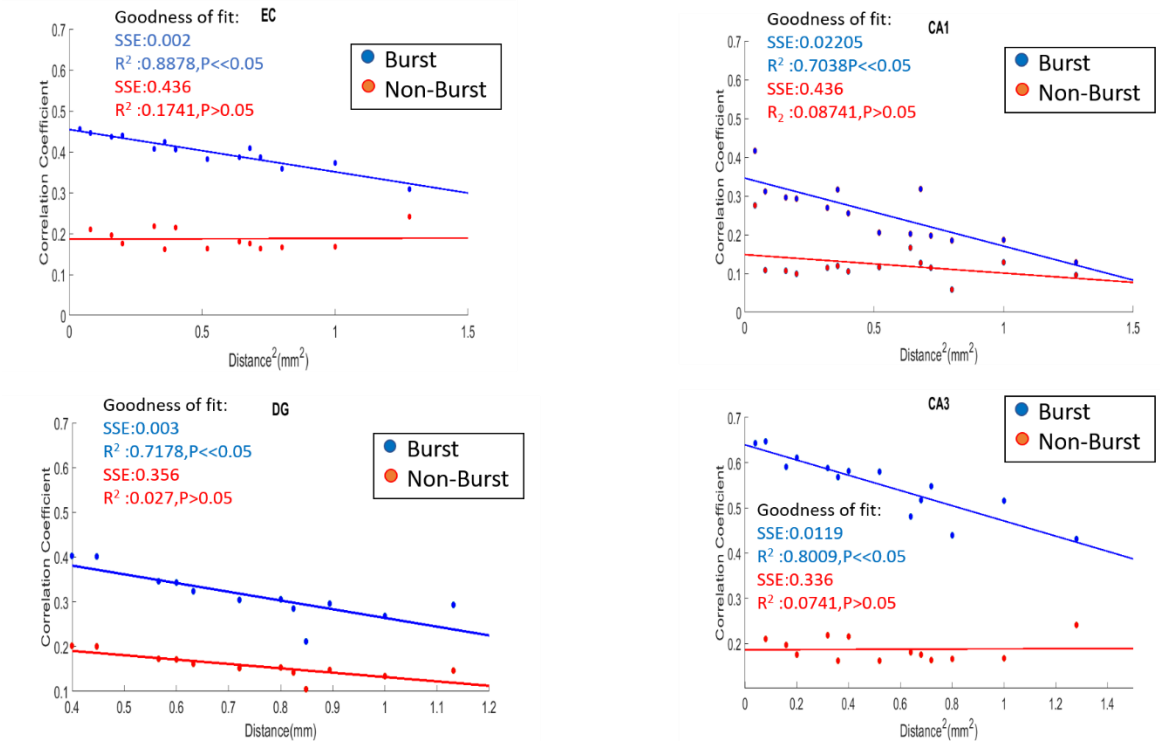

Figure 3.14: Theta Cross-Correlation coefficient declines between electrode pairs (n=5 arrays); Blue represents the burst theta correlation as a function of inter-electrode distance; The average correlation coefficient was computed at each unique distance; The CA3 region has the highest correlations for bursts and the CA1 the lowest, compared to the other regions. DG region has a best fit at correlation versus 1/distance and is different from the other sub-regions which vary as a function of 1/distance^2. Note: Red is theta correlation during non-bursts which is relatively independent of inter-electrode distance. There first observation at the shortest inter-electrode distance of 200 µm is 0.04 mm^2. File name for this figure is theta_distance.fig

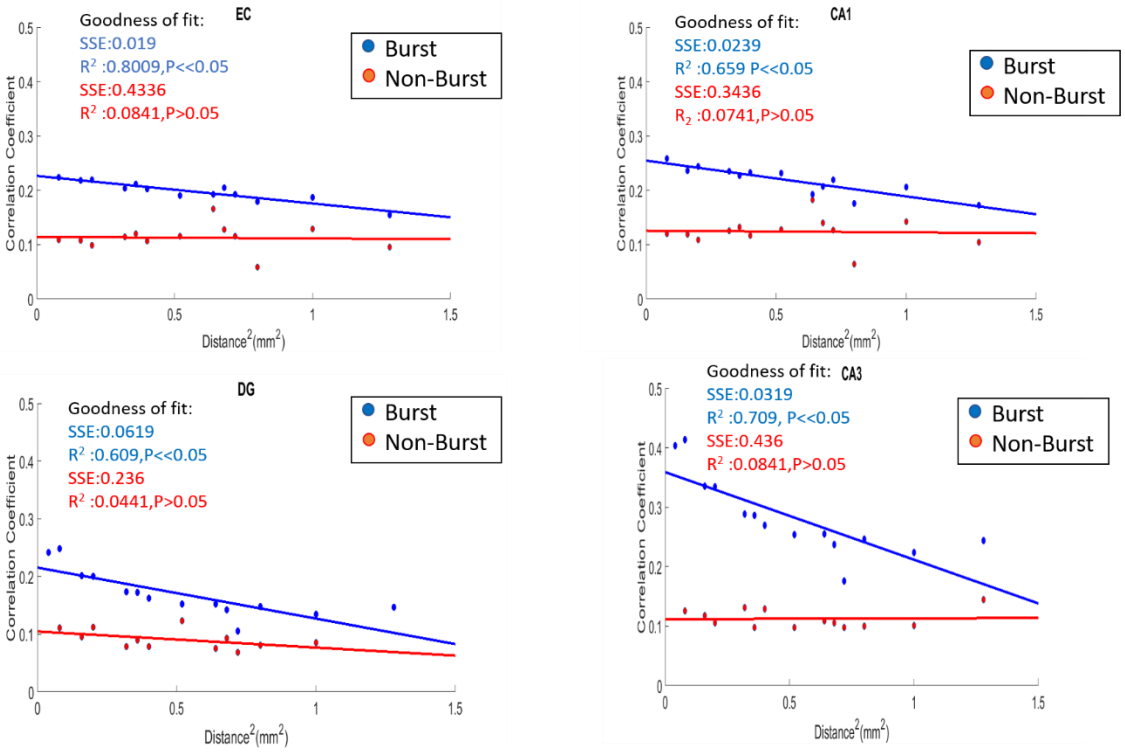

Figure 3.15: Gamma Cross-Correlation coefficient depends on the inverse-square of the spatial distance between electrode pairs (n=5 arrays); The average correlation coefficient was computed at each unique distance. Blue represents the burst theta correlation as a function of inter-electrode distance. Red is gamma correlation during non-bursts which is relatively independent of inter-electrode distance. Burst correlations with distance are highest in CA3 and lowest in DG. File name for this figure is gamma_distance.fig

However, the overall correlation of the gamma band across distance is lower than theta correlation as suggested by Fig 3.14, Fig 3.15. The line of best fit suggests that the relationship is consistent across sub-regions of the hippocampus, but the level of correlation differs depending on which frequency band is being represented and varies with the sub-region. The dependence of this correlation falloff function on anatomical sub-region appears strong in the CA3 with distance, also falloff is much stronger during burst than the non-bursts segments. The correlation in DG region however falls better at 1/r. The identification of functional areas of greater correlated power at certain frequencies with $1/r^2$ relationships suggests the fan out of the LFP from a strong source that is strong closer to the source and decrease with increase in inter-electrode distance.

Information processing and transmission comprises two stages (1) the computation that occurs in each of the subregion and (2) the transmission of the product of computation via the axons to the neighboring sub-region. In order to study the interaction between the sub-regions and the tunnels, the theta and gamma cross-correlation analysis was performed between the sub-region and the tunnels separated as source to tunnel and tunnel to target interactions as in Fig 3.16. An analog approach closely following Poli et al. [79] was used with LFP signals to compare the correlation between the LFP among axonal inputs (i.e. LFP in the tunnels) versus those among somata outputs (i.e. LFP in the target well). In pattern completion, the outputs are more similar than the inputs. In pattern separation, the outputs are less similar than the inputs [79]. In our system, higher correlations between axons in the tunnel (DG-CA3) and the target (CA3) than between the source (DG) and the axons (DGCA3) suggest a role of both theta and gamma LFP oscillations in pattern completion in CA3. A role for CA1 in pattern separation has been shown in animal models based on spike rates, but the contribution of LFP's is unknown [80]. We observed theta and gamma in the source (CA3) was more strongly correlated to the axons from CA3 to CA1)

than those of the axons from CA3 to CA1 to the target (CA1). This suggests a role of gamma and theta LFP's in pattern separation in the CA1 sub-region in support of the findings by Hanert et al [80].However, more rigorous analog and digital analysis must be carried to prove the involvement of CA1 in pattern separation

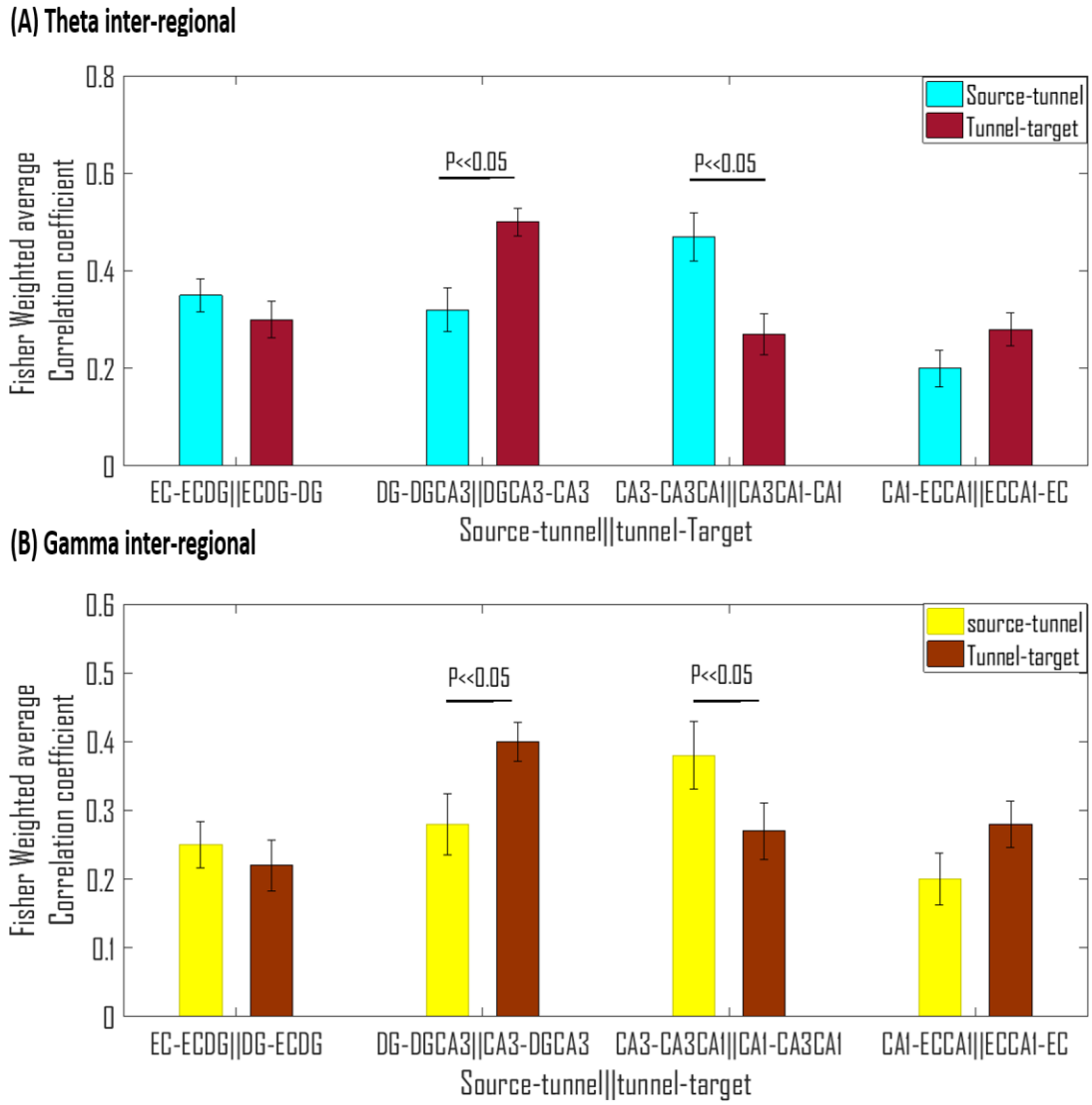

Figure 3.16: Correlation coefficient against the source to tunnel and tunnel to target interactions across the hippocampal sub-regions (a)Theta cross correlation and (b) Gamma Cross correlation. Note that high correlations between the tunnels from DGCA3 with CA3(Target) and CA3CA1 with CA3 (Source) sub-region. This might suggest a possible role of LFP synchrony in CA3 for pattern completion and in CA1 for pattern separation. File name for this figure is source_tunnel_target.fig

Chapter 4

DISCUSSION

The hippocampus is critically important to cognitive memory formation, as detailed from its role in anatomical, physiological, fMRI and behavioral studies. However, much remains to be learned in between the macro and single cell levels, specifically the encoding and transmission of information between subregions of the hippocampus. Working at the mesoscopic level also allows us to bridge the gap between the macroscopic measures of EEG, fMRI and the molecular levels. While microelectrodes and arrays have been used to monitor activity in behaving animals and in brain slices in in signal trains that filter out low frequency oscillations to focus on spikes [54], [55], there remains a need to develop a system that provides better access to the neuronal populations and enables single neuron monitoring of the axonal connectivity between the subregions and the source of the commonly used scalp EEG and intracranial ECoG field potentials. These methods rely on detection of local field potentials which are variously described as originating from parallel waves of fluctuating membrane potential, groups of neuronal spikes, collective synaptic potentials or volume conduction of aligned dipoles originating in the cortex and hippocampus [99]. Since all these mechanisms are unlikely to be simultaneously active, here, we have begun to identify which of these sources contribute most to different regions of a reconstructed hippocampus and question whether they are byproducts of the flow of spiking information or independent carriers of information.

The engineered living reconstruction of the hippocampal sub-regions used in this work is an extension of the 2 chambered device used by D Poli et al.,[61] and Bhattacharya et al.,[70]. We used a 4 chambered PDMS device, one for each sub-region (EC, DG, CA3, CA1) with micro-tunnel connectivity between the sub-regions thereby separating the axons from the cell bodies. The

5 x 10 μm micro-tunnels allow axonal growth and inhibit the migration of 15 μm diameter cell bodies or traversal by dendrites[62], [63] allowing insight into the role of axons in inter-regional communication. The uniqueness of this system thus enables us to separate the computations carried out in the sub-regions from the product of those computation in the axons and hence its transmission into the neighboring sub-regions. In vitro neuronal cell-culture preparations used in combination with this technology thus provides a straight forward way to manipulate and study the effects of connection strength upon network[81], [82]. The 4 chambered device over a standard MEA120 electrode thus allows the quantitation of the LFP oscillations, their dynamics and transmission at each step of the tri-synaptic circuit. In addition, it also provides the means to directly manipulate the structural properties of networks while simultaneously monitoring its effect on a network's functional dynamics. This device thus provides researchers a tool to understand network dynamics by having the ability to design both the degree and directionality of connectivity among multiple small neural populations.

Quantification of connection strength is one of the most fundamental steps towards a better understanding of a brain network's function [83], [84]. Coordinated activity within the hippocampal formation is thought to facilitate information flow and underlie information storage and is observable at multiple levels, from synchronized firing of individual neurons to population level oscillations at different frequencies [85]. For years we have known that neurons collectively have synchronous or oscillatory patterns of activity, the frequencies and temporal dynamics of which are associated with distinct behavioral states. Although the function of these oscillations has remained obscure, recent experimental and theoretical results indicate that correlated fluctuations might be important for a number of processes, such as attention, memory and learning, that control the flow of information in the brain. Synchrony is a form of temporal relationship between neurons

that has been intensely studied. As with ‘oscillations’ and ‘rhythmic activity’, the term synchrony encompasses a spectrum of neuronal behaviors with various spatial and temporal scales. Salinas and Sejnowski [86] labeled all these phenomena as temporally correlated activity, which describes a common feature: when two neurons are correlated, they do not fire independently of each other; when one fires, the other is more or less likely to fire. This is an extremely broad generalization, especially as the underlying mechanisms and potential functions can vary greatly. In order to understand the underlying mechanism, this work is based on analyzing the synchrony of the LFP oscillations at the population level which is considered as a possible mechanism to bind the cell assemblies together. The theta (4-11 Hz) and gamma (30-100 Hz) bands of oscillations were filtered and segmented into bursts and non-burst durations. We investigated the main hypothesis that LFP oscillations (theta and gamma) enable computation and communication in hippocampal networks, focusing on the cell assemblies (within a sub-region) and the axonal LFP (in the tunnels) and the relationship between the cell assemblies in the sub-region and the axonal tunnels. Donald Hebb hypothesized that the fundamental unit of brain operation is not the single neuron but rather the cell assembly-an anatomically dispersed but functionally integrated ensemble of neurons [87]. Despite the theoretical appeal of Hebb's idea and growing empirical evidence of assemblies, it remains unclear how diverse groups of neurons transiently coordinate their activity to form cell assemblies or functional networks [88]. This work addresses the question by considering that the individual neurons that compose an assembly act as a single functional unit through coordinated network activity which is one of the hypothesized roles of the LFP oscillations. The temporal synchrony in the neural oscillations are evidence to support a causal role for LFP synchrony to cause coordinated activity in the sub-regional neuronal population. In addition, according to the ‘cell assembly’ hypothesis, this transient synchrony of anatomically distributed groups of

neurons underlies information processing and cognitive mechanisms[87]. Brain rhythms may play a key role in coordinating neuronal ensembles[89], with a dynamic hierarchy of neuronal oscillations modulating local computation and long-range communication[90]. This hypothesis is supported by evidence that spiking activity depends on the local field potential (LFP) in the hippocampus[21], [91]. This study is also in agreement with the above hypothesis, whereby we see the spike of bursts associated with the LFP oscillations (Fig 3-8). Future studies will involve a more specific interventions and analysis of the relationship of the spikes with the LFP oscillations to definitively establish that LFP oscillations cause the spikes.

Spatial spread of LFP

This study also addressed the spatial spread function of the LFP signals towards understanding the relationship of neural frequency to information transmission. A current trend in the use of extracellular electrodes for *in vitro* and *in vivo* recordings of neuronal electrical activity is to increase spatio-temporal resolution to capture the dynamics of individual neurons or interactions within neuronal networks. We quantitatively defined a common inverse squared relationship of neural signal cross-correlation and spatial distance. with spike, but not with non-burst activity. The magnitude of an electric field from a point source or the fan out of dendritic branching and synapse in our planar network is expected to decrease as a function of one over distance squared [92]. In contrast, the field along an axon cable is expected to decrease as 1/distance and from the dipole source of aligned neurons as $1/\text{distance}^3$.

The major contributions to the observed spatial correlations, not including artifact or a common reference could be one of the following reasons. Firstly, there are functional correlations in neural networks due to synaptic connectivity. These functional correlations could appear in regions far away from each other because networks are not constrained to neighboring areas.

Secondly, there are correlations due to the properties of the underlying neurophysiological generators of electric signals, both spike after-potentials and EPSP's [93] [99]. However, the neural origins of the various frequency bands are still not clear. Importantly, our finding of stronger falloff of correlations during bursts activity suggests that LFP oscillations during bursts may add specificity to functional connectivity within the hippocampal sub-regions or that these oscillations may cause the bursts.

Origin of LFP's

Understanding the origin of these neural oscillations and significance of the synchrony among these LFP oscillations is equally important. The inverse problem theory helps in understanding both the synchrony and origin of the LFP oscillations. The inverse problem [94] arises when attempting to infer the microscopic variables from the macroscopic ones - in this case, inferring the origin of the LFP oscillations from the spatiotemporal profile, synchrony of the LFP - the underlying neuronal dynamics. Our findings suggest that theta or gamma band neural activity could possibly drive synchronization during information processing. The "gamma frequency hypothesis" implies that synchronized activity in the gamma range induces memory processes more successfully than both slower (e.g., beta-) and faster (e.g., ripple-) activity[95]. It has been proposed that neural firing synchrony may accomplish attentional selection in an effective manner, in particular perceptual selection[96]. Gamma synchronization during bursts observed in our study also supports Fell et al. [97] who reported phase synchronization of gamma activity not only seems to be a general mechanism underlying cortical information processing, but also appears to be particularly involved in attentional processes. Several studies in addition also report similar ideas about gamma synchronization being related to attention. Synchronization is thus an appealing mechanism for explaining how the hippocampus stores memories, processes

information, and it generally occurs on different timescales-or frequencies-of neural activity. In particular, gamma-band (30–100 Hz) synchronization is frequently invoked as a means for the hippocampus to communicate between sub-regions, since the fast nature of an oscillatory gamma signal is timed appropriately for rapid perceptual operations or induction of synaptic strengthening. Precise synchronization of neural firing within the millisecond range is associated with synchronization of gamma activity. However, the exact role of synchronized gamma activity with respect to different aspects of attentional processing like top-down versus bottom-up processing, early versus late selection, spatial versus object-based attention is unclear. Gamma synchronization between hippocampal and para-hippocampal regions have also shown to induce long term potentiation (LTP) in the CA3 region of the hippocampus[95]. Studies also suggest that synchronized neural activity in the gamma frequency range (around 30–100 Hz) plays a functional role for the formation of declarative long-term memories in humans. In the future, injecting gamma oscillations into the network to evoke greater synchronization or antiphase to inhibit it can test a causal gamma mechanism enabling transient associations of neural assemblies which thus may play a central role in information processing.

Synchrony of theta oscillations is necessary to understand the underlying mechanisms of hippocampal theta oscillations that play critical roles in higher brain functions, including spatial and episodic memory formation [36], [38]. They coordinate activities among hippocampus-associated brain regions, including entorhinal cortex and neocortex. Theta oscillations are also postulated to provide temporal references in the hippocampus and, thus, provide a potential mechanism for temporal coding of the relationships for spatial memories or events for episodic memories [1], [38], [98]. However, after decades of research, the origins of theta oscillations remain elusive. In order to guarantee a defined temporal order of memory processing,

synchronization in the gamma frequency range must be accompanied by ongoing theta oscillations. Theta oscillations are suggested to coordinate the encoding and retrieval of episodic and spatial memories [23]. Recent studies suggest that the theta frequency pacing of network excitability can provide temporal packaging and transfer of neuronal information.

LFPs in the brain were long thought to be primarily of synaptic origin [99]. As a consequence, many modeling studies focused on the extracellular fields induced by postsynaptic currents on the dendrites and the soma of a neuron [100], [101]. However, recent analyses and modeling efforts by Ray and Maunsell et al. [102], Schomburg et al. [103] and Ness et al. [104] have revealed that active, non-synaptic membrane currents can play an important role in generating population-level LFPs. Currents from the axon are still thought to be so small as to be of minor importance for the LFPs. However, our work has shown for the first time the clear presence of LFPs in the axons and a significant correlation between these LFP and bursts of spike. The contribution of the axonal currents to LFP has also been studied by McColgan et al. [105] and supports that axonal projections can contribute substantially to LFPs. The results quantitatively showed how the anatomy of axon terminal zones and the activity in axons determine its frequency-specific far-field contribution to the EFP. More importantly, their presence emanating from axons strongly suggests oscillations in membrane currents that could contribute to information processing and binding signals from different axons together to activate cell assemblies.

Electrochemical signal propagation is one of the properties that enables communication among neurons. In the hippocampus, such communication is essential for basic neural function, such as memorization or spatial navigation. To understand the mechanisms that regulate temporal coordination of neuronal activities in the hippocampus sub-regions and inter-regional

communication between them, we simultaneously recorded LFP and multiple single neuronal activities temporal relationships of neuronal activity between different subregions using the measure of cross-correlation. Computation of cross-correlation between the LFP oscillations and estimating the delay between the electrodes in the sub-regions and tunnels displayed non-zero delays of x-y ms. This could be supported by the studies by Mizuseki et al., [98] on principal cells in several mono-synaptically connected layers/regions fired with significantly longer theta phase offsets and temporal delays than would be expected by axonal conduction times. Synaptic delays and passive synaptic integration which could be attributed to the temporal windows set by the theta cycles allow for local circuit interactions. Qui et al. [106] also provide an explanation showing that neural signals can propagate by means other than synaptic transmission, gap junction, or diffusion. The population activity in a downstream layer not only reflects an upstream drive but also represents the result of autonomous local computation as can be seen from a cross-correlation analysis comparing the source to tunnel and tunnel to target interactions significantly noted between the DG - CA3 subregions and CA3 - CA1 subregions.

This study thus provides a detailed analysis showing that the synchrony of the neural oscillations is important for information processing and transmission. It also suggests a means for hippocampal information processing by binding of cell assemblies via slow wave synchrony.

Chapter 5

5.1 FUTURE WORK

Having studied the roles of theta and gamma synchrony in the hippocampal sub-regions separately. It is also important to understand the interaction of the theta and gamma oscillations since they are observed to co-exist in the hippocampal sub-regions. These oscillations interact by a mechanism called the cross-frequency coupling. This mechanism has also been suggested to form a code in order to represent multiple items in an ordered way as suggested by Lisman and Jensen [107]. Recent work also suggests that this coding scheme is important for the inter-regional communication and memory processes. Our 4 chambered hippocampal system will thus a provide a unique way to understand the cross-frequency coupling in the sub-regions and the axonal tunnels separately.

The origins of the LFP oscillation will also provide important step towards understanding the signal processing and routing of information in the hippocampus. Analyzing the phase relationships between the LFP oscillations and spikes will help establish causal link between them and if it is necessary for the network to produce the LFP oscillations for the propagation of the information.

Further, understanding the contribution of each individual sub-region will help to elucidate the role of each individual sub-region of the hippocampal sub-region in encoding and decoding of the information, memory formation and other cognitive processes. This can be done by omitting one of the 4 sub-regions to see the changes in the synchrony of the LFP oscillations in each of the sub-regions and tunnels.

5.2 LIMITATIONS

Despite the increase in use of such engineered models to elucidate the neuronal mechanisms and study of the neuronal networks, limitations persist since these 2D cultures do not take into consideration the 3D aspects of the cells in vivo. Often cell morphology and the growth of the axonal processes are restricted in a 2D cell culture and thus there is a shift to development of 3D cell cultures to better elucidate the computational and neurophysiological behavior of networks. Such 3D in vitro models are developed and investigated by many researchers [108], [109]. Future works will shift to using a 3D polydimethylsiloxane (PDMS) device to develop 3D cell cultures and study their computational and neurophysiological behaviors from a closer replica of the in vivo cell morphology and connections. Therefore, our findings encourage further analysis of the contribution to information processing and transmission processes among the cultured hippocampal neurons of the other sub-region through the analysis of theta and gamma coupling investigations and to understand the origin of these neural dynamics to decipher whether the LFP and the spikes are independent or if one causes the other.

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