The Functional Anatomy of Hippocampal Theta and Gamma Oscillations

by

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Abstract of the Dissertation

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This dissertation investigated how theta (4-10Hz) and gamma (40-100Hz) oscillations are coordinated across different hippocampal subnetworks during different behaviors. Using 96-site silicon probes to simultaneously record local field potentials (LFPs) and unit activity from dendritic and somatic layers of the dentate gyrus, CA3 and CA1 regions, I examined how local field potentials (LFPs) and unit activity changed the theta and gamma synchronization of hippocampal networks as a function of behavior. Specifically, I compared hippocampal activity of rats during performance of a hippocampus-dependent delayed spatial alternation task on a modified T-maze versus performance of non-mnemonic control tasks. I also compared hippocampal network activity during active waking behavior versus rapid eye movement (REM) sleep.

The first study examined how theta oscillations throughout the hippocampal subnetworks change during behavioral task performance. Although theta oscillations were generally highly coherent throughout the system, I found that the power, coherence and phase of theta oscillations fluctuated in a layer-specific manner, suggesting the presence of

multiple interdependent dipoles. I also found layer-specific changes in theta power and coherence during different portions of both alternation and control tasks, as well as a decreased phase lag between CA3 and CA1 theta oscillations on the center arm of the T-maze compared to other segments of the alternation and control tasks.

The second study examined changes in hippocampal gamma oscillations during performance of the alternation and control tasks. This study found that on the center arm of the T-maze, the power and coherence of gamma oscillations at the CA3-CA1 interface increased compared to other segments in the alternation task or compared to control task segments.

The third study examined changes in theta and gamma coordination of hippocampal networks during active waking versus REM sleep. I found increased dentate/CA3 theta and gamma synchrony, but decreased CA3-CA1 gamma coordination during REM sleep. I also detected phasic bursts of LFP power in the dentate molecular layer that divided REM sleep into phasic and tonic periods. Although tonic periods characterized the majority of REM sleep epochs (~95%), phasic periods exhibited transient increases in theta and gamma coordination among all of the hippocampal subregions.

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Section 1.0 - Introduction

1.1 - Overview

Coherent oscillations of cell assemblies have been proposed as an optimal mechanism for encoding and retrieving temporal information (Hasselmo et al. 2002; Lisman and Idiart 1995; Lisman 1999; Wallenstein et al. 1998). Theta (4-10Hz) and gamma (40-100Hz) oscillations are prominent network patterns in the rat hippocampus, thought to reveal synchronization between afferent and intrinsic hippocampal networks underlying the computational operation of the system (Buzsaki 2002; Buzsaki et al. 2003; Buzsaki and Draguhn 2004; Leung 1998). In humans, theta and gamma oscillations have been specifically associated with memory encoding and retrieval (Lee et al. 2005a; Raghavachari et al. 2001; Sederberg et al. 2003; Sederberg et al. 2007a). The amplitude of hippocampal oscillations (Berry and Seager 2001; Ekstrom et al. 2007; Kahana et al. 2001; Sinnamon 2005; Sinnamon 2006; Wyble et al. 2004) and the coherence between cortical and hippocampal oscillations (Fell et al. 2001; Fell et al. 2003a) have been shown to vary with task performance. The precise relationship between spiking of hippocampal neurons to the phase of hippocampal theta has been shown to carry information about spatial behavior (O'Keefe and Recce 1993; Skaggs et al. 1996) beyond the information carried by firing rate alone (Huxter et al. 2003; Jensen and Lisman 2000). Additionally, hippocampal cell assemblies coding for spatial position are coordinated at the time scale of gamma oscillations (Harris et al. 2003). Moreover, it has been shown that LTP is preferentially induced by stimulation at theta frequency (Greenstein et al. 1988; Larson and Lynch 1986) and depending on the phase of ongoing theta oscillations, stimulation can produce either LTP or LTD in hippocampal pyramidal cells (Holscher et al. 1997;

Huerta and Lisman 1995; Hyman et al. 2003).

The importance of theta and gamma oscillations in plasticity, transfer and coding suggests that these network rhythms offer a unique window into the manner by which hippocampal networks coordinate within and between different regions to perform diverse functions of the system. This thesis describes a set of experiments examining the anatomical distribution of theta and gamma oscillations throughout the different layers and regions of the hippocampus to investigate how the coordination of hippocampal networks changes during different behaviors. This work is introduced by a brief overview of the anatomy of the hippocampus, with a very basic outline of the stage and players in the hippocampal networks. Following is an introduction to the physiology of the hippocampal system and how coordinated theta and gamma oscillations emerge from the interplay between hippocampal principal cells and interneurons. Finally, I review the mnemonic role of the hippocampal formation. This includes a description of previously observed behavioral correlates of hippocampal theta and gamma oscillations, an overview of REM sleep, and support for specific functional roles of individual hippocampal subregions.

1.2 - Hippocampal Anatomy

The hippocampus is a three-layer cortical structure in the mammalian brain that receives convergent information from subcortical and polymodal association cortices (Amaral and Witter 1995). The primary cortical input to the hippocampus comes from the entorhinal cortex (EC), which receives olfactory (Haberly 2001) and gustatory (Kosar et al. 1986)

signals via the perirhinal cortex and visual input via the postrhinal cortex (Burwell and Amaral 1998). This broad convergence of inputs to the hippocampal formation offers a somewhat unique position in the brain to integrate diverse information and generate rich representations of experience.

1.2.1 - The tri-synaptic pathway

The hippocampus proper is commonly divided into CA3 and CA1 subregions which combine with the dentate gyrus to form a mainly feed-forward excitatory "tri-synaptic path" and a loop with the entorhinal cortex (Figure 1.1; Andersen et al. 1971 c.f. Amaral and Witter 1995). However, each of these subregions exhibit a laminar structure (Ramón y Cajal 1995) with specific external and recurrent inputs that set the stage for a diversity of neuronal computations. Layer II of the lateral and medial entorhinal cortex projects to the middle and outer dentate molecular layer (DGml), respectively, onto the dendrites of granule cells (Steward 1976). Granule cells, in turn, project large and powerful "mossy fiber" synapses onto the proximal apical dendrites of CA3 pyramidal cells in the stratum lucidum (Blackstad et al. 1970). CA3 pyramidal cells project to the CA1 stratum radiatum (CA1sr) on the middle dendrites of CA1 pyramidal cells (Ishizuka et al. 1990). CA1 then projects back to deep layers V and VI of the entorhinal cortex (van Groen and Wyss 1990) and then onto layer II and III neurons, thus completing the polymodal association "loop".

1.2.2 - More loops and shortcuts

In addition to this basic loop, the hippocampus also has a number of smaller excitatory

recurrent loops and short-cuts that can serve to integrate information and to self-organize population activity patterns (Lisman 1999; Traub et al. 2004). The most prominent excitatory loop is within the CA3 region, with CA3 pyramidal cells projecting back onto the CA3 middle dendritic layer in stratum radiatum, forming the single most numerous excitatory synaptic input to the region (Amaral and Witter 1995). Dentate granule cells form a disynaptic excitatory loop, projecting to mossy cells in the hilar region of the dentate gyrus (DGhr; Scharfman et al. 1990), which in turn project back to the dendrites of granule cells in the inner molecular layer of the dentate gyrus (Jackson and Scharfman 1996). Furthermore, the CA3 region also forms an excitatory loop with the dentate gyrus by projecting to granule cell dendrites in the inner molecular layer (Li et al. 1994) and possibly onto dentate mossy cells (Ishizuka et al. 1990). These recurrent loops are thought to form the basis for self-organizing network rhythms (Buzsaki 2002; Traub et al. 2004) and for auto-associative memory formation (Marr 1971; Rolls 1996; Treves 1995) within the hippocampal networks. There are also short-cuts through the tri-synaptic loop from Layer II EC to the distal pyramidal cell dendrites in CA3 and from layer III EC to the distal dendrites in CA1 stratum lacunosum moleculare (CA1lm; Steward 1976).

1.2.3 - A brief overview of inhibitory interneurons

In addition to the layer-specific excitatory pathways through the hippocampal formation, numerous specialized inhibitory interneurons shape the rhythmic activity patterns of the hippocampal network. Although there are notable differences between the dentate gyrus and CA regions, there are many interneurons that share common features among the different hippocampal regions. Like all cortical structures, each hippocampal region

contains multiple types of basket interneurons with cell bodies typically in or near the principal cell body layer, a dendritic extension similar to that of the principal cells, and formation of a basket-like axonal plexus around the somata of principal cells (Sik et al. 1995). Chandelier cells, also called axo-axonic cells, are similar in gross morphology in many ways to basket cells, but chandelier cells target principal cell axon initial segments (Somogyi et al. 1985), which may be directed specifically toward aborting neuronal output, while maintaining within-cell functions, such as back-propagation of action potentials to the dendrites (Somogyi and Klausberger 2005).

In addition, there are a number of dendritic targeting interneurons. Oriens lacunosum moleculare (O-LM) interneurons in CA1/CA3 and hilar perforant path-associated (HIPP) cells in the dentate gyrus have cell bodies and dendrites in the basal dendritic layer of principal cells and target the distal dendrites of principal cells in CA1/CA3lm and DGml, respectively (Freund and Buzsaki 1996). Another group of interneurons provide feed-forward inhibition of EC input to the distal dendrites in CA1lm and their counterpart, the MOPP (molecular layer perforant-path associated) cells, in the dentate gyrus (Freund and Buzsaki 1996). These inhibitory cells appear to preempt the incoming excitatory input directly to the principal cell dendrites (Buzsaki 1984), via fast acting AMPA receptors lacking GluR2 subunits (McBain and Fisahn 2001). HICAP (hilar commissural-associational pathway related) have cell bodies near the basal border of the somatic layers, with dendritic extension similar to principal cells, but with axons that target the mid-apical dendrites of principal cells (Sik et al. 1997). Although there may not be an exact analog to HICAP cells in the CA regions, the so-called bistratified cell has similar

somatic and dendritic arrangement, but in addition to targeting mid-apical dendrites with its axons, it also targets the basal dendritic layer stratum oriens (Sik et al. 1995).

Other interneurons in CA1 that do not yet have known analogs in the dentate gyrus include trilaminar cells (Sik et al. 1995), interneuron-selective interneurons (Acsady et al. 1996; Gulyas et al. 1996), back-projection interneurons from CA1 to CA3 (Sik et al. 1995), and interneurons at the CA1lm/sr border that target CA1sr/or dendrites. In fact, using dendritic and cell body location in combination with axonal targets as well as calcium buffers, peptides, and receptors expressed by the cells, some investigators have identified 16 distinct interneuron classes in CA1 alone (Somogyi and Klausberger 2005; Klausberger and Somogyi 2008). Although most systems neuroscientists suggest that it is the axonal target rather than cell body or even dendritic location that has the greatest impact on cell function (Dumitriu et al. 2007), it is likely that the great diversity of interneurons in the hippocampus play a crucial role in shaping the oscillatory activity and information processing of hippocampal networks.

1.2.4 - Subcortical input to the hippocampal formation

In addition to the prominent input from the entorhinal cortex, hippocampal networks receive substantial input from various subcortical nuclei. The most robust subcortical projection comes from the medial septum and diagonal band of Broca (MSDB), which projects both cholinergic and GABAergic fibers to most layers of the hippocampal formation (Amaral and Witter 1995). The locus coeruleus also projects fibers releasing norepinephrine (NE) broadly to many layers of the hippocampus and dentate gyrus, with

the densest projections to the hilus of the dentate gyrus, stratum lucidum of CA3 and stratum lacunosum-moleculare of CA3 and CA1 (Oleskevich et al. 1989). Serotonin (5HT) projections from the raphe nuclei are also widely distributed throughout hippocampal layers but especially dense in parts of the dentate hilar region and CA3/CA1lm (Amaral and Witter 1995; Vertes et al. 1999). Although some of these projections result in synaptic contacts, evidence suggests that in some areas, particularly stratum lacunosum-moleculare, there may be non-synaptic "loose-in-the-juice" modulation of local networks (Vizi and Kiss 1998). Dopamine fibers innervate the hippocampus very sparsely (Amaral and Witter 1995) and may primarily act at potassium channels (Pedarzani and Storm 1995). In addition to brainstem nuclei, hypothalamic nuclei, including the supramammilary nucleus, project predominantly to the dentate granule layer and inner third of the molecular layer (Vertes 1992). Further inputs terminate in CA1lm from the nucleus reuniens of the thalamus (Wouterlood et al. 1990) and from the amygdala to the ventral portions of CA1lm (Amaral and Witter 1995). As discussed in greater detail below, these various subcortical nuclei likely contribute to the generation and modulation of theta and gamma oscillations among the various hippocampal networks.

1.2.5 - Hippocampal efferents

The hippocampus targets numerous output structures that shape the functional role of the hippocampus in the brain. Although there are some efferent projections from other hippocampal regions, the lion's share come from CA1 (Amaral and Witter 1995). The output targets of the hippocampus vary systematically from the septal (also called dorsal

in rat and posterior/caudal in human) to the temporal poles (ventral in rat, anterior/rostral in human). A major output pathway of the hippocampus is in the deep layers of the entorhinal cortex, which in turn project to sensory association perirhinal and postrhinal cortices in the pathway from the septal pole of the hippocampus and to subcortical nuclei in the pathway from the temporal pole (Burwell 2000). Another major hippocampal output is via the subiculum, which projects to the nucleus accumbens, nucleus reuniens, mammilary nuclei, lateral septum and perirhinal cortex (Amaral and Witter 1995; van Groen and Wyss 1990). The septal two-thirds of the subiculum also sends projections to the retrosplenial cortex, while the more temporal portions project to the prefrontal cortices and the amygdala. The temporal one-third of CA1 also projects directly to the olfactory nucleus, olfactory bulb, lateral septal nucleus, nucleus accumbens, prefrontal cortex, amygdala and the hypothalamus (van Groen and Wyss 1990). Several hippocampal regions also send GABAergic projections to the MSDB, which may play an important role in coordinating the rhythm of theta oscillations (Freund and Buzsaki 1996).

1.3 - Hippocampal Physiology

Building on the anatomical framework described above, in this section I will review the basic physiological properties of neuronal activity and how groups of neurons cooperate to generate population patterns of activity.

1.3.1 - Unit activity

Information about the activity of single neurons in the hippocampus comes from both

intracellular and extracellular recording techniques. Intracellular and somatic and dentritic patch techniques have provided a great deal of information about the cellular mechanisms that underlie the integration inputs for spike generation. Likewise, extracellular recordings have provided a wealth of information about the manner in which populations of neurons change their collective activity in awake and freely behaving animals. Ranck (Ranck 1973) first characterized the difference between firing properties of interneurons in the hippocampus, firing tonically at 20+Hz, and principal cells that typically fire at low rates (~1Hz) with occasional complex spike bursts of 150Hz or more. Since then, it has been identified that pyramidal cells typically have wider action potentials than do interneurons (Csicsvari et al. 1999; Henze et al. 2000). While these gross characteristics of unit firing in the hippocampus have been useful to separate principal cells and interneurons using extracellular recordings (Csicsvari et al. 1999), more recent *in vivo* juxtacellular recordings have revealed a wide range of interneuron activity profiles in the hippocampus (Somogyi and Klausberger 2005; Klausberger and Somogyi 2008; Klausberger et al. 2003), each likely contributing a different voice to the activity of the network. Recent evidence further suggests that CA1 pyramidal cells may functionally segregate to play different roles in the ongoing network activity (Senior et al. 2008).

1.3.2 - Local field potential (LFP)

The summation of ionic currents across cellular membranes in the brain leads to large voltage fluctuations. These ionic fluxes can be generated from a number of sources including sodium spikes, calcium spikes, burst-induced hyperpolarization, intrinsic

voltage-dependent oscillations, and synaptic activity (Buzsaki et al. 2003). Action potentials generated from sodium spikes generate a large ion flux, but due to the short duration (<2 msec) and the capacitative (low-pass) filtering of the extracellular medium (but see Logothetis et al. 2007), these potentials do not exhibit a high degree of temporal or spatial summation, and therefore do not typically contribute substantially to the LFP. Calcium spikes, typically generated in the dendrites and much longer in duration (several tens of milliseconds), however, exhibit greater temporal and spatial summation properties and may contribute to the LFP more substantially (Magee and Johnston 1995; Schiller et al. 1997). Neuronal bursting can further generate an after-hyperpolarization resulting from the opening of calcium-mediated potassium channels (Hu et al. 2007). Intrinsic voltage-dependent membrane oscillations have been observed in hippocampal pyramidal cells (Hu et al. 2007; Kamondi et al. 1998; Leung and Yim 1986) that may contribute to the LFP provided that these fluctuations have some mechanism to be coordinated in time across neuronal populations to effectively summate. Synaptic activity, generating ionic flux across ligand-gated channels, is commonly believed to be the most significant source of LFP fluctuations (Buzsaki et al. 2003). Depending on the ionic nature of the synaptic activity, EPSPs generated by inward current or IPSPs generated by outward current create local sinks or sources, respectively, in the extracellular space lasting tens to one hundred milliseconds or more. Because the output of many neurons within neuronal networks are commonly synchronized in time (Csicsvari et al. 1999) and these outputs in cortical regions typically target specific anatomical layers with a parallel arrangement of long dendritic arbors, the ionic fluxes can create extracellular voltage fluctuations of up 2 millivolts in the rat.

1.4 - Hippocampal Network Activity

1.4.1 - Large-amplitude irregular activity

Based on the LFP and unit firing activity of neurons in the hippocampus, distinct states have been identified in the hippocampus. Large-amplitude irregular activity (LIA) accompanies "automatic" and consummatory behaviors such as grooming and eating, as well as the slow-wave stages of sleep (Buzsaki 1989; Vanderwolf 1969). LIA in the hippocampus is characterized by variable periods (100msec-10sec) of relative inactivity among pyramidal neurons punctuated by strong bursts of activity originating in the CA3 region (Csicsvari et al. 2000). The coordinated burst of CA3 pyramidal cells generates EPSP-related influx of ions into CA1 cells, creating a large (1-3mV) extracellular negativity in CA1 stratum radiatum that persists for roughly 100 milliseconds (see Figures 3.1, 4.1). This depolarization leads to the discharge of CA1 pyramidal cells and a brief emergence of a 120-200Hz "ripple" oscillation in the CA1 pyramidal layer generated by the synchronized feedback firing of local basket interneurons for the duration of the sharpwave induced depolarization of pyramidal cells (Buzsaki 1989; Buzsaki et al. 1992). These sharpwave/ripple complexes have been found to be correlated with up-down state transitions in the cortex (Isomura et al. 2006) and are believed to aid the transfer of processed packets of information to the cortex (Chrobak and Buzsaki 1994; Siapas and Wilson 1998; Ylinen et al. 1995a).

1.4.2 - Theta oscillations

Theta oscillations (4-10Hz) in the local field potential of the hippocampus, also called

rhythmic slow activity (RSA), appear in the presence of voluntary movements, arousal, and during REM sleep (Vanderwolf 1969; Bland 1986; Green and Arduini 1954; Sainsbury 1998). Theta oscillations have been seen in all mammals studied to date, including humans (Buzsaki 2002; Kahana et al. 2001), although the prevalence of theta during immobile arousal states (Vanderwolf 1969; Green and Arduini 1954), and possibly REM sleep (Bodizs et al. 2001; Cantero et al. 2003), varies to a large extent across species. Although theta oscillations are largest in CA1lm (Kamondi et al. 1998), they are present in every hippocampal region and have been observed in other structures, including the subiculum (Anderson and O'Mara 2003), neocortex (Kahana et al. 2001), amygdala (Pare et al. 2002), striatum (DeCoteau et al. 2007), medial septum (Vinogradova 1995), and hypothalamic nuclei (Vertes and Kocsis 1997), but the local generation must be carefully investigated to rule out effects of volume conduction (Sirota et al. In press).

1.4.3 - Mechanisms of theta oscillations

In understanding the cellular mechanisms that generate theta oscillations, it is important to distinguish the role of *current* generators and *rhythm* generators. Current generation refers to the mechanisms that create ionic flux (e.g. EPSPs and IPSPs), while rhythm generation refers to the network mechanisms that set the pace of the oscillatory current fluctuations. It has been previously observed that LFP theta oscillations exhibit a smooth phase shift across the different anatomical layers in the dorsal ventral axis of the hippocampus from CA1sp to the dentate gyrus (Kamondi et al. 1998; Bragin et al. 1995a; Lee et al. 1994; Fig. 2.2). Because a single current source would be expected to exhibit an

abrupt 180-degree phase shift, this result suggests at least two current generators, which is supported by studies showing that lesions of the entorhinal cortex and systemic injections of NMDA blockers reduce theta oscillations in some layers of the hippocampus, but not in other layers (Kamondi et al. 1998). Calculating the current source density (Mitzdorf 1985) to remove effects of passively conducted signals through the extracellular medium suggests an even greater number of current dipoles (Kamondi et al. 1998; Brankack et al. 1993; Buzsaki et al. 1986). To the extent that populations of similar neuronal types in the hippocampus work cohesively, it is plausible that there are as many current generators of theta oscillations as there are groups of neuronal types that exhibit layer specific projections and similar theta phase preference (Buzsaki 2002; Somogyi and Klausberger 2005; Csicsvari et al. 1999; Buzsaki et al. 1983; Ylinen et al. 1995b).

According to classic models, the *rhythm* of theta oscillations is generated by a pacemaker network in the MSDB (Petsche et al. 1962) and that rhythm is imposed on the hippocampus and related structures similar to the way a timing belt imposes order on the pistons and valves of a car engine. This idea of a single external clock setting the pace for hippocampal theta oscillations is supported by the fact that experiments lesioning the MSDB or cutting the fibria/fornix (Fi/Fx) fibers connecting the MSDB to the hippocampus abolish theta oscillations in the hippocampus (Stewart and Fox 1990). Furthermore, neurons in the MSDB have been shown to fire in rhythmic bursts with hippocampal theta (Brazhnik and Fox 1997; Dragoi et al. 1999) and continue to fire at theta frequencies even after FiFx lesions (Vinogradova 1995). Evidence has more

recently pointed to a role for the supramammilary nucleus and reticular pontine nucleus in pacing the theta frequency activity of the MSDB (Vertes and Kocsis 1997), but has still been argued to support the role of a theta clocking mechanism external to the hippocampus.

Some experiments have called into question the single oscillator model for the rhythm generation of hippocampal theta. Studies of hippocampal slices, absent any influence from outside brain regions, have found it possible to invoke network oscillations at theta frequencies using various pharmacological agents, including muscarinic and metabatropic glutamate receptor agonists (Traub et al. 2004; Gillies et al. 2002; Konopacki 1998; Whittington and Traub 2003). Furthermore, it has been found that in the hippocampal slice heterogeneous mechanisms underlie the generation of theta. Activation of muscarinic receptors via bath application of carbachol generates theta oscillations that are independent of $GABA_A$ mediated inhibition (Konopacki 1998), suggesting a role for intrinsic currents and/or recurrent network connectivity of pyramidal cells. Activation of theta oscillations with metabotropic glutamate receptors, on the other hand, induced O-LM interneurons to fire on each cycle of theta oscillations even when AMPA receptors were blocked (Gillies et al. 2002). These results suggest that previous lesions of the FiFx and entorhinal cortex (Kamondi et al. 1998; Stewart and Fox 1990) may have been cutting not only a rhythmic drive to the hippocampus, but also removing a depolarizing input that endows hippocampal neurons with the ability to entrain theta oscillations locally via inherent resonance properties of the neurons and local networks (Chapman and Lacaille 1999; Fellous and Sejnowski 2000; MacVicar and Tse 1989). *In*

vivo analysis has further supported the presence of multiple theta rhythm generators and suggested that the prevalence of these generators may be related to different behaviors and pharmacological constraints (Bland 1986; Kocsis et al. 1999; Vanderwolf 1988; Whishaw and Vanderwolf 1973). Despite the suggestion that there may be several behavior dependent rhythm generators of theta throughout the different layers and regions of the hippocampus, little attention has been given to systematically examine the behavior and layer specificity of hippocampal theta oscillations.

1.4.4 - Gamma oscillations

Gamma oscillations in the hippocampus are present during both theta and non-theta behavioral states (Csicsvari et al. 2003). During theta states, the amplitude of gamma oscillations are modulated by the phase of theta oscillations (Bragin et al. 1995a; Buzsaki et al. 1983; Gillies et al. 2002; Chrobak and Buzsaki 1998b) and two rhythms can been seen superimposed in the extracellular LFP and the intracellular membrane potential (Penttonen et al. 1998; Soltesz and Deschenes 1993). Furthermore, the frequencies of gamma and theta oscillations tend to vary together (Bragin et al. 1995a). Despite this comodulation, gamma oscillations exhibit a more local coherence than theta oscillation in the hippocampus (Bragin et al. 1995a), suggesting divergent function in coordinating hippocampal networks.

1.4.5 - Mechanisms of gamma oscillations

Similar to theta, gamma oscillations can be induced in the hippocampal slice preparation, but the induction and maintenance exhibit different mechanisms. Either the muscarinic

agonist, carbachol, or the glutamate receptor agonist, kainate, induce persistent gamma oscillations that require both AMPA-mediated excitatory transmission and $GABA_A$ mediated inhibition (Bragin et al. 1995a). Activation of metabotropic glutamate receptors or tonic depolarization by the addition of potassium to the bath, however, induces gamma oscillations that are resistant to AMPA receptor blockade, but still require $GABA_A$ signaling (LeBeau et al. 2002; Whittington et al. 1995). The role of $GABA_A$ mediated transmission in generating gamma oscillations is further emphasized by the fact that manipulation of the $GABA_A$ time constant with varying concentrations of pentobarbital concomitantly altered the frequency of network gamma oscillations (Whittington et al. 1995). Although different hippocampal neurons have been found to resonate at different frequencies due to unique intrinsic conductances, basket cells were found to resonate in the 30-50Hz gamma range (Pike et al. 2000). Evidence suggests similar $GABA_A$ mechanisms *in vivo.* Basket cells fire on the rising phase of local gamma, and gamma oscillations in the membrane potential of CA1 pyramidal cells exhibit a chloride reversal potential (Penttonen et al. 1998). In addition to the role of GABAa mediated inhibition, *in vitro* and *in vivo* studies suggest a role for gap juctions in coordinating network gamma oscillations (Buhl et al. 2003; Hormuzdi et al. 2001; LeBeau et al. 2003).

Evidence suggests the existence of at least two regional rhythm generators of gamma oscillations in hippocampal networks. Both the coherence of the intact system (Csicsvari et al. 2003) and lesions of the entorhinal cortex (Bragin et al. 1995a) suggest that the dentate gyrus provides one source of gamma oscillations that is dependent on the

entorhinal input, and the CA3 region provides an independent source of gamma oscillations. After entorhinal lesion, while dentate gamma power decreases, the gamma oscillations in the CA3 and CA1 regions actually increases in amplitude (Bragin et al. 1995a). Similar effects have been observed after transient stimulation-induced silencing of the entorhinal cortex (Leung 1987) and under the influence of urethane and ketamine anesthesia (Buzsaki et al. 1994). CA1 gamma oscillations appear to be somewhat dependent on the CA3 region because disconnection of the two regions in slice disrupts CA1 gamma, but leaves CA3 gamma intact (Fisahn et al. 1998). It is hypothesized that the ability of CA3 to self-organize gamma oscillations may result from the highly recurrent collateral system in this region (Csicsvari et al. 2003). Discharging CA3 pyramidal cells excite one another through this recurrent collateral system and can discharge local basket and chandelier cells to entrain local gamma oscillations. Because CA3 provides a primary excitatory input to CA1 pyramidal cells and CA1 basket cells actually receive more synapses from CA3 than CA1 pyramidal cells (c.f. Freund and Buzsaki 1996), the gamma oscillations generated in CA3 can serve to entrain the downstream CA1 network (Csicsvari et al. 2003). By coordinating across the different oscillators in the hippocampal formation, the entorhinal cortex and dentate gyrus can also transiently couple with the CA3 and CA1 networks (Chrobak and Buzsaki 1994; Csicsvari et al. 2003; Chrobak and Buzsaki 1998b), potentially serving diverse functions.

1.5 - Hippocampal Function

1.5.1 - The role of the hippocampus in episodic and semantic memory

The role of the hippocampus in memory was first proposed after broad aspiration lesions

of the medial temporal lobe left patient H.M. with severe anterograde amnesia (Scoville and Milner 1957). Since, neuropsychological studies in humans with more restricted lesions and focused lesion experiments in animals have revealed more specific information about the mnemonic role of the hippocampus in the formation and retrieval of declarative memories (Squire 1992). Declarative memories, i.e. those memories that can be consciously recalled, have been broadly divided into semantic and episodic memory. Semantic memory refers to memories for facts, such as who is the president elect of the United States, and depends primarily on parahippocampal structures including the entorhinal and perirhinal cortices (Squire 1992; Eichenbaum and Cohen 2001). Episodic memory refers to those memories that are embedded in a spatio-temporal context and are unique to an individual (Tulving 1972). These memories depend on the integrity of the hippocampus (Eichenbaum and Cohen 2001; Milner et al. 1998; Vargha-Khadem et al. 1997). Although there has been some debate as to whether non-human animals have true episodic memories (Tulving 1972), numerous studies have found animals can recall "what, where and when" information about unique experiences and use that information to solve various tasks (Clayton and Dickinson 1998; Clayton et al. 2001; Ergorul and Eichenbaum 2004; Fortin et al. 2002).

Convergent evidence from single unit recording studies supports the role of the hippocampus in episodic memory formation. Recording from rats, Wood et al. (Wood et al. 1999) found that different neurons in the hippocampus respond selectively to specific locations and odors in the environment as well as other specific task parameters. Several investigators have found that in hippocampus-dependent spatial alternation tasks, the

firing of both CA3 and CA1 neurons can predict the future choice (Ainge et al. 2007; Frank et al. 2000; Johnson and Redish 2007; Wood et al. 2000) as well as reflect past experience of the animal (Ferbinteanu and Shapiro 2003). Extending these findings, Pastalkova et al., (Pastalkova et al. 2008) found hippocampal neurons that spontaneously fire in time-dependent sequences during a wheel running delay period in a manner that predicted the future choice of the rat. Furthermore, recent recordings from the human hippocampus revealed that patterns of neuronal activity present during the encoding of novel cinematic audio-visual sequences were selectively reactivated during free recall prior to delivery of verbal reports (Gelbard-Sagiv et al. 2008).

In humans, gamma oscillations in the hippocampus also change in amplitude and coherence with other cortical areas during memory encoding and retrieval. Sederberg et al. (Sederberg et al. 2003; Sederberg et al. 2007a) have found that gamma oscillations in the hippocampus and medial temporal lobe exhibit increased power during the encoding of stimuli that will be subsequently correctly recalled. Another study found that the coherence of gamma oscillations between the hippocampus and rhinal cortices increases during successful encoding of later recalled stimuli (Fell et al. 2003a). Gamma oscillations in several medial temporal lobe locations were further found to increase during correct versus incorrect retrieval of prior memories (Sederberg et al. 2007a). The behavioral relationship of hippocampal gamma oscillations to behavior in non-human animals has received very little study to date.

1.5.2 - The role of the hippocampus in spatial memory

Parallel to the development of theories that the hippocampus is involved in declarative memory, other investigators have long suggested a role for the hippocampus in spatial navigation. Growing out of the cognitive map theory put forth by Tolman (Tolman 1948) to explain the spontaneous use of shortcuts by rats in various maze paradigms, (O'Keefe and Dostrovsky 1971) found so-called "place cells" in the hippocampus that fired bursts of activity in specific spatial locations in a given environment. Based on this compelling allocentric representation of space in the hippocampus O'Keefe & Nadel published their landmark book in 1978, *The Hippocampus as a Cognitive Map,* detailing the role of the hippocampus as the structure that mediates spatial memories in the brain. Since that time, numerous studies have found spatial memory deficits after hippocampal lesions (Nadel 1991; Nadel and Eichenbaum 1999) and place cells in numerous species, including humans (Ekstrom et al. 2003). More recently authors have proposed a reconciliation of these two views of hippocampal function, suggesting that the same circuitry that can integrate egocentric trajectory information into an allocentric spatial map, may also be suited to extract common aspects of episodic experience to form abstract semantic knowledge (Eichenbaum and Cohen 2001; Buzsaki 2005; Eichenbaum et al. 1999).

In rats, theta oscillations in the hippocampus have previously shown both amplitude and frequency increases as a function of running speed (Whishaw and Vanderwolf 1973; Arnolds et al. 1979; McFarland et al. 1975; Oddie and Bland 1998; Rivas et al. 1996). Theta coherence between the hippocampus and prefrontal cortex has been shown to increase during performance of a spatial working memory task (Jones & Wilson, 2005). Similarly, in humans, theta power in the hippocampus and medial temporal lobe increases

during spatial navigation (Caplan et al. 2001; Caplan et al. 2003; Cornwell et al. 2008; Ekstrom et al. 2005; Kahana et al. 1999). While these effects could be interpreted under the rubric of increased spatial processing, other studies in rodents have found changes in theta oscillations related to other contextual effects such as running to or away from reward, motivation or other task-related factors (Sinnamon 2005; Sinnamon 2006; Wyble et al. 2004). Furthermore, human hippocampal theta oscillations have also been found to increase in response to specific mnemonic stimuli (Ekstrom et al. 2007). Additionally, increases in hippocampal-rhinal theta coherence have been found to accompany successful versus unsuccessful encoding of memories (Fell et al. 2003b). During memory retrieval in humans, hippocampal theta has also been found to increase in amplitude (Ekstrom et al. 2007) and undergo a phase reset induced by probe stimulus presentation (Tesche and Karhu 2000). The wide ranging effects on theta oscillations have been proposed to fall generally under the framework of sensory-motor integration (Bland 1986), but the roles that theta and gamma synchrony in the hippocampus plays to coordinate various behaviors remain to be fully understood.

1.5.3 - Hippocampal function during REM sleep

Evidence from human and animal studies suggests that hippocampus-dependent memories are initially stored in the hippocampus, but are subsequently transferred to the cortex by a consolidation process (Buzsaki 1989; Eichenbaum and Cohen 2001; Squire 1994). Computational models have shown that consolidation processes allows new categories to be learned without catastrophic deconstruction of previously learned information (McClelland et al. 1995). It has been suggested that consolidation occurs

during slow-wave and rapid eye movement (REM) sleep (Buzsaki 1989; Vertes 2004; Walker and Stickgold 2004). In line with the sleep-consolidation hypothesis, a number of investigators have found evidence that hippocampal neurons replay patterns of waking activity during both slow-wave and REM sleep (Louie and Wilson 2001; Nadasdy et al. 1999; Skaggs and McNaughton 1996; Wilson and McNaughton 1994) and that experience-dependent gene expression is activated in the dentate gyrus during REM sleep (Ribeiro et al. 1999; Ribeiro et al. 2002).

During REM sleep, the hippocampus generates theta and gamma oscillations similar to the awake state. This superficial similarity between REM and waking states has commonly prompted the two states to be lumped together for analysis under the rubric of "theta behaviors" (Bragin et al. 1995a; Kocsis et al. 1999). However, it is likely that the regional coordination of hippocampal activity differs substantially between active waking and REM sleep. For instance, the activities of neuromodulatory systems are entirely different in REM sleep versus active waking behaviors. While ACh fibers exhibit high levels of release in the hippocampus during both REM and waking (Marrosu et al. 1995), norepinephrine (NE) and serotonin (5HT) levels in the hippocampus are high during waking, but very low during REM (Aston-Jones and Bloom 1981; Park et al. 1999). The dense projections of NE and 5HT to the dentate gyrus (Amaral and Witter 1995; Oleskevich et al. 1989; Loy et al. 1980; Oleskevich and Descarries 1990), suggests that activity in this region should be particularly affected by the different neuromodulator levels during REM versus active waking behavior.

In the rat, REM sleep is generally associated with a tonic 5-8Hz hippocampal theta and low muscle tone, but is interrupted by phasic muscle twitches associated with increased theta frequencies (Robinson et al. 1977). Interestingly, these phasic increases in hippocampal theta frequency during REM have recently been found to be coincident with pontine waves (or "pontine-geniculate-occipital spikes" in the cat and primates) (Karashima et al. 2005). The physiology of pontine waves (p-waves) has been studied in some detail (Datta 1997) and have been implicated in consolidation during REM sleep. Specifically, Datta (Datta 2000) showed that REM sleep after learning exhibited increased p-wave generation. Furthermore, learning deficits induced by REM sleep deficits can be mitigated by pharmacological activation of p-wave production (Datta et al. 2004). These data suggest that the phasic REM periods in the rat may be particularly important in REM consolidation processes. This is particularly interesting because humans may only have phasic bouts of hippocampal theta (Bodizs et al. 2001; Cantero et al. 2003) during REM sleep.

1.5.4 - Functional differentiation of hippocampal subregions

Anatomical specialization of the dentate gyrus, CA3 and CA1 hippocampal subfields suggests that the individual subfields may serve discrete computational functions (Marr 1971; Rolls 1996; Treves 1995). Until recently, this has largely been speculation based on network models, but selective lesion and knockout studies and single unit recordings from different hippocampal regions have begun to further specify the functions of hippocampal subregions.

Selective dentate lesions, as well as granule cells specific NMDA knockouts and unit recordings have suggested that the dentate gyrus is critical for the rapid encoding and pattern separation of spatial contexts (Leutgeb et al. 2007; McHugh et al. 2007). The role of the dentate gyrus in separating similar patterns (e.g. similar spatial contexts) has long been suggested by the large number of granule cells (1,000,000 in rat), compared to the input layer II EC network (200,000) cells or output CA3 network (300,000; Amaral and Witter 1995), and the relatively sparse coding of dentate granule cells (Jung and McNaughton 1993). Evidence further suggests that, while either lesions of the dentate gyrus or disconnection of the dentate and CA3 lead to impairments on indices of memory encoding, memory retrieval is relatively spared (Jerman et al. 2006; Lee and Kesner 2004b).

CA3 has been shown to be critical for associative (Gilbert and Kesner 2003; Rajji et al. 2006) and episodic memory formation (Hunsaker et al. 2008a; Li and Chao 2008). Furthermore, both lesion and knockout studies have shown that functioning of the CA3 region is necessary for the retrieval of associative memories (Brun et al. 2002; Nakashiba et al. 2008; Nakazawa et al. 2002; Steffenach et al. 2002), particularly when short delays are interposed (Kesner 2007). These findings complement the previous work of computational models, which have focused on the recurrent collateral system in CA3 as an ideal network for rapidly storing associative memories that could be later recalled via readout of the internally stored representations (Hasselmo et al. 2002; Lisman 1999; Marr 1971; Treves 1995; Rolls and Kesner 2006).

A variety of functions have been proposed for CA1 networks, but consensus has yet to emerge. Some models have proposed a role for CA1 in memory for sequences and stimuli ordered across time (Rolls and Kesner 2006), which has also received some experimental support (Hoge and Kesner 2007). Models have also proposed a mismatch function, comparing the output of associations stored in the CA3 network with those of direct layer III EC inputs (Hasselmo and Wyble 1997; Lisman and Otmakhova 2001), which has received some support from lesion experiments (Lee et al. 2005b) and single unit recordings (Lee et al. 2004; but see Leutgeb et al. 2004). A number of experiments have also suggested that CA1 is important for retrieval of memories (Hunsaker et al. 2008b; Hunsaker and Kesner 2008; Lee and Kesner 2004a). Understanding how the CA1 region integrates information from CA3 and EC inputs may be critical to understanding the mnemonic functions of the system.

1.6 - The Present Studies

Although the convergence of lesion, single unit and computational modeling studies provides a general framework for understanding hippocampal function, they are short on details that can link these various levels into a coherent mechanism. To begin to examine how the diverse hippocampal regions coordinate with one another to encode, integrate, consolidate, and retrieve hippocampus-dependent memories, I have conducted a set of experiments that examine how the diverse hippocampal subregions interact during performance of memory and control tasks and during offline processing in REM sleep. Using high density recordings of LFPs and unit activity with 96-site silicon probes, I recorded the activity from the different layers of the dentate gyrus, CA3 and CA1 to

examine how theta and gamma oscillations coordinate hippocampal activity under different behavioral conditions. Running rats on a hippocampus-dependent delayed spatial-alternation task (Ainge et al. 2007) and control tasks, I compared how theta and gamma power, coherence and phase changes during task performance. Because the alternation task requires rats to encode and retrieve information about their previous trajectories on the maze in order to maintain correct performance, these comparisons systematically investigated how hippocampal regions dynamically couple with one another during functional operation of the hippocampal system. I also examined how hippocampal regions coordinate with one another during phasic and tonic periods of REM sleep and compared these activity patterns to those observed during active waking. This comparison showed how hippocampal network coordination changes from online waking behavior to offline REM processing states and suggests a new framework for understanding hippocampal function during REM sleep.

1.7 – Figure Legends

Figure 1.1. Major excitatory pathways of the hippocampal circuit. Abbreviations: EC = entorhinal cortex; DG = dentate gyrus; CA = Cornu Ammonis; so = stratum oriens; sp = stratum pyramidale; sr = stratum radiatum; lm = stratum lacunosum moleculare; ml = molecular layer; gl = granule layer; hr = hilar region.

Section 2.0 - Behavior-dependent coordination of multiple theta dipoles in the hippocampus

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2.1 - Abstract

Theta (4-10Hz) oscillations in the hippocampus are thought to be important for plasticity, temporal coding, learning and memory. The hippocampal system has been postulated to have two (or more) rhythmic sources of theta oscillations, but little is known about the behavior-dependent interplay of theta oscillations in different subregions and layers of the hippocampus. We tested rats in a hippocampus-dependent delayed spatial alternation task on a modified T-maze while simultaneously recording local field potentials from dendritic and somatic layers of the dentate gyrus, CA3 and CA1 regions using high-density, 96-site silicon probes. We found that while theta oscillations were generally coherent throughout the hippocampus, the power, coherence and phase of theta oscillations fluctuated in a layer-specific manner, confirming the presence of multiple interdependent dipoles. Layer-dependent changes in the power and coherence of theta oscillations varied with aspects of both the memory and control (non-mnemonic) tasks, but only a small fraction of the variance could be explained by running speed or acceleration. Furthermore, the phase lag between theta oscillations in the CA3 and CA1 pyramidal layers was significantly smaller on the maze arm approaching the T-junction than on other arms of the alternation task or on comparable segments of control tasks. Overall, our findings reveal a consortium of layer-specific theta dipoles (current sinks and sources) generated by the rhythmic flow of ions into and out of hippocampal cells. Moreover, our data suggests that these different theta generators flexibly coordinate hippocampal regions and layers to support behavioral task performance.

2.2 - Introduction

The hippocampal theta rhythm is the most prominent clocking mechanism in the forebrain (Buzsaki 2002; Bland 1986; Vanderwolf 1988). Due to the architectonics of the hippocampus, the rhythm (5-10 Hz in the rat) can be readily detected as a macroscopic local field potential (LFP) in the dorsal hippocampus during exploratory behavior and sleep (Vanderwolf 1969; Grastyan et al. 1959). Theta oscillations are essential for the physiological operation of the hippocampus, and abolishing them results in severe behavioral deficits (Winson 1978). The phasic patterning of neuronal activity provides spatio-temporal coding of information in the hippocampus (O'Keefe and Recce 1993; Skaggs et al. 1996; Huxter et al. 2003; Huxter et al. 2008). Furthermore, theta oscillations support the compression of representations carried by neuronal spiking from the timescale of behavior (seconds) into short (milliseconds) timescales (referred to as "temporal compression"; Skaggs et al. 1996; Dragoi and Buzsaki 2006), which may be important for spike timing dependent plasticity (Magee and Johnston 1995; Jensen and Lisman 1996; Markram et al. 1997; Mehta et al. 1997) and for the proper temporal packaging and transfer of neuronal information (Hasselmo et al. 2002; Buzsaki 2005).

The macroscopic theta LFP is the result of coherent membrane potential oscillations across large numbers of neurons in all hippocampal subregions (Kamondi et al. 1998; Ylinen et al. 1995b; Soltesz and Deschenes 1993; Fox 1989). In the simplest model of theta generation, the medial septum functions as a temporal coordinator, or pacemaker (Lee et al. 1994; Stewart and Fox 1990), and coherently entrains hippocampal networks. From this perspective, there is a single global theta rhythm. However, numerous

experimental manipulations, including pharmacological interventions (Vanderwolf 1988), deafferentation (Bragin et al. 1995a; Buzsaki et al. 1983), inactivation (Mizumori et al. 1990), intracellular/intradendritic recordings (Kamondi et al. 1998; Fox 1989), multisite recording (Kocsis et al. 1999), current-source density (CSD) measurements (Bragin et al. 1995a; Brankack et al. 1993), in vitro studies (Traub et al. 2004; Gillies et al. 2002; Konopacki 1998; Whittington and Traub 2003) and computational modeling (Leung 1984; Rotstein et al. 2005), indicate that at least two (Bland 1986), but likely several (Buzsaki 2002) rhythm generating mechanisms and numerous theta current dipoles are at work.

Given the large number of anatomical substrates which can potentially generate theta currents, we hypothesized that theta oscillations do not function simply as a global clock, but instead, the varying coordination of the multiple sources of theta currents (Buzsaki et al. 1985) can serve different behaviors. To examine this hypothesis, we used high-density silicon probes to map the theta current distribution in the dentate-CA3-CA1 regions of the hippocampus (Montgomery et al. 2008) and examined the amplitude, frequency, phase and coherence relationships among the postulated dipoles during various aspects of behavior. We found that the power, coherence, and phase of theta oscillations exhibit layer-specific changes that depend on behavioral task demands.

2.3 - Methods

2.3.1 - Animals and Behavior

Four Long-Evans rats (male, 300-400g) were water-deprived and trained to run in a

hippocampus-dependent continuous delayed non-match to place ('alternation') task (Fig. 2.3A; Ainge et al. 2007) and a control task. All rats performed the alternation task well (>85% correct). Rats were initially shaped in the alternation task with no delay period and were required only to run on the maze to receive water reward. When consistent maze running and approach to the water points was established, the water delivery on the side arms was gradually withdrawn for trials in which the rat did not alternate. After alternation performance was established, a 10 second delay was interposed between each trial to require engagement of hippocampal networks. To maintain consistent running on the maze without exploratory interruptions, a small water reward was delivered in the delay area and increased water rewards were given at the appropriate water port for uninterrupted trajectories. After learning the alternation task, rats were trained on a control task. The control tasks (Fig. 2.5A) included a C-shaped (n = 2 rats) and a Z-shaped (n = 1 rat) linear track, requiring the rats to run back and forth for water reward, and a cue task (n = 1 rat) requiring the rat to run in a path similar to the alternation task, but on each trial the rat's trajectory was randomly cued left or right by a large block that was visible after the delay period. In fact, this block prevented the rat from fully entering one of the (right or left) maze arms, but we refer to it here as a "cue" because after initial training the rat ran swiftly through the T-junction onto the unblocked arm without stopping or attempting to enter the blocked arm. Rats were well-trained on both alternation and control tasks (at least 30 days training with 20-50 trials/task) prior to obtaining recordings described here in order to avoid novelty effects (Jeewajee et al. 2008). To constrain the behavioral and cognitive variability, trials in which the rat engaged in rearing, excessive sniffing, grooming or immobility were eliminated from further analysis.

2.3.2 – Surgery

After proficient maze running was established, recording and stimulation electrodes were implanted. A 96-site silicon probe was implanted on a movable drive in the right hemisphere parallel to the transverse axis of the hippocampus (45° parasagittal) with the outer shanks targeted at approximately AP -2.8mm, ML 2.7mm and AP -3.86mm, ML 1.64mm from bregma. Probes had recording sites spaced regularly over a 1.5mm x 1.5mm area with 6 shanks spaced at 300μm, each with 16 recording sites at 100μm spacing. DiI was applied to the probe prior to implantation to assist in histological analysis. A bipolar angular bundle (perforant path) stimulating electrode was implanted AP 1.0mm, ML -1.0mm from the junction between lambda and the right lateral ridge and DV -3.5mm from the dura. A bipolar commissural stimulating electrode was implanted at AP -1.2mm, ML -1.0mm from bregma and -3.8mm from the dura. Ground and reference screws were implanted in opposite hemispheres above the cerebellum.

2.3.3 - Electrode Localization

Post-mortem electrode location was verified using thionin, fluorescent Nissl (Invitrogen; Carlsbad, California) or DAPI (Invitrogen) staining in combination with DiI (Invitrogen) labeled electrode tracks. Prominent morphological features of the hippocampal anatomy were outlined for subsequent analysis of hippocampal physiological signals (Montgomery et al. 2008; Montgomery and Buzsaki 2007). LFP ripple bursts (120-250Hz) were detected on CA1 pyramidal layer sites and aligned to the depth negativity of the concomitant sharpwave for anatomical localization. Using a combination of the histology, ripple-triggered current source density (CSD), ripple power, perforant path evoked CSD, dentate spike-triggered CSD, and multiunit activity (Montgomery et al. 2008; Montgomery and Buzsaki 2007; Bragin et al. 1995b;

Csicsvari et al. 2003), the anatomical location of recording sites could be determined to a high degree of accuracy (Bragin et al. 1995a), estimated to be about ±30 μm. First, histological sections were oriented to vertically position the probe shanks and major anatomical features of the histology were outlined. Subsequently, the anatomical features were overlaid on plots of the major physiological features and stretched in the x and y axis to achieve alignment. These were aligned on several criteria: (1) spectral power of ripples were maximal in CA1 pyramidal layer and sharpwave sinks were maximal in CA1 stratum radiatum (Fig. 3.1,4.1;Csicsvari et al. 2000; Montgomery et al. 2008; Montgomery and Buzsaki 2007), (2) perforant path evoked currents and dentate spikes reversed polarity just dorsal to the dentate granule layer (Montgomery and Buzsaki 2007; Bragin et al. 1995b), (3) in response to perforant path stimulation, monosynaptic population spikes (~3 msec post stimulation) peaked in the dentate granule layer and subsequent disynaptic (~5 msec) population spikes peaked in the CA3 pyramidal layer (Fig. 3.1), (4) recording sites with high multi-unit activity reliably followed the cell body layers (Fig. 4.1 McNaughton and Barnes 1977).

2.3.4 - Data Acquisition and Analysis

Neurophysiological signals were acquired continuously at 20kHz on two synchronized 64-channel DataMax systems (16-bit resolution; RC Electronics Inc.). Local field potentials were down-sampled to 1.25kHz for further analysis. For tracking the position of the animals, two small light-emitting diodes (11cm separation), mounted above the headstage, were recorded by a digital video camera and sampled at 30 Hz. Stable recording sessions in which the silicon probe shanks spanned the CA1, CA3 and dentate gyrus somatic layers, were analyzed using custom-written Matlab-based software (Mathworks; Natick, MA). Recording site irregularities

(including cross-talk and excessive impedances) were *a priori* identified and removed from analysis using measures of coherence and normalized power similarity (Diba et al. 2005). Specifically, at several stereotaxic depths in the brain, we calculated the coherence and normalized power similarity for all channel pairs at different physiological frequencies. By averaging these measures across different positions of the electrode in the brain, including the neocortex and corpus callosum, the anatomical connectivity of the brain was averaged out to unveil the limitations of the recording sites. Outlier recording sites that exhibited concordance with other sites at exceptionally high (possibly due to cross-talk) or exceptionally low (due to excessive impedance) levels relative to baseline volume-conduction were designated as "bad sites" and removed from further analysis. The CSD was calculated by standard methods (2B – A – C, for 3 adjacent sites) (Mitzdorf 1985) and spatially smoothed using a normalized [1 2 1] filter (Isomura et al. 2006). Typically, a single bad site was surrounded by good sites and was linearly interpolated prior to CSD calculation to avoid false-positive detection of current sinks/sources. Two adjacent bad sites in the middle of a silicon probe shank were interpolated in four instances (each in different layers) in order to utilize remaining sites on the shank. CSD calculations centered on bad sites were excluded from further analysis. Occasionally, a group of nearby sites were defective and a portion of the silicon probe, or, in two cases, the entire silicon probe shank was excluded from analysis. In total, 11-19 LFP measurements and 5-19 CSD measurements were *a priori* selected from each layer for further analysis (see "Statistics" below and Montgomery et al. 2008; Montgomery and Buzsaki 2007). For unit analysis, the wide-band signals were digitally high-pass filtered (0.8–5kHz) and automatically spike sorted using KlustaKwik (Harris et al. 2000), followed by manual adjustment of the clusters (using the Klusters software package; Hazan et al. 2006). Pyramidal cells and interneurons were separated

on the basis of their auto-correlograms, waveforms and mean firing rates (Csicsvari et al. 1999; Bartho et al. 2004).

2.3.5 - Spectral Analysis

Theta power, frequency, coherence and phase analyses were performed using Morlet wavelet analysis (courtesy of Aslak Grinsted) and multi-taper Fourier analysis (Mitra and Pesaran 1999). To avoid contamination from frequency shifts of theta oscillations across behaviors, the value of each spectral measure was taken at the peak theta frequency calculated from a selected CA1lm channel (typically exhibiting the most robust spectral theta peak, see Fig. 2.1 legend for a list of anatomical abbreviations). Theta oscillations under the present behavioral conditions consistently had a spectral peak within the 6-12Hz range, most commonly 8-9Hz. Similar results were obtained using spectral estimates integrated over the 6-12Hz range. To avoid differential behavioral bias in the spectral estimate and statistical dependence due to overlapping time windows, on each trial a 0.5 second window was sampled separately from the center of each maze segment (as shown in Fig. 2.4A). Theta power, frequency, coherence and phase was independently calculated for each window, and tapered by a normalized Hann window to remove edge effects (center-weighted average; Mitra and Bokil 2008). Running speed and acceleration were similarly estimated by averaging over a tapered 0.5 second window. Coherence was calculated on the CSD estimates to avoid contamination from volume conduction (Fig. 2.1F) and was calculated for all channels with respect to one channel from each specified anatomical layer. To perform unit analysis, for each cell 0.5 second epochs with at least 4 spikes were detected. From these epochs, cells with at least 5Hz average firing were included in spectral analysis (similar results were obtained with different rate thresholds).

2.3.6 – Statistics

To perform group statistics, up to one site per silicon probe shank was *a priori* chosen to represent each anatomical layer (based on above electrode localization; also see Montgomery and Buzsaki 2007). Choosing one site/shank from the middle of each layer ensured greater statistical independence of layer sampling (at least 300 μm between sites) and prevented contamination from sites on the border between two layers. The spectral power was converted to decibels (10*Log10) and the coherence was transformed by the hyperbolic arc tangent such that the sample distributions would approximate a Gaussian (Thomson and Chave 1991). Circular theta phase data was linearlized for each channel pair by subtracting the trial-by-trial phase estimates in each behavioral condition, from the grand mean theta phase over all conditions. Fitting data from correct trials in the alternation task (errors were rare and often associated with exploratory behavior), the distribution of spectral estimates (power, frequency, coherence, phase) for each electrode site was fit with a linear model (MatLab, ANOVAN, Type 3 SS): $G = \beta_{contant} + \beta_{running\ speed} + \beta_{running\ acceleration} + \beta_{maze\ region\ (4\ arms)} + \varepsilon$

To perform group statistics across animals, resulting beta (β) values from one electrode site per shank per animal were sampled from each layer (Montgomery and Buzsaki 2007). The resulting sampled distribution of beta values from each anatomical layer was subsequently tested for a statistical difference from zero (non-parametric sign tests, $p<0.01$, Bonferroni corrected, typically n=5-19 for power/frequency comparisons, n=5-34 for coherence/phase comparisons). The assumptions of the general linear model, including normality of residuals, absence of interaction effects, and uncorrelated residuals, were assessed. For simple comparisons including only a single two-way

categorical variable (e.g. alternation versus control), a simple difference score across behaviors was calculated for each channel and the resulting distribution of scores within each layer was tested for a statistical difference from zero similar to above.

2.4 - Results

2.4.1 - Consortium of multiple dipoles generate field theta oscillations

LFPs and unit activity were recorded simultaneously from the dentate gyrus, CA3 and CA1 regions of the dorsal hippocampus using a 2-dimensional silicon probe array with 96 monitoring sites (Montgomery et al. 2008; Montgomery and Buzsaki 2007; Csicsvari et al. 2003). To observe sustained theta oscillations, rats (n=4) were tested during running on an elevated maze (Montgomery and Buzsaki 2007). Consistent with previous studies, the largest amplitude theta waves were observed in the CA1 str. lacunosum-moleculare (Fig. 2.1A, B; Brankack et al. 1993; Buzsaki et al. 1986). Theta oscillations during running were asymmetric, giving the appearance of saw-tooth waves and producing significant harmonics (16-20 Hz) in both power and coherence spectra (Fig. 2.1C). The waveforms varied as a function of both behavior and recording site, with the hilar region exhibiting the most sinusoid patterns. Although theta LFP and local currents (current source density, CSD; Mitzdorf 1985) were remarkably coherent across the CA1, CA3 and dentate regions (>0.7; Fig. 2.1C-F), attenuation of coherence with distance was lower within a layer versus across different layers (Fig. 2.1C-E). Whereas theta waves recorded from sites within the same layer were highly coherent (>0.98) irrespective of the distance, coherence between sites in different layers decreased substantially with distance (~0.03/mm; Fig. 2.1E). The within versus between layer coherence difference was statistically confirmed as significant interaction effect ($p=1.9e^{-9}$) using regression analysis, which compared

the attenuation of coherence as a function of inter-site distance for within-layer versus between-layer pairs (i.e. slopes in Fig. 2.1E were significantly different). A similar interaction effect was observed when only adjacent layers were included in the analysis ($p=2.9e^{-4}$). Furthermore, CSD signals exhibited a similar interaction effect ($p=2.7e^{-8}$), arguing against the possibility that these effects are merely due to the mixing of volume-conducted signals across the curvature of hippocampal layers. In fact, the contrast between the intralaminar and interlaminar distribution of coherence values was even more striking when the coherence of local currents, rather than that of the LFP, was used to generate the coherence maps (Fig. 2.1F). Given the laminar (layer-specific) distribution of intrahippocampal and extrahippocampal afferents (Amaral and Witter 1995), this observation suggests that many, if not all, hippocampal layers may contribute a somewhat independent theta dipole (Fig. 2.1C-F), and that it is the summation of these dipoles that results in the mean field (LFP).

The relative timing of theta current sinks and sources within the same anatomical layer were similarly homogeneous, while current sinks and sources were shifted in time across different layers. Figure 2.2A shows the peak-triggered average of theta oscillations recorded from different portions of the hippocampus. To further examine the multiple theta dipoles in the hippocampus, we mapped the distribution of phase for both LFP and extracellular CSD (Fig. 2.2B-D). The phase versus depth profile of theta LFP revealed a gradual phase reversal from the CA1 str. oriens to the str. lacunosum-moleculare (Hasselmo et al. 2002; Brankack et al. 1993; Buzsaki et al. 1986; Buzsaki et al. 1983; Winson 1978; Buzsaki et al. 1985; Leung 1984), with further phase shifts in deeper layers (Fig. 2.2B,C). Up to 270^{o} (-90^{o}) shifts between the CA1 pyramidal layer and the hilar region were previously observed (Buzsaki et al. 1986; Buzsaki et al.

1983). Because LFP can be contaminated by volume-conducted signals (Fig. 2.1F; Bland and Whishaw 1976), we also plotted the phase distribution of the theta CSD (Fig. 2.2B-D). Similar to LFP, a gradual phase reversal of the CSD traces was present from the CA1 str. oriens to the str. lacunosum-moleculare. In contrast to LFP, CSD phase plots showed large (up to 180^{o}) phase jumps between the CA1 str. lacunosum-moleculare and the middle molecular layer of the dentate gyrus and significant shifts between the molecular layer and the hilus/CA3 pyramidal layer. For group analysis, phase distributions were calculated between a chosen recording site in each of the anatomical layers and sites in the same and other layers (see statistical methods). As expected from the CSD coherence analyses, the theta phase differences within the same layer were consistently close to zero for both LFP and CSD (upward arrows in Fig. 2.2D). A notable exception was in the dentate molecular layer, which showed the lowest within-layer coherence (Fig. 2.1F) and large variability of theta current phases across experiments in both intralaminar and interlaminar comparisons (Fig. 2.2D). The distinct afferents to the outer, middle and inner portions of the molecular layers from the lateral and medial entorhinal cortex and the mossy cells of the hilus, respectively (Amaral and Witter 1995), may be the source of the this variability. Unfortunately, the 100-μm spatial resolution of the silicon probes used in this study was not sufficient to segregate these potentially independent theta dipoles (Fig. 2.S1).

To verify and further investigate the local generation of the layer-specific dipoles, we examined the theta phase relationship of neuronal spiking to local currents. As expected from previous experiments, principal cells and interneurons showed a wide range of phase relationships to the theta CSD from different layers (Klausberger and Somogyi 2008; Csicsvari et al. 1999). Theta phase histograms of example putative interneurons showed differential phase-locking to CSD

traces from different layers (Fig. 2.2E), with sharper tuning of some dentate interneurons to dentate/CA3 theta versus sharper tuning of some CA1 str. oriens interneurons to theta currents generated in the apical dendrites of CA1 (str. radiatum and str. lacunosum moleculare). Unit-CSD theta phase analysis further emphasized the multiplicity of phase shifted theta current dipoles in the dentate and CA3 regions, including clear phase reversal between theta currents in the molecular layer and str. lacunosum-moleculare and across the granule cell layer (Fig. 2.2E). Because probe shanks in all experiments were placed medially in the CA1-dentate axis and because the curved nature of the CA3 region confounds CSD analysis, the obtained information about CA3 theta dipoles was confined to the CA3c pyramidal layer. Future experiments may reveal the relationship among phase-shifted dipoles in the dendritic layers and in the different CA3 subregions. Overall, the above results support the hypothesis that LFP theta is a reflection of multiple theta dipoles (Fig. 2.1,2.2). In turn, the multiple sources of somewhat independent theta generation (Fig. 2.1C-E) imply that cooperation of different dipoles in various combinations can support a rich variety of behaviors.

2.4.2 - Layer-dependent modulation of theta oscillations by behavior

Previous research has shown that hippocampal field and unit activity can depend on behavioral movement patterns and speed (Whishaw and Vanderwolf 1973; Rivas et al. 1996; Buzsaki et al. 1985; Montgomery and Buzsaki 2007; McNaughton et al. 1983). To investigate this relationship further, we examined the relationships between physiological measures (theta power, frequency, coherence and phase) and behavior in a hippocampus-dependent delayed spatial alternation task (Fig. 2.3A; Ainge et al. 2007) and control tasks (Fig. 2.5). We first examined the effect of running speed. Because running speed varied systematically in different segments of the maze

(Fig. 2.3B,C), we applied statistical methods to disassociate the effects of motor behavior and environmental input variables on theta activity. Fitting theta power measurements from each recording site separately with a linear (regression) model including only running speed as an explanatory variable, we found that theta power in all CA1 layers reliably increased with higher speeds, while no other hippocampal layers showed significant changes with speed (Fig. 2.3D-F). We also found a significant correlation between running speed and theta frequency (Fig. 2.S4), but this effect was not layer specific (Kruskal-Wallis tests, $p>0.1$).

In addition to the speed-theta power relationship, we also observed that theta power exhibited a significant correlation with specific portions of the behavioral task, independent of running speed in those portions (Fig. 2.4; Fig. 2.S3). Figure 2.4A,B shows that example recording sites in CA1 str. radiatum and the dentate gyrus granule layer can exhibit very different theta power modulation during performance of the alternation task. To assess the contribution of different behavioral and environmental components to theta power fluctuations, theta power was fit separately for each recording site with a general linear model (GLM), including as explanatory variables, running speed, acceleration, and maze region (see Methods and Montgomery and Buzsaki 2007). In this expanded model, maze region explained substantially more theta power variance for most hippocampal layers (up to 30% of the variance), while running speed explained substantially less (~5%) and for a limited number of layers (Fig. 2.4C). Figure 2.4D shows that even after removing the contribution of running speed and acceleration, there was a robust and selective increase in CA1 str. radiatum theta power on the center arm of the maze (similar to the gamma power increase in this portion of the alternation task reported in Montgomery and Buzsaki 2007). Group statistics, shown in Fig. 2.4E and Fig. 2.S3A,B, confirm

that the theta power increases on the center arm of the alternation task were differentially expressed across the laminar anatomy of the hippocampus (Kruskal-Wallis tests; LFP, $p=1.9e^{-7}$; CSD, $p=3.9e^{-3}$). Theta frequency also increased on the center arm of the alternation task (Fig. 2.S4A,B), but unlike the layer-specific changes in theta power, the effect revealed no significant differences among hippocampal layers (Kruskal-Wallis tests, $p>0.1$)

Although the GLM statistics are designed to segregate individual explanatory variables (Rencher 2002), we performed additional control experiments to identify the contribution of speed, maze regions and other potential variables to theta oscillations. In addition to the alternation task, each rat was trained and tested on one of three control tasks (Fig. 2.5A). Similar to the alternation task, CA1 str. radiatum theta power increased significantly on the initial segment of the control task immediately after the rat was released from the delay area, in a manner that did not follow changes in running speed (Fig. 2.5B-D). Direct comparison of the center arm of the alternation task to the corresponding initial segments in the control tasks revealed that while LFP theta power was statistically higher in some layers in the alternation task, the effect did not appear in the current source density analysis. Thus, while speed and acceleration cannot fully explain the increased theta power on the center arm of the alternation task, the effect may not be directly related to the mnemonic components of the alternation task either. This is in contrast to previously observed gamma power changes in the CA3/CA1 axis, which were found to be specifically related to alternation task performance (Montgomery and Buzsaki 2007).

Comparing theta frequency in the alternation and control tasks, however, revealed significantly faster theta oscillations on the center arm of the alternation task than on comparable segments in the non-mnemonic control tasks (Fig. 2.S4C,D). This suggests that the increased mnemonic demands of the alternation task may have some effect on theta frequency. However, the effect revealed no significant differences among hippocampal layers (Kruskal-Wallis tests, $p>0.1$), suggesting that effects on theta frequency tend to occur more at a system-wide level.

Coherence of theta oscillations also showed layer and maze region specific changes. As can be seen in Figure 2.6A and Figure 2.S3C, the largest theta coherence increase between theta dipoles of different layers was present in the central arm. Significant changes in coherence were observed in multiple layer comparisons (Fig. 2.6B, C). The largest coherence changes occurred with the dentate hilar region, which could reflect several mechanisms involving the dentate gyrus and/or CA3 networks. However, in contrast to previous gamma coherence results (Montgomery and Buzsaki 2007), interlayer coherences were similar in the central arm of the alternation task and the initial segments of the control tasks (Fig. 2.6D). In addition to the center arm increase in coherence, there was a significant decrease in CA3sp theta coherence with most other layers and a wide-spread decrease in theta power when the rat approached the goal location in both alternation and control tasks (Fig. 2.S3, Fig. 2.4B,2.5C).

Because coherence is only an indicator of phase consistency, independent of the mean phase angle, we also examined whether the relative phase of theta oscillations in different hippocampal layers changed as a function of behavior. As shown in Figure 2.7A and C,

the phase offset between CA1sp and CA3sp theta was smaller in the center arm of the alternation task (approximately –90^{o}) than in other maze segments (-120^{o}). The anatomical profile of phase distribution in the central versus other segments further illustrates this shift (Fig. 2.7B,D) and indicates that the CA1 and CA3 regions may both contribute to the maze region-dependent phase shift. We also compared the theta phase shift between the alternation and control tasks (Fig. 2.7E-G). In contrast to the similarity in theta power and coherence across the alternation and control tasks, CA3sp theta phase relative to several other hippocampal layers was significantly different between the center arm of the alternation task and the initial segment of the control tasks (Fig. 2.7F). This task-dependent CA3-CA1 theta phase shift was consistently seen in all animals despite the fact that each rat ran one of three different control tasks. In the time domain, the example theta-peak triggered averages in Figure 2.7G show that the time lag between CA3sp and CA1sp theta oscillations was ~7 milliseconds shorter on the center arm of the alternation task. A similar CA3-CA1 theta phase shift was detected in the delay area, but was not found to be statistically reliable (Fig. 2.S4A,B). Additionally, the shift in the alternation task could not be explained by running speed or acceleration (Fig. 2.S4C) and comparing the initial segment versus other arms in control tasks revealed no significant theta phase shifts (Fig. 2.S4D).

2.5 - Discussion

Using high spatial resolution recording of extracellular LFP, we found that within the global theta rhythm framework, theta current dipoles generated in the different anatomical layers of the hippocampus can vary somewhat independently from one another. Layer-specific fluctuations of

theta power, intersite coherence and phase were observed to differentially accompany aspects of performance in memory and control tasks. Although the activities of hippocampal theta dipoles are often interrelated, the present data suggests that different dipole combinations may be used to support flexible processing within the global theta system.

2.5.1 - Multiple theta dipoles in the hippocampus

Theta currents may derive from intrinsic currents of neurons, such as burst-induced somatic hyperpolarization (Hu et al. 2007), dendritic Ca2+ spikes (Magee and Johnston 1995; Schiller et al. 1997), voltage-dependent membrane oscillations (Hu et al. 2007; Kamondi et al. 1998; Leung and Yim 1986), or from synaptic currents. Outside the somatic region, each anatomically defined layer is characterized by the termination zone of glutamatergic excitatory afferents from specific cell populations (Amaral and Witter 1995; Lopes da Silva and Arnolds 1978). The coherent activity of each of these excitatory inputs forms an active sink at confined dendritic domains of the cytoarchitecturally organized hippocampal neurons. Early pharmacological and lesion studies have indicated that hippocampal theta is not a single oscillator but a result of two or more oscillators and current dipoles (Bland 1986; Lee et al. 1994; Stewart and Fox 1990; Leung 1984; Lopes da Silva and Arnolds 1978; Lopes da Silva and Arnolds 1978; Kramis et al. 1975). Expanding on previous observations (Kamondi et al. 1998; Brankack et al. 1993; Buzsaki et al. 1986), our data reveal patterns of theta coherence and phase that are highly layer specific (Fig. 2.1,2.2), and suggest that many, if not all, hippocampal layers generate a theta current dipole that is somewhat independent from the global rhythm (Fig. 2.1-2.7). In fact, the number of current dipoles is actually higher than the number of layers because excitatory inputs to each layer are complemented by one or more families of interneurons with similar axonal projections (Freund

and Buzsaki 1996; Klausberger and Somogyi 2008). By activating GABA receptors, layer-specific inhibitory dipoles generate local outward theta currents and compete with or reinforce the inward-directed excitatory currents, depending on their phase relationship (Buzsaki 2002).

To explain the high within-layer theta coherence, one has to assume that the aggregate output of the afferent population is similar in each theta cycle, although individual neurons of the afferent population may vary in their theta phase preference. This was apparent in most layers, except for the dentate molecular layer. The large variability of theta phase and coherence in the dentate molecular layer (Fig. 2.1,2.2, Fig. 2.S1) may reflect the commixture of several different theta dipoles in this layer. These include putative dipoles elicited by projections from the medial and lateral entorhinal cortex to the middle and outer thirds of the molecular layer, respectively (McNaughton and Barnes 1977) and mossy cell-derived associational/commissural inputs to the inner third of the molecular layer (Amaral and Witter 1995; Steward 1976). An additional excitatory dipole may be formed by the theta-rhythmic supramammillary projection (Kocsis and Vertes 1997), terminating in the supragranular zone (Leranth and Hajszan 2007; Magloczky et al. 1994). This laminar organization of inputs is complemented by inhibitory theta dipoles formed by the layer-specific axonal arbors of HIPP (hilar perforant path-associated), HICAP (hilar commissural-associated pathway-related) and MOPP (molecular layer perforant path-associated) cells in the molecular layer, and basket and chandelier neurons with dense axonal terminals in the granule layer (Freund and Buzsaki 1996; Sik et al. 1997; Halasy and Somogyi 1993).

In addition to confirming the presence of independent sinks in CA1 str. lacunosum-moleculare

and the dentate molecular layer (Brankack et al. 1993; Buzsaki et al. 1986), we found that these sinks alternated in phase (Fig. 2.2). A prediction of these observations is that layer III and layer II neurons of the entorhinal cortex, innervating CA1lm and the DGml respectively, discharge on the opposite polarity of the theta cycle. The phase-alternating sources in these respective layers may also reflect passive return currents of phase-shifted sinks from other layers or may be due to IPSCs produced by layer-specific interneurons (Klausberger et al. 2003; Gillies et al. 2002; Klausberger et al. 2004). Supporting this latter hypothesis, O-LM interneurons (Sik et al. 1995; Lawrence et al. 2006) were found to discharge at times of the maximum current source in CA1 str. lacunosum-moleculare (Klausberger et al. 2003). In most experiments, the theta sinks of lacunosum-moleculare were continuous with sinks in the outer molecular layer and counter in phase with theta currents in the middle molecular layer (Fig. 2.2), suggesting that layer II lateral and medial entorhinal neurons may fire on different phases of the theta cycle. Further phase shifts of theta cycles were present between the middle molecular layer, granule cell layer, hilus and CA3 pyramidal layer (Fig. 2.2). Although the specific contribution of excitatory inputs and the numerous dentate interneurons (Sik et al. 1997; Halasy and Somogyi 1993; Amaral 1978) could not be identified, the observed phase shifts support the hypothesis that the large variability of theta phase in the molecular layer is due to the presence of multiple dipoles.

2.5.2 - Task-dependent modulation of theta oscillators

A large number of overt and covert behaviors have been associated with hippocampal oscillations. These vary from the input function-related orienting response, to sensorimotor coordination, explicit output functions such as non-automatic skeletal movement and to hidden variables including attention and volition (Buzsaki 2005; Miller 1991; Vanderwolf 2003). Theta

power has often been associated with the speed and/or acceleration of movement (Whishaw and Vanderwolf 1973; McFarland et al. 1975; Rivas et al. 1996). However, the robustness of this correlation varies extensively across studies. Most recent studies, on the other hand, report rather complex relationships, including contextual effects such as running to or away from reward, motivation or other task-related factors (Sinnamon 2005; Sinnamon 2006; Wyble et al. 2004). A robust observation in the present study is that task parameters (e.g. maze region, task type) accounted for a much higher variance of theta power, coherence and phase than running speed or acceleration (Fig. 2.4, Fig. 2.S3,2.S5). These results emphasize the impact of sensory and/or cognitive factors on hippocampal theta oscillations and show that failure to carefully isolate these different factors can result in spurious correlations.

Similar to previously reported results on gamma oscillations (Montgomery and Buzsaki 2007), the present results showed layer-specific increases in theta power and coherence on the center arm of the alternation task in a manner that could not be readily explained by running speed or acceleration (Fig. 2.4,2.6, Fig. 2.S3). Theta frequency also changed as a function of task performance, but exhibited more of a system-wide fluctuation, rather than layer-specific effects (Fig. 2.S4). Unlike gamma oscillations, theta power and coherence increased on the initial segment of the control tasks after release from the delay area (Fig. 2.5,2.6). These combined results suggest that CA3-CA1 gamma coordination may be related to mnemonic aspects of alternation task performance, such as memory retrieval (Montgomery and Buzsaki 2007), while the observed changes in theta power and coherence might be related to aspects of task performance such as route planning or other functions common to trial initiation in both alternation and control tasks. However, similar to the behavioral profile of CA3-CA1 gamma

coordination, CA3 theta oscillations exhibited a ~25 degree phase shift on the center arm of the alternation task that did not accompany trial initiation in the control tasks (Fig. 2.7, Fig. 2.S5). Computational models have suggested that encoding and retrieval may be separated on different phases of theta (Hasselmo et al. 2002) and behavior-dependent theta phase shifts have been previously reported during lever pressing versus running (Buzsaki et al. 1985) and as a function of encoding/retrieval (Manns et al. 2007) or learning/behavioral performance (Adey et al. 1960). Further experiments with higher spatial resolution sampling of LFP combined with unit recordings are needed to identify the mechanisms that govern coordination of different hippocampal regions during memory task performance.

The behavior-dependent layer specificity of theta oscillations also highlights the importance of recording theta oscillations from anatomically identified locations with a consistent (and distant) reference electrode in order to permit collected data to be compared across experiments and across laboratories. Locations such as the commonly used "fissure theta" may refer to any number of theta dipoles in neighboring layers, that can change with slight electrode movements (≤100μm) from day to day without experimenter's knowledge. On the other hand, the CA1 pyramidal layer is readily identifiable by the presence of unit activity and large "ripple" bursts during slow-wave sleep (Buzsaki et al. 1992; Ylinen et al. 1995b; Montgomery et al. 2008; Montgomery and Buzsaki 2007; Csicsvari et al. 2003) and can be used to calibrate LFP recordings in the hippocampus.

Overall, our study suggests that, rather than being a monolithic clock signal, hippocampal theta oscillations are created by a heterogeneous consortium of transmembrane currents, reflecting

layer-specific processing that can be modulated by extrahippocampal inputs or differential modes of operation within the hippocampus.

2.6 - Figure Legends

Figure 2.1: Layer specific synchrony of hippocampal theta oscillations. (A) One second epoch of LFP traces recorded from CA1stratum pyramidale (CA1sp) and str. lacunosum-moleculare (CA1lm) during running. (B) Example LFP traces (0.5 sec) from all 96 silicon probe recording sites (6 shanks at 300-μm spacing, each with 16 recording sites at 100 μm spacing). Traces are centered on the estimated recording location with respect to the hippocampal anatomy (gray lines) according to several measures of evoked and spontaneous activity, including perforant path stimulation, sharp wave-ripple activity and unit firing (Montgomery et al. 2008; Montgomery and Buzsaki 2007). (C) LFP coherence spectra between selected sites (shown in D) within the same layer (solid lines) and between different layers (dotted lines; ± 99% jackknife error bars). Note high within-layer coherence in the frequency range of theta (6-12 Hz) and its second harmonic. (D) Single animal example showing the anatomical profile of LFP theta coherence between selected reference sites (white rectangles) and all other recording sites. Dotted and solid rectangles indicate sites shown in C. Note the high coherence of theta oscillations between distant sites (1mm or more) within the same layer compared with the lower coherence between nearby sites (≤0.2mm) in different layers. (E) Group statistics showing the average within-layer (red) and between-layer (black) coherence as a function of distance between recording sites. The between-layer regression slope was significantly more negative than the within-layer slope ($p=1.9e^{-9}$). Similar results were obtained regardless of whether only channels in the middle of each layer were *a priori* selected from each shank and included in the analysis

(see Methods), or whether all functioning sites that could be *a priori* assigned to an identified layer were included (as shown in the regression plot). (F) Group data showing the average LFP (top) and current-source density (CSD; bottom) coherence for all layer-layer pairs. Note the high coherence values on the diagonal reflecting the strong within-layer coherence. Also note the higher LFP coherence between CA1or/sp and between DGgl/hr/CA3sp layers, likely reflecting volume conduction, compared to the more layer-specific coherence of the CSD traces. Abbreviations in this and subsequent figures: DG = dentate gyrus; CA = Cornu Ammonis; so = stratum oriens; sp = stratum pyramidale; sr = stratum radiatum; lm = stratum lacunosum moleculare; ml = molecular layer; gl = granule layer; hr = hilar region.

Figure 2.2. Phase relationships among multiple hippocampal theta current generators. (A) Single animal example of averaged LFP (black traces) and derived CSD (color; source=red; sink=blue) triggered on theta peaks from a selected CA1lm recording site overlaid on the hippocampal anatomy. (B) Theta phase shift (degrees) for all LFP (top) and CSD (bottom) sites with respect to a single CA1sp reference site (white rectangle). Note the highly consistent phase shifts in each layer. (C) Group data showing the average laminar phase shift of LFP (black) and CSD (red) theta oscillations with respect to a single CA1sp site for each animal. (D) Group data showing the LFP (top) and CSD (bottom) theta phase relationships between all layer-layer pairs. Size of the arrow reflects the phase consistency across layer-layer pairs. Note large arrows with near-zero phase offset between sites within the same layer. Inset, polar plots of specific layer-layer pairs. Note that the DGml shows a weak and variable phase relationship with other sites, possibly indicative of multiple theta dipoles in this layer (see also Fig. 2.S1). (E) Unit-CSD theta phase histograms for 4 example putative interneurons (recorded from CA3sp, DGgl, CA1or and

DGgl, respectively) in 2 animals (left and right columns). Note phase reversal of theta currents between CA1lm and DGml and between DGml and CA3sp (black arrows).

Figure 2.3. Layer-specific changes in theta power during task performance. (A) Rats were trained to perform a hippocampus-dependent, delayed spatial alternation task on a modified 'T-maze'. Gray blocks were used to hold the rat during the delay. Blue dots indicate water ports. (B) Example of running speed (cm/sec) as a function of spatial position during performance of the alternation task. (C) Group data histogram of running speeds observed in different regions of the maze (color-coded according to inset). (D) Example regression analysis examining the relationship between running speed and LFP theta power (decibels, dB, $10*\text{Log}_{10}$) from selected CA1lm and DGgl recording sites. Note the highly significant regression slope (beta value) between running speed and theta power in CA1lm, but nearly flat and non-significant slope for theta power in the DGgl. (E) Single animal example showing the anatomical profile of regression slopes between running speed and LFP theta power. (F) Group statistics calculated separately for LFP (top) and CSD (bottom) showing regression slopes between running speed and theta power for each layer (median beta ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests).

Figure 2.4. Maze region-dependent theta power fluctuations. (A) Single trial example of theta oscillations from recording sites in CA1sr and the DGgl. The raw LFP (gray) is overlaid on the spectrogram (color, same scale as in B). Black dotted lines delimit 0.5 second windows taken from each maze region for statistical analysis in C,D,E. Corresponding positions on the maze are shown above (red, sampled positions on each maze segment; gray, positions traversed during the

period shown in the spectrogram; black, positions traversed over the entire recording session). The 10-second delay period has been omitted from this plot. Note the increased theta power in CA1sr but not the DGgl on the center arm of the T-maze. (B) LFP theta power from the same layers as in A averaged over one recording session (20 trials) with respect to spatial position on the maze. (C, D) Fit of theta power with a general linear model (GLM) was used to assess the contribution of various overt and covert variables. (C) R-square of running speed and acceleration and maze region. Note that maze region explains substantial variance for CA1sr and some CA3sp/DGhr recording sites, while speed and acceleration explains very little (observe different scale bars). (D) Beta values showing changes in LFP theta power across the different maze regions for an example recording session. (E) Group statistics of theta power changes on the center arm versus other regions (center arm – other regions) across different anatomical layers (decibels, median ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests) for LFP (left) and CSD (right). Note that this comparison does not include running speed/acceleration as separate explanatory variables, but similar results were obtained when these variables were included (Fig. 2.S3A,B).

Figure 2.5. Performance in control tasks shows similar layer-dependent theta power fluctuations as the alternation task. (A) Control tasks with no dependence on the hippocampus (see Methods). Left, C-shaped linear track requiring the rats to run back and forth for water reward (n = 2 rats). Middle, Z-shaped linear track requiring the rat to run back and forth for water reward (n = 1 rat). Right, cue task requiring the rat to run in a path similar to the alternation task, but on each trial the rat's trajectory was randomly cued right or left by a large block (indicated by the dashed gray lines) that was visible after the delay period (n = 1 rat). Blue circles indicate location of water

reward. Solid gray lines indicate borders of the delay area(s). Dashed ovals indicate regions of control tasks that were compared to the center arm of the alternation task. (B) Average running speed maps (cm/sec) in each of the control tasks for trials with running directions shown in A (unidirectional running shown for C and Z shaped mazes and both right and left turn trials for the cue task). (C) Example of changes in LFP theta power from selected CA1sr sites during performance of control tasks on trials with running directions shown in A. Note increased theta power as the rat begins a journey to the reward port. (D) Example anatomical profile of the change in LFP theta power on the initial segment (dashed oval in A) versus other portions of the C-maze task (initial – other segments). Note the somewhat selective increase in CA1sr theta power on the initial segment similar to the center arm effect in the alternation task. (E) Group statistics comparing LFP (left) and CSD (right) theta power on the center arm of the alternation task versus the initial segment of each animal's respective control task (alternation – control, decibels, median ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests).

Figure 2.6. Layer specific changes in theta coherence on the center arm of the alternation task. (A) CSD theta coherence between a reference site in CA1sp (white rectangle in B) versus DGgl (top) or CA3sp (bottom) with respect to spatial position in the alternation task (color scale same as in B). (B) Example anatomical profile showing the change in CSD theta coherence between CA1sp and all other hippocampal recording sites on the center versus other maze arms (center arm – other regions). (C) Group statistics showing the center arm-associated change in CSD theta coherence between all hippocampal layer pairs. The color of each dot shows the median within-pair change in coherence and size of the dot indicates the significance of Bonferroni-

corrected non-parametric tests. The outlined comparisons correspond to the profile of changes in coherence with respect to the CA1sp shown in B. (D) Group statistics comparing CSD theta coherence between the center arm of the alternation task and initial segment of the control tasks.

Figure 2.7. Task-dependent CA3 theta phase shift. (A) CSD theta phase shift (degrees) between example CA1sp (reference) and CA3sp sites as a function of spatial position in the alternation task. (B) Anatomical profile showing the change in CSD theta phase between an example CA1sp reference site (white) and all other sites on the center arm of the alternation task (center arm – other regions). The outlined site corresponds to that shown in A. (C) Polar plot of theta phase (pooled over all four animals) between CA1sp and CA3sp in different regions of the alternation task (color-coded according to inset). Note the shorter phase lag between CA3 and CA1 theta on the center arm of the alternation task. (D) Group statistics showing the center arm-associated change in CSD theta phase between all hippocampal layer pairs. The color of each dot shows the median within-pair change in phase and size of the dot indicates the significance of Bonferroni-corrected non-parametric tests. The outlined comparisons correspond to the profile of changes in phase with respect to CA1sp shown in B. (E) CSD polar plot of phase (pooled over all four animals) between CA1sp and CA3sp on the center arm of the alternation task versus initial segment of control tasks. (F) Group statistics comparing CSD theta phase between the center arm of the alternation task and initial segment of the control tasks. (G) Example CSD traces (filtered 6-12Hz) from selected channels triggered by CA1sp theta peaks in the alternation and control tasks. Note in the alternation task how the CA3sp theta peak occurs ~7 milliseconds earlier (corresponding to 25 degrees at 9Hz) relative to the CA1sp theta oscillations.

Figure 2.S1. Large within-layer variability in the DGml (also see Fig. 2.2). (A) CSD theta phase histograms for CA1lm-DGml and DGml-DGml pairs, including all DGml sites within the *a priori* defined anatomical boundaries. This is in contrast to the data presented in the main figures, which only include a more limited sampling of one site per shank to represent each layer (see Methods). Note the large phase variability of theta currents both within the DGml and between the CA1lm and the DGml. This may be due to a mixture of theta dipoles within the DGml (see Discussion) and the 100 μm spatial resolution of the silicon probe may not be sufficient to isolate dipoles in the 3-sublayer dentate molecular layer. However, small movements of the probe across days provided indirect evidence for the presence of independent dipoles. (B and C) Coherence and phase plots on two consecutive recording days in which the probe position shifted by an estimated 60 μm. (B) CSD theta coherence between the highlighted DGml reference site (white) and all other sites. Note the stark change in DGml coherence with other hippocampal layers after only ~60um change in probe position. (C) CSD theta phase between the highlighted CA1lm reference site and other recording sites. The outlined DGml site shows a ~170 degree phase offset with CA1lm on day 1, but on day 2 the phase offset changes to ~90 degrees and neither adjacent sites on the same shank display the 170-degree phase shift. (D) Theta phase histograms of outlined sites in C. The complicated dipole structure in the DGml is further highlighted by the partially overlapping sets of channels in Fig. 2.6B,2.7B that exhibit behavior-dependent changes in theta coherence and phase.

Figure 2.S2. Running speed correlation with theta power in control tasks. Group statistics calculated separately for LFP (A) and CSD (B) showing regression slopes between running speed and theta power for each layer (median beta ± 95% bootstrapped error bars). Note the

generally weaker and more variable speed/power relationships (no comparisons reached statistical significance) across the different hippocampal layers in the control tasks as compared with the alternation task (Figure 2.3). This suggests that running speed *per se* is not the principal determinant of theta power changes in the hippocampus, but that under some behavioral conditions, speed may correlate with other factors that change with theta power. This notion is further supported by the fact that when running speed and maze region are both included in a linear model to explain theta power fluctuations (Fig. 2.4C), maze region explained substantially more variance for nearly all recording sites.

Figure 2.S3. Group statistics showing GLM analysis of the alternation task, including running speed, acceleration and each maze region as separate explanatory variables. Beta values for (A) LFP theta power and (B) CSD theta power (decibels, median ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests). (C) CSD theta coherence beta values. The color of each dot shows the median within-pair change in coherence and size of the dot indicates the significance of Bonferroni-corrected non-parametric tests.

Figure 2.S4. GLM analysis of behavior-dependent changes in theta frequency. (A,B) Theta frequency analysis of the alternation task, including running speed, acceleration and maze region as explanatory variables. Beta values for (A) LFP theta frequency and (B) CSD theta frequency (Hz, median ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests). Note that theta frequency changes by a small (<0.75Hz) but significant amount across maze regions even after removing effect of speed. Although some, but not all layers show a significant increase on the center arm, Kruskal-Wallis testing revealed that the changes in theta

frequency were not layer specific ($p>0.1$). Differences in theta frequency across layers could have been problematic for using a common theta frequency for spectral power, coherence, and phase estimates (see Methods), but the statistical similarity of theta frequency across layers supports the use of this method. This method is further supported by the fact that nearly identical results were obtained when spectral power, coherence, and phase estimates were averaged over the 6-12Hz theta range (data not shown). With more data it may be possible to distinguish slight differences in the layer-specifity of theta frequency changes during different behaviors.
(C,D) Theta frequency analysis of the alternation and control tasks including running speed and acceleration as separate explanatory variables. Beta values for (C) LFP theta frequency and (D) CSD theta frequency (Hz, median ± 95% bootstrapped error bars; *=$p<0.01$, Bonferroni corrected non-parametric tests). Note that theta exhibits a significantly higher frequency in most layers on the center arm of the alternation task compared to the initial segment of the control task. This alternation task increase in theta frequency appears to be a system wide effect, however, because there were no significant differences among the layers (Kruskal-Wallis test, $p>0.1$)

Figure 2.S5. The magnitude of theta phase shifts are maze region-dependent. (A) Maze region analysis of CSD theta phase shifts during performance in the spatial alternation task. The color of each dot shows the median within-pair change in coherence and size of the dot indicates the significance of Bonferroni-corrected non-parametric tests. To compare delay area activity with other maze regions, a 0.5 second window was taken from the end of the delay period immediately prior to exiting the delay area onto the center arm. Note that inclusion of the delay area into the maze region analysis did not substantially alter the pattern of phase shift results on the center arm. Furthermore, while the delay area itself does exhibit some phase shifting between

CA3sp/DGhr and other layers (also see Fig. 2.7A), these effects were not statistically significant ($p>0.01$). (B) Comparison of delay area activity in the alternation and control tasks. Note non-significant differences ($p>0.01$). These results do not rule out the occurrence of phase shifts among hippocampal theta oscillators during other portions of the delay period, but these effects and the presumed accompanying cognitive operations may not occur reliably at specific times in the delay area, whereas approaching the T-junction on the center arm may put a stronger demand on hippocampal operations. (C) Group statistics showing GLM analysis of the alternation and control tasks including running speed and acceleration as separate explanatory variables. Note that while this analysis extracts a relationship between acceleration and theta phase between particular layers, it also indicates that the CA3sp:CA1sp phase shift on the center arm of the alternation task cannot be simply accounted for by running speed or acceleration. (D) Group statistics comparing the initial segment to other arms in the control tasks (initial segment – other arms). Note that theta dipoles exhibited no significant phase shift during performance of the control tasks.

Section 3.0 - Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance

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3.1 - Abstract

The hippocampal formation is believed to be critical for the encoding, consolidation, and retrieval of episodic memories. Yet, how these processes are supported by the anatomically diverse hippocampal networks is still unknown. To examine this issue, we tested rats in a hippocampus-dependent delayed spatial alternation task on a modified T-maze while simultaneously recording local field potentials from dendritic and somatic layers of the dentate gyrus, CA3 and CA1 regions using high-density, 96-site silicon probes. Both the power and coherence of gamma oscillations exhibited layer-specific changes during task performance. Peak increases in the gamma power and coherence were found in the CA3-CA1 interface on the maze segment approaching the T-junction, independent of motor aspects of task performance. These results show that hippocampal networks can be dynamically coupled by gamma oscillations according to specific behavioral demands. Based on these findings, we propose that gamma oscillations may serve as a physiological mechanism by which CA3 output can coordinate CA1 activity to support retrieval of hippocampus-dependent memories.

3.2 - Introduction

Based primarily on lesion studies, a consensus has emerged that the hippocampal formation is critical for episodic memories (Squire 1992; Eichenbaum and Cohen 2001; Milner et al. 1998). In accord with lesion studies, the firing patterns of hippocampal and entorhinal neurons exhibit prospective and retrospective coding of episodic information (Frank et al. 2000; Wood et al. 2000; Ferbinteanu and Shapiro 2003; Shapiro and Ferbinteanu 2006). However, the specific roles of the various hippocampal subregions (Amaral and Witter 1995) responsible for encoding, consolidation and retrieval of memory traces have been long debated. Computational models (Wallenstein et al. 1998; Marr 1971; Rolls 1996; Treves 1995) have postulated the autoassociative recurrent network of the CA3 region as a suitable substrate for storing memories, which could be subsequently recalled via replay from CA3 to CA1. Circumscribed lesions in animals (Brun et al. 2002; Steffenach et al. 2002) and genetic manipulations (Nakazawa et al. 2002) also support the view that the integrity of the CA3 region is crucial in memory retrieval, but little is known about the physiological mechanisms of CA3-CA1 coordination that might support this process.

Although recordings from multiple single neurons can assess the output representations of a network, the currently available large-scale unit recording methods do not have the ability to effectively monitor how information transfer is coordinated across several neuronal networks (Buzsaki and Draguhn 2004). On the other hand, while local field potentials (LFP) lack single neuron resolution, they reflect the temporal synchrony of

local afferent activity and can effectively detect the changing *modes of operation* in local circuits (Bartos et al. 2007; Bressler and Kelso 2001; Varela et al. 2001). For example, synchronization of neuronal activity into coherent gamma frequency oscillations can serve to bind representations (Engel et al. 2001; Gray et al. 1989) and couple hippocampal and rhinal cortices during successful formation of declarative memories (Fell et al. 2001). To examine how regional networks within the hippocampal formation are dynamically coordinated during the retrieval process, we recorded LFPs from rats with high-density silicon probe arrays (Csicsvari et al. 2003) during performance of a hippocampus-dependent delayed spatial alternation task on a modified T-maze (Ainge et al. 2007). Because gamma oscillations in the hippocampus show clear regional and laminar patterns and the CA3 region is known to generate self-organized gamma oscillatory patterns (Isomura et al. 2006; Bragin et al. 1995a; Csicsvari et al. 2003; Mann and Paulsen 2005), we examined the laminar-specific power and coherence of gamma oscillations. Our results show that gamma frequency synchronization at the CA3-CA1 interface selectively increases on the center arm maze segment immediately preceding the T-junction, independent of the motor aspects of task performance. These findings signify that gamma oscillations can dynamically coordinate hippocampal networks according to behavioral demands. Based on these results, we hypothesize that gamma oscillations may provide a physiological mechanism by which CA3-CA1 activity is coordinated to support retrieval of hippocampus-dependent memories.

3.3 - Results

LFPs were recorded simultaneously in the dentate, CA3 and CA1 regions of the dorsal

hippocampus using a 2-dimensional silicon probe array with 96 monitoring sites. In agreement with previous observations, the *in situ* recording sites in the various regions and layers could be determined with high spatial resolution (±30 μm) using a combination of spontaneous LFP patterns, multiple unit activity, evoked potentials in response to perforant path and commissural stimulation and post-hoc histological identification of the anatomical position of each recording shank (Fig. 3.1; (Bragin et al. 1995a; Csicsvari et al. 2003); see Fig. 3.S2A for electrode positions in all animals).

To engage hippocampal networks, rats (n=4) were trained on a hippocampus-dependent, delayed spatial alternation task (Fig. 3.2A; (Ainge et al. 2007). This task requires rats to encode their spatial response on each trial and, after a 10 second delay, retrieve this information to appropriately choose the opposite arm from the one entered on the previous trial. Although retrieval of previous trial information could occur in this task at any time prior to crossing the T-junction, rats typically ran smoothly through the center arm and the T-junction in one swift trajectory, suggesting that retrieval processes likely occur before reaching the T-junction. All rats performed the task at high levels of proficiency (> 85% correct).

3.3.1 - Layer-specific gamma power increase on the center arm of the T-maze

We investigated the involvement of different hippocampal networks during performance of the spatial alternation task by measuring the power (amplitude) of gamma oscillations simultaneously in various layers, across different portions of the task. In agreement with previous findings, maximum LFP gamma power was recorded in the hilar region of the

dentate gyrus (Bragin et al. 1995a; Stumpf 1965). However, the relative power changes in the separate maze regions varied differentially across layers. As illustrated in Fig. 3.2B and C, the amplitude of the gamma oscillation (40-120 Hz; Fig. 3.S1), recorded from the middle of CA1 str. radiatum, showed a selective increase on the center arm that was not reliably observed in other hippocampal layers. Relative changes in gamma power were also observed in areas outside the CA1 str. radiatum in other segments of the maze, suggesting that the various hippocampal regions and afferents are involved in various task components (see Supplementary Movie 1) but the present behavioral paradigm did not reliably isolate these events.

Because the running pattern of the rat was not identical in the different segments of the maze and because previous research has shown speed and acceleration-dependent changes of hippocampal unit and field activity (Whishaw and Vanderwolf 1973; McNaughton et al. 1983), we first addressed the issue of whether overt motor behavior could account for the fluctuations in gamma power. Gamma power measurements were fit separately for each recording site with a general linear model (GLM), including as explanatory variables, running speed, acceleration, and maze region (see Methods). Similar to an analysis of covariance, GLM analysis dissects the trial-by-trial variance in gamma power measurements that can be accounted for by the variables of interest (Rencher 2002; Jacobs et al. 2006). GLM analysis showed that gamma power only weakly correlated with running speed and acceleration, as indicated by the consistently low r-square values ("explained variance" <10%) across all recording sites (Fig. 3.3A). Maze region, on the other hand, could explain over 30% of the observed variance in

gamma power at a number of sites. Especially striking was the degree to which the effect of maze region respected the laminar anatomy of the hippocampus, with the highest r-square value confined to the CA1 str. radiatum. Beta values of the GLM analysis, showing the direction and magnitude of the change in gamma power for each maze region (Fig. 3.3B), revealed increased gamma power in CA1 str. radiatum on the center arm of the maze. Because the dominant projection to CA1 str. radiatum derives from the CA3 region (Amaral and Witter 1995), the increase of gamma power in this layer suggests an enhancement of CA3 output to CA1 on the center arm of the T-maze.

To statistically compare task-related changes in the anatomical distribution of gamma power across animals, recording sites were assigned to specific layers (as shown in Fig. 3.S2B) and the distribution of beta values from each layer across all 4 rats was tested for reliable behavior-related changes (two-tailed t-tests). Figure 3.3C shows group statistics revealing differential increases in gamma power across hippocampal layers on the center arm versus other arms of the T-maze. Although significant increases in gamma power were observed in several hippocampal layers on the center arm of the maze, the gamma power increase in CA1 str. radiatum was larger than in all other layers except for the CA3 pyramidal layer (ANOVA, Tukey post-hoc test, $p<0.05$). Further statistical analysis, including the effect of running speed, acceleration and all maze regions is shown in Figure 3.S4. We also tested whether gamma patterns were different on “error” versus “correct” trials (data not shown), but found no reliable difference – possibly due to the low number of error trials, high behavioral variability on error trials and/or the foiled engagement of cognitive processes, e.g. retrieval mechanisms that return the wrong

information.

Even though the center arm increase in LFP gamma power shown in Figure 3.3C was concentrated in specific layers, the LFP may be contaminated by volume conduction of activity in other layers. To reduce the contribution of possible volume conducted fields, we first performed a one-dimensional current source density (CSD) analysis on the LFP data and repeated the GLM calculations on the derived data (Fig. 3.3D). This analysis revealed that the increased gamma oscillations in the CA3 pyramidal layer and CA1 str. radiatum were generated by local currents rather than volume conducted from the dentate gyrus or direct entorhinal input to the CA1 region (i.e., str. lacunosum-moleculare). CSD gamma power showed significant increases in the dentate molecular layer, possibly reflecting a change in entorhinal input, local circuitry or CA3 back-projections to the inner molecular layer of the dentate gyrus (Li et al. 1994). Gamma power also increased in the CA1 pyramidal layer, possibly reflecting the entrainment of CA1 gamma oscillations by CA3 input (Csicsvari et al. 2003; Fisahn et al. 1998) or str. radiatum return currents unmasked from the volume conduction bias caused by anatomical curvature.

3.3.2 - Enhanced CA3-CA1 gamma coherence on the center arm

Since power analysis suggested the engagement of specific intrahippocampal networks on the center arm of the T-maze, we further examined the functional connectivity by analyzing the coherence of gamma oscillations between hippocampal regions during alternation task performance. The mean gamma coherence was highest between sites within the same layer (see “Constant” term, Fig. 3.S4C), revealing that within-layer

processing is highly coordinated even over large physical distances (>1.5mm). Figure 3.4A shows the changes in CSD gamma coherence between a reference site in the CA3 pyramidal layer and two example sites in CA1 str. radiatum and the dentate molecular layer, averaged with respect to spatial position on the T-maze (see also Fig. 3.S3). The anatomical distribution of the center arm-related changes in gamma coherence between the CA3 pyramidal layer and all other hippocampal recording sites are shown in Fig. 3.4B (see also Fig. 3.S4, Movie 3.S2). Gamma coherence between site pairs was separately fit with GLM statistics for all rats. The matrices in Figure 3.4C and D show the group statistics for gamma coherence between all hippocampal layer pairs. Extending the gamma power results, the coherence analysis showed increased coordination of intrahippocampal circuits, reflected by the maximal gamma coherence increase between the CA3 and CA1 regions on the center arm of the maze.

3.3.3 - Task-specific enhancement of CA3-CA1 gamma synchrony

Although the GLM statistics are specifically designed to dissect individual explanatory variables (Rencher 2002), we performed additional control experiments to isolate the hypothesized mnemonic contribution to the observed changes in gamma oscillations. In addition to the alternation task, each rat was trained and tested on one of three control tasks (Fig. 3.S5) possessing no memory requirement and no dependence on the hippocampus in order to control for motor and non-mnemonic cognitive aspects of task performance. Similar to the alternation task, in each control task the rat was held in a small delay area and then released to run for water reward. The initial segment of these control tasks showed similar speed and acceleration profiles to the center arm of the

alternation tasks (Fig. 3.S5). Thus, comparison of these initial maze segments of control tasks to the center arm of the alternation task controls for basic route 'planning' operations that may accompany the start of a journey, and further controls for the effects of speed and acceleration. As evidence against these alternatives, we found significantly higher gamma power in the CA1 str. radiatum LFP in the alternation task relative to tasks with no mnemonic demand (Figure 3.5A; also see Fig. 3.S6). Separate analysis of CSD traces also revealed increased gamma power in the CA1 pyramidal layer and confirmed that the str. radiatum gamma oscillation was locally generated, indicating that the LFP gamma power effects in CA1 str. lacunosum-moleculare were likely due to volume-conducted contamination from str. radiatum (Figure 3.5B). Analysis of gamma coherence revealed enhanced CA3-CA1 coordination in the alternation task compared to controls. Smaller increases in coherence between CA1 and other layers were also observed, but notably, effects were weak with the CA1 str. lacunosum-moleculare and between the CA3 pyramidal layer and dentate layers, indicating that the other effects may have been mediated multisynaptically via the dominant changes in CA3-CA1 synchrony. These results provide evidence that gamma coordination of CA3-CA1 networks is enhanced by the specific cognitive demands of the delayed alternation, independent of motor performance.

3.4 - Discussion

The major finding of these experiments is that gamma oscillations are differentially modulated by aspects of behavior in the various hippocampal networks. Both the power and coherence of gamma oscillations at the CA3-CA1 interface were selectively

enhanced by the cognitive demands of the spontaneous alternation task.

Gamma oscillations have been suggested to assist various operations, including the binding of sensory attributes (Gray et al. 1989), synaptic plasticity (Bragin et al. 1995a), working memory (Lisman and Idiart 1995; Howard et al. 2003), sensory-motor coordination (Engel et al. 2001), attention (Fell et al. 2003a), formation of cell assemblies (Harris et al. 2003), memory coding (Sederberg et al. 2003; Fell et al. 2001; Sederberg et al. 2007b), and even consciousness (Llinas et al. 1998; Sauve 1999). These diverse putative functions indicate that gamma oscillations *per se* cannot be explicitly assigned to specific behavioral events. Instead, changes in the gamma oscillations recorded from a particular brain structure may be taken as an indicator that the supporting circuitry is engaged in a particular mode of computation.

3.4.1 - CA3 and CA1 networks are dynamically coordinated by gamma oscillations

Gamma oscillations have diverse expression throughout the hippocampal networks. They are generated by a consortium of mechanisms and depend primarily on the activity of fast spiking basket neurons and $GABA_A$ receptor-mediated inhibition and AMPA receptor-mediated excitation (Traub et al. 2004; Buzsaki et al. 1983; Whittington et al. 1995; Bartos et al. 2007; Traub et al. 1996; Wang and Buzsaki 1996; Whittington et al. 2000). Gamma oscillations in the CA1 str. lacunosum-moleculare and the dentate gyrus are mainly under the control of entorhinal input (Bragin et al. 1995a; Charpak et al. 1995). However, evidence from *in vitro* and *in vivo* studies suggests that the CA3 region can generate gamma oscillations from the interactions between CA3 pyramidal cells and

basket interneurons (Bragin et al. 1995a; Csicsvari et al. 2003; Mann and Paulsen 2005). The gamma phased-locked firing of CA3 projection neurons can in turn entrain the CA1 network (Csicsvari et al. 2003; Fisahn et al. 1998). We found dynamic changes in the power and coherence of CA3 and CA1 gamma oscillations. Some analyses also implicated specific layers in the dentate gyrus, though future experiments with dual entorhinal-hippocampal recordings will be needed to precisely determine to the extent to which these effects were related to direct entorhinal involvement, local activity or CA3-dentate projections. Since our recordings principally sampled the CA3b,c subregions, we cannot speculate about the role of the CA3a subregion from these data, given the differential wiring patterns of these subregions (Li et al. 1994). Nevertheless, our results provide further support for a role of the CA3 region in generating self-organized gamma activity, independent of the gamma oscillations in other hippocampal regions. Additionally, our results provide evidence that the temporal coordination of CA3 and CA1 networks is dynamically regulated via gamma synchronization, according to behavioral demands.

3.4.2 - Mnemonic function of CA3-CA1 gamma coupling

Numerous lesion studies in humans and other animals link hippocampal networks to memory (Eichenbaum and Cohen 2001; Milner et al. 1998). Previous computational models (Hasselmo et al. 2002; Marr 1971; Rolls 1996; Treves 1995), lesion studies (Brun et al. 2002; Steffenach et al. 2002) and genetic knockout studies (Nakazawa et al. 2002) have implicated CA3-CA1 communication in retrieval of hippocampus dependent memories. We found a consistent pattern of changes in the power and coherence of

gamma oscillations, showing increased synchrony in the CA3-CA1 interface on the center arm of the T-maze. Using statistics and analysis of control tasks to rule out the contribution of overt behavior and other non-mnemonic variables, we found that the increased CA3-CA1 gamma coordination was specifically enhanced on the center arm of the T-maze by cognitive aspects of alternation task performance. Performance of the delayed spatial alternation task requires, on each trial, that rats retrieve information about the previous journey in order to choose the opposite arm upon reaching the T-junction. Although the exact spatial position(s) at which retrieval processes occur likely varies from trial to trial (as does the CA3-CA1 gamma synchrony increase seen in the Supplementary Movies), the fact that rats typically run through the center arm and the T-junction in one swift trajectory suggests that retrieval and decision-making usually occurs on the maze prior to the T-junction. This is corroborated by previous studies finding that turn-selective neuronal firing emerges in the CA1 network during traversal of the center arm in similar tasks (Frank et al. 2000; Wood et al. 2000; Ferbinteanu and Shapiro 2003; Shapiro and Ferbinteanu 2006, but see Ainge et al. 2007; Bower et al. 2005). Based on our results and convergent evidence pointing to the mnemonic role of the hippocampus and its subregions, we hypothesize that the increased CA3-CA1 gamma coordination on the center arm of the T-maze may reflect retrieval of previous trial information. Overall, our findings suggest that gamma oscillations may provide a mechanism by which hippocampal networks can dynamically coordinate to perform specific mnemonic operations.

Our results also posit specific predictions for future experiments investigating the

changes in CA3 and CA1 unit firing that accompany retrieval processes. The observed increase in CA1 str. radiatum gamma power on the center arm of the T-maze is suggestive of accompanying changes in bursting and/or gamma synchrony of CA3 units. Increased bursting leads to a non-linear increase in neurotransmitter release (Lisman 1997), thereby increasing post-synaptic currents and the amplitude of LFP oscillations. Similarly, increased synchrony of unit firing increases the amplitude of LFP oscillations through greater temporal summation of post-synaptic currents (Buzsaki et al. 2003; Robbe et al. 2006). Local modulation of specific CA1 layers could also facilitate CA3 inputs, but this must occur at fast (ostensibly ionotropic) receptors to mediate the observed gamma fluctuations. The observed increase in gamma coherence between CA3 and CA1 suggests that the synchrony between CA3 and CA1 unit firing increases at short (monosynaptic) and gamma timescales during the retrieval process. Because hippocampal principal neurons fire in restricted spatial locations and because the AMPA receptor-mediated effects between pyramidal cells are weak (Sayer et al. 1990), assessment of the CA3-CA1 connections by monitoring unit activity would require recording very large numbers of neurons simultaneously. In contrast, using LFP recording, the gamma power and coherence provide information about the collective behavior of neurons and the mode of operation of a given network.

In summary, our findings show that gamma oscillations dynamically coordinate the activity of hippocampal networks during performance of a hippocampus-dependent memory task. The most robust change in network activity was increased gamma coordination between CA3 and CA1 regions during the portion of the task associated

with the retrieval of prior experience. While the specific content of the retrieved memory may not be accessible without large-scale single cell recordings, our results show that monitoring gamma oscillations with sufficient spatial resolution and proper behavioral control is an effective tool for identifying specific circuit dynamics involved in cognitive operations.

3.5 - Methods

3.5.1 - Animals and Behavior

Four Long-Evans rats (male, 300-400g) were water-deprived and trained to run in a hippocampus-dependent continuous delayed non-match to place ('alternation') task (Ainge et al. 2007) and a control task. All rats performed the alternation task well (>85% correct). The control tasks included a C-shaped (n = 2 rats) and a Z-shaped (n = 1 rat) linear track, requiring the rats to run back and forth for water reward, and a cue task (n = 1 rat) requiring the rat to run in a path similar to the alternation task, but on each trial the rat's trajectory was randomly cued left or right by a large block that was visible after the delay period. To constrain the behavioral and cognitive variability, trials in which the rat engaged in rearing, excessive sniffing, grooming or immobility were eliminated from further analysis.

3.5.2 - Spectral and GLM Analysis

Gamma band (40-120Hz) power analysis was performed using the filter-rectify-smooth method and spectral power and coherence analysis was performed using Morlet wavelet analysis (courtesy of Aslak Grinsted; see Supplementary Material). Fitting data from

correct trials in the alternation task (errors were rare and often associated with exploratory behavior), the distribution of spectral estimates (either power or coherence) for each electrode site was fit with a linear model (MatLab, ANOVAN, Type 3 SS): $G = \beta_{contant} + \beta_{running\ speed} + \beta_{running\ acceleration} + \beta_{maze\ region\ (4\ arms)} + \varepsilon$

To perform group statistics across animals, resulting beta (β) values from one electrode site per shank per animal were sampled from each layer (Fig. 3.S2B). The resulting sampled distribution of beta values from each anatomical layer was subsequently tested for a statistical difference from zero (t-tests, $p<0.001$, Bonferroni corrected). The normality assumption of the t-test and assumptions of the general linear model, including normality of residuals, absence of interaction effects, and uncorrelated residuals, were assessed. Similar analyses were performed to compare physiological parameters on the center arm of the alternation task to those in the corresponding region of a control task (Fig. 3.S5).

3.6 - Figure Legends

Figure 3.1. On-line calibration of recording sites. Ninety six-site silicon probe with recording pads spaced regularly over 1.5mm x 1.5mm area. The probe sites have a vertical spacing of 100μm and a horizontal spacing of 300μm. The anatomical position of recording electrodes in the different layers in the dentate gyrus, CA1, and CA3 regions of the hippocampus was determined using DiI labeling of electrode tracks (A) matched with known electrophysiological and anatomical characteristics of the hippocampus for each recording session. (B,C) Event triggered averages of the LFP (traces) and CSD (color) centered on the estimated position from which the activity was recorded. (B) Ripple-

triggered average responses (centered on ripple event, n=179). Note the large sink (blue) associated with a sharp wave in CA1 str. radiatum. (C) Average evoked activity in response to perforant path stimulation (left aligned to stimulation, n=3). Note the large sinks in the molecular layer of the dentate gyrus.

Figure 3.2. Changes in gamma power during performance of a delayed spatial alternation task. Rats were trained to perform a hippocampus-dependent, delayed spatial alternation task on a modified 'T-maze' (A). (B) Single trial example of the task-related fluctuations in LFP gamma oscillations from recording sites positioned in CA1 str. radiatum (CA1 rad) and the dentate molecular layer (DG mol). The filtered (40-120Hz) gamma trace (gray) is overlaid on the normalized spectrogram (color). Note burst of gamma power in CA1 str. radiatum but not the dentate molecular layer on the center arm of the T-maze. (C) Normalized LFP gamma power averaged over one recording session (20 trials) from the same layers with respect to spatial position on the maze.

Figure 3.3. Gamma power on the center arm is maximally expressed in the CA3-CA1 associational system. Fit of gamma power with a general linear model (GLM) was used to assess contribution of various overt and covert variables. (A) R-square of running speed and acceleration (Accel) and maze region. Note that maze region explains substantial variance for CA1 str. radiatum recording sites, while speed and acceleration explains very little. (B) Beta values showing changes in LFP gamma power (in decibels) across the different maze regions for a single recording session. (C, D) Group statistics of gamma power changes on the center arm versus other arms across different anatomical

layers (decibels, +/- std error; $p<0.001$, Bonferroni corrected t-tests) for LFP (C) and CSD (D). See also Fig. 3.S4.

Figure 3.4. CA3-CA1 gamma coherence is enhanced on the center arm. (A) Spatial distribution of gamma coherence between the CA3 pyramidal layer and example sites in CA1 str. radiatum (top; shown in B) and the dentate molecular layer (bottom). (B) Center arm change in gamma coherence between a CA3 pyramidal reference site (circle) and all other hippocampal recording sites averaged over an entire recording session (20 trials). (C,D) Group data. Change in gamma coherence between all hippocampal layer pairs on the center arm of the maze (C). Note peak increases in coherence at the CA3-CA1 interface. (D) Bonferroni corrected p-values. Note most significant values between CA3-CA1 layers. See also Fig. 3.S4.

Figure 3.5. Enhanced CA3-CA1 gamma coordination on the center arm cannot be explained by motor performance. Gamma power and coherence on the center arm of the alternation task was compared to the corresponding region of a control task (see Fig. 3.S5). (A, B) Group data show increases in gamma power (decibels, +/- std error) of the LFP (A) and CSD (B) in the alternation versus control tasks ($p<0.001$, Bonferroni corrected t-tests). (C) Group data for gamma coherence difference between the alternation task and control tasks and corresponding Bonferroni corrected p-values (D) from t-test comparisons in (C). See also Fig. 3.S6.

Figure 3.S1. Anatomical distribution of power spectral density of LFP (A) and CSD (B)

recorded during alternation task performance. Traces reflect power (in decibels) averaged over a single recording session (20 trials). A prominent wide 'hump' or power enhancement between 40 Hz and 120 Hz from the 1/f power baseline with a peak centered around 80Hz was observed in all animals and defined the 'gamma band'. This power increase was most prominent in CA1 str. radiatum and the hilus. The choice of this band does not affect our main findings because similar results were obtained with more restricted bandwidths centered around 80Hz.

Figure 3.S2. Layer sampling and illustration of GLM analysis. (A) Approximate electrode positions in each of the four rats. The exact vertical position of the electrodes with respect to the hippocampal layers was determined *a priori* for each recording day (as described in Fig. 3.1, Detailed Methods). Recording sites with excessive impedance or crosstalk were identified *a priori* and eliminated from further analysis (as described in Detailed Methods; Diba et al. 2005). The second silicon probe shank in the first animal was missing before implantation. (B) The gamma power from one site from each shank in each anatomical layer was separately fit with a general linear model and the resulting distribution of beta values (up to 6 shanks x 4 animals) were tested for a significant difference from zero using Bonferroni corrected t-tests. To perform group statistics across animals, resulting beta (β) values from one electrode site per shank per animal were sampled from each layer. This sampling avoids underestimation of effects due to categorization of anatomical transition zones and ensures at least 300 μm spacing between sites in a given layer, minimizing statistical dependence due to volume conduction. In some cases numerically large, layer-specific effects (e.g. the increased

CSD gamma power in the CA3 pyramidal layer shown in Figure 3.5B) may have reached statistical significance and further supported our conclusions by additional layer sampling. Because the distribution of beta values rather than each individual beta value was tested for a significant difference from zero, homogeneity in the variance of GLM residuals was not a precondition for statistical interpretation.

Figure 3.S3. Phase locking of CA3 and CA1 gamma oscillations on the center arm of the T-maze. A) Single trial example of coherence (color) and phase (gray x's, averaged for each time point over 40-120Hz gamma band). Note the reliable CA3-CA1 phase relationship on the center arm compared to substantial phase variability on other portions of the maze. B) Examples of filtered CA3 and CA1 CSD traces during high and low coherence epochs (σ = extracellular conductance). Although the CA3-CA1 phase relationship is easily observed in the frequency domain, it is more difficult to assess in the time domain.

Figure 3.S4. Group statistics showing GLM analysis of the alternation task, including running speed, acceleration and each maze region as separate explanatory variables. Beta values for (A) LFP gamma power and (B) CSD gamma Power (decibels, +/- std error; Significance: $p<0.001$, t-tests Bonferroni corrected). (C) CSD gamma coherence mean beta values and (D) Bonferroni corrected p-values from t-tests. The "constant" term (left column) reveals the basal level of gamma power (A,B) or inter-layer coherence (C) after the relationship with the explanatory variables is accounted for. Note that significant gamma power increase was present in the molecular and granule cell layer in the center

arm (see also Fig. 3.3). This increase could reflect the CA3-dentate backprojection (Li et al. 1994) or could be brought about by local activity or entorhinal input (Chrobak and Buzsaki 1998a). The latter effect can be resolved by simultaneously recording currents from both dentate and entorhinal cortical areas. For the present analysis we only included maze regions in which the rat was actively running on the maze to avoid contamination from sharpwave/ripple brain states. However, additional analysis of gamma activity (not shown) including the water ports and the last 0.5 seconds of the delay period did not change the central findings of the study (as indicated by the example plots in Figures 3.2C and 3.4A).

Figure 3.S5. Motor performance in the delayed alternation and control tasks. (A) Delayed alternation task (n = 4 rats). (B-D) Control tasks with no memory requirement or dependence on the hippocampus. (B) C-shaped linear track requiring the rats to run back and forth for water reward (n = 2 rats). (C) Z-shaped linear track requiring the rats to run back and forth for water reward (n = 1 rat). (D) Cue task requiring the rat to run in a path similar to the alternation task, but on each trial the rat's trajectory was randomly cued right or left by a large block (indicated by the dashed gray lines) that was visible after the delay period (n = 1 rat). These tasks had similar overt behavioral requirements to the alternation task, but have no memory requirement and no dependence on the hippocampus. Blue circles indicate location of water reward. Solid gray lines indicate borders of the delay area(s). Dashed ovals indicate regions of comparison between tasks.

Figure 3.S6. Group statistics showing GLM results comparing the center arm of the

alternation task and to the corresponding region of control tasks, including running speed and acceleration as explanatory variables. Beta values for (A) LFP gamma power and (B) CSD gamma Power (decibels, +/- std error; Significance: $p<0.001$, t-tests Bonferroni corrected). (C) CSD gamma coherence mean beta values and (D) Bonferroni corrected p-values from t-tests. The "constant" term (left column) reveals the basal level of gamma power (A,B) or inter-layer coherence (C) after the relationship with the explanatory variables is accounted for. The constant term of the coherence (C, see also Figure 3.S4) highlights the layer specificity of layer-layer coherence and the accuracy of our *a priori* layer assignment (see Figure 3.1). The sites identified as CA3 are highly coherent with other CA3 sites and dentate sites are highly coherent with other dentate sites but they show much less coherence between the regions. In fact, the CA3 pyramidal layer, on average, has numerically higher gamma coherence with its output in CA1 str. radiatum than with the geometrically intervening dentate sites (C). These results show that our identified CA3 sites share a common network synchrony not shared by nearby dentate sites. These findings favor CA3 specific effects, as opposed to contamination by activity of the surrounding dentate gyrus. Parsimony also suggests that the increased CA3-CA1 synchrony on the center arm of the alternation task can explain the increased coherence between CA1 and other layers for the following reasons: 1) there is no significant increase in dentate-CA3 coherence, 2) CA3-CA1 coherence shows the largest increase, suggesting its primacy, 3) a change in CA1 LM-CA1 str. pyramidale gamma coherence is notably absent, 4) the higher power gamma oscillations in CA1 str. radiatum could lead to greater return currents in CA1 LM, which may possibly account for the CA1 LM-CA1 str. radiatum coherence.

Movie 3.S1. Layer-specific changes in gamma power during alternation task performance. Three consecutive, correct trials of alternation task performance with epochs in the delay area and water port area removed. Left panel, current position (red dot) and trajectory of the rat (gray dots) on the maze. Right panel, normalized LFP gamma power (z-score) for each of the 96 recording sites (colored pixels) aligned to the anatomy of the hippocampus (gray lines). Note the degree to which fluctuations in gamma power respect the laminar anatomy of the hippocampus. The transient burst of gamma power in CA1 str. radiatum reliably accompanies traversal of the center arm but not other maze areas. Note on the 3rd trial how the rat is distracted while starting down the center arm and the CA1 str. radiatum gamma power increases only after the rat reengages in performance of the task. Movie is playback ¼ speed; real time is displayed beneath the left panel.

Movie 3.S2. Changes in gamma coherence during alternation task performance. The same three trials as Supplementary Movie 1. Left panel, current position (red dot) and trajectory of the rat (gray dots). Right panel, normalized CSD gamma coherence (x-μ) for each recording site (colored pixels) referenced to a CA3 pyramidal layer recording site (white dot) aligned to the anatomy of the hippocampus (gray lines). Although coherence measures commonly exhibit more “noise” than power values, CA3-CA1 gamma coherence clearly shows a selective enhancement on the center arm of the maze. Movie is playback ¼ speed; real time is displayed beneath the left panel.

3.7 - Detailed Methods

3.7.1 - Behavioral Training

Rats were initially shaped in the alternation task with no delay period and were required only to run on the maze to receive water reward. When consistent maze running and approach to the water points was established, the water delivery on the side arms was gradually withdrawn for trials in which the rat did not alternate. After alternation performance was established, a 10 second delay was interposed between each trial to require engagement of hippocampal networks. To maintain consistent running on the maze without exploratory interruptions, a small water reward was delivered in the delay area and increased water rewards were given at the appropriate water port for uninterrupted trajectories. After learning the alternation task, rats were trained on one of the control tasks.

3.7.2 - Surgery

A 96-site silicon probe was implanted on a movable drive in the right hemisphere parallel to the transverse axis of the hippocampus (45° parasaggital) with the outer shanks targeted at approximately AP -2.8, ML 2.7 and AP -3.86, ML 1.64 from bregma. DiI was applied to probe prior to implantation to assist in histological analysis. A bipolar angular bundle (perforant path) stimulating electrode was implanted AP 1.0 mm, ML -1.0 mm from the junction between lambda and the right lateral ridge and DV -3.5 mm from the dura. A bipolar commissural stimulating electrode was implanted at AP -1.2 mm, ML -1.0 mm from bregma and -3.8 mm from the dura. Ground and reference screws were implanted in opposite hemispheres above the cerebellum.

3.7.3 - Electrode Localization

Post-mortem electrode location was verified using thionin, fluorescent Nissl (Invitrogen, Carlsbad, California) or DAPI (Invitrogen) staining in combination with DiI (Invitrogen) labeled electrode tracks. Prominent morphological features of the hippocampal anatomy were outlined and aligned with analysis of hippocampal physiological signals, including: ripple-triggered CSD, perforant path evoked CSD (Fig. 3.1, Csicsvari et al. 2003), ripple power, dentate spike-triggered CSD (Bragin et al. 1995b), and multiunit activity (data not shown).

3.7.4 - Data Acquisition and Analysis

Neurophysiological signals were acquired continuously at 20 kHz on two synchronized 64-channel DataMax system (16-bit resolution; RC Electronics Inc.). Local field potentials were down-sampled to 1.25kHz for further analysis. For tracking the position of the animals, two small light-emitting diodes (11-cm separation), mounted above the headstage, were recorded by a digital video camera and sampled at 30 Hz. Stable recording sessions in which the silicon probe shanks spanned the CA1, CA3 and dentate gyrus somatic layers, were analyzed using custom-written Matlab-based software (Mathworks, Natick, MA). Recording site irregularities (including cross-talk and excessive impedances) were *a priori* identified and removed from analysis using measures of coherence and normalized power similarity (Diba et al. 2005). Current source density was calculated by standard methods (Mitzdorf 1985) and spatially smoothed, interpolating bad channels.

3.7.5 - Spectral Analysis

Coherence was calculated on the CSD estimates to avoid contamination from volume conduction and coherence was calculated for all channels with respect to one channel from each specified anatomical layer. To avoid differential bias in the spectral estimate and statistical dependence due to overlapping time windows, on each trial a 0.5 second window was sampled separately from each maze arm (as shown in Fig. 3.2B). The spectral power and coherence was independently calculated for each window, tapered by a normalized Hanning window to remove edge effects, and averaged over the gamma frequency band. Running speed and acceleration were similarly estimated by averaging over a tapered 0.5 second window. The spectral power was converted to decibels ($10*\text{Log}_{10}$) and the coherence was transformed by the hyperbolic arc tangent such that the sample distributions would approximate a Gaussian (Thomson and Chave 1991).

Section 4.0 - Theta and gamma coordination of hippocampal networks during waking and REM sleep

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4.1 - Abstract

REM sleep has been considered a paradoxical state because, despite the high behavioral threshold to arousing perturbations, gross physiological patterns in the forebrain resemble those of waking states. To understand how intrahippocampal networks interact during REM sleep, we used 96-site silicon probes to record from different hippocampal subregions and compared the patterns of activity during waking exploration and REM sleep. Dentate/CA3 theta and gamma synchrony was significantly higher during REM sleep compared to active waking. In contrast, gamma power in CA1 and CA3-CA1 gamma coherence showed significant decreases in REM sleep. Changes in unit firing rhythmicity and unit-field coherence specified the local generation of these patterns. Although these patterns of hippocampal network coordination characterized the more common tonic periods of REM sleep (~95% of total REM), we also detected large phasic bursts of local field potential (LFP) power in the dentate molecular layer that were accompanied by transient increases in the firing of dentate and CA1 neurons. In contrast to tonic REM periods, phasic REM epochs were characterized by higher theta and gamma synchrony among the dentate, CA3 and CA1 regions. These data suggest enhanced dentate processing, but limited CA3-CA1 coordination during tonic REM sleep. In contrast, phasic bursts of activity during REM sleep may provide windows of opportunity to synchronize the hippocampal trisynaptic loop and increase output to cortical targets. We hypothesize that tonic REM sleep may support offline mnemonic processing, while phasic bursts of activity during REM may promote memory consolidation.

4.2 - Introduction

Sleep is not a homogeneous state, but is composed of various stages of slow-wave sleep and rapid eye movement (REM) sleep (Borbely and Tobler 1989; Steriade and Llinas 1988). REM sleep is characterized by muscle atonia, saccadic eye movements, a host of autonomic effects and, at least in humans, dreaming (Aserinsky and Kleitman 1953; Berger and Oswald 1962; Dement and Wolpert 1958; Hobson and Pace-Schott 2002; Llinas and Pare 1991). There is considerable debate on the function of REM sleep (Buzsaki 1989; Vertes 2004; Walker and Stickgold 2004; Crick and Mitchison 1983; Muzur 2005; Siegel 2001), and the only real consensus is that we need a better understanding of the physiological changes accompanying REM sleep to inform our functional theories (Walker and Stickgold 2004; Gottesmann 2001). Despite presumed functional differences in brain activity between the waking state and REM sleep, available physiological evidence indicates global electroencephalogram (EEG) similarities in forebrain areas (Vertes and Kocsis 1997; Llinas and Pare 1991; Wehrle et al. 2007). Both waking and REM sleep are associated with theta oscillations in the hippocampus and "desynchronized" low-amplitude, high-frequency EEG activity in the neocortex (Grastyan and Karmos 1961; Jouvet 1999; Lissak et al. 1962), leading many investigators to lump these states together for analysis. Furthermore, brain reactivity to external stimuli during REM sleep is more similar to waking responsiveness than that observed during slow-wave sleep (Wehrle et al. 2007; Bastuji and Garcia-Larrea 1999). These similarities are surprising since waking and REM forebrain activity profiles are accompanied by strikingly different firing patterns of serotonergic (McGinty and Harper 1976), catecholaminergic (Chu and Bloom 1973) and histaminergic (Sakai et al. 1990) neurons.

Given the specific innervation of hippocampal networks from hindbrain modulator systems (Amaral and Witter 1995) and the shift in subcortical neuromodulator tone between REM and waking states, we hypothesized that there may be hitherto undetected state-dependent changes in hippocampal network activity that may inform our functional understanding of the hippocampus during REM sleep. To test this hypothesis, we used high-density silicon probes to record local field potentials and unit activity from the different layers of the dentate gyrus, CA3 and CA1 hippocampal regions across periods of active waking and REM sleep. Recording simultaneously from the different hippocampal subregions permitted us to investigate how hippocampal networks dynamically coordinate with one another in different behavioral states. We found that, while the dentate and CA3 became more highly synchronized by theta and gamma oscillations during REM sleep, CA3-CA1 gamma coordination was significantly reduced during REM sleep compared to during active waking. Furthermore, we found phasic bursts of activity during REM sleep in which theta and gamma oscillations in the dentate, CA3 and CA1 were transiently synchronized, and the output firing of CA1 neurons was transiently increased. We hypothesize that our observations may reflect enhanced processing of hippocampus-dependent memories in the dentate and dentate/CA3 interface during REM sleep, with brief windows during which these associations may be transmitted through the trisynaptic loop to downstream cortical targets.

4.3 - Methods

4.3.1 - Animals and Behavior

LFP and unit activity was recorded from four Long-Evans rats (male, 300-400g) during active waking behavior and REM sleep. Rats were water-deprived and trained to run on a modified linear track and in a continuous delayed non-match to place (‘alternation’) task for water reward

(Montgomery and Buzsaki 2007). Immobility periods (<5cm/sec) and consummatory behaviors during which hippocampal networks shift to a sharpwave state were excluded from analysis. Data were combined across the two tasks to represent "active waking". REM sleep recordings were performed in the animals' home cages before and after maze running. Once theta activity emerged in the sleeping rat, the experimenter verified that the rat was still in sleeping posture, typically curled up in one of the corners. Occasionally, behavioral signs of phasic REM sleep were present, including limb movements and twitching of whiskers (Vanderwolf 1969). REM sleep was detected semi-automatically using the ratio of the power within the theta band (4-12Hz) to the power in nearby bands (1-4Hz, 12-30Hz; Fig. 4.S1; Csicsvari et al. 1999; Louie and Wilson 2001). Visual inspection of whitened (using a low order autoregressive model; Mitra and Pesaran 1999) power spectra and raw traces was used to manually include brief fluctuations in the theta/delta ratio nested in long periods of REM sleep (~10sec to several minutes) and to exclude portions that were interrupted by CA1 pyramidal layer ripple oscillations (120-250Hz; Buzsaki et al. 1992) occurring during slow wave sleep or muscle artifacts (most prominent above 250Hz) that commonly accompany waking after the termination of REM sleep. REM periods were cross-validated with experimenter notes taken while watching the rat and listening to online LFP/unit recordings.

4.3.2 - Surgery

After proficient maze running was established, recording and stimulation electrodes were implanted. A 96-site silicon probe was implanted on a movable drive in the right hemisphere parallel to the transverse axis of the hippocampus (45° parasagittal) with the outer shanks targeted at approximately AP -2.8mm, ML 2.7mm and AP -3.86mm, ML 1.64mm from bregma.

Probes had recording sites spaced regularly over a 1.5mm x 1.5mm area with 6 shanks spaced at 300µm, each with 16 recording sites at 100µm spacing. DiI was applied to the probe prior to implantation to assist in histological analysis. A bipolar angular bundle (perforant path) stimulating electrode was implanted AP 1.0mm, ML -1.0mm from the junction between lambda and the right lateral ridge and DV -3.5mm from the dura. A bipolar commissural stimulating electrode was implanted at AP -1.2mm, ML -1.0mm from bregma and -3.8mm from the dura. Ground and reference screws were implanted in opposite hemispheres above the cerebellum.

4.3.3 - Electrode Localization

Post-mortem electrode location was verified using thionin, fluorescent Nissl (Invitrogen; Carlsbad, California) or DAPI (Invitrogen) staining in combination with DiI (Invitrogen) labeled electrode tracks. Prominent morphological features of the hippocampal anatomy were outlined for subsequent analysis of hippocampal physiological signals (Fig. 4.1A). LFP ripple bursts (120-250Hz) were detected on CA1 pyramidal layer sites and aligned to the depth negativity of the concomitant sharpwave for anatomical localization (Fig. 4.1B). Using a combination of the histology, ripple-triggered current source density (CSD), perforant path evoked CSD, ripple power, dentate spike-triggered CSD, and multiunit activity (Fig. 4.1, Montgomery and Buzsaki 2007; Bragin et al. 1995b; Csicsvari et al. 2003), the anatomical location of recording sites could be determined to a high degree of accuracy (Bragin et al. 1995a), estimated to about ±30 µm.

4.3.4 - Data Acquisition and Analysis

Neurophysiological signals were acquired continuously at 20kHz on two synchronized 64-channel DataMax systems (16-bit resolution; RC Electronics Inc.). Local field potentials were

down-sampled to 1.25kHz for further analysis. For tracking the position of the animals, two small light-emitting diodes (11cm separation), mounted above the headstage, were recorded by a digital video camera and sampled at 30 Hz. Stable recording sessions in which the silicon probe shanks spanned the CA1, CA3 and dentate gyrus somatic layers, were analyzed using custom-written Matlab-based software (Mathworks; Natick, MA). Recording site irregularities (including cross-talk and excessive impedances) were *a priori* identified and removed from analysis using measures of coherence and normalized power similarity (Diba et al. 2005). The CSD was calculated by standard methods (Mitzdorf 1985) and spatially smoothed. Typically, a single bad channel was surrounded by good channels and was linearly interpolated prior to CSD calculation to avoid false-positive detection of current sinks/sources. Two adjacent bad channels in the middle of a silicon probe shank were interpolated in four instances (each in different layers) in order to utilize remaining channels on the shank. CSD calculations centered on bad channels were excluded from further analysis. Occasionally, a group of nearby channels were defective and a portion of the silicon probe, or, in two cases, the entire silicon probe shank was excluded from analysis. In total, 11-19 LFP measurements and 5-19 CSD measurements were *a priori* selected from each layer for further analysis (see "Statistics" below and Montgomery and Buzsaki 2007). For unit analysis, the wide-band signals were digitally high-pass filtered (0.8–5kHz) and automatically spike sorted using KlustaKwik (Harris et al. 2000), followed by manual adjustment of the clusters (using the Klusters software package; Hazan et al. 2006). Pyramidal cells and interneurons were separated on the basis of their auto-correlograms, waveforms and mean firing rate (Csicsvari et al. 1999; Bartho et al. 2004). Bursting was quantified as the fraction of interspike intervals shorter than or equal to 6 milliseconds (Harris et al. 2001). To reduce noise contamination of bursting analysis, cells with overall firing rates < 0.1Hz were

excluded (similar results were obtained with rate thresholds from 0.01-0.5Hz).

4.3.5 - Spectral Analysis

To obtain independent spectral estimates, LFP data from waking and REM was divided into non-overlapping one second windows. Theta (4-12Hz) and gamma band (40-120Hz) power and coherence analysis was performed using Morlet wavelet analysis (courtesy of Aslak Grinsted) and multi-taper Fourier analysis (Mitra and Pesaran 1999). Coherence was calculated on the CSD estimates to avoid contamination from volume conduction and was calculated for all channels with respect to one channel from each specified anatomical layer. The spectral power was converted to decibels (10*Log10) and the coherence was transformed by the hyperbolic arc tangent such that the sample distributions would approximate a Gaussian (Thomson and Chave 1991). For each cell, one second epochs with at least 4 spikes were detected. From these epochs, cells with at least 5Hz average firing were included in spectral analysis (similar results were obtained with different rate thresholds). Phasic REM was detected using a two standard deviation threshold of the integrated spectral power (0-250Hz) of the whitened signal (Mitra and Pesaran 1999), averaged across dentate molecular layer sites. Threshold crossings were subsequently subjected to a local maxima search in a +/- one second window to ensure the peaks of phasic bursts were detected. Theta wavelength variability was investigated in the time domain by detecting peaks of the filtered (4-12Hz) LFP and quantified using temporal autocorrelations and the second temporal derivative of spike times (i.e. the change in inter-peak interval).

4.3.6 - Statistics

To perform group statistics, up to one site per silicon probe shank was *a priori* chosen to

represent each anatomical layer (based on above electrode localization; also see Montgomery and Buzsaki 2007). Choosing one channel ensured greater statistical independence of layer sampling (at least 300μm between sites) and prevented contamination from sites on the border between two layers. For each site, the difference across behaviors (e.g. REM versus waking) was calculated and the distribution of difference scores was tested against the null hypothesis of zero change. LFP results were tested using Bonferroni corrected t-tests after checking assumptions of normality and homogeneity of variance. Unit results were tested using Bonferroni corrected non-parametric within-cell Kruskal-Wallis and post-hoc tests.

4.4 - Results

LFPs and unit activity were recorded simultaneously from the dentate gyrus, CA3 and CA1 regions of the dorsal hippocampus using a 2-dimensional silicon probe array with 96 monitoring sites. In agreement with previous observations, the *in situ* recording sites in the various anatomical regions and layers could be determined with high spatial resolution (±30 μm) using a combination of spontaneous LFP patterns, multiple unit activity, evoked potentials in response to perforant path or commissural stimulation, and post-hoc histological identification of the anatomical position of each recording shank (Fig. 4.1A,B; Csicsvari et al. 2003; Montgomery and Buzsaki 2007). To observe sustained theta/gamma oscillations, rats (n=4) were tested during active waking on an elevated maze and during REM sleep in the home cage (see methods). To confine our analysis to theta associated behaviors, maze epochs associated with occasional stops and other consummatory behaviors, as reflected by <5 cm/sec locomotion speed, were excluded from the analysis. REM sleep was identified by large changes in the LFPs from large amplitude slow oscillations and ripples associated with slow-wave sleep (Isomura et al. 2006) to theta

rhythm in the absence of locomotion or overt movements.

4.4.1 - Increased dentate-CA3 synchrony and decreased CA3-CA1 synchrony during REM sleep

Although theta/gamma oscillations were continuously present during both waking and REM sleep states, striking differences in their regional distributions were obvious, even by visual inspection of the raw LFP traces (Fig. 4.1C,D). During REM sleep, 6-8Hz theta waves and high frequency gamma oscillations dominated in the dentate, while waking activity was accompanied by smaller amplitude 8-10Hz theta waves and gamma activity in this region. To quantify these effects, the power of LFP theta (4-12Hz) and gamma (40-120Hz) oscillations were calculated separately for each recording site in both states (Fig. 4.2A) and the power differences between the states displayed on a pseudo-color scale (Fig. 4.2B). To exclude potential contamination of LFP by volume-conducted fields, an identical analysis was also carried out on the calculated current-source density traces (see Methods). Group comparisons of both LFP and CSD confirmed that theta and gamma power was significantly enhanced during REM sleep in the dentate gyrus. Despite similar CA1 theta power in REM and wake, the second and third harmonics of theta oscillations, reflecting theta wave shape asymmetry, were typically higher during running segments (Fig. 4.1C,D; 4.2A, Buzsaki et al. 1986; Terrazas et al. 2005). However, CA1 showed significantly decreased gamma power during REM sleep (Fig. 4.2C,D). Coherence analysis supported and extended the conclusions drawn from the power analyses. In general, during REM sleep, theta coherence significantly increased among the different layers of the dentate and CA3 and apical dendritic layers of CA1, whereas theta coherence across CA1 str. pyramidale, radiatum and str. lacunosum-moleculare decreased significantly (Fig. 4.3). Similarly, gamma coherence during REM sleep significantly increased between the dentate

molecular layer, hilar region and CA3c pyramidal layer. Gamma coherence in the CA3-CA1 axis, on the other hand, diminished during REM sleep, with highly significant decreases among CA1 layers and between the CA3 pyramidal layer and CA1 str. pyramidale and radiatum.

The frequency of theta oscillations was significantly higher during running in the maze than during REM sleep (Fig. 4.4G; $p<0.001$). Differences in waking and REM theta frequency have been reported previously (Louie and Wilson 2001), though other findings (Leung 1984) suggest the effect may depend on the specific waking behavior. However, the power and coherence observations summarized above cannot be simply explained by the specific motor behaviors in the waking rat since similar results were obtained when running speeds and differences in theta frequency were also considered (Fig. 4.S2,4.S3). The frequency and layer specificity of these effects, in addition to the CSD results (Fig. 4.2,4.3), further subdue any potentially confounding effects of volume conduction. Overall, the findings reflect a fundamental difference in network operation between REM sleep and waking states. During REM sleep there is a high degree of synchronous processing in the dentate/CA3 regions, especially at theta frequencies, while during waking there is a shift to greater gamma-mediated coordination between CA3 and CA1.

4.4.2 - Phasic activation of hippocampal networks during REM sleep

The frequency and power of theta and gamma oscillations were more variable during REM sleep than while running in the maze (Fig. 4.4F-H; Fig. 4.S4). Previous work has shown that sudden increases in theta frequency and power during REM sleep are associated with clusters of eye movements, ponto-geniculo-occipital (PGO) spikes, muscle twitches, accelerated or irregular heart rate and respiration (Robinson et al. 1977; Karashima et al. 2005; Lerma and Garcia-Austt

1985; Rowe et al. 1999; Sakai et al. 1973; Sano et al. 1973; Sei and Morita 1996; Valle et al. 1992), and distinguished these episodes as phasic REM sleep from the tonic REM epochs, characterized by steady muscle atonia. Examining the laminar profile of LFP activity during REM sleep, we found phasic increases in the power and frequency of both theta and gamma oscillations, most prominent in the molecular layer of the dentate gyrus (Fig. 4.4). These phasic power increases had spectral peaks in the theta and gamma bands, though the power often showed broad-band increases, up to nearly 250Hz. Examining several combinations of spectral parameters, we found that these phasic bouts of activity were best characterized by increases in the integrated power (0-250Hz) averaged across dentate molecular layer recording sites (Fig. 4.4B). Using a threshold of 2 standard deviations, we detected 131 phasic bouts (typically lasting 1-2 seconds) out of a total of 3337 seconds of REM sleep (3.9% across all animals). Theta frequency during phasic REM epochs was comparable with theta present during running behavior and significantly higher than during tonic REM sleep (Fig. 4.4D,G; $p<0.001$). These phasic LFP changes were consistently associated with increased unit activity in the dentate gyrus and CA1, but not in CA3 (Fig. 4.4E, 4.6A, Fig. 4.S6). In addition to the distinct phasic REM epochs, theta power also showed variability within tonic REM periods. This theta power fluctuation during tonic REM may reflect "replay" of waking patterns (Louie and Wilson 2001), or, alternatively, a modulation by underlying slower rhythms (Leopold et al. 2003). Another major difference between the waking state and REM sleep was the higher variability of the theta wave periods during tonic REM (Fig. 4.4F,H). This was not simply due to gradual or systematic shifts in frequency because comparing adjacent wave-by-wave periods revealed a significantly larger theta wave variability in tonic REM compared to phasic REM sleep and waking theta (Fig. 4.4H; $|\Delta\text{wavelength}|$, $p=0$, Kruskal-Wallis and post-hoc tests).

4.4.3 - Phasic REM epochs transiently synchronize DG, CA3 and CA1 networks

Because of the significant differences between phasic and tonic stages of REM sleep, we analyzed the changes in the anatomical profile of theta and gamma power and coherence patterns separately for each state. Since REM sleep is dominated by tonic REM activity with only short interspersed episodes of phasic patterns (Hobson and Pace-Schott 2002), the wake versus tonic REM analyses yielded essentially the same results (Fig. 4.S5) as the wake versus entire REM sleep sessions (Fig. 4.2,4.3). Compared to tonic REM periods, phasic REM epochs showed increased theta and gamma power throughout most hippocampal layers (Fig. 4.5A). Comparing phasic versus tonic REM also showed increased theta coherence among CA1 layers and between the dentate and CA1, possibly indicative of more coherent inputs from layers 2 and 3 of the entorhinal cortex to the dendrites of the dentate and CA1 respectively. Gamma coherence increased among nearly all hippocampal regions during phasic REM suggesting that while tonic REM is associated primarily with dentate/CA3 gamma synchrony, phasic epochs may provide short windows in which the dentate, CA3 and CA1 transiently synchronize. These highly significant results were obtained despite the relatively small number of detected phasic epochs, indicating robust changes in hippocampal network coordination during phasic REM sleep. Using alternative phasic detection criteria (e.g. REM epochs with theta frequency in the highest 5 percentile) yielded similar effects (data not shown).

The coordination of hippocampal networks during phasic REM epochs also showed differences from the waking state (Fig. 4.5B). Theta power was significantly greater in phasic REM than during wake across nearly all hippocampal layers and theta coherence was increased among

layers of the dentate, CA3, and apical dendritic layers of CA1 during phasic REM. Gamma power also showed large increases in the dentate gyrus during phasic REM, though a slight but significant decrease in CA1 str. radiatum compared to the waking state ($p<0.01$). Similarly, gamma coherence showed significant increases among dentate layers and between the dentate and CA3/CA1 layers during phasic REM. Although there was a decrease in gamma coherence between CA1 str. radiatum and str. pyramidale, there was no significant difference in CA3-CA1 gamma coherence ($p>0.05$) between phasic REM and waking, possibly suggesting a differential contribution of CA1 apical, basal, and perisomatic compartments in mediating CA3-CA1 gamma coordination during phasic REM. In total, these data suggest that during phasic REM, dentate/CA3 networks are even more synchronized by both theta and gamma oscillations than during tonic REM sleep. Furthermore, during phasic epochs, coordination between dentate, CA3 and CA1 networks is transiently reinstated.

4.4.4 - Changes in single unit firing properties between waking and REM sleep states

Although the spacing of the recording sites of the 96-site silicon probe is not ideal for single unit separation (Wilson and McNaughton 1993), we could nevertheless isolate several putative principal cells and interneurons in various regions and layers (Csicsvari et al. 2003). We recorded from a variety of cell types in different layers (Fig. 4.S6), but for statistical analysis we confined our analysis to putative CA1 and CA3 pyramidal cells and fast firing, putative dentate granule layer interneurons (Fig. 4.6). The discharge rates of dentate area putative interneurons and CA1 pyramidal cells mirrored the changes of LFP power, being highest during phasic REM sleep (Fig. 4.6A). In contrast, the activity of CA3 pyramidal cells was significantly lower during tonic REM compared to waking state while phasic REM sleep rates showed intermediate values.

The incidence of "burst" discharges (defined as spikes at <6 msec intervals; Ranck 1973; Harris et al. 2001) of dentate interneurons and CA1 principal cells were higher during phasic REM than during tonic REM or waking behavior (Fig. 4.6B), while CA3 pyramidal cells showed the highest incidence of bursting during tonic REM sleep. These results show that CA1 and dentate neurons selectively increase output firing and bursting during phasic REM, while CA3 firing rates and bursting change somewhat independently of firing in other hippocampal networks.

Rhythmic discharge activity of hippocampal neurons was congruent with the power changes of the LFP and CSD. We assessed rhythmic firing of neurons by calculating rate-normalized power spectra of unit firing patterns (Fig. 4.6C; Jarvis and Mitra 2001).The low number of spikes recorded during the short and sparsely occurring phasic REM epochs precluded quantitative analysis of firing patterns during phasic REM sleep. In accord with the field analysis, dentate interneurons and CA3 pyramidal cells showed increased theta rhythmicity during tonic REM compared to the waking state, while CA1 pyramidal cells showed no significant difference between these two states. Furthermore, CA3 and CA1 neurons showed greater gamma rhythmicity during active waking than during tonic REM, congruent with the field results and underscoring the generation of these patterns by local networks. Finally, we analyzed the degree to which neuronal firing phase-locked with extracellular currents recorded from other hippocampal layers (Fig. 4.6D). Although firing rates of CA3 and CA1 neurons were too low to obtain reliable results, dentate interneurons showed significantly increased theta coherence with CSD traces across most hippocampal layers during tonic REM sleep compared to waking. Dentate interneurons showed a split effect of unit-CSD gamma coherence, with significantly increased coherence with dentate recording sites and significantly decreased coherence with CA1

str. radiatum during tonic REM. Thus, the unit results support and extend the LFP analysis and show how firing patterns of single neurons coordinate within and across hippocampal networks during active waking and REM sleep.

4.5 - Discussion

Numerous functions have been postulated for REM sleep since its discovery over 50 years ago (Aserinsky and Kleitman 1953), but the best consensus is that we need a better understanding of REM sleep physiology to shape our functional theories (Walker and Stickgold 2004; Gottesmann 2001). REM sleep is not only the state with the highest threshold to arousing perturbations from the body and the environment, but it also minimizes the brain's ability to control the skeletal muscle system (Chase and Morales 1990). This dramatically different behavioral state has been assumed to be "paradoxically" associated with seemingly identical physiological forebrain patterns as in the fully waking brain (Llinas and Pare 1991; Jouvet 1999; Steriade 2000). The present results, however, reveal distinct changes in the coordination of hippocampal networks across REM and waking states, implicating fundamentally different intrahippocampal processing in the waking and sleeping animal.

The state-dependent shifts in theta and gamma coordination of hippocampal sub-regions is summarized in Figure 4.7. Overall, we found increased dentate/CA3 theta and gamma synchrony during REM sleep compared to active waking behavior. In contrast, gamma power in CA1 and CA3-CA1 gamma coherence showed significant decreases in REM sleep. Changes in unit firing rhythmicity and unit-field coherence further specified the local generation of these patterns. Although these patterns of hippocampal network coordination characterized the more common

tonic periods of REM sleep (~95% of total REM), we also detected large phasic bursts of LFP power in the dentate molecular layer, accompanied by transient increases in the firing of dentate and CA1 neurons. In contrast to tonic REM periods, these phasic REM epochs were characterized by higher theta and gamma synchrony among the dentate, CA3 and CA1 regions. Although phasic REM patterns, exhibiting high CA3-CA1 gamma coherence and relatively consistent theta-wave periods, were more similar to waking activity than activity during tonic REM sleep, phasic REM sleep also significantly differed from patterns of the waking state.

4.5.1 - Critical role of the CA3region in coordinating hippocampal networks

The role of the CA3 region in these operational shifts in hippocampal processing is likely to be critical. Gamma rhythmicity of CA3 unit firing decreased during tonic REM, likely contributing to the decreased CA1 gamma power and CA3-CA1 gamma coherence (Bragin et al. 1995a; Csicsvari et al. 2003; Fisahn et al. 1998; Mann and Paulsen 2005). Interestingly, compared to the waking state, during tonic REM sleep there is no change in CA3 gamma power and a significant increase in gamma coherence between the dentate molecular layer and the CA3 pyramidal layer. These results suggest that during tonic REM sleep, there may be a state-dependent shift in the contribution of local CA3 perisomatic inhibition and other proximal inputs (e.g. mossy fiber input) to local CA3sp gamma currents, and this shift may result in a failure to entrain output spiking. Furthermore, while CA3 pyramidal cells show less gamma rhythmicity during tonic REM, they show increased theta rhythmicity. Similarly, local CA3 theta currents show increased coherence with specific dentate and CA1 layers, perhaps reflecting enhanced coherence of the layer 2 and layer 3 entorhinal cortical inputs (Chrobak and Buzsaki 1998b). These combined results suggest that the CA3 region defaults to the broader theta rhythm to mediate coordination

of hippocampal networks during tonic REM at the expense of the temporally and spatially more precise gamma coordination. These findings may be contrasted with the theta-gamma changes in the absence of entorhinal inputs. Following surgical removal of the entorhinal cortex, the decreased theta power in the DGml and CA1lm is associated with a remarkable increase of CA3 gamma power and increased gamma coherence between CA3 and CA1 regions (Bragin et al. 1995a). These combined findings support the hypothesis that both the dentate and the CA3 networks contribute to gamma oscillations (Bragin et al. 1995a; Csicsvari et al. 2003).

4.5.2 - Mechanisms of hippocampal activity patterns during REM sleep

A prominent difference between REM sleep and the waking state is a change in subcortical neuromodulator tone. While the concentration of acetylcholine (ACh) in the hippocampus is high during both REM sleep and active waking (Marrosu et al. 1995), norepinephrine, serotonin and histamine are high during waking, but nearly absent during REM sleep (Aston-Jones and Bloom 1981; Park et al. 1999; Brown et al. 2001). Noradrenergic, serotonergic and histaminergic fibers densely innervate specific hippocampal layers (Amaral and Witter 1995; Inagaki et al. 1988) and each of these modulators exert numerous actions on hippocampal neuronal firing (Vizi and Kiss 1998; Nitz and McNaughton 1999), synaptic transmission (Brown et al. 1995; Brown and Haas 1999; Segal 1981c; Segal 1981a; Segal 1981b), neuronal resonant properties (Hu et al. 2002), and LFP theta and gamma oscillations (Hajos et al. 2008; Kocsis et al. 2007; Krause and Jia 2005). These subcortical mechanisms likely explain the difference between waking and tonic REM sleep. However, hippocampal patterns underlying phasic episodes of REM sleep differed from both active waking and tonic REM sleep. A potential explanation is that pontine waves during phasic REM episodes fire brainstem cholinergic neurons (Datta 1997) and are associated

with increased power and frequency of hippocampal theta oscillations (Karashima et al. 2005). Previous work, however, has shown that phasic epochs of hippocampal theta during sleep, similar to movement-associated theta in the waking animal, are resistant to muscarinic ACh receptor blockade (Robinson et al. 1977; Kramis et al. 1975), though nicotinic receptors may play a role in phasic REM periods (Reinoso-Suarez et al. 2001). Additionally, while serotonergic neuron firing is inversely related to the occurrence of pontine waves (McGinty and Harper 1976), noradrenergic neurons in the locus coeruleus fire short bursts of activity correlated with pontine waves (Chu and Bloom 1973). These bursts may release sufficient amount of the critical neurotransmitter to transiently reinstate synchrony among hippocampal networks and perhaps exert similar effects on neocortical circuits as well.

4.5.3 - Functional implications of REM/Waking changes in hippocampal network synchrony

Greater LFP synchrony reflects higher temporal coincidence of synaptic activity (Buzsaki and Draguhn 2004), promoting greater plasticity and spike transmission and has been suggested to reflect enhanced local processing (Engel et al. 2001; Gray et al. 1989). Consistent with this, previous results have shown greater plasticity related gene expression in the dentate gyrus during REM sleep (Ribeiro et al. 1999), which may result from increased dentate synchrony in this state. More broadly, during REM sleep the dentate may engage pattern separation (Rolls 1996; Leutgeb et al. 2007) and/or recombination of previous memories to facilitate novel inferences (Walker and Stickgold 2004). Interestingly, during tonic REM, CA3-CA1 gamma coherence significantly decreases, while theta coherence is maintained or slightly increased. Since theta oscillations exhibit a more global synchronization across the extent of the hippocampus than gamma oscillations (Buzsaki 2002; Bragin et al. 1995a), this difference between theta and

gamma may reflect a decrease in the extent to which local assemblies can transmit specific information across these networks (Harris et al. 2003), while maintaining global synchronization of the system. From this perspective, we may speculate that increased temporal coordination and neuronal firing in the dentate area during tonic REM sleep may bring about plastic changes within this region but these effects are not transmitted through CA3 to CA1 and downstream targets. The altered balance of input of CA3 and the entorhinal inputs to CA1 may further explain the theta phase reversal of specific CA1 pyramidal cells during REM sleep (Poe et al. 2000).

We also found phasic periods of REM sleep that transiently increased theta and gamma synchrony in the dentate, CA3 and CA1 regions, in contrast to the limited CA3-CA1 gamma coherence during tonic REM sleep. Recent work has implicated the pontine waves associated with phasic REM as important for hippocampus-dependent memory consolidation (Datta et al. 2004; Datta et al. 2005; Mavanji et al. 2004). Reinstatement of CA3-CA1 communication during phasic bouts of REM sleep may provide windows of opportunity for information encoded in the dentate during tonic REM to be replayed back to the neocortex (Louie and Wilson 2001). Because these phasic epochs transiently reestablished waking-like communication between the hippocampal regions and increased output to the neocortex, it is also possible that these short but powerful episodes are responsible for generating the dream content of sleep. These phasic windows are of particular interest in light of recent observations in depth recording from the hippocampus in human epileptic patients. In contrast to the continuous presence of theta throughout REM sleep in lower animals, theta oscillations in these human subjects were only observed in short bouts lasting a second or so (Cantero et al. 2003; but see Bodizs et al. 2001).

Thus, it is possible that in humans only the phasic REM-associated, large amplitude theta bouts are detectable.

Overall, our data shows that hippocampal subregions exhibit dynamic oscillatory coupling at theta and gamma frequencies. Even within "theta behaviors", hippocampal networks engage differential coupling patterns that may reflect specific modes of operation. The present data reveal large-scale changes in hippocampal network coordination between active waking, tonic and phasic REM sleep and cast a new light on hippocampal function during REM sleep.

4.6 - Figure Legends

Figure 4.1. Identification of recording site locations and laminar specific changes in hippocampal network synchrony during REM sleep versus active waking behavior. (A,B) Anatomical positions of recording sites were localized for each recording session by aligning the histology with several spontaneous and evoked activity patterns (see Methods). (A) Histological sections with DiI labeling of electrode tracks (yellow) overlaid with estimated locations of single units (red points; location estimated from waveform amplitudes; putative cell types: triangle=principal cell, circle=interneuron, diamond=unclassified) and average perforant path evoked potentials (black traces, n=3). Note the EPSP-associated negativity in the dentate molecular layer, and monosynaptic and disynaptic population spikes in the dentate and CA3 respectively resulting from perforant path stimulation. (B) Ripple-triggered average LFP responses (black traces, centered on ripple event, n=179) overlaid on normalized ripple power (color, 120-250Hz) and outline of histological features (gray lines). (C,D) Example of raw local field

potential traces recorded during REM sleep and running in a maze. (C) One second LFP traces from CA1 stratum radiatum (CA1sr) and the dentate granule layer (DGgl). (D) LFP activity (0.5 seconds overlapping with trace in C) centered on the anatomical location from which the trace was recorded. Note the higher theta and gamma oscillation amplitudes on dentate recording sites during REM sleep, but the larger gamma in CA1sr during wake. Signals were spatially interpolated over *a priori* identified defunct recording sites (see methods).

Figure 4.2. Region specific changes in hippocampal theta and gamma power during REM sleep versus active waking behavior. (A) Example power spectra from one animal (decibels, +/- SEM across recording sites) of local currents recorded from CA1 stratum radiatum (CA1sr, n=6 sites) and dentate molecular layer (DGml, n=4 sites) sites during a single recording session including REM and waking. Theta (4-12Hz) and gamma (40-120Hz) frequency ranges are highlighted in gray. (B-D) Changes in the power (amplitude) of theta (left column) and gamma (right column) oscillations across REM and waking states (REM – Wake). Positive values indicate increases during REM sleep compared with active waking behavior. (B) Anatomical profile showing state-dependent changes in LFP power for each recording site from one recording session. (C,D) Group statistics showing changes in LFP (C) and current source density (CSD, D) power across different layers of hippocampal subregions (decibels, +/- SEM; $p<0.01$, Bonferroni corrected t-tests). Calculating $dB = 10*\log_{10}(Voltage^2)$, a 1-4dB change is equivalent to a 12-58% change in the voltage amplitude of the raw LFP oscillation. Abbreviations in this and subsequent figures: DG = dentate gyrus; CA = Cornu Ammonis; so = stratum oriens;

sp = stratum pyramidale; sr = stratum radiatum; lm = stratum lacunosum moleculare; ml = molecular layer; gl = granule layer; hr = hilar region.

Figure 4.3. Behavior dependent changes in theta and gamma coherence across hippocampal regions. (A) Example coherence spectra from one animal (+/- SEM across recording site pairs) between CA3sp and CA1sr (top, n=6 pairs) and between CA3sp and DGml (bottom, n=4 pairs) during REM and waking. Note the peak in gamma coherence ~80Hz that varies differently between site pairs across behavioral states. Coherence peaks in the 15-30Hz range during waking likely reflect increased theta harmonics during running behavior (see Fig. 4.1,4.2, Buzsaki et al. 1986; Terrazas et al. 2005). (B, C) Changes in CSD theta (left column) and gamma (right column) coherence across REM and waking states (REM – Wake). (B) Group statistics showing REM sleep associated change in theta and gamma coherence between all hippocampal layer pairs. The color of each dot shows the average within-pair change in coherence and size of the dot indicates the significance of Bonferroni-corrected t-tests. The outlined column of dots corresponds to the profile of changes in coherence with respect to the CA3 pyramidal layer (CA3sp). (C) Example anatomical profile of the changes in coherence with respect to the CA3 pyramidal layer (outlined white pixel) from one recording session.

Figure 4.4. Identification of phasic activity bursts in the dentate gyrus. (A) Whitened spectrogram of LFP recorded from the dentate molecular layer showing a typical period from REM sleep. Note the transient increases in the power and peak frequency of both theta and gamma oscillations. The gamma power increase in the whitened spectrum

typically peaked around 100Hz, but the elevated power in these bursts often extended up to around 250Hz. (B) Phasic bursts of activity in REM sleep were detected using a threshold of two standard deviations from the average molecular layer integrated whitened power (0-250Hz). (C) Typical example of the dentate molecular layer LFP during a detected burst of activity. (D) LFP power spectra in the dentate molecular layer compared across different behavioral states (decibels, mean +/- SEM across 18 DGml recording sites). (E) Granule layer multiple unit firing rates increased during detected phasic bursts (n=13, +/- SEM). (F-H) Lower theta frequency and greater wave-by-wave variability during low power "tonic" periods than during detected phasic periods or active waking behavior. (F) Example autocorrelogram of theta peaks. (G) Group data histogram of peak theta frequencies calculated from one second spectral estimates. (H) Wave-by-wave change in theta wavelength (second temporal derivative of theta peak times) with bootstrapped 95% confidence bars.

Figure 4.5. Changes in hippocampal network coordination during phasic REM versus tonic REM and active waking. Changes in theta and gamma synchrony (left and right panels of each subplot respectively) between phasic and tonic REM sleep (A-C, Phasic – Tonic) and between phasic REM and active waking (D-F, Phasic – Waking). Group statistics show changes in CSD power (A,D; decibels, +/- SEM, Bonferroni corrected t-tests) and coherence (B,E; dot color, mean within site change; dot size, significance of Bonferroni-corrected t-tests). (C,F) Examples from one recording session showing anatomical maps of coherence changes with respect to a single reference site (white pixel) illustrating the highlighted group of layer pairs (dotted outline) in (B,E).

Figure 4.6. Behavior dependent changes in hippocampal unit firing. (A-C) Changes in firing properties of CA1 pyramidal cells (pyr, top row), dentate granule layer interneurons (int, middle row), and CA3 pyramidal cells (bottom row) during active waking (black), phasic (red) and tonic (cyan) REM sleep. Changes in (A) firing rate (n: CA1=28, DG=10, CA3=27), (B) bursting (fraction of ISIs < 6 milliseconds; (Harris et al. 2001) n: CA1=18, DG=10, CA3=18), and (C) unit-firing theta (4-12Hz) and gamma (40-120Hz) rhythmicity (rate normalized spectral analysis, (Jarvis and Mitra 2001); n: CA1=15, DG=10, CA3=13). (D) Changes in unit-CSD coherence across active waking and tonic REM sleep. Top row, one-second example of a putative interneuron and simultaneously recorded CSD from the dentate granule layer. Note the high degree of unit firing theta rhythmicity and phase-locking to local currents. Middle row, average coherence spectrum of all granule layer interneurons with dentate hilar region CSD traces. Bottom row, group statistics showing theta and gamma coherence changes between granule layer interneuron firing and CSD traces recorded from all hippocampal layers (n: CA1=15, DG=10, CA3=13). Due to small sampling and potential contamination from non-stationarity effects, phasic REM was excluded from unit spectral analyses. CA1 and CA3 pyramidal cells were further excluded from coherence analyses due to insufficient firing rates. See Methods for firing rate inclusion criteria of bursting and spectral analyses. All results were statistically tested using Bonferroni-corrected within-cell non-parametric Kruskal-Wallis and post-hoc tests; * $p<0.05$.

Figure 4.7. Summary of hippocampal network coordination during active waking and

tonic and phasic REM sleep. Bar height reflects within-region synchrony changes, combining the effects of CSD power, within-region CSD coherence, unit rhythmicity, and within-region unit-CSD coherence. Arrow thickness reflects between-region coordination changes, combining CSD coherence and unit-CSD coherence between different hippocampal regions. Note the increase in dentate synchrony and dentate-CA3 coordination at theta and gamma frequencies during tonic REM sleep compared to active waking. In contrast, gamma synchrony in CA1 and CA3-CA1 gamma coordination was significantly lower during tonic REM sleep than active waking. Phasic REM, on the other hand, is accompanied by high theta and gamma synchronization throughout the tri-synaptic circuit.

Figure 4.S1. Detection of REM sleep. Whitened spectrogram of CA1lm LFP recorded while the rat was in the home cage. Black line shows the ratio of theta power (4-12Hz) to power in nearby frequency bands (1-4Hz, 12-30Hz). Dotted lines mark the beginning and end of the identified REM period. SPW: power increase associated with sharp wave ripples. Blue trace shows 10 seconds of raw LFP recorded during the transition from slow-wave sleep (SWS) to REM sleep. Note large amplitude spindles, likely transmitted from the neocortex, typical for SWS-REM transition (Gottesmann et al. 1998). Note the strong peak in the 4-12Hz band during REM with brief increases in theta power and frequency during phasic bursts. REM often terminated with 200Hz+ EMG activity, volume conducted from the nuchal and jaw muscles, when the rat briefly awoke, yawned, jaw clenched and stretched. Sleep recordings were monitored online via visual monitoring of the rat and audio amplification of the LFP. When the characteristic audio

indicated an LFP transition to theta, the time was noted and the animal was visually inspected for signs of sleep (eyes closed, relaxed posture, motionlessness occasionally interrupted by muscle twitches) and subsequent wake times were noted (eyes open, often posture adjustment). Offline, REM periods were initially detected using the power ratio of theta to nearby bands (Csicsvari et al. 1999; Louie and Wilson 2001). Spectrograms and raw traces of these periods were subsequently examined by eye to adjust start/end times and to include brief dips in the theta power ratio embedded in long REM periods. Identified REM periods were checked against experimenter notes to determine validity of spectral criteria.

Figure 4.S2. Hippocampal network changes during waking versus REM sleep after restricting to epochs with overlapping theta frequency. To rule out the contribution of different theta frequency distributions between active waking and REM sleep (Fig 4.4G), we selected one second epochs in which the peak theta frequency was in a maximally overlapping interval (7.6-8.1Hz) between the two states. (A) Changes in theta and gamma CSD power (decibels, +/- SEM; $p<0.01$, Bonferroni corrected t-tests) and (B) changes in theta and gamma CSD coherence (dot color, mean within layer change; dot size, significance of Bonferroni-corrected t-tests). Overall, the pattern of results from this comparison is very similar to that comparing across all theta frequencies during REM and waking (Fig. 4.2,4.3). This suggests that the primary difference in hippocampal network coordination results from a qualitative state-dependent shift rather than numerical changes resulting from differences in theta frequency. Although it is somewhat difficult to assess slightly weaker effects because this could result from reduced sampling of the

data, it is interesting to note that some effects got stronger in this restricted comparison. For example, theta power in CA1 layers (sr and lm) and gamma coherence with specific dentate layers showed a significant increase during REM sleep in this analysis. These effects may reflect changes in hippocampal network coordination that are correlated with changes in theta frequency. However, these effects may also result in part from subthreshold phasic REM events among these higher frequency REM periods or due to specific behaviors correlated with lower frequency waking periods.

Figure 4.S3. Changes in hippocampal network coordination during slow-moving waking periods versus REM sleep. To assess whether our results were dependent on the contribution of high-speed running epochs, we reanalyzed the REM sleep versus waking comparison after selecting only those waking epochs in which the rat was moving 5-10 cm/sec. (A) Distribution of running speeds averaged over one second epochs for all animals (running speeds < 5 cm/sec excluded; blue, 5-10 cm/sec; gray, >10 cm/sec). (B) Changes in theta and gamma CSD power (decibels, +/- SEM; $p<0.01$, Bonferroni corrected t-tests) and (C) changes in theta and gamma CSD coherence (dot color, mean within site change; dot size, significance of Bonferroni-corrected t-tests). Overall, despite utilizing only ~15% of the waking data, the pattern of results from this comparison is very similar to that comparing REM and waking across all running speeds, suggesting that the primary difference in hippocampal network coordination results from a qualitative state-dependent shift in between REM sleep and active waking. There are a few interesting differences between this analysis and previous analyses (e.g. differences in theta power/coherence with CA1sr/lm and some modest differences in gamma

coherence with CA1), but future studies will be needed to determine the extent to which these are genuine running speed effects or due to other components of task performance that are correlated with changes in running speed (Wyble et al. 2004; Whishaw and Vanderwolf 1973; Montgomery and Buzsaki 2007).

Figure 4.S4. Comparison of phasic activation during REM sleep versus waking behavior. (A,B) Integrated power, averaged over identified dentate molecular layer sites, and plotted as a function of time. Several short REM and waking periods were concatenated from recordings gathered during one recording session. (A) Whitened spectral power (see methods) integrated over 0-250Hz similar to Figure 4.4B. (B) Non-whitened spectral power integrated over 40-120Hz range. "Phasic" epochs during REM and waking (red dotted lines; 41 during REM sleep versus 13 during wake) were detected using a 2 standard deviation threshold (dotted black line). From the same data, using a higher threshold of 2.5 standard deviations, 23 phasic activations were detected during REM and zero during waking. Note that the detection metrics in (A) and (B) showed similar peak times during REM sleep. During waking, the presence of large theta harmonics (reflecting wave shape asymmetry, see Fig. 4.1,4.2) and effects of specific whitening parameters increased the baseline whitened integrated (0-250Hz) spectral power. However, when analyzing only REM periods, the whitened 0-250Hz power provided lower noise for detecting phasic activations during REM.

Figure 4.S5. Changes in hippocampal network coordination during tonic REM sleep versus active waking. (A) Changes in theta and gamma CSD power (decibels, +/- SEM;

$p<0.01$, Bonferroni corrected t-tests) and (B) changes in theta and gamma CSD coherence (dot color, mean within site change; dot size, significance of Bonferroni-corrected t-tests). Note that the pattern of results from this tonic versus wake comparison is nearly identical to the all REM versus wake comparison. This is not surprising given that tonic REM sleep accounts for the vast majority of total REM (~95% using our phasic REM detection criteria).

Figure 4.S6. Firing rates of units recorded from different hippocampal subregions across REM and waking behaviors. Location of maximal spike amplitudes was used to determine layer origin of each unit (rows) and aspects of unit firing and wave shape (see Methods) were used to separate principal cells and interneurons (columns). Each plot shows the median firing rate from active waking, phasic and tonic REM (+/- 95% bootstrap confidence intervals; n, number of recorded neurons of each type).

Section 5.0 - General Discussion

Using high density LFP and unit recordings, the studies described in this thesis examined how the hippocampal networks dynamically coordinate with one another at theta and gamma frequencies under different behavioral conditions.

5.1 - Theta coordination of hippocampal networks during behavioral task performance

"The theta rhythm" has been long thought of as the central clocking mechanism of the hippocampal system, imposed on the hippocampal formation by the medial septum (Lee et al. 1994; Stewart and Fox 1990) and perhaps the supramammillary nucleus (Vertes and Kocsis 1997). The assumption that theta is a monolithic rhythm in the hippocampus (and persistence in the face of evidence to the contrary; Kamondi et al. 1998; Bland 1986; Lee et al. 1994) has very commonly led researchers to use a single bipolar electrode placed somewhere in the hippocampus to measure the amplitude and/or phase of "hippocampal theta" across different behavioral conditions (c.f. Kahana et al. 2001). Although this method has been recognized as a source of experimental variability for over 25 years (Robinson 1980), the present work clearly shows that the power, coherence, and phase of theta oscillations in the hippocampus change in a layer- and behavior-specific manner. In addition to important methodological implications, this work suggests that theta oscillations should be somewhat reconceived not as a rigid clock signal, but as a loose framework that allows different layers and regions within the hippocampus to flexibly coordinate with one another depending on the specific processing functions of the system. In fact, the present work suggests that each anatomical layer (and possibly each

current dipole) can generate a slightly different theta oscillation that can become more or less synchronized to different parts of the system. Because current dipoles derive from numerous sources, including burst-induced somatic hyperpolarization (Hu et al. 2007), dendritic Ca^{2+} spikes (Magee and Johnston 1995; Schiller et al. 1997), voltage-dependent membrane oscillations (Hu et al. 2007; Kamondi et al. 1998; Leung and Yim 1986), and synaptic currents generated from a diversity of layer-specific excitatory and inhibitory inputs (Amaral and Witter 1995; Freund and Buzsaki 1996; Klausberger et al. 2003; Gillies et al. 2002; Klausberger et al. 2004), the present results suggest a large degree of computational flexibility.

The present results further highlight the importance of separating the contribution of different behavioral factors toward physiological properties of the system. Numerous studies have found correlations between theta power and running speed (Whishaw and Vanderwolf 1973; McFarland et al. 1975; Rivas et al. 1996). Similarly, in the present study, when running speed alone was used to explain changes in theta power, significant correlations between the two variables were observed for several hippocampal layers. However, adding maze region as an explanatory variable revealed that much of the variance previously explained by running speed could be better explained by maze region. These results emphasize the impact of sensory and/or cognitive factors on hippocampal theta oscillations (Sinnamon 2005; Sinnamon 2006; Wyble et al. 2004) and show that failure to carefully isolate these different factors can result in spurious correlations. It should be noted here that finding the ‘best’ behavioral correlates of neuronal activities is a very difficult problem. For example, it is possible that running

speed and acceleration do not correlate with the concurrent (zero time-lag) theta power, but correlate with the theta power occurring one second earlier or one-second later. In order to statistically test every possible contributing variable, impractically large datasets are required.

As an alternative, the present study also compared memory versus non-mnemonic control tasks to determine which physiological parameters relate to mnemonic function. While changes in theta power and coherence on the initial maze segment could not be explained by concurrent running speed or acceleration, they also did not differentiate between the different tasks. It is possible that there is some unaccounted for aspect of overt behavior other than concurrent running speed/acceleration that can explain these changes in theta oscillations. Alternatively, the power and coherence of theta oscillations in various layers may contribute to aspects of route planning or sensory-motor integration (Bland 1986) or other functions that accompany trial initiation in a variety of tasks. CA3-CA1 theta oscillations, however, exhibited a phase shift that was specific to the center arm of the alternation task and did not accompany comparable regions of the control tasks. This extends some previous findings of theta phase shifts (Buzsaki et al. 1985; Manns et al. 2007; Adey et al. 1960) with both anatomical and behavioral specificity. Anatomically, the present data indicate that it is the CA3 pyramidal layer that is changing theta phase relative to other hippocampal theta oscillations. Theta oscillations in CA3sp are likely created by a combination of current sources including local inhibition (Freund and Buzsaki 1996) and dentate mossy terminals in the immediately adjacent stratum lucidum (Amaral and Witter 1995). The mechanism of the phase shift could derive from phase

changes and/or changes in the amplitude balance between these two dipoles.

Behaviorally the CA3 theta phase shift effect is especially interesting because performance of the delayed spatial alternation task requires, on each trial, that rats retrieve information about the previous journey in order to choose the opposite arm upon reaching the T-junction. Although rats with hippocampal lesions can perform the alternation task as well as sham animals when no delay is interposed, a two second delay drops lesion performance to ~68% (compared to ~83% for controls) and a ten second delay further drops lesion performance to chance {476 Ainge,J.A. 2007;}. Because animals were run on blocks of both delay and non-delay trials on each day of testing, these data suggest that the hippocampal lesions specifically disrupt the mnemonic ability of the animals rather than strategy selection or other non-specific aspects of performance in the alternation task. Although the exact spatial position(s) at which retrieval processes occur likely varies from trial to trial, the fact that rats typically run through the center arm and the T-junction in one swift trajectory suggests that retrieval and decision-making usually occurs on the maze prior to the T-junction. This is corroborated by previous studies finding that turn-selective neuronal firing emerges in the CA1 network during traversal of the center arm in similar tasks (Frank et al. 2000; Wood et al. 2000; Ferbinteanu and Shapiro 2003; Shapiro and Ferbinteanu 2006, but see Ainge et al. 2007; Bower et al. 2005). Furthermore, convergent evidence from computational models (Wallenstein et al. 1998; Marr 1971; Rolls 1996; Treves 1995), lesion (Brun et al. 2002; Steffenach et al. 2002), and genetic manipulations (Nakashiba et al. 2008; Nakazawa et al. 2002) support the view that the integrity of the CA3 region is crucial in memory

retrieval. Although the present experiments do not rule out alternative cognitive processes, such as decision making or heightened attention, the role of the hippocampal formation in episodic memory (Squire 1992; Eichenbaum and Cohen 2001; Milner et al. 1998) suggests a mnemonic interpretation of the present results.

5.2 - Task-dependent coordination of hippocampal gamma oscillations

Gamma oscillations in the hippocampus have received much less study than the larger and slower theta oscillations, especially in relation to behavioral and cognitive correlates. While the power of local gamma oscillations is modulated by the phase of theta (Bragin et al. 1995a) and both oscillations in the dentate gyrus are under the control of the entorhinal input (Kamondi et al. 1998; Bragin et al. 1995a; Charpak et al. 1995), the two oscillations diverge in a number of ways. Principally, CA3 networks have the ability to generate self-organized gamma oscillations through interactions between CA3 pyramidal cells and basket interneurons (Bragin et al. 1995a; Csicsvari et al. 2003; Mann and Paulsen 2005) and entrain the downstream CA1 network (Csicsvari et al. 2003; Fisahn et al. 1998). This CA3-CA1 gamma interaction also appears in my data as a local maximum in the grand mean coherence matrix (Fig 3.S4), but there is also a clear influence of layer-specific gamma coordination exhibited by high within-layer coherence.

Gamma oscillations also diverge from theta in the behavioral profile exhibited in mnemonic and non-mnemonic behavioral tasks. While the power and coherence of gamma oscillations show clear changes in the coordination patterns of hippocampal networks during memory task versus control task performance, theta by comparison

shows much less difference. This difference between theta and gamma suggests that the same neurons may be able to link different functions by coordinating with one another at different frequencies. Another possibility is that a specialized set of pyramidal cells may be specifically involved in gamma frequency processing (Senior et al. 2008).

The combined results on theta and gamma oscillations also prompt some degree of speculation about how the two effects may be related. Specifically, comparing the center arm of the alternation task to control tasks, there is increased CA3-CA1 gamma coordination as well as a phase shift in CA3sp theta oscillations relative to other layers. Since CA3 gamma oscillations self-organize from the interaction between pyramidal cells and basket cells (Bragin et al. 1995a; Csicsvari et al. 2003; Mann and Paulsen 2005), one possibility is that when increased engagement of basket cells in CA3sp enhance local gamma, they also change the balance of local current dipoles, resulting in a theta phase shift. In this scenario, the gamma increase drives the theta phase shift. However, it is also possible that a phase shift of the incoming theta currents (e.g. from dentate granule cells into CA3 stratum lucidum; Amaral and Witter 1995) drives a change in the CA3 network dynamics to self-organize gamma oscillations. Unfortunately, the number of neurons recorded in the present study was insufficient to address this potentially interesting link between CA3 gamma generation and the observed phase shift in local CA3 theta oscillations.

5.3 - Coordination of hippocampal theta and gamma oscillations during REM sleep versus active waking behavior

The present study also investigated whether the anatomical profile of theta and gamma coordination changes across active waking and REM sleep behaviors. Although forebrain LFPs look paradoxically similar across waking and REM sleep (Aserinsky and Kleitman 1953; Berger and Oswald 1962; Dement and Wolpert 1958; Hobson and Pace-Schott 2002; Llinas and Pare 1991), obvious functional differences suggest that there are likely to be changes in the coordination of forebrain networks. Despite decades of research on REM sleep, little consensus has emerged on either the function or on the critical physiological differences between REM and waking (Walker and Stickgold 2004; Gottesmann 2001).

The present study revealed a number of interesting changes in hippocampal network coordination during REM sleep that may have functional implications. As previously suggested (Robinson et al. 1977; Karashima et al. 2005; Lerma and Garcia-Austt 1985; Rowe et al. 1999; Sakai et al. 1973; Sano et al. 1973; Sei and Morita 1996; Valle et al. 1992), REM could be divided into tonic periods interrupted by phasic bursts of activity that we found could be most easily detected as a broad-band increase in LFP power in the dentate molecular layer. During tonic REM sleep, both theta and gamma power increased in the dentate gyrus compared to active waking. This increased dentate synchrony may help to explain the increased plasticity related gene expression in this region during REM sleep (Ribeiro et al. 1999). However, CA3/CA1 gamma coordination decreased significantly during tonic REM compared to active waking. This is in contrast to the synchrony of theta oscillations between CA3 and CA1, which increased slightly during tonic REM compared to waking. Functionally, these changes suggest that dentate

processing may be somewhat enhanced during tonic REM sleep, but that the gamma synchronization of specific neuronal assemblies (Harris et al. 2003) between CA3 and CA1 may be somewhat limited. Instead, CA3/CA1 networks may default to theta coordination which exhibit a more global synchronization across the extent of the hippocampus than gamma oscillations (Buzsaki 2002; Bragin et al. 1995a). Phasic bursts of activity, however, increase both theta and gamma coordination throughout the tri-synaptic loop of the hippocampal formation, which may permit consolidation of hippocampus dependent memories (Datta et al. 2004; Datta et al. 2005; Mavanji et al. 2004).

Because the anatomically specific neuromodulatory input to the hippocampus shows pronounced differences between REM and active waking behavior, it is tempting to relate the above findings to what is known about the effects of neuromodulators in the hippocampus. Since serotonin (5HT) and norepinephrine (NE) input to the hippocampus are both high during active waking behavior and low during REM sleep (Aston-Jones and Bloom 1981; Park et al. 1999), I will focus on studies examining the effect of 5HT and NE on hippocampal activity. Please note that this discussion is entirely speculative and is intended to provide a framework to begin thinking about what changes at the synaptic and cellular level may be driving the changes in network coordination during REM versus active waking behavior.

5.3.1 - Increased theta/gamma synchrony between the dentate gyrus and CA3 during REM

Serotonin and norepinephrine densely innervate the dentate gyrus (Amaral and Witter 1995; Loy et al. 1980), where synaptic and non-synaptic varicosities (Vizi and Kiss 1998) release neurotransmitter onto both principal cells and interneurons (Milner and Bacon 1989a; Milner and Bacon 1989b; Oleskevich et al. 1991). In hippocampal slices and *in vivo* it has been shown that NE increases dentate theta and gamma oscillations (Brown et al. 2005; Hajos et al. 2003), while 5HT decreases theta power in CA3 and the dentate gyrus (Krause and Jia 2005; Hajos et al. 2003). The reduction in 5HT during REM sleep may thereby explain my data showing increased theta/gamma power in the dentate gyrus during REM sleep. However, decreased 5HT may not explain the decrease in CA3 gamma power. Furthermore, reduced 5HT would also be expected to increase dentate interneuron firing (Nitz and McNaughton 1999; Gulyas et al. 1999), which was not observed, suggesting the influence of other factors. It has also been shown that NE presynaptically decreases dentate granule mossy fiber input to CA3 (Scanziani et al. 1993). The dense innervation of NE fibers in the CA3 proximal dendrites (stratum lucidum), where dentate mossy fibers terminate on CA3 pyramidal cells (Amaral and Witter 1995; Loy et al. 1980), gives NE a unique position to modulate the connectivity between the dentate gyrus and CA3. Accordingly, higher levels of NE during waking could help explain the observed reduction in dentate/CA3 theta and gamma coordination.

5.3.2 - Increased gamma oscillations in CA1 during locomotor behavior

The increased gamma oscillations in CA1 str. oriens, pyramidale, and radiatum during active waking could be due to diverse actions of increased NE and 5HT. Norepinephrine enhances bursting of CA3 neurons through action at beta receptors (Jurgens et al. 2005).

Since CA3 projections to CA1 heavily target stratum radiatum (Ishizuka et al. 1990), this increased CA3 bursting could explain the greatly enhanced gamma oscillations in this layer. However, other studies have reported that NE reduces EPSPs and population spikes in response to str. radiatum stimulation (Vizi and Kiss 1998). In addition to acting through CA3 cells, NE is known to excite several types of interneurons that may play a role in increasing CA1 gamma oscillations (Bergles et al. 1996). Serotonin, on the other hand, increases evoked population spikes in CA1 (Freund and Buzsaki 1996). This, and the fact that serotonin innervation of CA1 str. radiatum and oriens is greater than NE innervation of these layers (Mongeau et al. 1997), suggests a role for 5HT in the observed increase in gamma oscillations. Serotonin neurons are known to innervate specific types of interneurons in CA1 (Gulyas et al. 1999). Serotonin excites CCK positive basket cells, calbindin positive Schaffer collateral-associated interneurons and calretinin positive interneuron-selective interneurons via 5HT3 receptors. The cell bodies of the calbindin positive Schaffer collateral-associated interneurons resides in distal str. radiatum and has axons that terminate on pyramidal cell dendrites in CA1 str. radiatum and oriens (Somogyi and Klausberger 2005). These cells could increase gamma oscillations during locomotor behavior if 5HT tuned their firing to gamma frequencies. Alternatively, disinhibition via activation of interneuron selective interneurons could also lead to the observed increase in CA3/CA1 gamma coherence. Although the above detailed observations are difficult to combine into an all-inclusive coherent framework for understanding REM sleep, bridging the gap between these cellular and molecular changes and the network level changes observed in the present study will likely be crucial to developing better models of hippocampal function during REM sleep.

5.3.3 - *Increased tri-synaptic coordination during phasic REM epochs*

Pontine waves during phasic REM episodes are associated with the firing of brainstem cholinergic neurons (Datta 1997) as well as increased power and frequency of hippocampal theta oscillations (Karashima et al. 2005). However, previous work has shown that phasic epochs of hippocampal theta during sleep are resistant to muscarinic ACh receptor blockade (Robinson et al. 1977; Kramis et al. 1975). This suggests that if ACh plays a role in increased hippocampal synchrony during phasic REM, that it may be predominantly through nicotinic receptors (Reinoso-Suarez et al. 2001). Furthermore, noradrenergic neurons in the locus coeruleus fire short bursts of activity correlated with pontine waves (Chu and Bloom 1973), which may also serve to transiently increase inter-regional coordination among hippocampal networks.

Overall, the present dissertation used high density LFP recordings to investigate how the hippocampus uses oscillations to synchronize its diverse networks. These data revealed that both theta and gamma oscillations exhibit a high degree of layer-specific synchronization that can be flexibly coordinated depending on behavioral requirements of the system. This highlights the importance of behavior in studying network and system level functioning of the brain. These data further show that hippocampal networks can simultaneous synchronize at theta and gamma oscillations frequencies and yet these rhythms may serve different functions within the system.

Section 6.0 – Bibliography

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Section 7.0 – Appendix

Section 8.0 – Curriculum Vitae

Sean M. Montgomery

Born

- Nevada City, CA — May 26, 1977

Education

- Nevada Union High School — 1995, Grass Valley, CA
- Reed College: B.A., Psychology — GPA 3.32 — 1999, Portland, OR
- Rutgers University: Ph.D., Neuroscience — GPA 3.985 — 2009, Newark, NJ

Employment

- Research Assistant, Eichenbaum Laboratory, Boston University — 6/99 to 8/01

Fellowships

- Center for Neuroscience at University of Pittsburgh Fellowship — 6/98 to 8/98

Awards

- Reed College President's Commendation for Academic Excellence — 1999
- NSF Graduate Research Fellowship honorable mention — 2001
- NDSEG Graduate Research Fellowship finalist — 2001
- The Neurosciences Graduate Student of the Year Award — 2001-2002
- Reinvest in Rutgers Competitive Academic Scholarship — 2001-2003
- Integrative Neurosci Minisymposium - Best Poster Presentation — 2004
- Integrative Neurosci Minisymposium - Best Oral Presentation — 2005

Leadership Roles

- Integrative Neuroscience Program Student Body President — 2002-2007
- Integ Neurosci Student-Postdoc Research Social Coordinator — 2002-2007