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Intrahippocampal Synchrony and Memory for Items in Spatiotemporal Context

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M.A., Emory University, 2012

B.A., SUNY Buffalo, 2010

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An abstract of

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Abstract

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By John B. Trimper

The hippocampus is a region of the brain known to play a central role in declarative memory, or memory for facts and events, in humans and other mammals. In particular, the hippocampus is believed to be especially important in binding memories for items with memories for the spatial and temporal context in which they are encountered. The hippocampus is composed of multiple anatomically distinct subregions, including dentate gyrus, CA3, CA1, and subiculum. A fundamental question is how each subregion coordinates with the others to enable binding items and spatiotemporal context in service of declarative memory. Three experiments were conducted with rats to investigate this question. Neural data was recorded simultaneously from each of four hippocampal subregions as rats performed object recognition memory tasks that also tested memory for spatial and temporal contexts. In the first experiment, results demonstrate that the pattern of neural interactions throughout the hippocampal subregions during novel object exploration is distinct from patterns of neural interactions associated with locomotive states and in a manner that may facilitate memory encoding (Chapter 3). In experiment 2, analyses revealed differences in oscillatory interactions, particularly in the slow gamma range (30-55 Hz), at memory encoding relating to the degree to which rats remembered objects' spatial locations in addition to objects' identities (Chapter 4). Chapter 5 reports elevated slow gamma within the hippocampus at test that may relate to retrieval of an object memory cued by a repeated temporal context. The findings here demonstrate a relationship between slow gamma oscillations in the hippocampus and memory for items in spatiotemporal context and mark a significant advancement for the field, both with regard to technical approach and in further elucidating how the hippocampal subregional network state differs by behavioral state and memory state. The results described here advance our understanding more broadly of the brain mechanisms underlying memory for items in spatiotemporal context in humans and other mammals.

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Chapter 1

General Introduction

The hippocampus is a region of the brain known to be important for declarative memory, or memory for facts and events (Squire, 1992). Early work in humans (Scoville & Milner, 1957) and rats (O'Keefe & Dostrovsky, 1971) suggested substantial differences in hippocampal function between the two species, with the human hippocampus underlying declarative memory and the rodent hippocampus supporting spatial navigation. The diverging ideas, however, have now given way to consensus that the hippocampus contributes similarly to memory in both species (Squire, 1992), possibly by offering a contextually relevant map—be it spatial, temporal, or other—into which memories for experiences, such as encounters with particular items, can be integrated (Buzsaki & Moser, 2013; Schiller et al., 2015). An unanswered question is how precisely the hippocampus underlies the binding of memories for items within spatiotemporal context and, moreover, how best to characterize hippocampal function in a way that applies equally well across all mammals.

Anatomy of the Hippocampal Memory System

Figure 1.1 shows an illustrated schematic of the functional anatomy of the hippocampal memory system and associated cortical regions. The hippocampus—which is composed of multiple subregions, including dentate gyrus, CA3, CA1, and subiculum—sits at a privileged position, such that it is the site of convergence for two partially segregated functional and anatomical pathways, carrying spatial and nonspatial information separately (Manns & Eichenbaum, 2006; Witter et al., 2000). In monkeys, the dorsal visual stream, thought to be important for visually-guided actions, projects to the parahippocampal cortex (postrhinal in rats) which then projects to the lateral entorhinal cortex. The ventral visual stream, which is thought to be important for object identification, projects to the perirhinal cortex which then projects to the medial entorhinal cortex (Suzuki & Amaral, 1994). Although rats do not exhibit the same dorsal versus ventral visual stream segregation, the rat postrhinal and perirhinal cortices receive disproportionate spatial and nonspatial information, respectively, and exhibit patterns of

connectivity with the entorhinal cortex similar to those observed in monkeys (Burwell & Amaral, 1998). Entorhinal efferents from the lateral and medial areas converge upon the hippocampus, providing the components necessary for combining spatial and nonspatial representations within the hippocampus.

The anatomical organization of the hippocampus is highly conserved across the mammalian taxon (Manns & Eichenbaum, 2006). Projections between the hippocampal subregions are primarily unidirectional, from dentate gyrus to CA3 to CA1 to subiculum, though each subregion also receives entorhinal input directly (Amaral & Witter, 1995; Witter & Amaral, 1991; Witter, 1993). Entorhinal projections from the medial and lateral areas arrive at the hippocampus with patterns of connectivity that differ by subregion (Witter et al., 2000). For example, in dentate gyrus and CA3, medial and lateral entorhinal projections converge upon the same neurons, whereas in CA1 and subiculum, medial and lateral entorhinal afferents arrive at anatomically disparate locations. These differences in entorhinal connectivity may relate to distinct memory functions being performed by each subregion.

The hippocampal subregions also exhibit substantial differences in internal organization. For example, dentate gyrus possesses an especially large number of neurons (i.e., 10^6 in the rat) (Witter, 1993). Likewise, dentate gyrus is one of only two regions in the adult brain continually producing new cells (Kempermann et al., 2004), a process termed "neurogenesis." CA3 exhibits a unique projection system termed "recurrent collaterals" in which axons from CA3 pyramidal cells loop back upon other pyramidal cells bilaterally within CA3 (Amaral & Witter, 1989; Ishizuka et al., 1990). CA1 cells project to subicular cells in a manner that preserves their segregated entorhinal input (Amaral et al., 1991). As with extrinsic connectivity, these differences in internal organization have been suggested to underlie differential contributions made by each subregion to declarative memory processing (Kesner & Rolls, 2015).

Hippocampal Function

Early on, it was discovered that hippocampal damage in humans leads to profound and selective impairments in declarative memory. For example, patient H.M.'s hippocampus and associated cortical structures were bilaterally removed as a young man as treatment for pharmacologically intractable epilepsy (Scoville & Milner, 1957). Though H.M. could no longer form new memories for daily events (declarative memories), he improved over time at tasks associated with other forms of memory. For example, H.M. improved at tasks of procedural memory, such as tracing the outline of a star while viewing the paper and his hands only through the mirror (Milner, 1962). Subsequent studies of patients with more selective damage confirmed that damage to the hippocampus was sufficient to produce impairments of the sort observed with H.M, though additional damage to cortical regions outside of the hippocampus produced more severe impairments (Squire et al., 2004).

Early work with rats suggested that the rat hippocampus may not share the same function as the hippocampus in humans. In 1971, John O'Keefe and his graduate student Jonathan Dostrovsky demonstrated the existence of neurons within the rat hippocampus that respond selectively when rats occupy discrete regions of space, or "place cells" (O'Keefe & Dostrovsky, 1971). Though a single place cell is only preferentially active for a small section of an enclosure, if one records simultaneously from many of these neurons as rats ambulate throughout the environment, a pattern of activity is revealed such that, across cells, the entire area is represented. Additional evidence for the preferential role of the rat hippocampus in spatial representation came from lesion studies. Damage to the hippocampus in rats produces substantial impairments in tasks with a spatial component (Aggleton et al., 1986; Becker et al., 1980).

Subsequent work revealed many similarities between hippocampal function in rats and humans. First, it became clear that hippocampal lesions in rats produce impairments on many tasks that do not include a spatial component (Eichenbaum et al., 1988; Meck et al., 1984; Rudy & Sutherland, 1989). Second, it was discovered that the hippocampus in humans also exhibits

place cells (Ekstrom et al., 2003) and, thus, the hippocampal representation of space is not unique to rats. Third, it is now understood that hippocampal place cell activity in rats can be modulated by nonspatial information as well as rats' current locations (Manns & Eichenbaum, 2009; Smith & Mizumori, 2006; Wood et al., 2000)

What, then, might the prominent spatial representations in the hippocampus be offering to declarative memory? One idea is that hippocampal representations of space present a foundation in which to integrate memories for items with memories for their spatial context (Eichenbaum et al., 1999; Buzsaki & Moser, 2013; Schiller et al., 2015). Indeed, when hippocampal damage is present, humans (Crane & Milner, 2005; Holdstock et al., 2002; Konkel et al., 2008), monkeys (Bachevalier & Nemanic, 2008; Parkinson et al., 1988), and rats (Gilbert & Kesner, 2002; Gilbert & Kesner, 2004; Langston & Wood, 2010) show impairments on learning item-place associations.

Along with spatial context, the hippocampus may also contribute to remembering events in temporal context, with temporal context being defined here as other events that occur in close temporal proximity (Howard & Kahana, 2002; Manns et al., 2015). For example, if, this past Friday, you went to the new theatre in town, then for dessert at your favorite restaurant, these events may be bound together in memory such that subsequent visits to the new theatre cue memory for desert at your favorite restaurant.

How might hippocampal activity underlie such a function? As sensory experience changes over time, so too must cortical representations for experience, and therefore, the information conveyed from cortex to the hippocampus. Similar to how the hippocampus may bind items and spatial information in memory, so too might it bind representations for multiple experiences that occur in close temporal proximity to one another. Mechanisms that might underlie such a function include modification of synaptic strength within the hippocampus (Bi & Poo, 1998) or adding a temporal tag via recently born neurons (Aimone et al., 2010). Indeed,

lesions to the hippocampus in rats have been found to impair memory for temporal order (Fortin et al., 2002; Hoang & Kesner, 2008; Hunsaker et al., 2008).

Functional Anatomy of the Hippocampal Subregions

An important question then is how the hippocampal subregions—dentate gyrus, CA3, CA1, and subiculum—differentially contribute to binding memories for events to their spatiotemporal context, and, further, how the subregions interact with one another in service of this process. In the following sections, empirical evidence and hypotheses for each hippocampal subregion's involvement in declarative memory processing is reviewed. Special focus is given to rodent work, as this species has been the subject of the majority of experimental work in the domain of hippocampal subregional analyses. A discussion of hippocampal subregion CA2, whose functions are just beginning to be elucidated (Dudek et al., 2016), is beyond the scope of this review.

Dentate Gyrus

Given the especially large number of neurons present in dentate gyrus (Witter, 1993), one function that is often attributed to this subregion is the orthogonalization of incoming representations in the service of creating distinct hippocampal representations for distinct, though possibly highly similar, experiences (Kesner & Rolls, 2015). This function is often referred to as "pattern separation" and theoretically allows for non-redundant representations of incoming information in downstream CA3. The very large number of neurons within dentate gyrus permit distinct populations of neurons to be active for only marginally different inputs. Such a function may facilitate the disambiguation of encounters with novel items from other items encountered previously in the same spatial location.

Neurogenesis in dentate gyrus has also been suggested to play a role in pattern separation. One hypothesis for how neurogenesis mechanistically supports pattern separation is that neurogenesis adds a temporal tag to newly encoded memories. Granule cells, the principal

excitatory neuron of dentate gyrus, are hyper-excitable before reaching maturity, allowing for groups of newborn neurons to preferentially represent experiences occurring within their transient developmental window (Aimone et al., 2006; Rangel et al., 2014).

Support for the role of dentate gyrus and neurogenesis in temporal pattern separation comes from a recent study by Rangel et al. (2014) utilizing in vivo electrophysiological techniques to simultaneously record the activity of large groups of neurons from this subregion. The authors reported that, when rats' experiences with distinct environments were separated by a three week temporal lag, distinct groups of dentate neurons were preferentially active in distinct environments. However, when the temporal lag between distinct environmental exposures was shortened, overlap between active neuronal populations within the dentate gyrus increased. Thus, the similarity of dentate representations for these environments was correlated with the temporal lag between environmental exposures. Importantly, when neurogenesis was experimentally disrupted, activity patterns for a long temporal separation looked similar to those associated with a short temporal separation. Further evidence has been provided by lesion studies reporting a disruption of temporal associations between events by dentate gyrus lesions (Morris et al., 2013). Activity in dentate gyrus may therefore contribute to remembering items within the temporal context in which they are encountered.

Several additional studies bolster a role for dentate gyrus in spatial pattern separation. Gilbert et al. (2001) reported that lesions to dentate gyrus led to impairments in memory for objects bound to particular locations, an effect that negatively correlated with distance between the test objects and the foils such that increasingly similar spatial locations were associated with more severe impairments. Clelland et al. (2009) reported a similar pattern of spatial pattern separation impairments after disrupting neurogenesis in dentate gyrus. Therefore, dentate gyrus may be particularly important for remembering associations between items and spatial locations, especially when spatial locations are similar.

CA3

CA3's recurrent collateral system has been extensively modelled as playing an integral role in retrieving complete memory representations given partial cues, a function termed "pattern completion." This same system has also been suggested to be important for the rapid formation of associative memories, or representations for the learned relationship between two or more unrelated items (Rolls, 1987; Rolls, 1989a,b,c,d; 1990a,b; for review, Kesner & Rolls, 2015). These functions may be particularly important for retrieving an item encounter memory given a spatial or temporal cue, and for learning the association between items and their spatial or temporal context.

Physiological evidence for a role of CA3 in pattern completion was provided by Vazdarjanova & Guzowski (2004) who used an immediate-early gene visualization approach that allows for the identification of neuronal populations activated at two distinct time points [i.e., catFISH (Guzowski & Worley, 2001)] to show that CA3 neurons, relative to CA1 neurons, had higher overlap in their activity between modestly different environments. Similarly, Lee et al. (2015) reported in vivo electrophysiological evidence that distal CA3 (near CA1), where recurrent collaterals are the strongest, maintained coherent representations of the environment despite modifications. Mice genetically modified to lack NMDA receptors in CA3, which are known to be physiologically important for synaptic strengthening in relation to learning and memory, were unable to complete a memory task when familiar cues were removed, suggesting these mice lacked the ability to retrieve the required memory representation given only partial retrieval cues (Nakazawa et al., 2002).

CA3's role in the rapid formation of associative memories is also supported by several experimental findings. In a go/no-go task in which rats must learn that the presence of an object or odor in a particular location means the rat should displace the stimulus for a reward but the presence of the object or odor in a separate location means rats should withhold action, CA3

lesions produced impairments in learning both object-place and odor-place pairings (Gilbert & Kesner, 2003). Likewise, Kesner et al. (2008) found that CA3 lesions impaired associative memory encoding on an object-cued spatial location recall task and a spatial-cued object recall task, where learning unique stimuli configurations was required on each trial.

To conclude, empirical evidence bolsters a role for CA3 in making important contributions to retrieving complete memories given degraded or partial input, and in the rapid formation of associative memories. These functions suggest CA3 is particularly important for remembering items in conjunction with their spatiotemporal context, and at both retrieval and encoding.

CA1

Memory functions often attributed to CA1 include contributions to temporal context processing and to efficiently recoding representations conveyed from CA3 to allow for more efficient recall and reactivation of the originally active cortical representation (Kesner & Rolls, 2015).

Some of the earliest evidence of temporal context influencing representations in CA1 was provided by Manns et al. (2007). The authors reported that neuronal activity patterns in rats gradually changed over the course of successive encounters with a five odor sequence in a way that might provide a mechanism for binding representations of each odor to a gradually changing temporal context. Importantly, the degree of change across the sequence encounters predicted memory performance in the subsequent test phase, in which rats were asked to choose which of two odors had been presented earlier in the sequence.

A substantial obstacle for this body of research was to demonstrate that these neurons preferentially related to the passage of time, rather than spatial location, given the well-established role of the hippocampus in representing spatial information (Derdikman & Moser, 2010). Indeed, place cells are highly prominent in CA1 in particular (Mizuseki et al., 2012). Thus,

Manns et al. (2007) also analyzed spatial representations over successive odor encounters and found that, in opposition to temporal context representations, the degree of change in spatial representations across the study phase were not predictive of success at test.

Subsequent research on how CA1 neurons might code for temporal information revealed the existence of neurons now deemed "time cells" whose activity while rats remain fixed in a single location during a delay period appears to track the passage of time, and moreover, contains information about tasks to be completed after the delay period (Eichenbaum, 2014; MacDonald et al., 2011; Pastalkova et al., 2008). For example, Pastalkova et al. (2008) found that CA1 neurons reliably fired in different sequences while rats ran on a running wheel based on whether rats were to turn right or left on a t-maze after the running wheel delay period. MacDonald et al. (2011) furthered this finding to the nonspatial domain. On a task in which rats were exposed to an object, then asked, after a delay period, to choose the odor that had been previously paired with that object, CA1 neurons fired in sequences during the delay period in a way that related within and across trials to the specific object-odor pair.

CA1 is also associated with a second neuronal activity pattern referred to as "reactivations" in which recently active neuronal sequences are replayed rapidly, at up to 200x their activation in real-time (Buzsaki, 1986). These reactivations, which occur primarily during slow wave sleep and quiet rest, are believed to play an integral role in memory consolidation and cortical reactivation of representations associated with the original sensory experience (for review, Buzsaki, 2015). Interruption of these transient bursts of spiking activity, which occur in conjunction with equally short-lasting high-frequency (150 – 400 Hz) bursts of oscillatory activity termed "sharp wave ripples," has been experimentally demonstrated to impair learning on a spatial alternation task (Jadhav et al., 2012). Subsequent work from the same group recording in vivo electrophysiological activity simultaneously from both CA1 and prefrontal cortex demonstrated that sharp-wave ripples in CA1 occur in a coordinated fashion with transient

modulations of neuronal activity in prefrontal cortex (Jadhav et al., 2016), thus providing experimental evidence linking sharp waves in CA1 and cortical reactivation.

In sum, evidence supports a role for CA1 in contributing to representations for spatial and temporal context, as well as efficiently recoding hippocampal representations for cortical reactivation. This activity may be particularly important for structuring neural representations of temporal context and for subsequent retrieval of memories about items bound to a particular spatiotemporal context.

Subiculum

Relative to the three previously discussed hippocampal subregions, subiculum has received far less research attention, with some debate as to whether subiculum should be considered a part of the hippocampus at all (O'Mara et al., 2001). Subicular cytoarchitectonics, however, are similar to that of the other hippocampal subregions, also exhibiting a three-layered allocortical structure (Amaral & Witter, 1995; O'Mara et al., 2001). Likewise, subiculum shares with CA1 a similar proximal-distal segregation in medial and lateral entorhinal cortical afferents, in addition to receiving dense and robust projections from CA1 (Amaral et al., 1991; Naber et al., 2001; Witter et al., 1989). Subiculum also serves as a major output structure of the hippocampus with projections to a diverse array of targets including the prefrontal cortex, amygdala, nucleus accumbens, and hypothalamaus (Witter, 2006). In lieu of these points and others, Aggleton & Christiansen (2015) offered the thought: "[Given that] the subiculum is… at the heart of the 'connected hippocampus'… only by understanding the subiculum can the rest of the hippocampus be understood." For all of these reasons, subiculum will be treated here as a hippocampal subregion.

What, then, might be subiculum's contribution to declarative memory processing? Considerable evidence points to a role for subiculum in spatial navigation (O'Mara et al., 2009), and, therefore, in contributing to the neural code for binding experiential memories to spatial

context. For example, Kim et al. (2012) report that subicular neurons, like CA1 neurons, display theta phase precession, a spike-phase relationship pattern in which successive spikes from single neurons occur at increasingly earlier phases of the ongoing theta rhythm (6-12 Hz) in a way that relates to the rat's past, present, and future spatial location.

Lesions to subiculum disrupt spatial navigation (Morris et al., 1990; Potvin et al., 2007). Interestingly, one study reported spatial navigation impairments produced by lesions to the subiculum alone were comparable to lesions to the entire remainder of the hippocampus when rats were required to navigate in the dark, but subicular lesioned animals were less impaired than animals with the remainder of their hippocampus lesioned when tested during the day (Potvin et al., 2007). A possible explanation for this finding is that subiculum is particularly important in self-referential (idiothetic) spatial navigation. When the room was dark and rats were required to rely on internal representations of their position within the environment, subicular lesions were more detrimental. In line with this idea, some neurons within the subiculum fire in such a way as to code for rats' head direction or for a combination of spatial location and head direction (Muller et al., 1991), providing an important component to an idiothetic neural map of space.

Though subiculum has not received the same degree of research attention as the other hippocampal subregions, research is beginning to coalesce around the idea that subiculum is preferentially involved in idiothetic spatial navigation. Such a function may be particularly important when remembering items within their spatial context, as understanding not just where an item is located within the broader environment, but how that item is positioned in relation to the viewer, is an important component of memory for events in spatiotemporal context.

Subregional Interactions

Beyond considering each hippocampal subregion in isolation, an important question to ask is how the subregions interact with one another in the service of memory. One research approach that has emerged as particularly useful for studying interactions is recording in vivo

electrophysiological activity simultaneously from multiple subregions and asking how action potentials and local field potentials, which reflect the summed electrical activity of large groups of neurons (Buzsaki et al., 2012), interact with one another across region pairs. While many of the actual in vivo electrophysiological recording methods have been in place for quite some time, only recently has data handling capacity increased to the degree where large-scale simultaneous recordings from multiple brain regions and their analyses are possible (Buzsaki, 2004).

Local field potentials in the hippocampus, like in other parts of the brain, exhibit rhythmic fluctuations in voltage, termed oscillations. Oscillations are thought to be important for facilitating communication between brain regions (Fries, 2005; Fries, 2015) and for functionally grouping active neuronal ensembles (Buzsaki & Draguhn, 2004; Fries et al., 2007). One idea is that when oscillations are synchronized across two unidirectionally connected brain regions, spikes from the upstream region will be more likely to arrive at the downstream region when both areas are maximally depolarized (i.e., at the oscillatory peak). This alignment between spikes and oscillatory phase may, thus, provide a mechanism for upstream activity to be maximally effective in eliciting a downstream effect (Fries, 2005). Due to an intricate balance between inhibitory and excitatory currents underlying rhythm generation (Buzsaki & Wang, 2012), only neurons that are maximally excited within the brief depolarization window of each oscillatory cycle will be able to fire before all others are inhibited. Oscillations, therefore, also provide a mechanism for suppressing the activity of nonessential or mildly excited neurons, while supporting the functional grouping of those neurons most excited or important for the computational task at hand.

Five oscillatory frequency bands are most notable in the hippocampal local field potential: theta (6-12 Hz), beta (13-30 Hz), slow gamma (30-55 Hz), fast gamma (65-90 Hz), and sharp-wave ripples (150-400 Hz). With some overlap, each is associated with its own neurobiological underpinnings (Buzsaki et al., 2012; Buzsaki & Draguhn, 2004; Colgin, 2016; Kopell et al., 2000). For example, theta is most prominent within CA1 and subiculum, relative to

CA3 and dentate gyrus, and is at least partially driven by inhibitory interneurons in the medial septum, termed "pacemaker cells," that project to the hippocampus (Colgin, 2013; Hangya et al., 2009; Toth et al., 1997). Lesioning entorhinal-hippocampal projections reduces fast gamma oscillations in CA1, but leaves slow gamma oscillations intact (Bragin et al., 1995), suggesting slow and fast gamma oscillations in CA1 arise from distinct inputs (Bragin et al., 1995; Colgin et al., 2009; Schomburg et al., 2014) and may therefore relate to the communication of different types of information (Colgin et al., 2009; Colgin & Moser, 2010; but see Buzsaki & Schomburg, 2015). Beta is associated with distinct synchronization properties, as compared to the gamma ranges, and may be better suited for facilitating communication when axonal projections possess longer conduction delays (Koppell et al., 2000). Finally, hippocampal sharp-wave ripples, briefly alluded to above in the section on CA1, are transient (~100 ms) bursts of high frequency activity initiated by activity in CA3 and associated with the rapid replay of recently active neuronal sequences (Buzsaki, 1986; Buzsaki, 2015).

Recording simultaneously from multiple hippocampal subregions and analyzing interactions is an approach just beginning to gain prominence in the literature. As such, experimental data for review is limited and comes almost exclusively from simultaneous recordings from CA3 and CA1. That said, some findings are of particular note.

Montgomery and Buzsaki (2007) recorded local field potentials simultaneously from CA3 and CA1 during performance of a spatial memory task. The authors found that gamma coherence, broadly spanning both slow and fast gamma ranges, increased during the decision making phase of the task. Likewise, Carr et al. (2012) recorded local field potentials simultaneously from CA3 and CA1 and reported that slow gamma oscillations in CA3 and CA1 became increasingly coherent before sharp-wave ripple associated reactivations in CA1. The levels of synchrony in the slow gamma range related to the quality of the subsequent reactivations. These results suggest gamma interactions between CA3 and CA1 can function to

facilitate memory retrieval processes.

Trimper et al. (2014) also recorded local field potentials simultaneously from CA3 and CA1 in rats while the animals performed a novel object recognition memory task. The authors found that coherence between CA3 and CA1 in the slow gamma range increased markedly during novel object exploration. Moreover, coherence between CA3 and CA1 was greater when rats subsequently demonstrated good memory for the objects relative to when rats subsequently demonstrated poor memory. These results suggest that interactions between CA3 and CA1 as measured by slow gamma coherence may also be important during the formation of recognition memories. It may be the case that slow gamma within the hippocampus can act as a universal mediator of hippocampal function. Several questions were left open by the study by Trimper et al. (2014), however, including what information in particular was being remembered and how other hippocampal subregions might be contributing to the process.

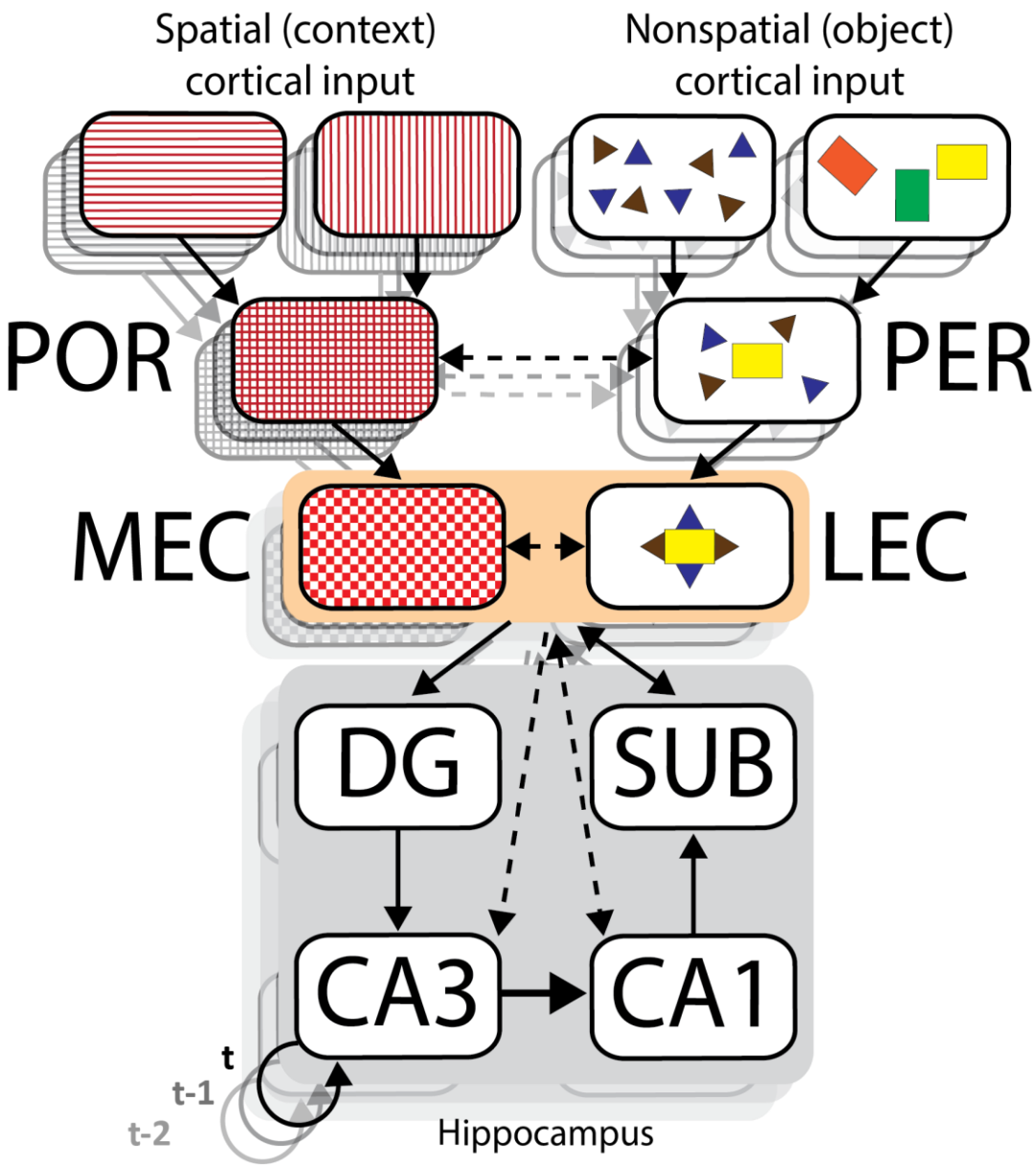
Summary

The hippocampus is a region of the brain known to be important in forming memories for events in spatiotemporal context. The functional anatomy of the structure is ideal for accomplishing such a task. The hippocampus possesses readily modifiable synapses and a recurrent collateral system ideally positioned at the site of convergence for two largely distinct neural pathways, carrying spatial and nonspatial neural information separately. Though many ideas have been proposed, how the hippocampal subregions and interactions between them differentially relate to the formation and retrieval of memories for items within spatiotemporal context remains poorly understood.

In the following pages, three experiments are described in which neuronal activity was recorded simultaneously from hippocampal subregions dentate gyrus, CA3, CA1, and subiculum while rats performed variants of object recognition memory tasks designed to probe memory for objects within spatiotemporal context. To our knowledge, this is the first report of its kind detailing

recordings simultaneously made from each of these four locations. Analyses focus on asking how interactions between the subregions, and activity within each subregion on its own, differ based on the content being remembered as well as which memory processes might be underway. Experiment 1 asks how the hippocampal network state during novel object exploration might differ in a way that extends above and beyond the well-established relationship between hippocampal activity and locomotion (Chapter 3). Experiment 2 asks how hippocampal subregional activity differs during object exploration based on whether, and to what degree, rats remember an item's spatial context (Chapter 4). Experiment 3 asks how the hippocampal oscillatory network state during locomotion might be augmented by simultaneously retrieving an object memory cued by a repeated temporal context (Chapter 5).

Figure 1.1. Illustration of the hippocampal role in associating spatial and nonspatial information across time. Cortical representations for contextual information, such as spatial location, are conveyed to the hippocampal formation via a separate route relative to cortical representations for nonspatial information, such as objects. Spatial information is transferred via the postrhinal cortex (parahippocampal cortex in primates) to the medial entorhinal cortex, while nonspatial information is preferentially conveyed from the perirhinal cortex to the lateral entorhinal cortex. These two streams converge at the hippocampus, which processes information in a largely unidirectional fashion from dentate gyrus to CA3 to CA1 to subiculum, possibly with each subregion contributing uniquely to memory processing. As representations change over time, graphically represented by gradually fading black and white images behind the primary schematic, the hippocampus may likewise function to associate these events across time with one another.


Spatial (context)
cortical input
Nonspatial (object)
cortical input
POR
PER
MEC
LEC
DG
SUB
CA3
CA1
t
t-1
t-2
Hippocampus

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Chapter 2

General Method

This chapter describes common experimental methods shared by each of the three subsequently described experiments (Chapters 3, 4, and 5).

Subjects

All experiments utilized male Long-Evans rats, individually housed (12h light/dark cycle; testing during light phase) with free access to water and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight (~400g). Neuronal data for all experiments came from the same six rats. An additional eight rats were included for behavioral data only for the temporal context repetition experiment (Chapter 5). All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

Behavioral Training for Object Recognition Memory Tasks

All three experimental tasks, described in Chapter 3, 4, and 5, required rats to run consecutive clockwise laps around an elevated circular track (diameter = 91.5cm/ track width = 7 cm) for small chocolate sprinkle rewards at the completion of each lap. Rats were trained daily to perform these laps up to criteria (80 laps in 40 minutes), a process lasting approximately five weeks. Throughout the training process, rats were additionally habituated to touching of their heads in anticipation of the neural recording experiments. After surgical implantation of a chronic neural recording assembly (see below), rats were re-trained daily up to criteria, at which point performance was maintained with approximately twice-weekly training sessions until recording tetrodes were in position. One day before initial testing, rats were exposed to objects placed on retractable flaps adhered to the perimeter of the elevated track, for the purpose of reducing potential neophobia at test related to rats never having encountered any objects before along the track. Importantly, at test, the degree of exploration of objects was at the rats' discretion, relying on rats' innate curiosity and preference for novelty, and never rewarded, encouraged, or otherwise manipulated by experimenters.

Objects

Objects were randomly pulled from a set of approximately 320 unique objects (but note adjustments based on size below), with up to four duplicates of each unique object. All objects were originally purchased from a local store to be used solely for object recognition memory testing with rats in our laboratory. Objects ranged in size from approximately 7 x 7 x 7 cm to 17 x 17 x 10 cm. Object size was equated within trials to control for exploration time effects related to this factor. Objects were randomly assigned to experimental conditions. All objects were novel to rats at the beginning of testing, and were washed immediately after testing, with all duplicates of that object, to limit scent marking and ensure all duplicates of the same objects were handled similarly. Objects were adhered to retractable flaps on the outside of the elevated circular track using Velcro.

Surgery and Positioning of Recording Tetrodes

Stereotaxic surgery was performed after rats were deeply anesthetized with isoflurane (1–3% in oxygen) and given buprenorphine (0.05 mg/kg) as an analgesic. Rats were implanted with a custom chronic electrophysiological recording headstage that contained up to 32 independently movable tetrodes. Tetrodes were funneled through two stainless steel cannulae (14 gauge and 17 gauge) to concentrate their positioning over the hippocampal subregions of interest—dentate gyrus, CA3, CA1, and subiculum. Craniotomies spanned an area from approximately 2.6 to 6.4mm posterior to bregma and 1.3 to 4.2 mm lateral to the central suture, with tetrodes typically falling within 3 to 6 mm posterior to bregma and 1.8 to 3.8 mm lateral to the central suture. Each tetrode consisted of four 12.5 µm nichrome wires whose tips were plated with gold to reduce the impedance to 200 kΩ at 1 kHz. Rats were monitored in the lab for several hours after surgery, and daily for the following three days. Additional doses of buprenorphine (0.05 mg/kg) were given immediately after surgery and the following morning. Meloxicam (Metacam) was administered immediately after surgery (0.75ml) and each of the two following mornings for pain relief.

Following a one-week recovery period after surgery, tetrode positioning in the pyramidal layers of CA3, CA1, and subiculum and the granule cell layer of dentate gyrus occurred over several weeks and was assisted by known electrophysiological hallmarks [e.g., dentate spikes (Bragin et al., 1995), sharp-wave ripples (Buzsaki., 1986)]. A stainless steel screw implanted in the skull above the cerebellum served as the reference for local field potentials during recording, whereas a tetrode within the hippocampus but without single units served as the reference for spike channels. Tetrodes were never turned prior to testing on days in which experiments were performed, though minor adjustments were made after test sessions to maintain good single unit isolation for the following days.

Data Acquisition

Rat behavior during experimental sessions was recorded using a digital video camera mounted above the circular track at a frame-rate of 30 frames per second. Local field potentials (sampling rate = 1,500 Hz; bandpass filter = 1-400 Hz) and action potentials (bandpass = 600-600 Hz) were acquired using NSpike data acquisition system (nspike.sourceforge.net). Action potentials recorded on the same tetrode were separated into distinct units by visual inspection of several waveform characteristics across the four wires (e.g., spike amplitude, waveform shape) using Offline Sorter (Plexon Inc.).

Histology

After experiments were completed, a 20–40 µA current was passed through each recording tetrode for 20-40 s while rats were under anesthesia immediately prior to euthanizing the rat, with the resulting brain lesions serving as confirmation of tetrode position. Transcardial perfusions were performed with 0.9% saline followed by 4% formalin. Brains were extracted and allowed to sit for several days in 4% formalin solution. Brains were moved to a 40% sucrose solution for approximately 72 hours, until brains sank to the bottom of the container, at which point brains were sliced into approximately 70 µm coronal slices and mounted on glass

microscope slides. Brains were left for several days to dry in an 37° C oven, then Nissl stained with a cresyl violet solution.

Figure 2.1 shows the locations of local field potential tetrodes for each rat by subregion, as verified post-mortem through histology. For local field potential analyses in CA3, CA1, and subiculum, one tetrode in the middle third of each region's transverse axis (proximal to distal relative to dentate gyrus) was selected for each rat. This intermediate portion along the proximal/distal axis was selected because the intermediate portion of CA3 projects directly to the intermediate portion in CA1 which projects to the intermediate portion of subiculum, and because this portion of each of the regions receives input from both lateral and medial entorhinal cortex (Witter and Amaral, 2004). The intermediate portion of dentate gyrus was not selectively targeted as dentate cells project to the entire transverse extent of CA3 (Swanson et al., 1978; Gaarskjaer, 1986).

Statistical Reporting Format

Unless otherwise noted, all figures and central tendency reporting is provided as mean plus and minus the standard error of the mean.

Behavioral Analyses

Experimental videos were scored using custom written software. A behavioral flag was assigned to each event of interest (e.g., lap start and end times, object exploration initiation and offset). Rats were considered to be exploring objects only when their noses were within approximately 1 cm of the object and rats were exhibiting signs of active investigation (e.g., whisking). Exploration events including excessive chewing were discarded and data for that trial were not used. For analyses of rat locomotion, we tracked rats' position within the videos in Cartesian coordinates using custom written software in MATLAB (Mathworks) which detected the centroid of two LEDs affixed to the recording headstage on rats' heads.

Neural Data Analyses

Local Field Potential Analyses

Figure 2.2 shows a schematic of the local field potential analysis procedure, moving from raw local field potentials to statistical evaluation. All data analyses were performed using custom written code in MATLAB (Mathworks). Local field potential analyses were additionally assisted by an open source library of functions that implemented a multitaper fast Fourier transform method for calculating coherence and other spectral estimates (Chronux: Bokil et al., 2010). The multitaper approach was used because it has several advantages over a standard (single taper) fast Fourier transform for most oscillatory ranges of interest, including reduced variance and bias in the resulting spectral estimates (Bokil et al., 2010). For a sample of local field potentials of duration T seconds, 2TW − 1 orthogonal tapers [discrete prolate spheroidal sequences, also referred to as Slepian sequences; (Slepian, 1978)] were used that were well concentrated in the frequency bandwidth −W to +W. Unless noted otherwise, sliding 0.5 s windows with step size of 0.05 s was used to calculate spectral estimates to reduce the possible complication of nonstationarity in the data (Mitra and Pesaran, 1999). To ensure adequate spectral resolution within each frequency range of interest, we employed separate taper parameters for the theta range and below (5 – 13 Hz) relative to 13 Hz and above (13 – 90 Hz). For 3 – 13 Hz, we used a frequency half bandwidth of 1 Hz (-1 Hz to +1 Hz) and a single taper for each 0.5 s section of data. For 13 Hz and above, we used a frequency half bandwidth of 6 Hz (-6 Hz to +6 Hz), enabling the use of five well-concentrated orthogonal tapers for each 0.5 s section of data. To account for possible bias in spectral metric calculation, in cases where an uneven number of trials were present across conditions within a rat, a subsampling procedure where trials for each condition were subsampled down to the lowest number of trials present across conditions was performed. Subsampling was repeated 1,000 times, or the max allowable number of times when the max number of unique subsamples was less than 1,000. The final values for each condition were then calculated by averaging across these subsampling iterations.

Spectral Metrics

Spectral power, also referred to as spectrum or auto-spectra, is a metric providing information about the prevalence of oscillatory activity at each frequency within a local field potential sweep. Power is calculated as the product of the complex Fourier coefficients multiplied by their complex conjugate. Power was log-transformed to account for a 1/frequency distribution, and converted from bels to decibels by multiplying log transformed values by ten.

Coherence is a metric for covariance of phase and amplitude between two local field potentials. It is calculated as the absolute magnitude of coherency, which is cross spectrum normalized by the product of the two auto-spectra (i.e., power for each local field potential). Coherence was Fisher transformed to stabilize variance at the tails of the distribution, thus explaining why values greater than 1 are observed when coherence is particularly strong.

Statistical Analyses of Spectral Metrics by Frequency

Evaluation of statistically significant differences across conditions and subregions/subregion pairs in spectral measures by frequency was performed using a cluster based permutation approach similar to that described previously (Maris et al., 2007; Maris & Oostenveld, 2007), but adapted here for more than a single independent variable and more than two levels of each variable.

A description of the procedure is as follows. For each frequency bin, an F ratio was calculated. For questions regarding interactions between subregion/subregion pairing and condition, the F ratio was calculated with a two-way repeated measures analysis of variance (ANOVA) with subregion/subregion pairing as one factor and condition as a second. For questions regarding an effect of condition *within* subregion/subregion pairing, a one-way ANOVA was employed with condition as the sole factor. This procedure produced a vector of F values spanning all frequency bins under consideration.

F ratios were then converted to p values corresponding to the lower tail of the F

distribution. This procedure inverts the p value from its typical usage. In other words, a p value typically expressed as 0.05 would here be represented as 0.95. Thus, higher values indicate a lower statistical probability of occurrence. This procedure produced a vector of p values spanning all frequency bins under consideration.

All p values greater than 0.90 were then identified and only consecutive groups of those p values of at least a pre-defined length were further considered (two consecutive points for below 13 Hz, four consecutive points for above 13 Hz). P values within each identified cluster of points were summed, such that a single sum was recorded for each cluster of sufficient length.

These cluster sums recorded from the nonrandomized data were then compared to the maximum cluster sums recorded from each of 1,000 randomizations. This comparison against a random distribution essentially asks: is the difference across conditions present within this particular frequency range greater than the difference you might observe by chance?

When looking for significant differences across conditions within a subregion/subregion pairing, conditions were randomized within rats. When looking for significant interactions between subregion/subregion pairing and condition, both subregion/subregion pairing and condition were randomized within rats. Just as with the non-randomized data, cluster sums were identified in the averages across rats. Cluster sums from the non-randomized data greater than the 97.5^{th} percentile for the randomized cluster sums were denoted as significant. A cutoff of 97.5 was used, rather than 95, as clusters from two separate frequency ranges were statistically evaluated (3-13 Hz and 13-90 Hz).

Spiking Analyses

For all spiking analyses, only putative pyramidal (CA3, CA1, subiculum) or granule (DG) neurons were considered. Putative interneurons, identified by a firing rate of greater than 4 Hz or a spike auto-correlogram differing considerably from that associated with hippocampal pyramidal neurons, were excluded. For comparisons of firing rates across conditions, units were

excluded from consideration if they did not emit at least 50 spikes across all conditions.

For analyses of spike-phase relationships, neurons were required to fire at least 50 action potentials per condition to be evaluated for significant phase modulation, following the procedure employed by Mizuseki et al. (2012). Significant phase modulation was said to be present for a given neuron if a Rayleigh's Z-Test for circular non-uniformity returned a p-value of less than 0.05. To evaluate whether or not the percent of neurons significantly modulated by phase differed from the percent expected by chance, the actual percent of significantly modulated neurons was compared to the percentages attained from 1,000 shuffles, where, in each of the shuffles, the number of neurons and action potentials was held constant, but spike phase was randomly drawn from a uniform circular distribution. Strength of phase modulation was assessed with pair-wise phase consistency (Vinck et al., 2010), which quantifies the consistency of angular phase preference for each possible pair of action potentials, thus avoiding the bias associated with mean resultant length.

When assessing spike-phase relationships with nonstationary rhythms (e.g., beta, slow gamma, fast gamma), only spikes occurring when these oscillations are prominent can be considered, as failure to pre-select periods of strong oscillatory activity can lead to spurious detection of spike-phase relationships (Colgin et al., 2009). Thus, when assessing spike-phase relationships to frequency ranges above theta, which is consistently strong throughout the rat hippocampus, we filtered each local field potential in the frequency range of interest, then extracted an amplitude envelope for the local field potential via a Hilbert transform and detected periods of time in which beta and gamma rhythms were strong for further consideration. We defined oscillatory events as time points in which the amplitude envelope surpassed an edge threshold of at least 1 standard deviation above average and a peak of at least 1.5 standard deviations above average. Oscillatory events were required to be at least three cycle lengths long, with the cycle length defined by the average frequency for that range. For example, when looking

for events in the slow gamma range (30-55 Hz), detected events were required to last at least 70.587 ms in duration, or three full cycles of a 42.5 Hz rhythm, the average frequency of a slow gamma oscillation. Events occurring within 3 average cycle lengths of one another were considered to be the same event.

Spike-phase alignment to the hippocampal theta rhythm was assessed in relation to theta recorded from the pyramidal layer of CA1, rather than in relation to each subregion's local theta oscillation. The theta oscillation is largely coherent throughout the hippocampus but most readily visible in CA1. Likewise, this procedure allowed for more direct comparisons of spike-phase relationships across subregions. As theta in CA1 is known to exhibit an asymmetric saw-toothed shape rather than a sinusoidal rhythm, we followed the protocol established by Belluscio et al. (2012) when defining the borders between phase components (e.g., peak, falling, trough, rising). In brief, phase centers, established as the peak, trough, and zero crossings of the local field potential time series, are first found for a narrowly filtered theta band (6 -12 Hz). The local field potential is then re-filtered in a broader band (3 - 20 Hz) and phase centers established from the narrow band are re-defined to be the closest peaks, troughs, and zero-crossings detected in the broader band.

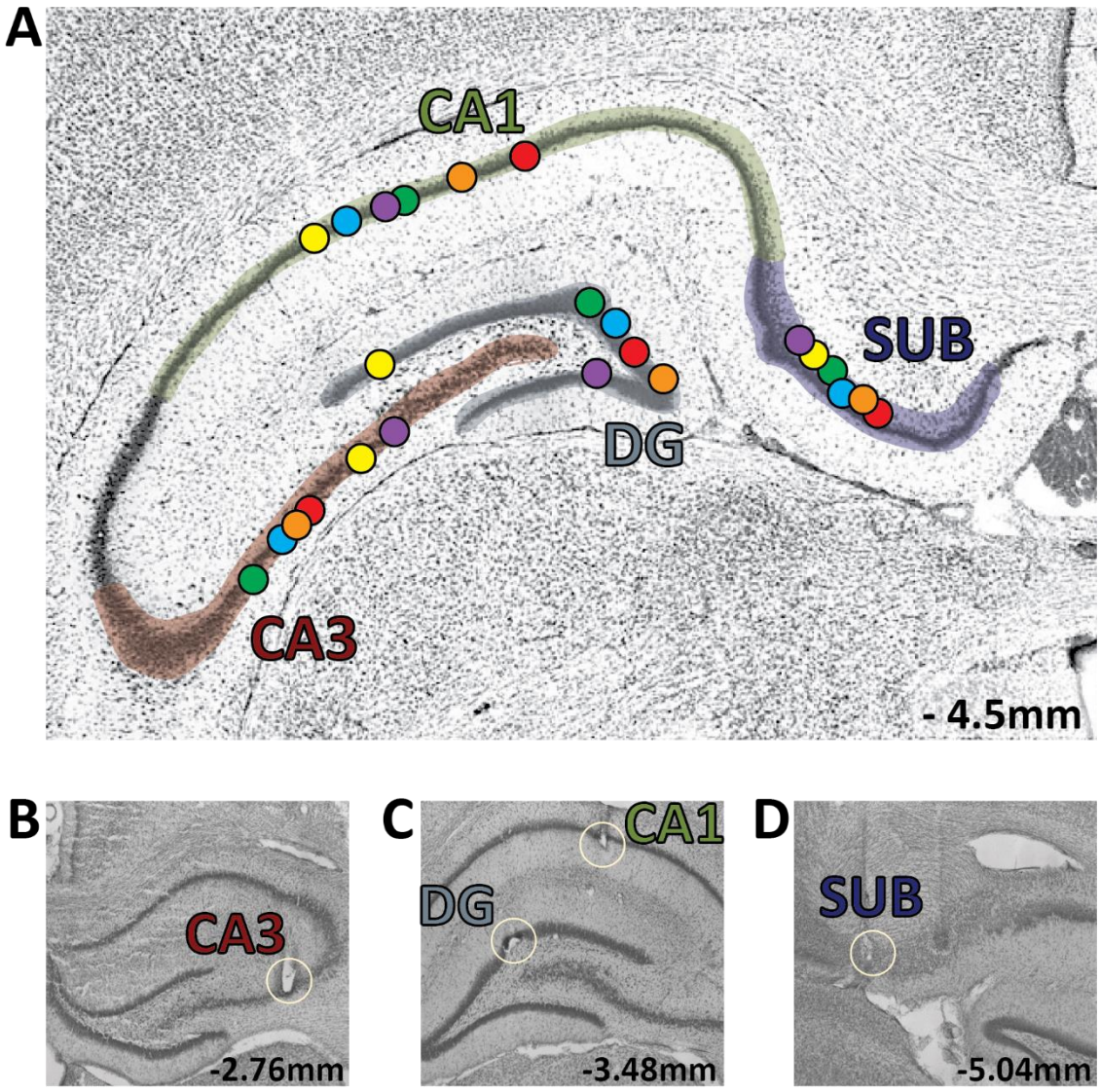
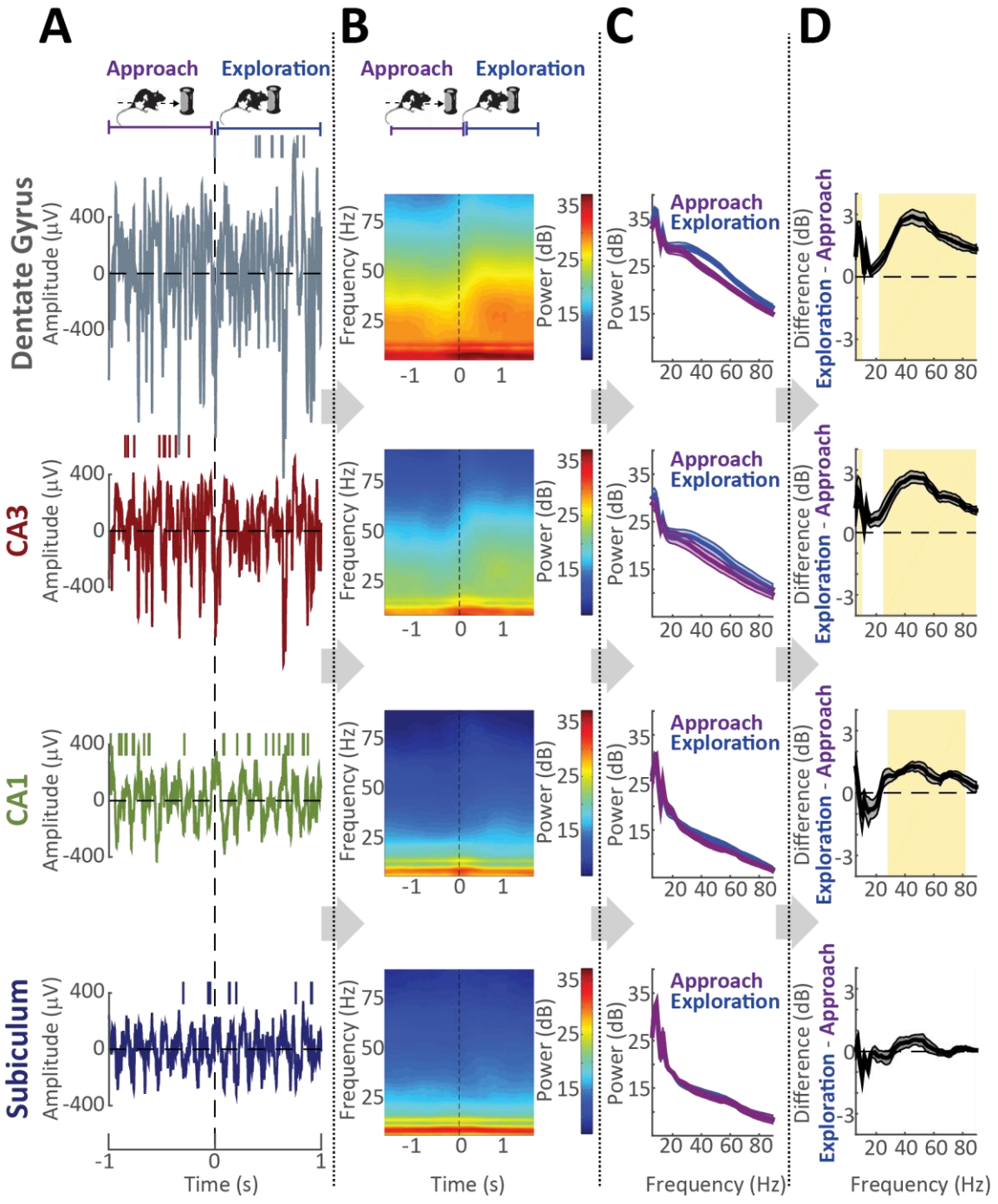


Figure 2.1. Anatomical locations of local field potential recording sites from each of four subregions within the hippocampus. Panel A shows the approximate local field potential recording sites in each subregion for each rat, with each rat specified by a unique color. Recording sites are shown on a single coronal section for clarity. Panels B, C, and D show actual recording sites in each of the four subregions, all from a single rat.

Figure 2.2. Illustrative schematic for local field potential analysis processing procedure. See text for details. Panel A shows example peri-event (+/- 1 second) local field potentials and action potentials, indicated by vertical ticks above local field potential sweeps, for each subregion, time-locked to the initiation of object exploration (onset = 0 seconds). Moving window spectral estimates, as exemplified in Panel B with moving window spectrograms for each subregion, were calculated first. Spectral power was then averaged across time for each time-window of interest [here, Approach (purple) and Exploration (blue)] to arrive at power by frequency values for each condition (Panel C). The power difference between conditions was calculated for each rat (the average of which is plotted in Panel D) and statistical analyses were performed on the average of these difference scores. Yellow rectangles indicate frequency bins found to differ significantly across conditions.


A
B
C
D
Approach
Exploration
Dentate Gyrus
CA3
CA1
Subiculum
Amplitude (μV)
400
0
-400
Frequency (Hz)
75
50
25
Power (dB)
35
25
15
-1
0
1
Time (s)
Approach
Exploration
20 40 60 80
Frequency (Hz)
Difference (dB)
Exploration - Approach
3
0
-3

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Chapter 3

Distinct Hippocampal Network State

During Novel Object Exploration

Abstract

Hippocampal activity in rats is known to relate closely to locomotion. In comparison, very little is known about hippocampal activity in rats during encoding of memories for individual items such as novel objects. The present study employed in vivo electrophysiology to simultaneously record local field potentials and action potentials from hippocampal subregions dentate gyrus, CA3, CA1, and subiculum as rats locomote around an empty track and as rats explore novel objects, a behavior likely related to the memory encoding. We report here dramatic differences in oscillatory activity by behavioral state that extend beyond a relationship with locomotion. Slow gamma (30 – 55 Hz) is strongest in dentate gyrus, CA3, and CA1 during object exploration; beta (13-30 Hz) is most prevalent while rats remain stationary; locomotive states are accompanied by strong hippocampal theta (6-12 Hz). These results reflect the first characterization of hippocampal activity simultaneously recorded from four hippocampal subregions during a task related to recognition memory, and underline the importance of considering object exploration as a behavioral state unique from the cessation of locomotion. Further, these results address an important gap in data regarding the hippocampal network state during encoding of memory for novel items and provide a basis for subsequent experiments to ask how this network state might differ based on the success of memory encoding.

Since the discovery of neurons in the hippocampus that fire selectively when rats occupy discrete regions of the environment (O'Keefe & Dostrovsky, 1971), the hippocampal role in navigation has been intensely studied and well documented (Derdikman & Moser, 2010; Buzsaki & Moser, 2013). An essential component of investigating the hippocampal contribution to navigation has been to assess hippocampal activity patterns as rats ambulate throughout novel environments and formulate a so called "cognitive map" (O'Keefe & Nadel, 1978), or neural representation of their surroundings. While many have investigated spiking activity in relation to navigation (Bieri et al., 2014; Johnson & Redish, 2007; Skaggs et al., 1996), others have gone on to characterize how oscillatory activity within the hippocampus varies as rats traverse these environments (Belluscio et al., 2012; Colgin et al., 2009), and, further, how that oscillatory profile varies with speed of locomotion (Ahmed & Mehta, 2012; Slawinska & Kasicki, 1998; Zheng et al., 2015).

Indeed, substantial differences in hippocampal oscillatory activity are present across speeds of locomotion. For example, in CA3 and CA1, the frequency of the hippocampal theta oscillation (6-12Hz) correlates with running speed during spontaneous locomotion (Slawinska & Kasicki, 1998) as does the frequency and amplitude of hippocampal fast gamma (65-90 Hz) (Ahmed & Mehta, 2012; Zheng et al., 2015). Though slow gamma (30-55 Hz) does not shift in frequency, its amplitude negatively correlates with running speed, such that slow gamma in CA3 and CA1 is stronger while rats remain stationary relative to while locomoting (Zheng et al., 2015).

In addition to its prominent contributions to spatial memory, the hippocampus is known to play a major role in nonspatial memory for items (Eichenbaum et al., 1999). How hippocampal oscillatory activity patterns relate to memory for individual items, however, has received relatively less research attention than activity during locomotive states. Thus, in the present study, one goal was to expand upon the body of work characterizing the hippocampal activity state

during locomotion by adding to it a thorough characterization of hippocampal activity as rats engage in events that may relate to the formation of recognition memories—namely, object exploration—and to examine how the activity patterns associated with object exploration differ from those associated with various locomotion speeds.

As newer technologies have emerged for studying neural function with higher anatomical resolution, an increased appreciation has been garnered for differences in function that may be present amongst the hippocampal subregions—dentate gyrus, CA3, CA2, CA1, and subiculum (Gilbert et al., 2001; Kesner et al., 2004; Small et al., 2000). Trimper et al. (2014) reported that oscillatory synchrony, or coherence, between two subregions of the rat hippocampus, CA3 and CA1, increased markedly while rats explored novel objects. Further, the degree of coherence was stronger for items which the rats subsequently remembered. Here we expand upon this work by additionally recording from two more hippocampal subregions, dentate gyrus and subiculum, as well as CA3 and CA1, in an effort to better understand how each subregion may be differentially involved in novel object exploration relative to other behavioral states. We report that spiking analyses revealed modest differences in neuronal firing rate and spike phase-relationships between locomotive versus non-locomotive states. Oscillatory analyses, however, revealed striking differences by subregion and behavioral state that extended beyond a relationship with locomotion. Novel object exploration was associated with a network state very different from that observed during locomotion or the cessation of locomotion, characterized by the strong presence of slow gamma within the hippocampus. These results underscore the importance of considering hippocampal activity during novel object exploration as a unique network state that relates to the encoding of memories for individual items.

Method

Subjects

Subjects were six male Long-Evans rats, cared for as described in General Methods

(Chapter 2).

Experimental Task

Rats alternated between completing laps around the elevated circular track with no objects present, and laps around the track with two novel objects present. Novel objects were always presented in the 10 o'clock and 2 o'clock positions relative to the central stem of the track at 6 o'clock. Up to 24 trials were performed per rat per day across up to five days of testing, with the number of trials and experimental sessions limited by rats' willingness to explore objects during the initial presentation on object lap 1.

Segregation of Activity into Behavioral States

Figure 3.1, Panel A, shows how behavioral states were classified. We separated rats' activity on blank laps into periods of time in which the rat was not locomoting (Stationary) and periods of time in which the rats were locomoting (Run). To accomplish this task, spatial coordinate data, gathered as described in General Method (Chapter 2), and local field potential data on blank laps were divided into 250 ms segments. Stationary bouts were defined as 8 consecutive 250 ms segments in which rats moved less than 10 cm/s. Run bouts were defined as 8 consecutive 250 ms segments in which rats moved more than 10 cm/s. A threshold of 10 cm/s, rather than 0 cm/s, was chosen to allow for small head movements and rearing in the Stationary condition. Exploration bouts were defined as period of time lasting at least 2 s in which rats were consistently engaging in active investigation of novel objects, while Approach bouts were defined as the 2s of time immediately preceding exploration onset. Figure 3.2, Panel A, shows averaged exploration times by rat for all initial novel object exploration bouts lasting at least 2 seconds.

Data Analysis

Analyses followed the procedures outlined in General Method (Chapter 2).

Results

Speed of Locomotion

Figure 3.2, Panel B, shows rats' locomotion speeds in cm/s for each of the four behavioral states. A one-way repeated measures ANOVA indicated locomotion speeds differed significantly across conditions [$F(3,15) = 132.41$, $p < 0.001$]. Adopting a Bonferroni corrected alpha of 0.0125 for four comparisons, results indicated Stationary (2.7 +/- 0.171 cm/s) differed significantly from Run (38.2 +/- 2.72 cm/s) [$t(5) = -12.5$, $p < 0.001$], Exploration (12.2 +/- 1.54 cm/s) differed significantly from Approach (32.2 +/- 2.63 cm/s) [$t(5) = -15$, $p < 0.001$], Stationary differed significantly from Exploration [$t(5) = -5.670$, $p = 0.002$], and Approach did not differ significantly from Run [$t(5) = 3.71$, $p = 0.014$]. Thus, behavioral classification procedures correctly sorted locomotive and exploratory states as anticipated.

Firing Rate

We recorded several well isolated putative pyramidal (CA3, CA1, subiculum) or granule cells (dentate gyrus) from each of the subregions targeted. Across rats, cell counts were 39, 123, 261, and 39 for dentate gyrus, CA3, CA1, and subiculum, respectively.

Figure 3.3 shows average firing rates for each subregion's principal neurons by behavioral state. A one-way repeated measures ANOVA across behavioral states for each subregion revealed that firing rate differed significantly only for CA1 [Huynh-Feldt $F(2.455, 638.414) = 18.283$, $p < 0.001$], with firing rates being greatest for states in which the rats were actively locomoting (i.e., Run and Approach). Firing rates differences in dentate gyrus approached the Bonerfonni corrected alpha level (0.0125) [Greenhouse-Geisser $F(1.540,58.508) = 4.502$, $p = 0.023$], with the numerically greatest firing rate observed during Exploration. P values for CA3 and subiculum did not approach significance ($p = 0.082$ and $p = 0.303$, respectively). The finding that firing rates in CA1 are higher when rats are locomoting has been documented previously (Ahmed & Mehta, 2012; McNaughton et al., 1983; Zheng et al., 2015).

Spike-Phase Modulation

To avoid spurious conclusions based on volume conduction, one must verify the local

nature of the signal by demonstrating a relationship between local field potentials and local neuronal firing (Buzsaki et al., 2012). Figure 3.4 shows, without categorization by behavioral state, the percent of neurons in each subregion significantly modulated by the phase of oscillations in each prominent frequency range within the hippocampus [theta (6-12 Hz); beta (13-30 Hz); slow gamma (30- 55 Hz); fast gamma (65-90 Hz)], both in relation to the local oscillations (i.e., oscillations emitted from the spiking subregion) and in relation to the downstream oscillations (i.e., oscillations emitted from the subregion efferent to the spiking subregion). Spikes were compared to downstream oscillations based on the commonly accepted idea that action potentials sent from upstream regions influence the excitatory post synaptic potentials, and therefore oscillations, in downstream regions. For all subregions and all frequency ranges of interest, the percent of total neurons significantly modulated by oscillatory phase is significantly different from chance (~5%). Thus, in general, hippocampal pyramidal and granule cells exhibited strong relationships with local field potentials in both the spiking region itself and the downstream region at all frequency ranges of interest.

To assess spike-phase relationships to nonstationary rhythms (e.g., beta, slow gamma, fast gamma), one must pre-select periods of time in which oscillatory power is sufficiently strong to avoid spurious results (Colgin et al., 2009). As this process necessarily discards a large number of action potentials, we were unable to assess spike-phase relationships across conditions with any frequency range above theta due to too small a number of spikes.

Figure 3.5, Panel A, shows the percent of neurons in each subregion significantly modulated by theta phase and split by behavioral state. All bars are significantly greater than chance (~5%), though in no subregion did the percent of neurons significantly modulated by theta phase differ across behavioral states.

Figure 3.5, Panel B, shows pairwise phase consistency, a metric quantifying the consistency of spike-phase alignment, for each subregion and each behavioral state. In dentate

gyrus only, pairwise-phase consistency differed significantly across conditions, according to a Bonferonni corrected alpha of 0.0125, [$F(3,53) = 7.0176$, $p < 0.001$], such that dentate gyrus pairwise phase consistency was highest for Run and Approach, relative to states in which the rat is not locomoting around the track [other subregions: CA3: $F(3,123) = 1.395$, $p = 0.248$; CA1: $F(3,286) = 0.376$, $p = 0.770$; SUB: $F(3,45) = 2.823$, $p = 0.0494$].

The average theta phase angle at which spikes were emitted differed significantly in CA1 only, according to Watson-Williams Tests for equality of circular averages [DG: $F(3,53) = 1.25$, $p = 0.314$; CA3: $F(3,123) = 2.33$, $p = 0.078$; CA1: $F(3,286) = 5.20$, $p = 0.002$]. Angles are reported in degrees with peak equal to 0°, falling equal to 90°, trough equal to 180°, and rising equal to 270°. The Watson-Williams test results were inapplicable to subiculum data due to too weak of an average phase preference (i.e., mean resultant length < 0.45). In CA1, the average theta phase angle was between the trough and falling phase of the theta wave when rats were Stationary (mean angle +/- standard deviation = 163.60° +/- 64.794°), but shifted modestly more towards the rising phase when rats were in more active states, including Run (207.06° +/- 64.794°), Exploration (209.49° +/- 61.404°), and Approach (182.37° +/- 55.413°). Thus, modest differences are present in both firing rate and spike-phase alignment that appear to relate primarily to whether or not rats were in a locomotive state.

Power and Coherence

Figures 3.6 and 3.7 show spectral power and coherence, respectively, by frequency from 5 – 90 Hz. According to both metrics, the spectral profile throughout the hippocampal network of subregions differs dramatically and significantly across the behavioral states considered.

Figure 3.6 shows spectral power across behavioral states for dentate gyrus, CA3, CA1, and subiculum. Large statistically significant differences in power between behavioral states were observed for each subregion (Fig 3.6, B). Specifically, in dentate gyrus, all frequencies considered (5-90Hz) differed significantly across behavioral states, while for CA3, all but a small

band from 10.25 - 14.65 Hz reached statistical significance. In CA1, all frequencies from 24.9 – 90 Hz differed significantly, and in subiculum, two distinct ranges (5.89 – 10.25 Hz and 45.41 – 90 Hz) reached significance independently. Likewise, a behavioral state by subregion interaction is present in the theta band (7.32-10.25 Hz) and from 13.18 to 90 Hz. For both dentate gyrus and CA3, spectral power in the theta range (6-12Hz) was greatest for Exploration, next strongest for Approach and Run, and lowest for Stationary. CA1 and subiculum revealed different patterns, where Approach and Run showed the strongest levels of theta, Exploration was slightly less, and Stationary far lower. In dentate gyrus, CA3, and subiculum, the beta range (13 – 30 Hz), specifically beta2 (23 – 30 Hz) was strongly elevated during Stationary epochs. Conversely, Approach and Run appear to have maintained the highest beta levels in CA1. Finally, perhaps most strikingly apparent from the figures, is the relatively high levels of gamma, both slow (30 – 55 Hz) and fast (60 – 90Hz) for Exploration in dentate, CA3, and CA1, and the relatively low levels of fast gamma for Stationary in CA1 and SUB.

Large differences were also observed when power was averaged across behavioral states for each subregion (Figure 3.6, B). A main effect of subregion is present at all frequency bins considered (5 – 90 Hz), indicating spectral power varied strongly by subregion, with differences topping out near 13 dB in the slow gamma range between dentate and subiculum. Panel D shows mean hippocampal power, averaged across subregions, for each behavioral state. A main effect of behavioral state is present from 5.89 to 11.72 Hz and from 16.1 to 90 Hz, indicating average hippocampal power differed significantly across behavioral states at almost all frequency ranges. Exploration was associated with relatively high levels of both slow and fast gamma relative to the other three behavioral states. The average hippocampal spectral profile for Stationary is most elevated in the beta range, while both locomotive states (Run and Approach) are associated with relatively strong levels of theta.

Figure 3.7 shows coherence across behavioral states for dentate gyrus and CA3, CA3 and

CA1, and for CA1 and subiculum. An interaction is present at most frequencies considered (5.89 – 62.99 Hz), indicating the pattern of coherence by frequency differs uniquely across behavioral states and subregion pairs. Coherence between dentate gyrus and CA3, which differs significantly across conditions from 13.18 to 90 Hz, looks highly similar to the patterns observed in dentate gyrus and CA3 spectral power, with strong levels of gamma associated with Exploration and beta associated with Stationary. Coherence between CA3 and CA1 is notably similar across conditions, aside from the significantly elevated slow gamma (32.23 – 49.8 Hz) for Exploration relative to the other three behavioral states. Coherence differences for CA1 and subiculum, too, are appreciably absent with the blatant exception of substantially elevated theta and beta (7.32 – 26.37 Hz) for locomotive states (i.e., Run and Approach) relative to non-locomotive states (i.e., Stationary and Exploration). As the beta profile follows theta exactly, it likely reflects a harmonic of the theta range.

Discussion

Analyses revealed large, statistically-significant differences in the hippocampal network state during object exploration compared to time periods in which rats remained stationary or periods of locomotion (Run and Approach). Figure 3.8 shows a summary of the spectral results and highlights the most notable spectral features of each behavioral state considered. Novel object exploration was associated with high levels of gamma, particularly in the slow gamma range, in dentate gyrus, CA3, and CA1. Locomotion was associated with high levels of theta in CA1 and subiculum. Stationary epochs were associated with high levels of beta in dentate gyrus and CA3.

Slow Gamma During Novel Object Exploration

Exploration of novel objects in the current data set was associated with increased gamma power, in both the slow and fast gamma ranges, in dentate gyrus, CA3, and CA1. Moreover, we observed an increase in slow and fast gamma coherence between dentate gyrus and CA3, and increased slow gamma coherence between CA3 and CA1. These findings substantially expand

upon previous work reporting increased slow gamma coherence between CA3 and CA1 during novel object exploration (Trimper et al. 2014) by extending the findings to include two additional primary hippocampal subregions and their interactions.

An interesting question, then, is why gamma activity in particular is associated with novel object exploration. What computational advantages might be offered by oscillatory activity at the gamma frequency range? Gamma oscillations have been suggested to play an important role in the formation of functional cell ensembles, or groups of simultaneously active neurons functionally representing individual chunks of information (Buzsaki & Chrobak, 1995). The balance of inhibition and excitation underlying gamma rhythm generation provides a mechanism for temporally grouping the most strongly excited neurons within each gamma cycle, while suppressing neurons that are less excited (Wang & Buzsaki, 2012). Thus, gamma oscillations during novel object exploration may provide the temporal coordination necessary to exchange information about item encounters within and across hippocampal subregions, while suppressing irrelevant activity. Related, gamma oscillations in visual cortex have been proposed to play a role in binding information simultaneously represented by distinct groups of neurons (Gray & Singer, 1989). It may be the case that the strong presence of gamma during novel object exploration serves to bind neural representations for both spatial context and item identity information in the service of associative memory formation.

An additional question involves why gamma activity increases in dentate gyrus, CA3, and CA1, but not in subiculum. Dentate gyrus may be particularly involved due to the necessity of orthogonalizing the incoming information in service of creating a unique representation in downstream CA3 (Kesner & Rolls, 2015). Rats encounter many objects in these locations over the course of experimental sessions, and thus separating the pattern associated with this encounter from those associated with other encounters may be particularly important. CA3 activity may, likewise, be especially important for the rapid formation of a memory for the object encounter

within that particular location (Kesner & Rolls, 2013). Recurrent collaterals in CA3 have been extensively modeled as contributing to this process (Rolls, 1987; Rolls, 1989a,b,c,d; 1990a,b)—a suggestion which lesion data supports (Gilbert & Kesner, 2003; Kesner et al., 2008).

An exciting idea, given that slow gamma coherence between CA3 and CA1 is similarly low for all behavioral states except novel object exploration, is that only when a significantly stimulating event occurs, such as a novel object encounter, is the connection between CA3 and CA1 heightened, presumably to enhance communication between the subregions (Fries, 2005; Fries, 2015) in the service of memory. CA1 is hypothesized to play a role in the efficient recoding of representations in CA3 for the purpose of reactivation of the cortical ensembles originally active during the initial sensory experience (Kesner & Rolls, 2015). Perhaps during the formation of a hippocampal representation for the novel object encounter in CA3, communication with CA1 becomes increasingly important relative to during the other behavioral states, as further processing is essential for subsequent retrieval of the newly formed memory.

Hippocampal Theta is Elevated During Locomotion

The most notable spectral feature present during locomotive epochs, including both Run and Approach, is the elevated presence of theta oscillations. Theta in CA1 and subiculum exhibits a well-studied relationship with locomotion, increasing in amplitude and frequency as speed of locomotion increases (Bender et al., 2015; Buzsaki & Moser, 2013). Theta during locomotion may function primarily to coordinate sensory representations of the environment, which necessarily require faster processing during locomotion as the immediate spatial environment changes more rapidly (e.g., Skaggs et al., 1996).

An interaction between behavioral state and subregion in the theta range, however, points to potentially interesting functional differences between theta in CA1 and subiculum versus theta in dentate gyrus and CA3. In dentate gyrus and CA3, theta power is highest during exploration and *second* highest for locomotive states. Theta in dentate gyrus and CA3 may offer the

additional function of coordinating neuronal ensembles in relation to memory for the novel object encounters. Indeed, several studies have linked hippocampal theta to memory (Hyman et al., 2003; Larson et al., 1986; Macrides et al., 1982; Winson, 1978), as well as, in particular, to synaptic plasticity within the dentate gyrus (Orr et al., 2001). Given the strong simultaneous presence of gamma and theta oscillations in dentate gyrus and CA3 during novel object exploration, an interesting idea is that theta offers a mechanism for temporally ordering the information represented by successively active, gamma-coordinated cell ensembles (Colgin et al., 2010; Lisman & Jensen, 2013; Tort et al., 2009)

Stationary Epochs Are Associated with Elevated Beta Activity

While rats remained stationary, relative to when rats were locomoting or exploring novel objects, beta power in dentate gyrus and CA3, but also subiculum and to a smaller degree CA1, was elevated. This beta elevation is likewise apparent in coherence between dentate gyrus and CA3, and, to a lesser extent, between CA3 and CA1. In the motor system, it has been proposed that beta oscillations relate to maintaining the current motor plan or cognitive state (Engel & Fries, 2010)—an intriguing hypothesis that may be in line with the presently observed elevated beta when rats remained stationary.

Beta oscillations in the hippocampus, relative to theta (Buzsaki, 2002; Colgin, 2013) and gamma (Wang & Buzsaki, 1996; Colgin & Moser, 2010), have received far less attention. Whereas gamma oscillations are purportedly maximally suited to anatomically local organization of functional neuronal ensembles (Buzsaki & Chrobak, 1995), beta oscillations are believed to play an important role in neuronal communication when axonal projections possess longer conduction delays (Koppell et al., 2000). In line with this idea, beta power in dentate gyrus has been found to increase during odor sampling (Vanderwolf, 2001; Martin et al., 2007), with beta in the dentate gyrus temporally led by beta in the olfactory bulb (Gourevitch et al., 2010).

In an odor-place association task, Igarashi and colleagues (2014) found that the degree of

beta coherence between CA1 and lateral entorhinal cortex correlated with learning across successive encounters. Interestingly, in the present study, object exploration was associated with gamma oscillations, rather than beta oscillations, raising the interesting possibility that the mode of investigation employed by the rats—that is, visual or tactile relative to olfactory—plays a role in whether the beta or gamma band become elevated within the hippocampus (but see Rangel et al., 2015).

In Rangel et al. (2015), beta oscillations in the dentate gyrus increased as rats stopped to explore objects, but not when rats came to a stop without objects present. In the current data set, beta was elevated when rats were stationary with no objects present. A possible explanation for the discord is that is that stationary epochs in the current experiment, even though no objects were present on the track, included periods of active olfactory investigation, whereas those in Rangel et al. (2015) were associated with a greater degree of inactivity. It may be that with a task better suited to segregate inactive epochs from olfactory investigation epochs, we would observe the degree of hippocampal beta remaining low when rats were inactive and stationary, but elevated when rats were actively sniffing and stationary.

Conclusion

In conclusion, we report here striking differences in the hippocampal oscillatory network state during novel object exploration, relative to during locomotive and stationary behavioral states. Novel object exploration was associated with marked increases in hippocampal gamma, particularly slow gamma, possibly in relation to the encoding of memories for individual item encounters. A remaining open question is how the network state expressed during novel object exploration might relate to subsequent memory for the events, and, furthermore, how that activity pattern might vary based on the content of the memory formed.

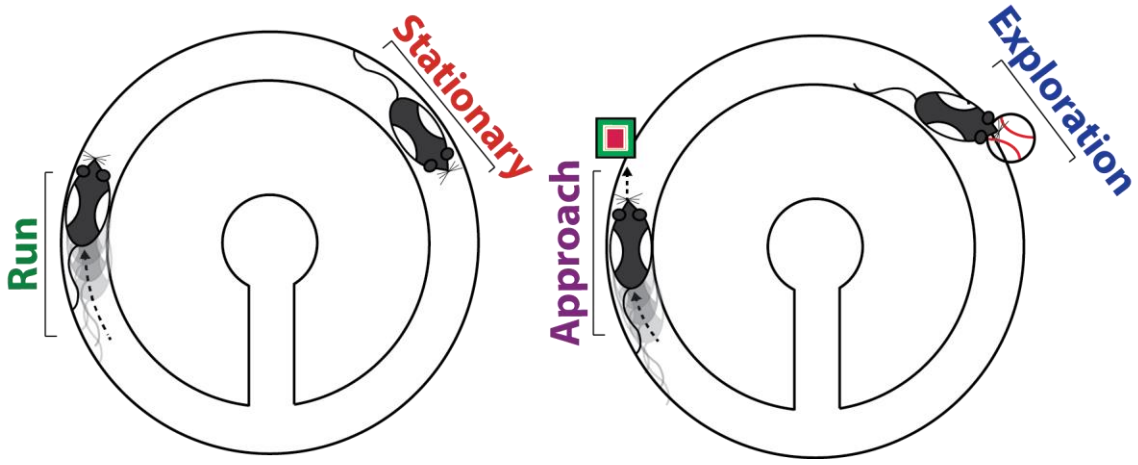


Figure 3.1. Illustrated schematic of behavioral states. Run (>10 cm/s) and Stationary (<10 cm/s) behavioral states were considered only on laps in which no objects were present on the track. Approach was defined as the period 2 seconds before object exploration (Exploration) was initiated. Exploration was defined as time periods in which rats engaged in active exploratory activities with their noses remaining within ~1cm of the object and oriented towards the object.

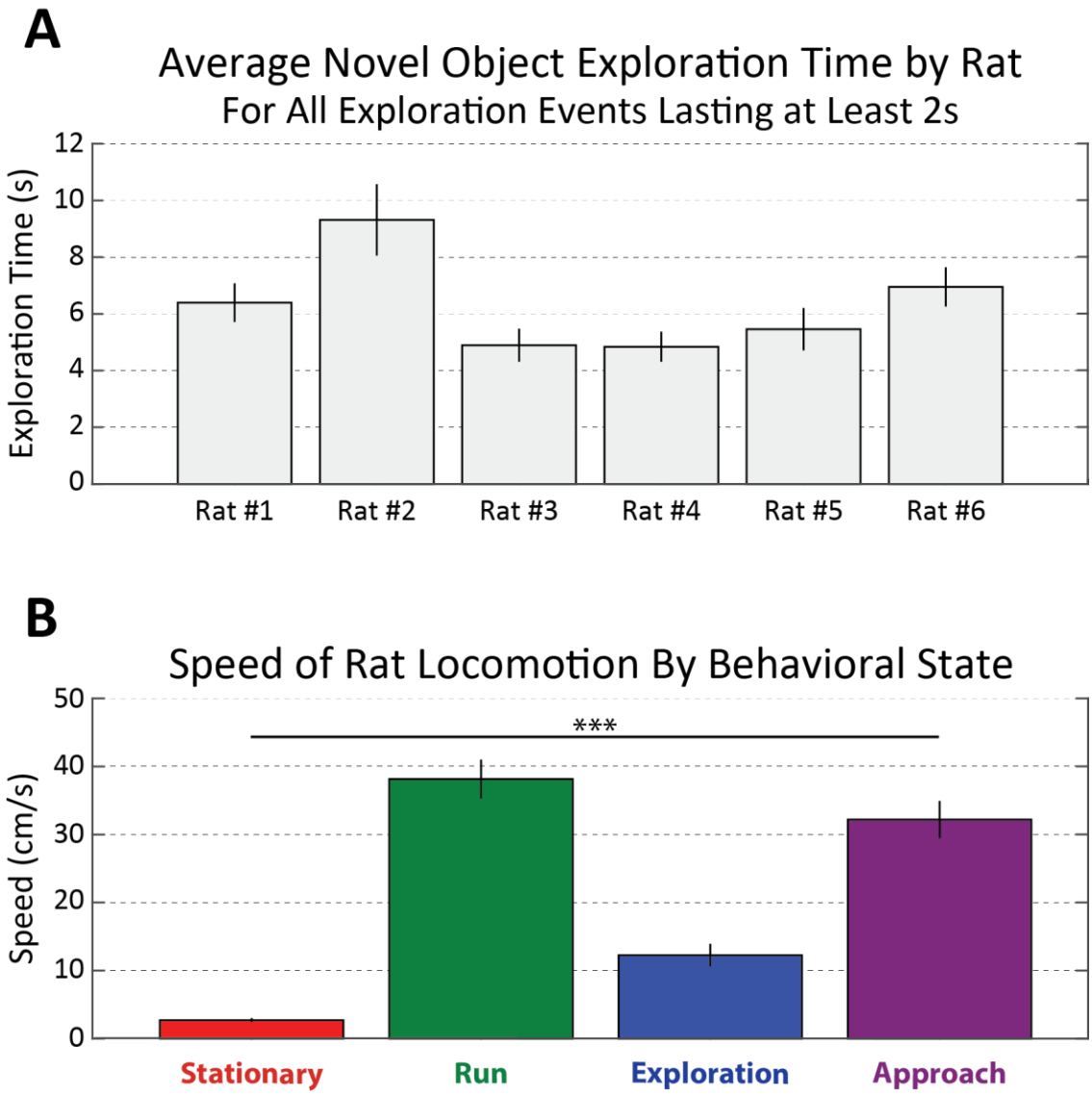


Figure 3.2. Behavioral activity by behavioral state. Panel A shows average novel object exploration duration by rat for all events considered in neural analyses. On average, rats demonstrated strong evidence of engagement with novel objects, with averages ranging from 4.841 +/- 0.498 s to 9.31 +/- 1.225 s. Panel B shows that speeds of locomotion differed significantly across behavioral states ($p < 0.001$), with Run and Approach differing significantly ($p = .0134$), Stationary and Exploration differing significantly ($p = 0.002$), and Run + Approach differing significantly from Stationary + Exploration ($p < 0.001$).

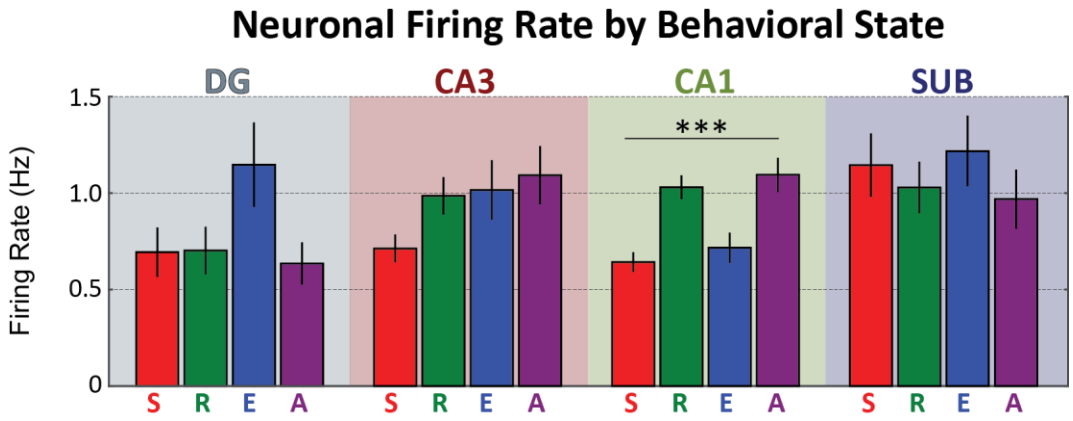


Figure 3.3. Firing rate differs by behavioral state and subregion. After correcting for alpha-inflation, neuronal firing rates differed significantly only in CA1 ($p < 0.001$), reflected in Approach (A) and Run (R) being associated with higher firing rates than Exploration (E) and Stationary (S). Neuronal firing rates in dentate gyrus (DG) approached significance ($p = 0.023$), but failed to surpass it.

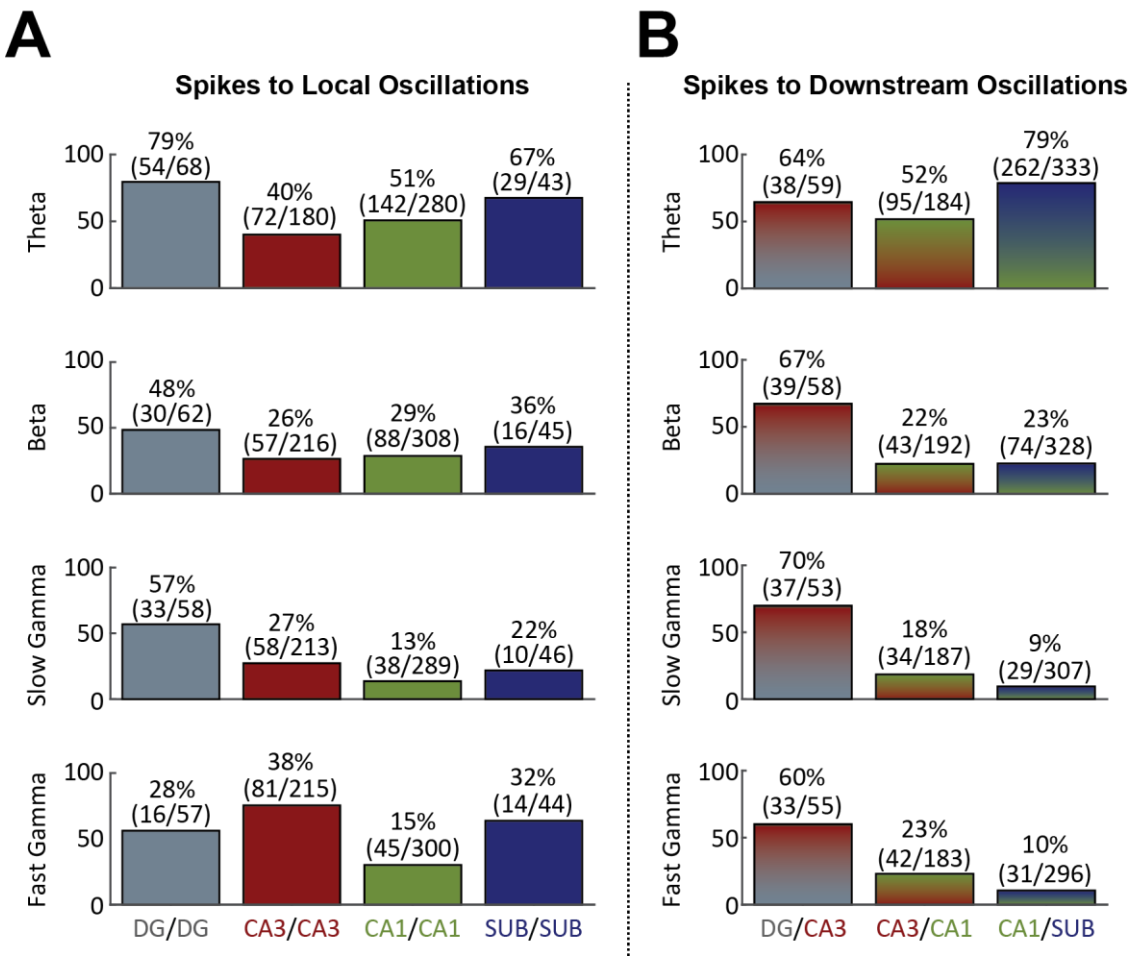


Figure 3.4. Neuronal spiking is significantly modulated by the phase of local and downstream oscillations. Panel A shows results for neuronal spiking in relation to the local field potential of the subregion emitting the action potentials (e.g., DG/DG shows dentate gyrus spikes compared to dentate gyrus local field potential), while Panel B depicts results from analyses of neuronal spiking in relation to oscillations in the downstream subregion (e.g., DG/CA3 shows dentate gyrus spikes compared to CA3 local field potentials). Above each bar, the percent of neurons significantly phase modulated, as indicated by a p-value of less than 0.05 on a Rayleigh's Z-Test for circular non-uniformity, is displayed along with the ratio of significantly modulated neurons to total neurons recorded. All bars are significantly different from chance as indicated by a bootstrapping permutation test in which spike phases were randomly shuffled 1,000.

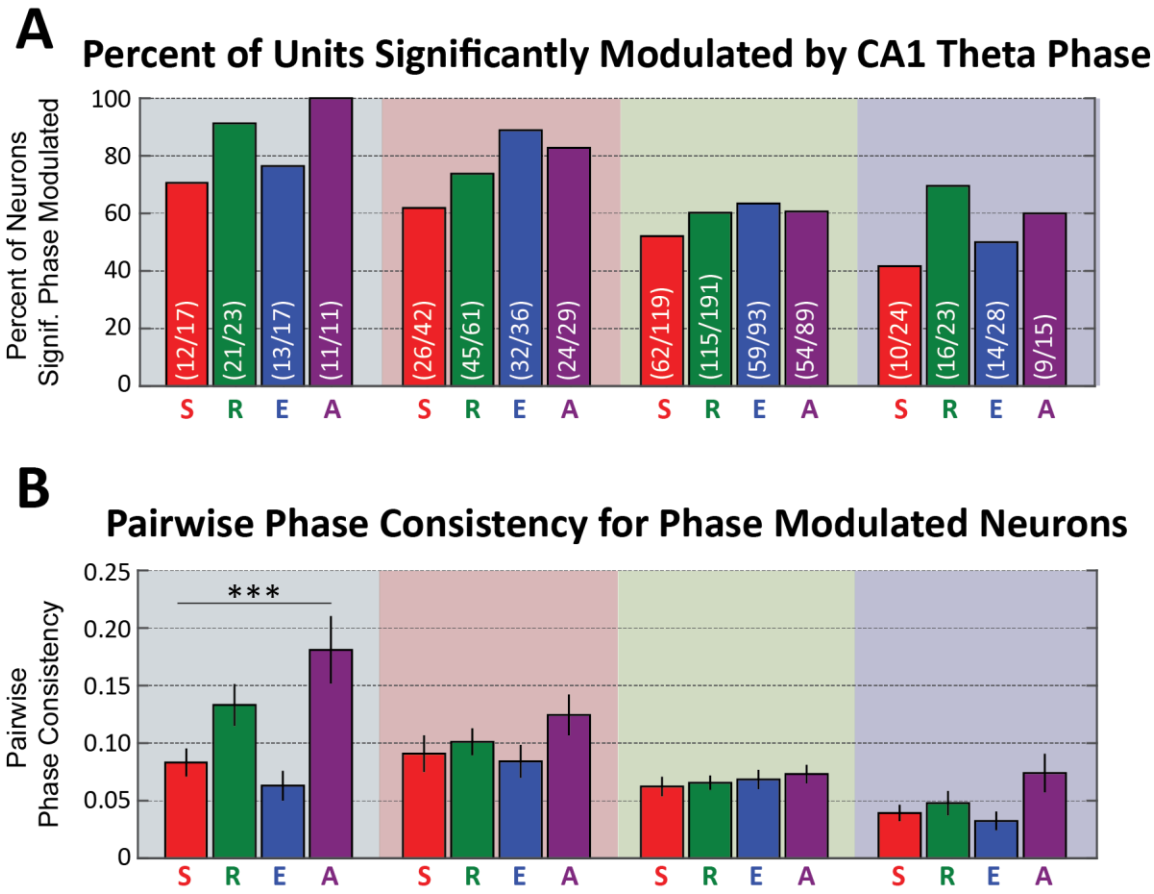


Figure 3.5. Spike-phase modulation differs by behavioral state. Panel A shows the percent of neurons by subregion significantly modulated by the phase of theta in CA1 for Stationary (S), Run (R), Exploration (E), and Approach (A) behavioral states. In all subregions and conditions, values are significantly different from chance, but in no subregion do they differ significantly across conditions. Panel C shows pairwise phase consistency, a measure for how consistently neuronal spikes occur at a particular phase of the oscillation, for neurons in each subregion in relation to theta in CA1. In dentate gyrus (DG) only, the pairwise-phase consistency differs significantly across conditions ($p < 0.001$), such that it is highest for Run and Approach, relative to states in which the rat is not locomoting around the track.

Figure 3.6. Spectral power differs strikingly by behavioral state and subregion. Panel A shows power by behavioral state for each subregion. Panel B shows power by behavioral state for each subregion plotted as each condition's difference from the average across conditions, where a difference score was first calculated for each rat and then the average was calculated across rats. Yellow rectangles indicate frequency bins which differed significantly across conditions. Asterisks at top indicate frequency bins associated with a statistically significant interaction between behavioral states and subregions. Note the strong slow gamma during Exploration, strong beta during Stationary epochs. Panel C shows power for each subregion, averaged across behavioral states. Significance markers here indicate a main effect of subregion. Spectral profiles differ greatly across subregions. Panel D shows whole-hippocampal power, averaged across subregions, for each condition, plotted as the difference from the average across conditions. Significance here indicates a main effect of condition in the frequency bins indicated. Note non-uniform y-scales across panels, with ranges chosen for best visualization of the differences within that panel.

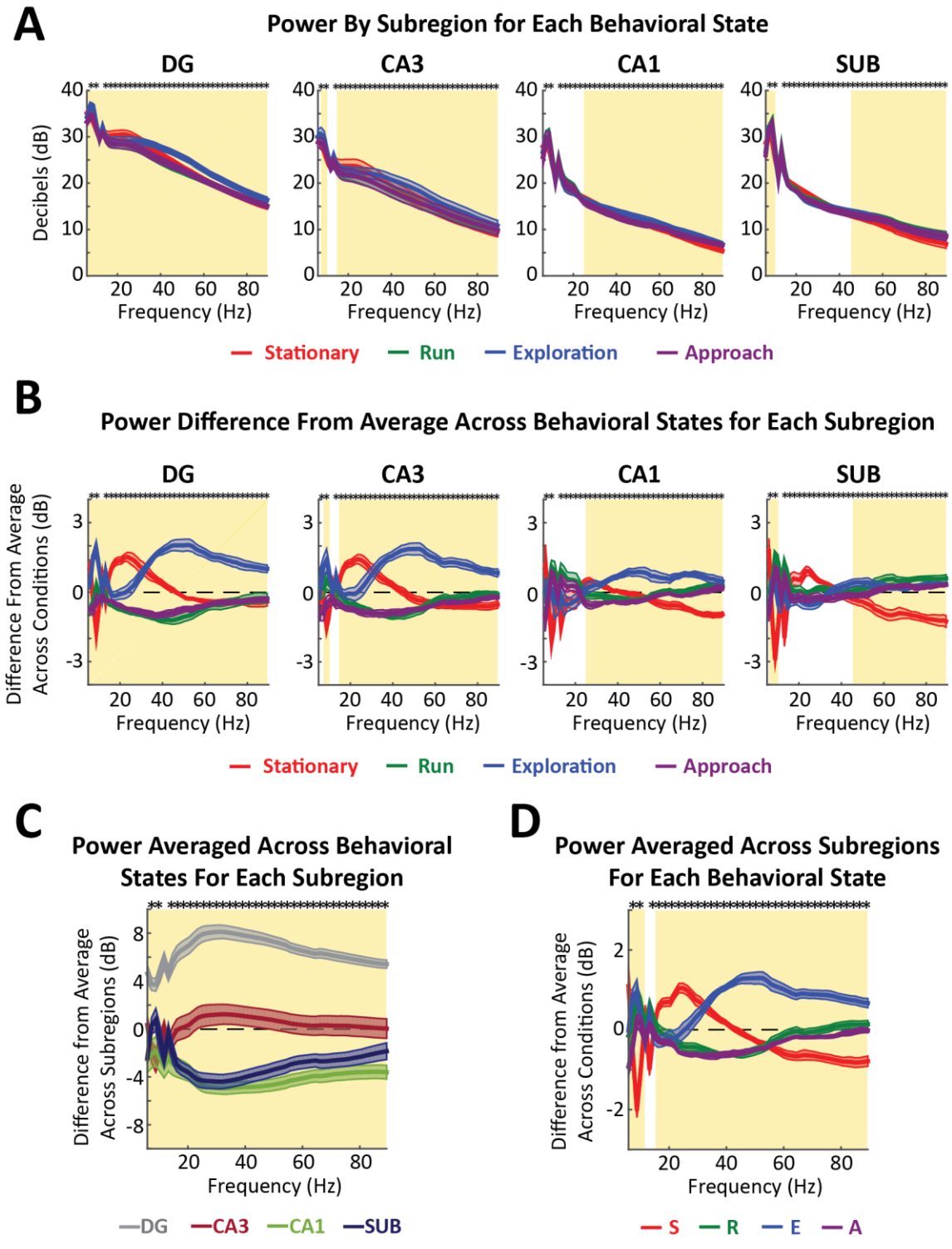
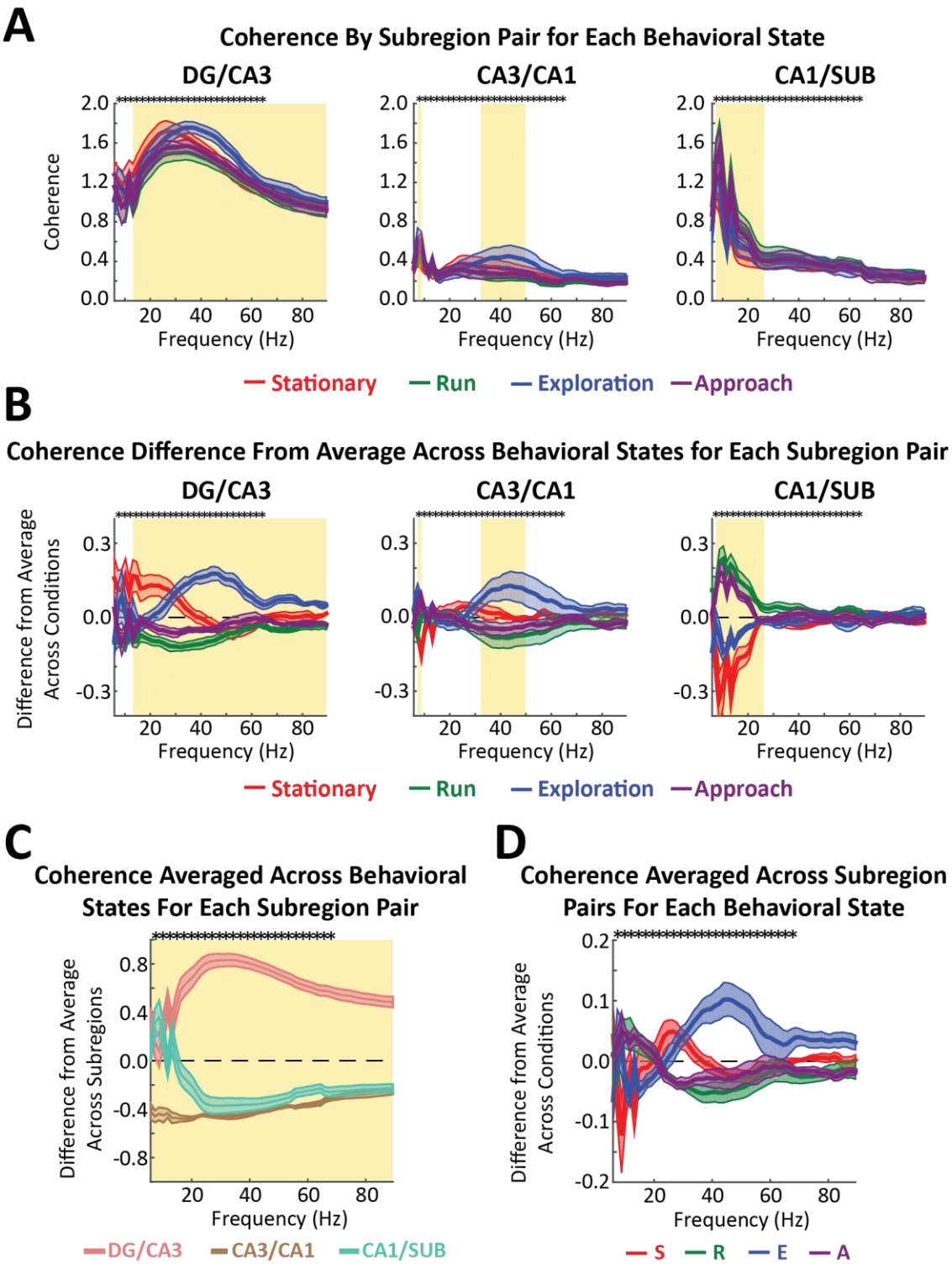

A
Power By Subregion for Each Behavioral State
DG
CA3
CA1
SUB
Decibels (dB)
Frequency (Hz)
Stationary
Run
Exploration
Approach
B
Power Difference From Average Across Behavioral States for Each Subregion
Difference From Average Across Conditions (dB)
C
Power Averaged Across Behavioral States For Each Subregion
Difference from Average Across Subregions (dB)
DG
CA3
CA1
SUB
D
Power Averaged Across Subregions For Each Behavioral State
Difference from Average Across Conditions (dB)
S
R
E
A

Figure 3.7. Coherence differs by behavioral state and subregion pair. For all panels, significance markers (i.e., yellow rectangles and asterisks) are as specified for Figure 3. Panel A shows coherence by behavioral state for each subregion pair. Panel B shows the same data plotted as each condition's difference from the average across conditions, where differences were first calculated for each rat then averaged across. Note the strong slow gamma coherence in DG/CA3 and CA3/CA1 during Exploration, strong DG/CA3 beta during Stationary epochs, and strong theta in CA1/SUB during Run and Approach epochs. Panel C shows power for each subregion pair, averaged across behavioral states. Panel D shows coherence averaged across the three subregion pairs for each condition, plotted as the difference from the average across conditions. As in Figure 3, y-scales vary by panel for optimal visualization.


A
Coherence By Subregion Pair for Each Behavioral State
DG/CA3
CA3/CA1
CA1/SUB
Coherence
Frequency (Hz)
Stationary
Run
Exploration
Approach
B
Coherence Difference From Average Across Behavioral States for Each Subregion Pair
Difference from Average Across Conditions
C
Coherence Averaged Across Behavioral States For Each Subregion Pair
Difference from Average Across Subregions
DG/CA3
CA3/CA1
CA1/SUB
D
Coherence Averaged Across Subregion Pairs For Each Behavioral State
S
R
E
A

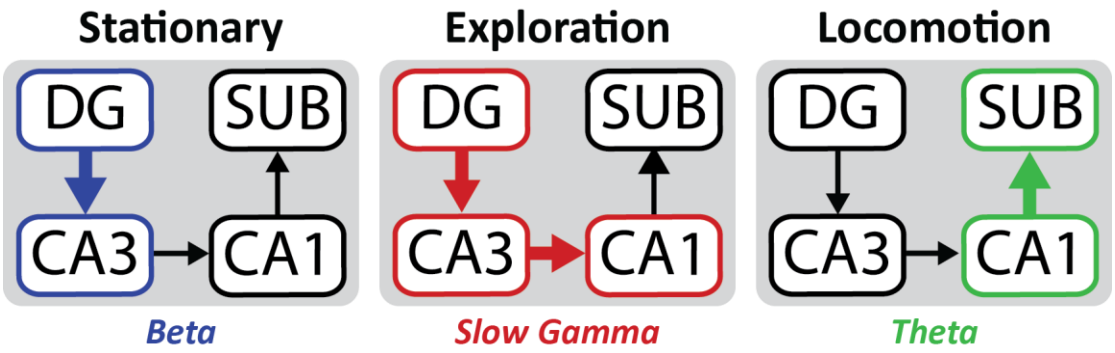


Figure 3.8. Summary illustration for network state differences by behavioral state. Analyses revealed markedly different oscillatory network states for each of the behavioral states assessed. Stationary epochs were best characterized by strong beta (blue) in DG and CA3. Exploration bouts were best characterized by strong slow gamma (red) in DG and CA3, but also between CA3 and CA1. Locomotive states (i.e., Run and Approach) were best characterized by strong CA1/SUB theta (green).

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Chapter 4

Intrahippocampal Synchrony and Memory for Objects in Spatial Context

Abstract

The hippocampus is a region of the brain believed to be important for remembering items with their spatial contexts. The hippocampus is composed of multiple subregions—dentate gyrus, CA3, CA1, and subiculum—and how in particular subregional activity differentially relates to remembering items in spatial context remains unclear. The present experiment utilized a novel approach of recording in vivo electrophysiological activity simultaneously from dentate gyrus, CA3, CA1, and subiculum as rats performed an object recognition memory task designed to examine the influence of spatial context on recognition memory. Local field potential analyses revealed that oscillatory interactions in the slow and fast gamma ranges differentially related to the encoding of object memories based on the degree to which rats also remembered items' spatial locations. Hippocampal slow gamma power during initial object exploration was greater when rats subsequently demonstrated memory for items plus their spatial contexts, relative to memory for items only, but higher for both subsequent memory conditions relative to when rats showed poor memory for the objects. The observed patterns of oscillatory activity differed by subregion and subregional interaction, such that the effects were observed numerically in slow gamma power in dentate gyrus and CA3, fast gamma power in CA1, and slow gamma coherence between CA1 and subiculum. The degree of slow gamma at test in dentate gyrus and CA3 also related to the degree to which object presentations included a novel object or spatial component. The findings here represent a significant advancement for the field by demonstrating that hippocampal activity differentially relates to remembering item encounters based on the degree of spatial information also remembered, and that this effect differs by hippocampal subregion.

The hippocampus is known to play an important role in remembering items in their spatial contexts (Eichenbaum et al., 1999). Lesions to the hippocampus in rats (Gilbert & Kesner, 2002; Langston & Wood, 2010), monkeys (Bachevalier & Nemanic, 2008; Parkinson et al., 1988), and humans (Olson et al., 2006) lead to impairments in learning the relationship between objects and their spatial locations. A remaining question is how activity within the particular hippocampal subregions—dentate gyrus, CA3, CA1, and subiculum—may differentially relate to this process.

Anatomical data suggest that hippocampal subregions differ in their contributions to remembering objects with their spatial locations (Kesner & Rolls, 2015). Nonspatial and spatial information arrive at the hippocampus via two partially segregated anatomical pathways, carried from the lateral and medial entorhinal cortices respectively (Manns & Eichenbaum, 2006; Witter et al., 2000), and their patterns of overlap differ across the hippocampal subregions. In dentate gyrus and CA3, lateral and medial entorhinal projections terminate on the same groups of cells. In CA1 and subiculum, lateral and medial entorhinal projections terminate on anatomically segregated areas (Witter et al., 2000). It may be the case that these differences in connectivity lead to dentate gyrus and CA3 being better adapted than CA1 and subiculum for combining information about items and spatial locations in the service of memory, whereas CA1 and subiculum may be better able to maintain separate representations for each component.

Lesion studies also support a functional segregation of activity amongst the hippocampal subregions in relation to remembering objects bound to particular locations. For example, Gilbert and Kesner (2003) reported that CA3 lesions, but not CA1 lesions, produced impairments in learning object-place associations. Lee & Kesner (2002, 2003) reported that disrupting activity in CA3 led to rats being impaired on a spatial delayed non-match to sample task, but that CA1 lesions had no effect. Lee et al. (2005) reported that dentate gyrus and CA3 lesions, but not CA1 lesions, impaired rats' abilities to recognize that objects had moved to new locations.

One technique particularly well suited to address this question of subregional differences during the processing of object-in-location memories is in vivo electrophysiology. Previously, Trimper et al. (2014) recorded in vivo electrophysiological activity simultaneously from CA3 and CA1 in rats while the animals performed a novel object recognition memory task. The authors reported that oscillatory synchrony, or coherence, in the slow gamma range (30 -55 Hz) between CA3 and CA1 increased markedly while rats were exploring novel objects. The degree of coherence during novel object exploration was greater when rats subsequently demonstrated good memory for the objects, relative to when rats subsequently demonstrated poor memory. Unanswered questions include how activity in dentate gyrus and subiculum may also relate to this process, and, furthermore, how the oscillatory differences observed related to memory for objects versus memory for objects with their spatial locations.

The present experiment sought to address this question of how hippocampal subregional activity differentially relates to memory for objects and spatial context. Local field potentials in dentate gyrus, CA3, CA1, and subiculum were recorded simultaneously as rats performed an object recognition memory task. Oscillatory analyses revealed that hippocampal slow gamma power, especially in dentate gyrus and CA3, was greatest during novel object exploration when rats subsequently remembered objects with their spatial locations (Object-in-Location), relative to when rats did not show memory for objects' spatial locations (Object Only). Both subsequent memory conditions were associated with greater levels of slow gamma power than when rats did not demonstrate memory for the objects (Poor). Interestingly, a similar pattern was present for fast gamma (55-90 Hz) power in CA1, and slow gamma coherence between CA1 and subiculum. At test, slow gamma power in dentate gyrus and CA3, but not CA1 and subiculum, was found to relate to the amount of object and spatial context information repeated from study, such that more novelty in object identity or spatial location was associated with a greater degree of slow gamma power. The results here indicate hippocampal activity differs by subregion and oscillatory

frequency range based on the degree to which rats remember objects with their spatial contexts.

Method

Subjects

Subjects were six male Long-Evans rats, cared for as described in General Methods.

Behavioral Task

Figure 4.1, Panel A, shows an illustrated schematic of the behavioral task. Rats ran clockwise laps around an elevated circular track for a reward of a few chocolate sprinkles at the completion of each lap. Each trial consisted of a single lap around the track with no objects present (blank lap) followed by three laps with objects present in the 10 and 2 o'clock positions, relative to the inner stem of the track at 6 o'clock. On the first object lap (lap 1), rats encountered two novel objects. On Lap 2, rats encountered duplicates of the same objects from lap 1 in the same positions. Duplicates were employed to avoid scent marking. On lap 3, rats encountered one of two new object configurations. Either one object was replaced with a duplicate in the same location (Repeat) while the other was replaced with a novel object (Novel), or the two objects were repeated again, but in swapped locations (Switch). Trials alternated in a 2:1 fashion, such that there were two Switch trials for every one Repeat/Novel trial. The locations for the Repeat and Novel objects were counter-balanced across trials. Rats performed up to 72 trials across up to 5 days of testing, with up to 24 trials on a single day. The number of trials and test sessions was limited by the quality of recordings and rat performance (i.e., willingness to explore objects at study on lap 1).

The task is based on rats' preference for novelty. Rats explore novel objects to a greater degree than repeated objects, and thus the reduction in exploration across successive encounters with a particular object can be interpreted as evidence of memory for that object. Object recognition memory tasks based on spontaneous preference for novelty have been widely used (Bass et al., 2012; Bass et al., 2014; Clark et al., 2000; Ennaceur & Delacour, 1988; Galloway et

al., 2014; Manns et al., 2015; Trimper et al., 2014).

Analyses

One question was whether a neural signature during initial object exploration might be revealed that relates to whether rats formed an associative memory for objects plus their locations (Object-in-Location Memory), rats remembered the object's identity without a simultaneous location memory (Object-Only Memory), or rats failed to show evidence of encoding a strong memory for the encounter (Poor Memory). To that end, neural data during the initial object encounter on lap 1 of Switch trials was sorted based on the pattern of exploration observed across the following two laps.

If rats reduced their exploration duration from lap 1 to lap 2 by *less than* 50%, the lap 1 exploration event was categorized as Poor memory. If rats reduced their exploration of an object from lap 1 to lap 2 by *at least* 50%, then explored that object on lap 3 to a *greater* extent than that rat's average exploration time for Repeat objects, the lap 1 exploration event was categorized as an Object-in-Location memory. If rats reduced their exploration of an object from lap 1 to lap 2 by at least 50%, but then explored the object on lap 3 *less* than their average exploration time for Repeat objects, that lap 1 exploration event was categorized as an Object-Only memory. Lap 3 exploration in Switch conditions was compared to the average exploration duration for Repeat objects based on the idea that the lap 3 Repeat exploration duration would, on average, represent a combination of Object-in-Location memories and Object-Only memories. Thus, a lap 3 exploration time in the Switch condition greater than the average lap 3 Repeat exploration duration might indicate Object-in-Location memories while a lower exploration duration might indicate Object-Only memories. Analyses then asked how exploration times differed by condition (i.e., Object-in-Location vs. Object-Only vs. Poor memory), and how neural data during the initial 1.5s of exploration for those objects on lap 1 differed by condition.

Results

Average Exploration Times by Lap and Trial Type

Figure 4.1, Panel B, shows the exploration time results for the behavioral task. Rats explored novel objects on lap 1 (2.478 +/- 0.580 s) for a significantly greater duration than the repeated objects on lap 2 (0.725 +/- 0.141 s) [$t(5) = 4.498$, $p = 0.006$], thus evidencing memory for the objects on average. Exploration times on lap 3 varied by object condition [$F(2,10) = 10.93$, $p = 0.003$], such that the average exploration times for Novel items (1.566 +/- 0.482 s) were greater than the average exploration times for Switch items (0.8361 +/- 0.192 s) [$t(5) = 3.196$, $p = 0.024$], which were greater than the average exploration times for Repeat objects (0.475 +/- 0.074 s) [$t(5) = 3.446$, $p = 0.018$]. To ensure that initial object encounters were not impacted by lap 3 manipulations, we verified that lap 1 exploration times did not differ when split by lap 3 conditions [$F(2,10) = 1.780$, $p = 0.218$] , nor did lap 2 exploration times [$F(2,10) = 2.597$, $p = 0.124$].

Neural Results for Lap 3 Object Exploration Split by Trial Type

Figure 4.2, Panel A, shows spectral power for dentate gyrus, CA3, CA1, and subiculum during the initial 1 second of object exploration on lap 3 for Repeat, Switch, and Novel objects. A significant interaction is present in the slow gamma range (30.76 – 49.8 Hz) driven by significant differences across conditions in dentate gyrus (24.9 – 58.59 Hz) and CA3 (24.9 – 51.27 Hz) combined with the lack of an effect in CA1 and subiculum. For both dentate gyrus and CA3, slow gamma is highest for Novel, next highest for Switch, and lowest for Repeat objects. Panel B shows hippocampal power for dentate gyrus, CA3, CA1, and subiculum averaged across conditions. A main effect is present across subregions for all frequency bins considered (5 – 90 Hz), indicating hippocampal power differs strongly by subregion at all frequencies considered. Panel C shows hippocampal power averaged across subregions for each condition. A main effect is present across conditions from 24.9-58.59 Hz, reflected in average hippocampal slow gamma power being strongest for Novel objects, second strongest for Switch objects, and lowest for

Repeat objects.

Neuronal firing rate did not differ across conditions for any subregion (all p values > 0.397). For this comparison of neural activity during lap 3 object explorations, and for all subsequent comparisons within this chapter, we were unable to assess the possibility of spike-phase differences across conditions due to an insufficient number of action potentials.

Exploration Times by Subsequent Memory

Figure 4.3 shows exploration times by lap (Panel A) and the percent change in exploration times across laps (Panels B and C) for the objects categorized as Object-in-Location, Object-Only, and Poor memory objects. Rats significantly reduced their exploration times from lap 1 to lap 2 for Object-in-Location objects [Lap1: 9.448 +/- 1.711 s; Lap 2: 1.599 +/- 0.381 s; $t(5) = 5.306$, $p = 0.003$] and Object-only Objects [Lap 1: 6.110 +/- 0.752 s; Lap 2: 0.941 +/- 0.175 s; $t(5) = 8.547$, $p < 0.001$], but not for Poor memory objects [Lap 1: 4.617 +/- 1.147 s; Lap 2: 4.550 + /- 1.078 s; $t(5) = 0.296$, $p = 0.780$] (Figure 4.3, A). This finding validates that the 50% exploration time reduction criteria used to separate Object-in-Location and Object-Only memory objects from Poor memory objects effectively sorted events as desired.

Rats significantly increased their exploration time durations from lap 2 to lap 3 for Object-in-Location objects [Lap 2: 1.599 +/- 0.381 s; Lap 3: 5.405 +/- 0.9207 s; $t(5) = -4.819$, $p = 0.005$], but not for Object-Only objects [Lap 2: 0.941 +/- 0.175 s; Lap 3: 0.995 +/0 .362 s; $t(5) = -0.256$, $p = 0.807$]. In line with this point, lap 3 exploration times for Object-in-Location (5.405 +/- 0.9207 s) and Object-Only (0.995 +/0 .362 s) memory objects differed significantly from one another [$t(5) = 6.693$, $p < 0.001$], as did the percent change from lap 2 to lap 3 (Object-in-Location: 341.1 +/- 99.58%; Object-Only: -5.414 +/- 17.8%; $t(5) = -3.461$, $p = 0.018$]. Lap 3 exploration times for Poor memory objects were not considered. These findings validates that lap 3 exploration time criteria employed to segregate Object-in-Location from Object-Only memory objects sorted categorized events as desired at the behavioral level.

Figure 4.3, Panel C, shows that the degree of reductions in exploration time from lap 1 to lap 2, as measured in percent of change from lap 1, differed significantly across conditions [$F(2,10) = 86.06$; $p < 0.001$]. Follow-up contrasts indicated that the reduction for Object-in-Location memories (-82.98 +/- 3.796%) did not differ significantly from that associated with Object-Only memories (84.91 +/- 1.645%) [$t(5) = -0.620$, $p = 0.563$], suggesting initial memory strength was similar across Object-in-Location and Object-Only memory objects. The average reduction for both Object-in-Location and Object-Only memory objects considered together (83.942 +/- 2.476%) was significantly greater than the percent change observed for Poor memory objects (0.790 +/- 7.072%) [$t(5) = 9.706$, $p < 0.001$], suggesting memory strength for Object-in-Location and Object-Only memory objects was significantly stronger than that associated with Poor memory objects. Notable is that lap 1 exploration times also differed significantly across subsequent memory conditions [Object-in-Location: 9.448 +/- 1.711 s; Object-Only: 6.11 +/- 0.753 s; Poor: 4.617 +/- 1.147 s; $F(2,10) = 9.413$, $p = 0.005$], a point that will be addressed later in the chapter.

Neural Results by Subsequent Memory

Figure 4.4 shows spectral power for dentate gyrus, CA3, CA1, and subiculum during the initial 1.5s of object exploration on lap 1 for subsequent Object-in-Location, Object-Only, and Poor memory objects. A main effect is present across conditions, primarily in the slow gamma range (27.83-39.55 Hz), reflected in highest hippocampal power averaged across subregions for subsequent Object-in-Location memory objects, second highest for subsequent Object-Only memory objects, and lowest slow gamma power for subsequent Poor memory objects (Figure 4.4, C). The same pattern is visible numerically, though nonsignificantly, within subregion for dentate gyrus and CA3. In CA1, slow gamma is largely equal for subsequent Object-in-Location and Object-Only memory objects but lowest for Poor memory objects. Spectral power is similar in the slow gamma range for all three conditions in subiculum. A significant condition by subregion

interaction is present in the fast gamma range from 67.38 to 74.71 Hz. In CA1, fast gamma power is strongest for subsequent Object-in-Location memory objects, second strongest for Object-Only memory objects, and weakest for subsequent Poor memory objects. An effect in the fast gamma range is numerically absent in each of the other dentate gyrus, CA3, and subiculum. Figure 4.4, Panel B, also shows a main effect of subregion present at all frequency bins under consideration, from 5 – 90 Hz, again emphasizing the substantial differences in spectral power present across subregions.

Figure 4.5, Panel A, shows coherence between dentate gyrus and CA3, between CA3 and CA1, and between CA1 and subiculum during the initial 1.5 s of object exploration on lap 1 for subsequent Object-in-Location, Object-Only, and Poor memory objects. A within-subregion pair effect is present in slow gamma coherence between CA1 and subiculum from 29.3 to 41.02 Hz, reflected in the higher slow gamma coherence for subsequent Object-in-Location memory relative to the largely similar slow gamma coherence for subsequent Poor and Object-Only memory objects. Similar to spectral power, a main effect is present across subregion pairs, as presented in Panel B, for all frequency bins under consideration (5 – 90 Hz), though no main effect is present across conditions (Figure 4.5, Panel C).

We were unable to assess neuronal firing rate across subsequent memory conditions due to an insufficient number of action potentials across conditions during the time window of interest.

Neural Activity During Lap 1 Object Encounters by Exploration Duration

Given that lap 1 exploration times differed across subsequent memory conditions, a possibility remained that the observed subsequent memory neural differences might be solely the product of some unaccounted for factor such as initial interest or attention. Thus, it was important to ask how neural activity during the first few moments of exploration might differ just based on exploration duration, rather than what type of memory is subsequently expressed. To that end, we

divided lap 1 exploration times into Low Exploration (1 – 2.5 s), Medium Exploration (2.5 – 5.0s), and High Exploration (5.0 – 7.5 s), and asked how the neural activity during the first 1 second of exploration differed across conditions. Here, we used a 1 second time window, rather than the 1.5 second window employed before, due to a 1.5 second window leaving too few trials in the low exploration condition.

Figure 4.6 shows exploration times on lap 1 and lap 2 for High, Medium, and Low Exploration object encounters. As expected given that we divided conditions by initial exploration time, lap 1 exploration times differed significantly across conditions [$F(2,10) = 49.75$, $p < 0.001$], with High Exploration objects being explored for the longest duration (6.739 +/- 0.601 s), Medium Exploration objects having the second longest durations (4.305 +/- 0.463 s), and Low Exploration objects being associated with the lowest exploration durations (2.275 +/- 0.266 s). Lap 2 exploration times did not differ across conditions (High: 1.698 +/- 0.373 s; Medium: 1.677 +/- 0.702 s; Low: 1.366 +/- 0.219 s; $F(2,10) = 4.713$, $p = 0.036$], but percent reduction from lap 1 to lap 2 *did* differ significantly (High: 73.72 +/- 5.907%; Medium: 62.08 +/- 12.96%; Low: 30.92 +/- 8.059%) [$F(2,10) = 4.713$, $p = 0.036$], with percent reduction in exploration scaling with the degree of initial exploration.

Figure 4.7 shows spectral power for dentate gyrus, CA3, CA1, and subiculum during the first 1 second of exploration for Low, Medium, and High Exploration objects. No significant differences are present within any subregion. A significant subregion by condition interaction is present, however, from 65.92 – 76.17 Hz, reflected by spectral power being strongest in this range in dentate gyrus and CA3 for Medium exploration events, strongest activity within this range in CA1 for High exploration events, and strongest activity within this range in subiculum for Low exploration events. As shown in Panel B, a significant main effect is present across subregions for all frequency bins considered (5-90 Hz), though no main effect of conditions is present, as shown in Panel C.

Figure 4.8 shows spectral coherence between dentate gyrus and CA3, between CA3 and CA1, and between CA1 and subiculum during the first 1 second of exploration of High, Medium, and Low Exploration objects (Panel A). No significant differences are present across conditions within any subregion pair, nor any main effects of condition when coherence is averaged across subregion pairs (Panel C). Thus, analyses of neural activity by exploration duration cannot account for the significant differences observed in analyses of Object-in-Location versus Object-Only versus Poor subsequent memory.

Discussion

The hippocampus is important for remembering items in their spatial locations. The present experiment sought to understand if and how the hippocampal subregions—dentate gyrus, CA3, CA1, and subiculum—might be differentially involved in this memory process by recording simultaneously from all four of these subregions as rats performed an object recognition memory task. Neural analyses revealed several differences across subregions. First, slow gamma power in dentate gyrus and CA3 was strongest when rats encountered a novel object, second strongest when rats encountered a repeated object in a novel location, and lowest when rats encountered a repeated object in a repeated location. Second, hippocampal slow gamma during novel object exploration was greatest when rats subsequently demonstrated memory for objects and their locations, relative to memory for the objects only. Moreover, both of these subsequent memory conditions were associated with a greater degree of slow gamma than when rats subsequently demonstrated poor memory for the objects.

Slow Gamma in Dentate Gyrus and CA3 at Test Reflects the Degree of Novelty

Slow gamma power in dentate gyrus and CA3 was strongest when rats encountered novel objects, second strongest when rats encountered repeated objects in new locations, and lowest when rats encountered repeated objects in repeated locations. One account for this pattern of results is that slow gamma in dentate gyrus and CA3 reflects the encoding of novel associations

between object identities and spatial locations. When objects are entirely novel, the strongest degree of activity is observed, possibly reflecting the encoding of a new memory for the new object and its location. When objects were repeated, but the spatial location is novel, the second strongest degree of activity is observed. This activity may reflect the updating of memory for that object being presented in a new location. When objects are repeated in repeated locations, the degree of slow gamma activity is lowest, as no novel information is presented and therefore no new information must be encoded. This suggestion is consistent with the results presented by Trimper et al. (2014) who suggested that the elevated slow gamma coherence between CA3 and CA1 during novel object exploration related to memory encoding, but advances the findings by demonstrating that activity at encoding may also vary with the content of information being encoded.

Hippocampal Gamma During Encoding Relates to Subsequent Memory Strength and Content

Hippocampal gamma during novel object exploration was greatest when rats subsequently demonstrated a memory for the object and its location, second greatest when rats subsequently demonstrated a memory for the object only, and lowest when rats did not demonstrate memory for the object encounter. The pattern of activity differed across subregions, such that the differences were most prominent in slow gamma power in dentate gyrus and CA3, fast gamma power in CA1, and slow gamma coherence between CA1 and subiculum. In line with the suggestions offered for the aforementioned slow gamma differences observed on lap 3, the degree of slow gamma activity during novel object exploration may relate to the encoding of associative memories for objects and their locations, as well as to the strength of the memory being encoded.

The finding that gamma activity was lowest when rats subsequently did not show evidence of memory supports the idea that hippocampal slow gamma relates to the strength of

initial memory encoding. This result is in line with previous reports that hippocampal gamma synchrony during encoding is higher for subsequent good memories relative to subsequent poor memories in monkeys (Jutras et al., 2010) and rats (Trimper et al., 2009). However, behavioral evidence of memory strength, as assessed by percent reduction from lap 1 to lap 2, did *not* differ between subsequent Object-in-Location and Object-Only memories, yet gamma activity *did* differ. This result suggests that, in addition to memory strength, hippocampal gamma also relates to the encoding of associations between objects and their locations. This result is in line with the interpretation offered previously for the gamma differences present at test. Moreover, the finding of elevated activity associated with subsequent object-only memories relative to poor memories suggests hippocampal involvement when rats form memories for items even void of a strong contextual component.

Gamma Oscillations Facilitate Associative Memory Encoding

Both sets of analyses suggest that hippocampal gamma relates to the encoding of memories for objects in their spatial locations. Interestingly, the pattern of results differed by subregion and frequency range, such that slow gamma power differed in dentate gyrus and CA3, fast gamma power differed in CA1, and slow gamma coherence differed between CA1 and subiculum. Unanswered questions are why do gamma oscillations in particular relate to associative memory encoding and why does the pattern of activity differ across subregions.

Gamma oscillations likely offer an important computational advantage to the coordination of hippocampal neuronal activity in relation to associative memory encoding. Gamma oscillations are thought to be important for the formation of functional cell ensembles (Buzsaki & Draguhn, 2004), or the coordination of transiently active neurons whose activity together represents a chunk of related information. In the visual system, gamma oscillations have been suggested to be important for binding multiple representations across active cell groups (Gray & Singer, 1989). Thus, gamma oscillations within the hippocampus may facilitate the

coordination of neuronal activity in the service of rapidly binding neural representations for object identity and object location in memory.

The finding that slow gamma activity in dentate gyrus and CA3 is elevated in relation to associative memory encoding is in line with current hypotheses in the field regarding these subregions' contributions to declarative memory. The dentate gyrus to CA3 network has been implicated in playing an important role in the orthogonalization of incoming sensory information for the purpose of rapidly creating non-overlapping memory representations within the hippocampus (Kesner & Rolls, 2015). Slow gamma oscillations, which can arise from CA3 (Bragin et al., 1995; Schomburg et al., 2014), likely contribute to the temporal coordination of neuronal activity in service of these functions.

Fast gamma in CA1 is thought to arise from entorhinal inputs to the hippocampus (Bragin et al., 1995; Schomburg et al., 2014). As such, it has been hypothesized that fast gamma oscillations in CA1 may relate to the selective routing of sensory information from entorhinal cortex to CA1 in the service of memory encoding (Colgin et al., 2009; Colgin & Moser, 2010). The finding that fast gamma in CA1 is greatest during events linked to associative memory encoding is in line with this hypothesis, as both object location and object identity information are being received and processed by the hippocampus. When less information is being remembered, such as in the Poor memory or Object-Only memory conditions, less sensory information may be conveyed, and therefore, less fast gamma is present.

Slow gamma coherence between CA1 and subiculum is elevated when rats are encoding Object-in-Location memories, relative to when rats form Object-Only or Poor memories. The finding that slow gamma coherence between CA1 and subiculum is only elevated for memories containing a spatial context component perhaps reflects subiculum's importance in processing idiothetic spatial information (Potvin et al., 2007). Subiculum possesses neurons which code for rats' head direction and spatial location (Muller et al., 1991). Likewise, damaging subiculum is

particularly disruptive to spatial navigation when rats must rely on internal cues (Potvin et al., 2007). It may thus be the case that this subregion is particularly engaged when rats form memories involving a spatial component because subicular representations of space in relation to the animal itself likely form an integral component of learning about the rats' environment.

Evidence suggests that subiculum is capable of independently generating both fast and slow gamma rhythms (Jackson et al., 2012). Subiculum is also the primary output region of the hippocampus, with dense reciprocal connections with the entorhinal cortex (Witter, 2006). Therefore, enhanced subicular gamma activity in relation to associative memory encoding could represent a feedback or calibration loop between entorhinal cortex and hippocampus that could function to align hippocampal representations with cortical representations. Such a function could facilitate subsequent cortical reactivation in the service of memory retrieval. Future work recording simultaneously from subiculum and entorhinal cortex will be required to assess such a possibility.

In Trimper et al. (2014), we observed that slow gamma coherence between CA3 and CA1 in rats increased markedly as rats explored novel objects positioned around an elevated circular track. The degree of coherence related to the strength of the subsequently demonstrated memory. In an attempt to coalesce the observed results with a hypothesis in the field regarding slow gamma's possible role in memory retrieval (Colgin et al., 2009), we suggested the possibility that the observed slow gamma coherence between CA3 and CA1 might be a product of the rats retrieving a memory for an object previously bound to that location, rather than encoding a new memory for the novel object. The present results speak against this explanation of slow gamma categorically relating to retrieval, as the degree of hippocampal slow gamma power and coherence between CA1 and subiculum was *lowest* during encounters with repeated objects in repeated locations, the situation most readily related to a possible retrieval event. Likewise, slow gamma power in dentate gyrus and CA3 was *highest* during lap 3 novel object encounters, the

situation is most readily related to encoding.

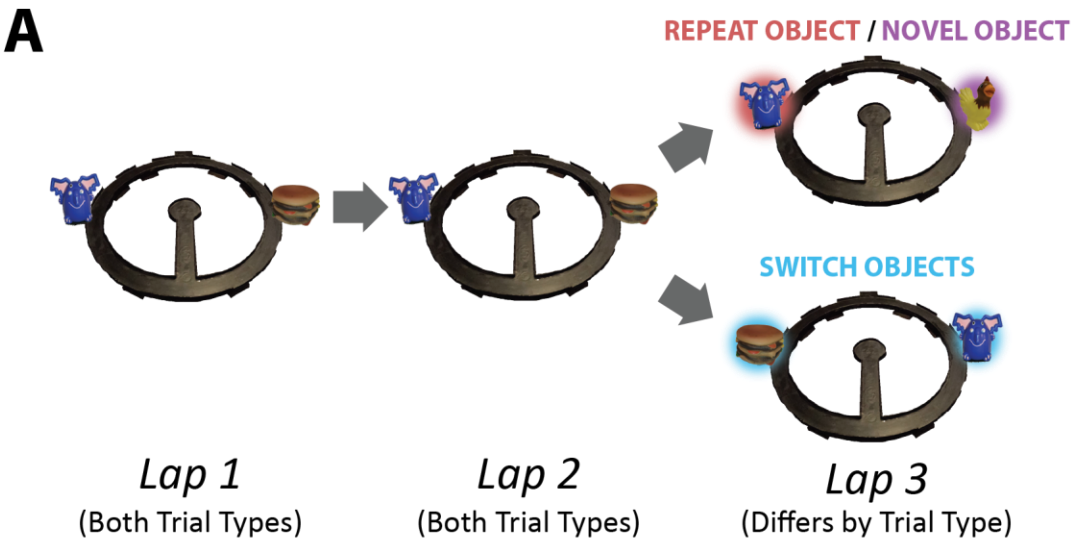
Hippocampal gamma power was elevated during novel object exploration when rats subsequently demonstrated Object-Only memory relative to when rats subsequently demonstrated Poor memory. This finding suggests that the hippocampus may be engaged during memory encoding even when the memory does not include a strong contextual component. Whether or not the hippocampus is involved in remembering nonassociative object recognition memories has been considerable empirical work (Davachi et al., 2003; Manns et al., 2003; Jackson & Schacter, 2004; Sauvage et al., 2008; Wais et al., 2006) and a rich history of debate (Aggleton & Brown, 1999; Wixted et al., 2010; Yonelinas et al., 2010). While the findings of current experiment cannot conclusively support either hypothesis, one interpretation for the observed result is that the elevated gamma power for Object-Only memory encoding reflects engagement of the hippocampal circuitry that fails to surpass some undefined threshold for associative memory formation. Support for this idea comes from the discord between the pattern of results observed for power and coherence. Whereas power is elevated for Object-Only memories relative to Poor memories, coherence between CA1 and subiculum, between dentate gyrus and CA3, and averaged across subregion pairs is largely overlapping for Object-Only and Poor memory formation. Perhaps power in this situation reflects the subthreshold engagement of hippocampal circuitry, and only when activity surpasses that threshold are interactions between subregions heightened in the service of associative memory formation. Thus, whether or not the hippocampus is *necessary* for nonassociative recognition memory cannot be addressed here, but the data suggests that the hippocampus is at least engaged to some degree in processing information related to the formation of nonassociative recognition memories.

Conclusion

The findings from the present study support the idea that gamma oscillations in the hippocampus can facilitate the encoding of memories for objects and their spatial locations. These

results reflect a significant advancement for the field of hippocampal oscillatory analyses by adding to it a thorough characterization of how the oscillatory profile during object exploration, a behavioral state distinct from the cessation of locomotion (Chapter 1), is further augmented by memory, and moreover, how the oscillatory pattern differs across four of the primary hippocampal subregions. Future work will additionally ask how action potentials are coordinated within the hippocampal network during associative versus nonassociative memory formation, how optogenetic disruption of the hippocampal circuitry augments this activity, and how activity is coordinated between the hippocampus and interconnected cortical regions.

Figure 4.1. Task schematic and exploration times. Panel A provides an illustrative schematic of the object-in-location task. On lap 1, rats encountered two novel objects. On lap 2, rats encountered duplicates of those objects in the same location. On lap 3, rats encountered one of two trial types. In Repeat Object/Novel Object trials, one object was replaced with a duplicate in the same location (Repeat Object, red) whereas the other was replaced with a novel object (Novel Object, purple). The positions of Novel Objects and Repeat Objects were counter balanced across trials. On Switch trials, objects were repeated from lap 2 but switched locations (Switch Objects, blue). There were two Switch Objects trials for every Repeat Object/Novel Object trial. Panel B shows that rats significantly reduced their exploration times from lap 1 to lap 2 ($p = 0.006$), indicating memory for the objects presented. On lap 3, rats explored Novel objects significantly more than Switch objects ($p = 0.024$), and Switch objects significantly more than Repeat objects ($p = 0.018$), indicating that rats responded to the novelty of locations in the Switch location and thus that rats, on average, possessed memory for the objects' prior locations.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SWITCH OBJECTS
Lap 1
(Both Trial Types)
Lap 2
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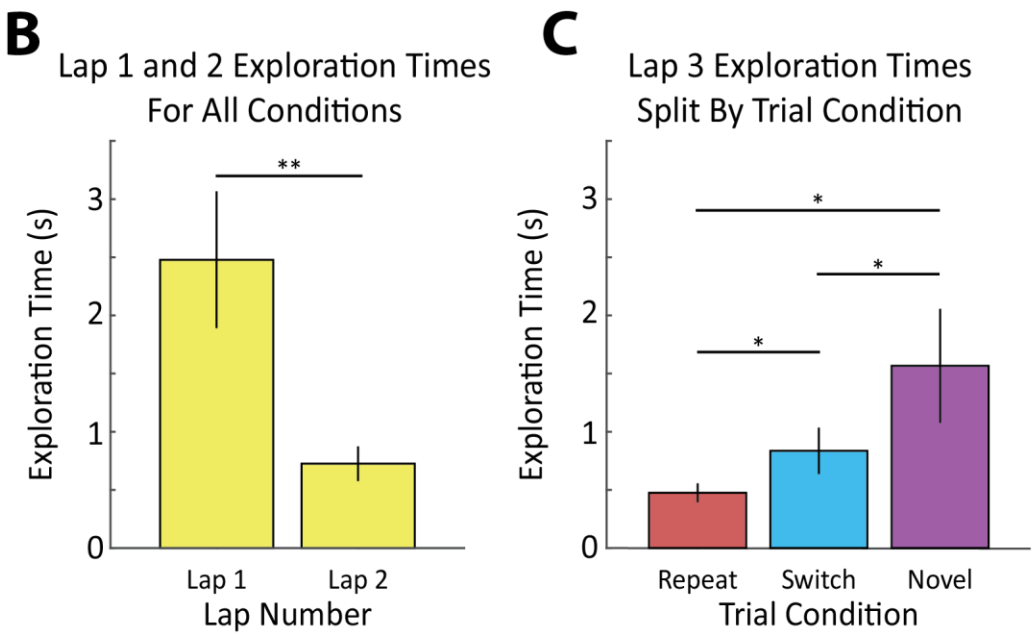
Lap 3
(Differs by Trial Type)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Lap 1 and 2 Exploration Times
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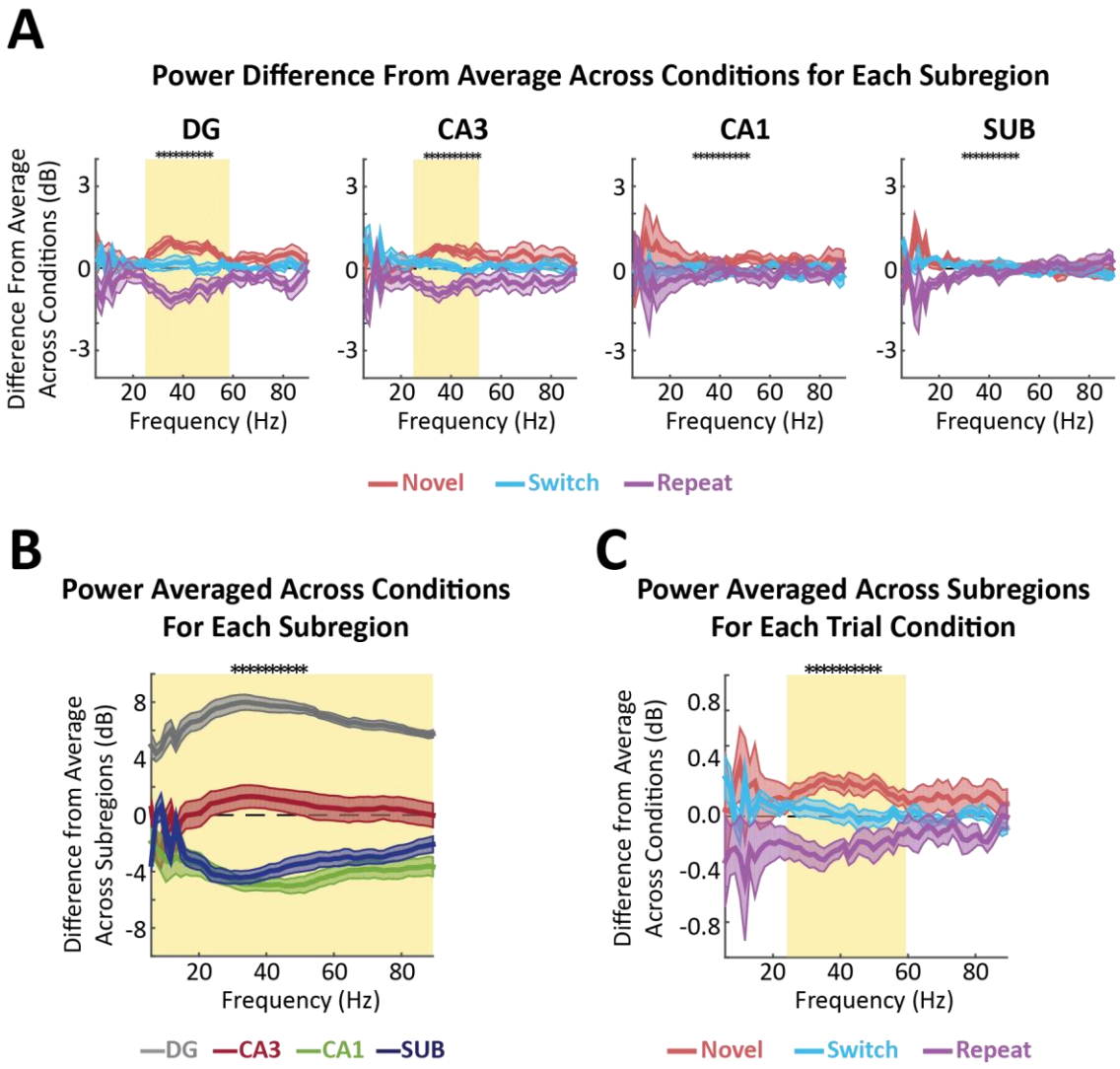


Figure 4.2. Spectral power differs during exploration of lap 3 objects split by object condition. Panel A shows spectral power for each subregion and each object condition, plotted as the difference from the average across conditions. Dentate gyrus (DG) and CA3 power in the slow gamma range is highest during Novel object exploration, next highest during exploration of Switch objects, and lowest during exploration of Repeat objects. As in previous figures, yellow rectangles mark significant differences across conditions, while asterisks mark a significant subregion by condition interaction. Panel B shows power averaged across conditions for each subregion. Panel C shows power averaged across subregions for each trial condition. A similar pattern of results to that observed within dentate gyrus and CA3 is visible here.

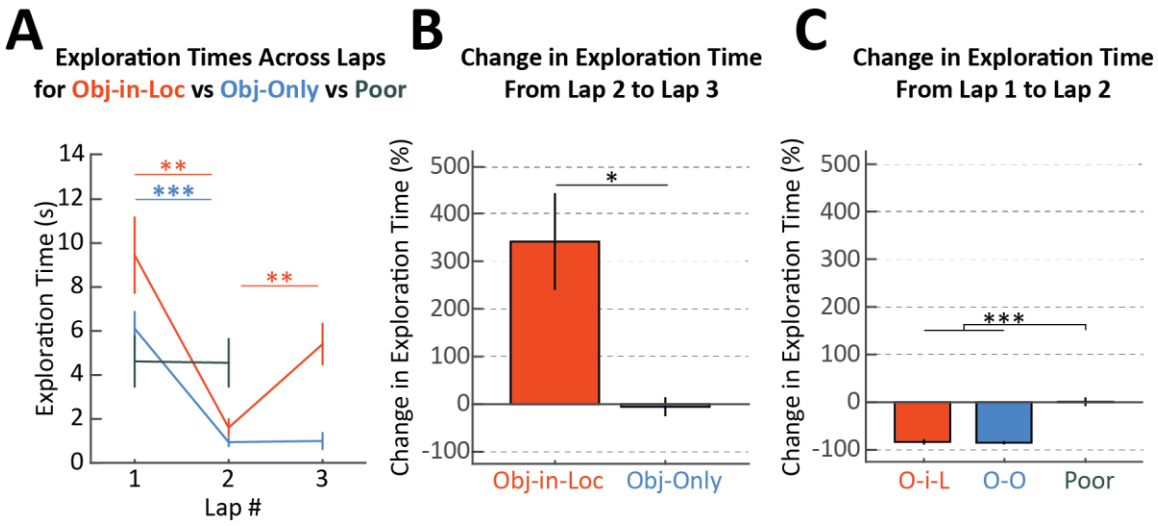


Figure 4.3. Exploration times for Object-in-Location versus Object-Only versus Poor memory objects. Panel A shows exploration times by lap for each Object-in-Location (orange), Object-Only (blue), and Poor (gray) memory objects. For Object-in-Location and Object-Only memory objects, rats significantly reduced their exploration times from lap 1 to lap 2. Rats significantly increased exploration times from lap 2 to lap 3 for Object-in-Location memory objects, but not Object-Only memory objects. Exploration times for Poor memory objects were not considered on lap 3. Panel B shows percent change in exploration time from lap 2 to lap 3 as a function of lap 2 exploration times for Object-in-Location (orange) and Object-Only (blue) memory objects. Percent change from lap 2 to lap 3 different significantly between the two conditions. Panel C shows percent change in exploration time from lap 1 to lap 2 as percent of lap 1 exploration time. Rats reduced their exploration times for Object-in-Location and Object-Only memory objects to a greater extent than for Poor memory objects.

Figure 4.4. Spectral power differs during lap 1 object exploration for subsequent Object-in-Location versus Object-Only versus Poor memory objects. Panel A shows power during the initial 1.5s of object exploration on lap 1, split by whether rats subsequently demonstrated Object-in-Location (orange), Object-Only (blue), or Poor (gray) memory for those objects. Power is plotted as the difference from average across conditions. A significant interaction is present in the fast gamma range from 67 to 75 Hz. Panel B shows spectral power averaged across conditions for each subregion. Substantial and significant differences are present at all frequency bins under consideration. Panel C shows average hippocampal power plotted as difference from average across conditions. A significant main effect of condition is present in the slow gamma range from 28 to 40 Hz, reflected in highest slow gamma power for Object-in-Location memory objects, second highest slow gamma power for Object-Only memory objects, and lowest slow gamma power for Poor memory objects.

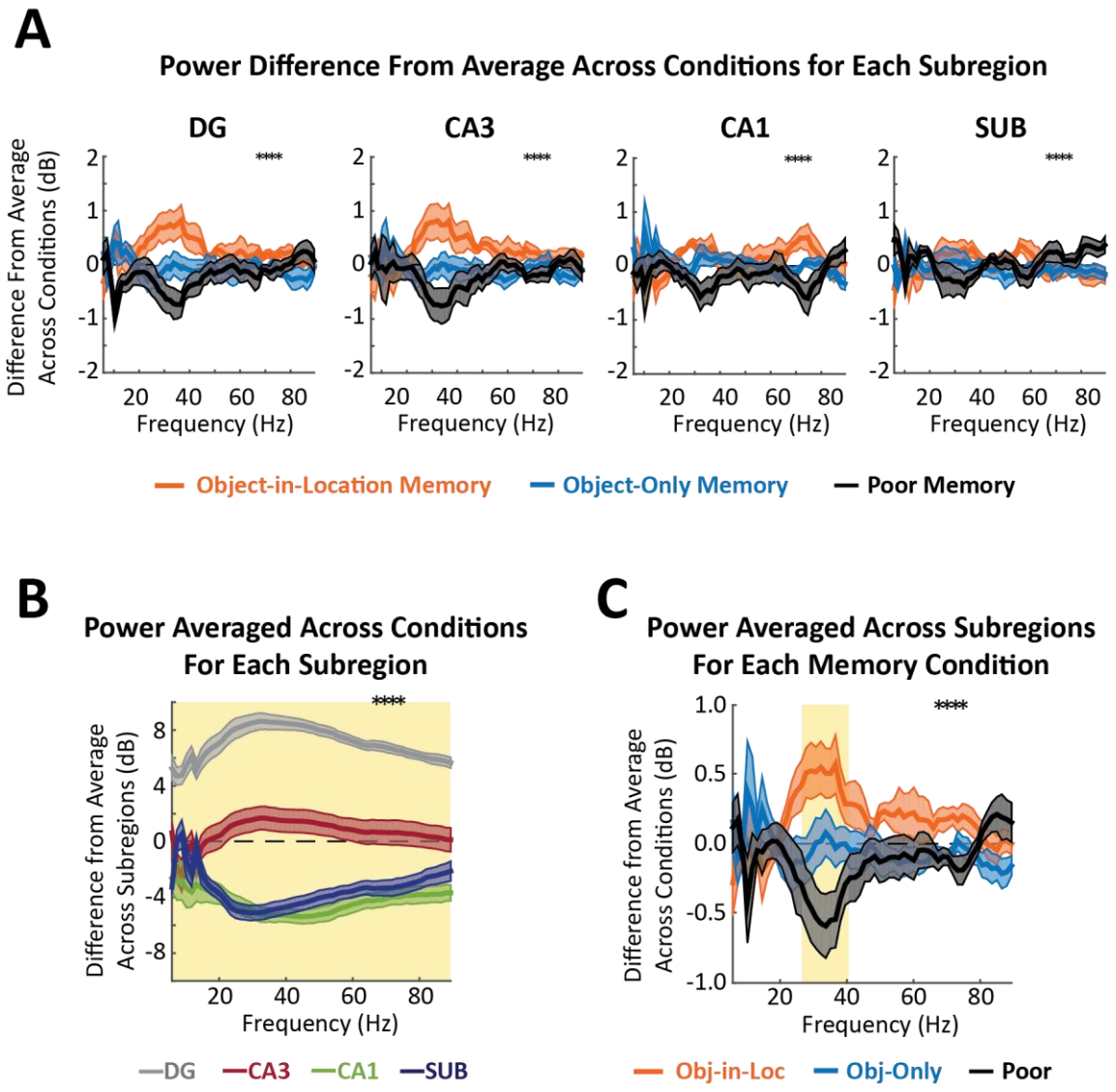
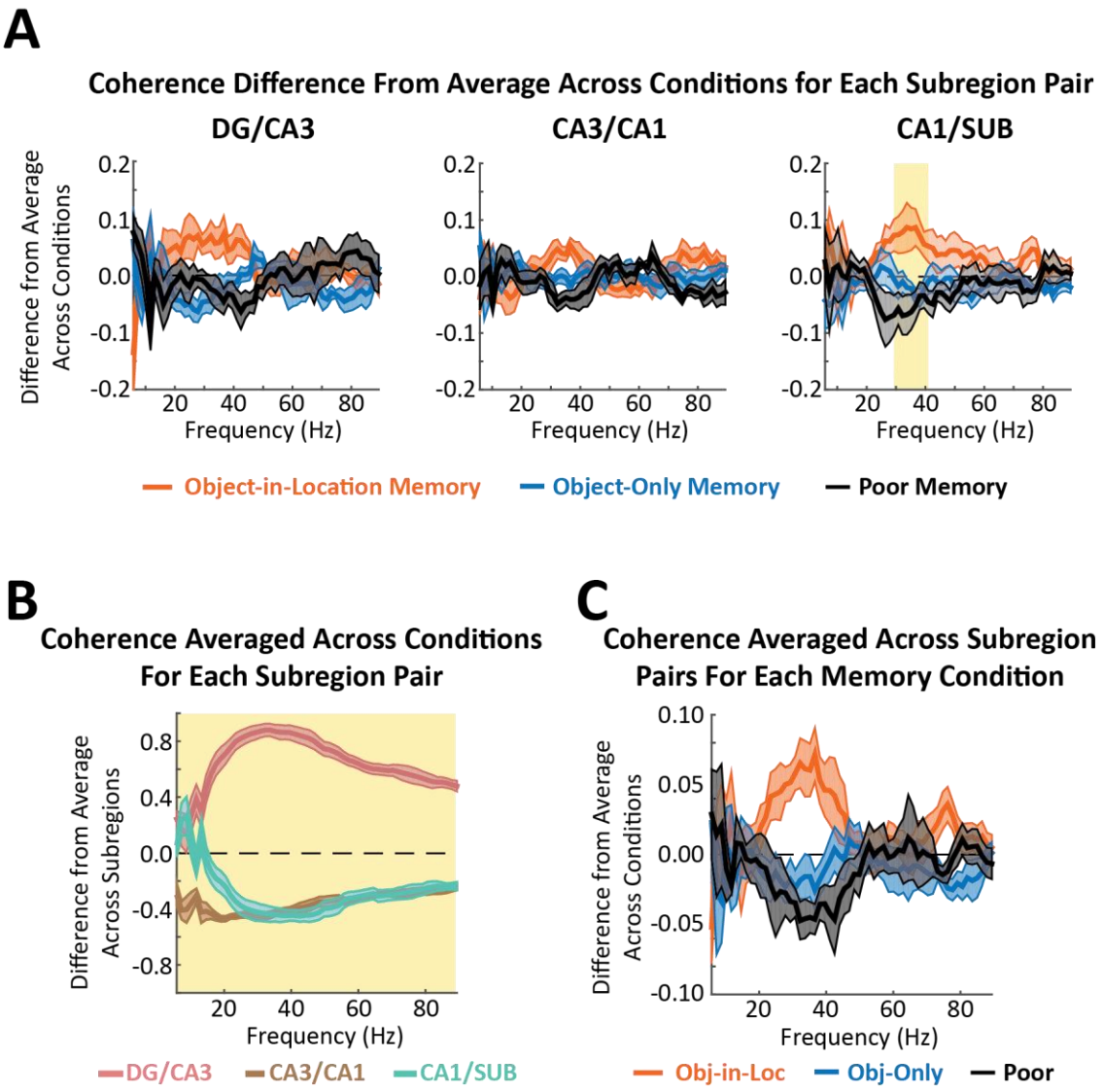

A
Power Difference From Average Across Conditions for Each Subregion
DG
CA3
CA1
SUB
****
Difference From Average Across Conditions (dB)
Frequency (Hz)
Object-in-Location Memory
Object-Only Memory
Poor Memory
B
Power Averaged Across Conditions For Each Subregion
Difference from Average Across Subregions (dB)
DG
CA3
CA1
SUB
C
Power Averaged Across Subregions For Each Memory Condition
Difference from Average Across Conditions (dB)
Obj-in-Loc
Obj-Only
Poor

Figure 4.5. Coherence differs during lap 1 object exploration for subsequent Object-in-Location versus Object-Only versus Poor memory objects. Panel A shows coherence during the initial 1.5s object exploration on lap 1, split by whether rats subsequently demonstrated Object-in-Location (orange), Object-Only (blue), or Poor (gray) memory for those objects. Coherence is plotted as the difference from average across conditions. Yellow rectangles mark frequency bins that differ significantly across conditions. Coherence between dentate gyrus and CA3 (DG/CA3) in the slow gamma range (29-41 Hz) differs significantly across conditions, with slow gamma coherence for Object-in-Location memory objects being higher than slow gamma coherence for Object-Only and Poor memory objects. A similar, though nonsignficiant, pattern is present in coherence between dentate gyrus and CA3. Panel B shows coherence averaged across subsequent memory conditions for each subregion pair. Significant differences are present at all frequency bins considered. Panel C shows coherence for each conditions averaged across subregion pairs. Numerical, though nonsignificant differences, are present in the slow gamma range following the pattern described for coherence between CA1 and subiculum.


A
Coherence Difference From Average Across Conditions for Each Subregion Pair
DG/CA3
CA3/CA1
CA1/SUB
Difference from Average Across Conditions
0.2
0.1
0.0
-0.1
-0.2
20
40
60
80
Frequency (Hz)
Object-in-Location Memory
Object-Only Memory
Poor Memory
B
Coherence Averaged Across Conditions For Each Subregion Pair
Difference from Average Across Subregions
0.8
0.4
0.0
-0.4
-0.8
Frequency (Hz)
DG/CA3
CA3/CA1
CA1/SUB
C
Coherence Averaged Across Subregion Pairs For Each Memory Condition
Difference from Average Across Conditions
0.10
0.05
0.00
-0.05
-0.10
Frequency (Hz)
Obj-in-Loc
Obj-Only
Poor

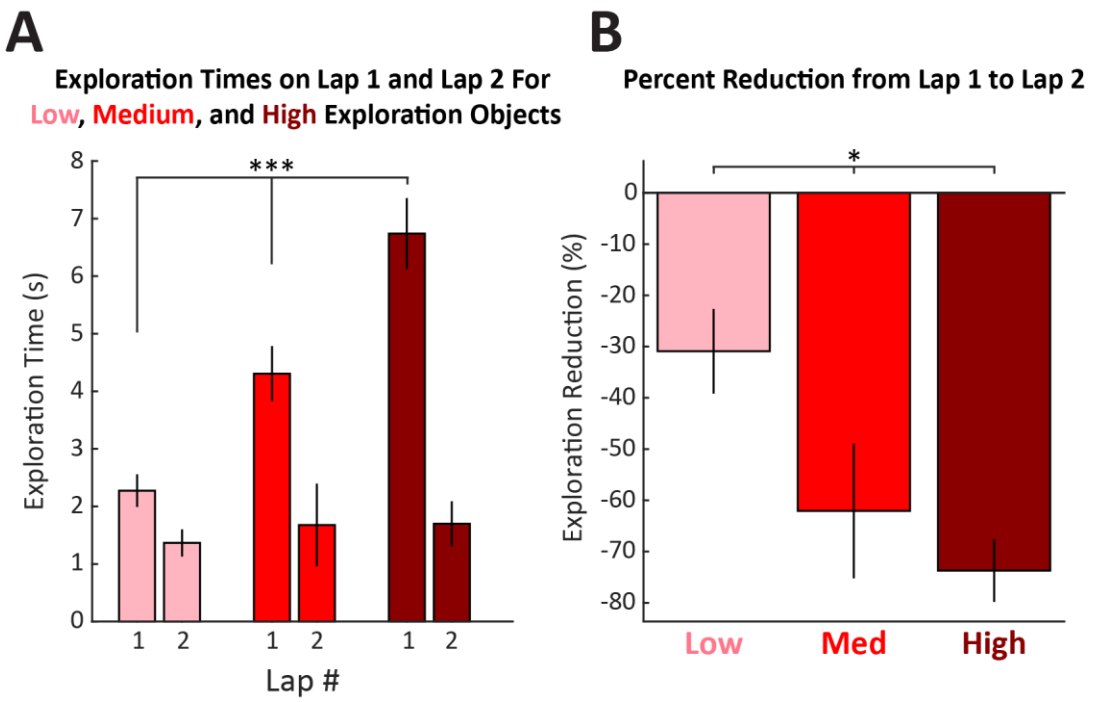


Figure 4.6. Exploration times and percent change across laps for Low, Medium, and High exploration objects. Panel A shows average exploration times on lap 1 and lap 2 for objects grouped as low (pink), Medium (red), and High (maroon) exploration objects. As anticipated, lap 1 exploration times differ significantly across conditions ($p < 0.001$). Exploration durations on lap 2 were not significantly different, though, as shown in Panel B, percent reductions from lap 1 to lap 2, plotted as percent of lap 1, did significantly differ across conditions ($p = 0.036$).

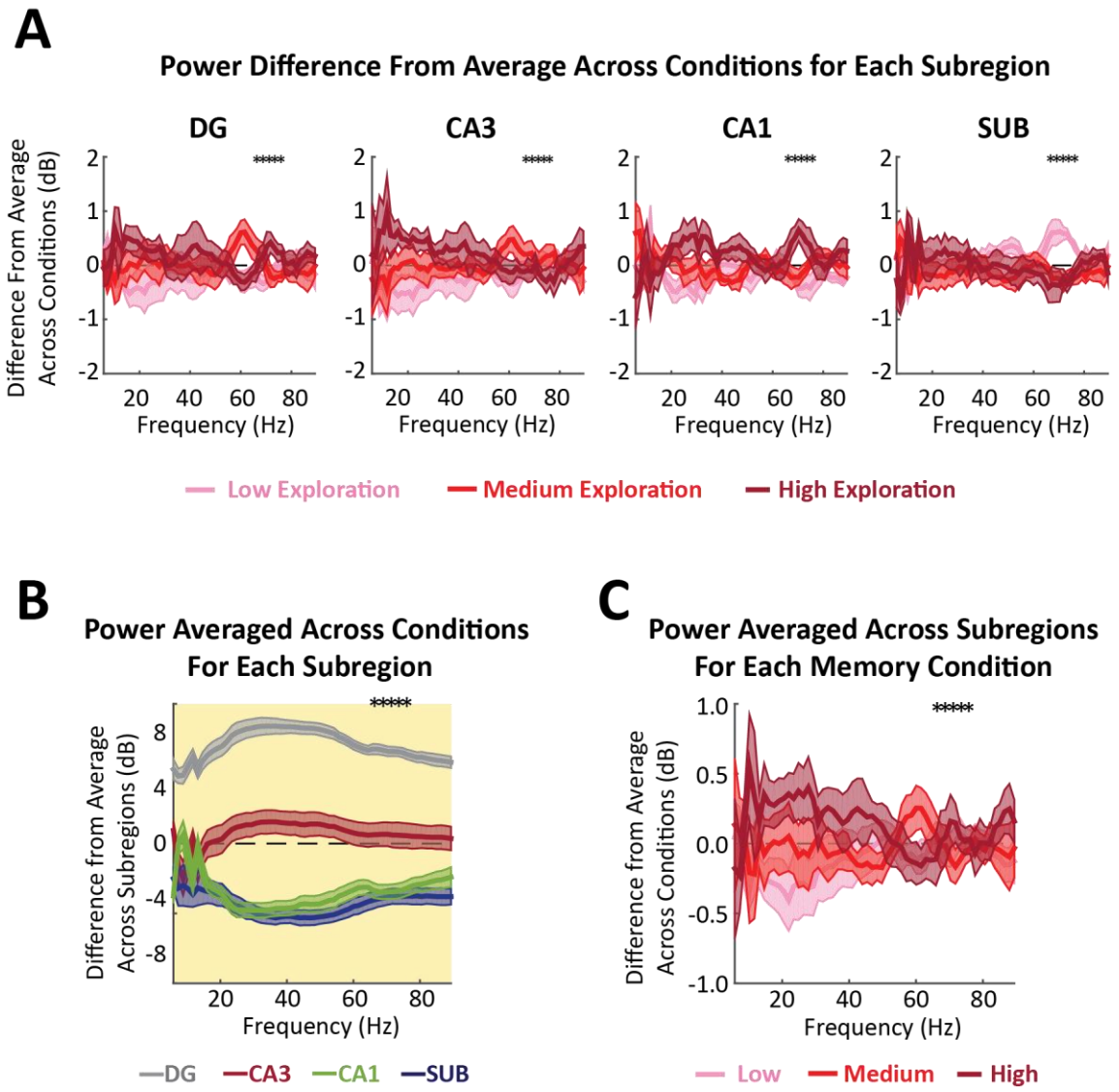


Figure 4.7. Spectral power is largely similar during the initial 1 s of novel object exploration regardless of ultimate exploration time duration. Panel A shows spectral power for each exploration duration condition [Low (pink), Medium (red), High (maroon)]. A significant subregion by exploration time interaction is present in the fast gamma range from 65.92 to 76.17 Hz, with patterns differing substantially across subregions. Panel B shows power averaged across exploration time conditions for each subregion, and reveals significant differences, again, at all frequency bins under consideration. Panel C shows average hippocampal power for each exploration time condition. No main effect of condition is present.

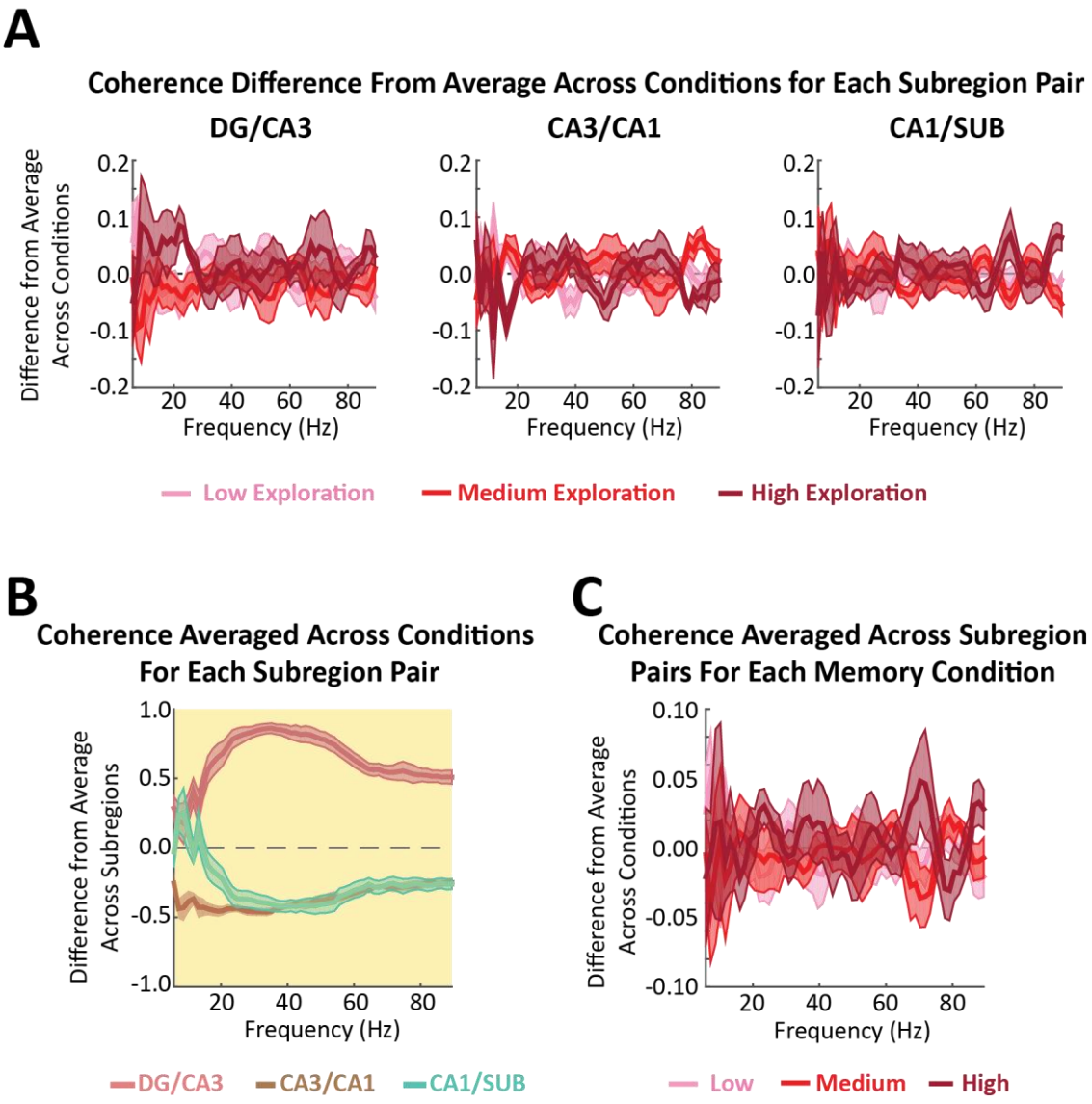


Figure 4.8. Coherence does not differ by exploration time duration. Panel A shows coherence for each subregion pair during the initial second of object exploration on lap 1 split by ultimate exploration time duration [Low (pink), Medium (red), High (maroon)]. No significant differences across conditions are present. Panel B shows coherence averaged across conditions for each subregion pair. Again, substantial and significant differences are present at all frequency bins under consideration. Panel C shows coherence averaged across subregion pairs for each exploration time condition. No statistically significant differences are present.

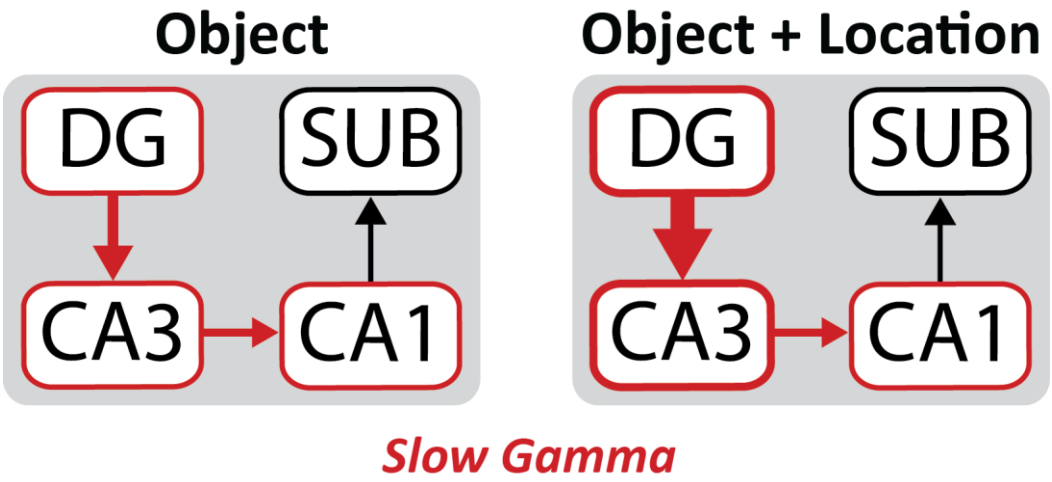


Figure 4.9. Summary illustration of subregional network state differences. The degree of slow gamma (red) in dentate gyrus (DG) and CA3, both during the initial encoding event and during later encounter with objects, is augmented by the involvement of contextual memory, such that when an object is explored and an object-in-location memory is formed, dentate gyrus and CA3 show stronger slow gamma relative to when an object-only memory is formed. Likewise, at test, the degree of slow gamma scales with the amount of novel information, such that novel objects are associated with a greater degree of slow gamma than repeated objects in novel locations, and repeated objects in novel locations are associated with greater slow gamma than repeated objects in repeated locations.

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Chapter 5

Intrahippocampal Synchrony and Memory for Objects in Temporal Context

Abstract

The hippocampus is important for remembering events in spatial context. Recent research suggests that the hippocampus may also be important for remembering events in temporal context. One definition of temporal context is events that occur in close temporal proximity to one another. The current study asked what hippocampal mechanisms in rats might underlie remembering events in temporal context. Local field potentials were recorded simultaneously from hippocampal subregions dentate gyrus, CA3, CA1, and subiculum while rats' memory for trial-unique three-object sequences was tested. Behavioral analyses revealed evidence of rats' memory for the third item in the sequences being cued by repeated presentation of the first two items. Oscillatory analyses revealed elevated slow gamma power within the hippocampus following rats' encounter with the first two items in the repeated sequence, relative to following rats' encounters with two novel items. This elevated slow gamma power may underlie the retrieval of an item memory cued by a repeated temporal context, with numerically elevated activity in dentate gyrus and CA3 perhaps relating to additional computational expense in the service of pattern completion. The results advance the field by demonstrating hippocampal involvement in memory retrieval cued by a repeated temporal context and by furthering a role for slow gamma in the hippocampus as a mechanism underlying memory processing.

Memories for events that occur in close temporal proximity to one another often become intertwined. For example, imagine you visit a new breakfast place one day, then get a flat tire on the way home afterwards. Your memory for the breakfast place may now be bound to your memory for the flat tire such that future visits to the restaurant cue—like it or not— your memory for the flat tire.

Events occurring close in time to one another can be considered a form of temporal context. Similar to how the location of a restaurant and the company present can be integrated into a memory as contextual information, so too can other events occurring in the nearby temporal vicinity. This notion of a running average of recent experience has a long history in research (Bower, 1972; Estes, 1955; McGeoch, 1932) and has been developed into a formal model called the temporal context model (TCM: Howard & Kahana, 2002), which, in conjunction with other related models, has been successful in explaining an array of memory phenomenon (Howard et al., 2005; Howard et al., 2009; Polyn et al., 2009; Sederberg et al., 2008; Sederberg et al., 2011). The model was developed to explain effects observed in word recall tasks, but more generally detailed how new items become bound to their temporal context and how repeated items can cue the temporal context with which they are associated.

Recently, Smith et al. (2013) developed a task with humans that probed the ability of a repeated temporal context to cue memories for items associated with it. The authors presented participants with images successively and asked participants to make an indoor/outdoor judgement about each one to ensure participants were attending to each stimulus. Unbeknownst to participants, images were organized into groups of three. After participants had been exposed to a sufficient number of these novel image triplets, one of four experimental manipulations was introduced on each successive triplet presentation, where either: (1) all three images were repeated from a prior occurrence; (2) the first two images were novel, but the third was repeated; (3) the first two images were repeated but the third was novel; or (4) all three images were novel.

The motivation for the task was to ask if repeating the first two images from a triplet, which served as the temporal context, could cue memory for the third item originally paired with it. Indeed, analyses revealed that encountering a repeated temporal context (i.e., the first two images repeated) strengthened memory for the third item originally paired with it *even when the third item was not actually presented for a second time*. This result suggests that encountering the repeated temporal context led participants to incidentally retrieve information about the third item.

In an effort to replicate the finding with a species better suited to neurobiological investigations, Manns et al. (2015) developed an analogous task for use in rodents. On each trial, rats ran two laps around an elevated circular track for a small chocolate sprinkle reward at the completion of each lap. On the first lap of each trial, rats always encountered three novel objects. On lap 2, one of four experimental conditions was employed, their design analogous to those used by Smith et al. (2013) with humans, where either: (1) all three objects were repeated; (2) the first two objects were novel, but the third was repeated; (3) the first two objects were repeated but the third was novel; or (4) all three objects were novel. Behavioral analyses revealed that rats explored novel objects significantly more when the first two objects were repeated relative to when the first two objects were novel. This result suggests that encountering the repeated first two objects (i.e., repeated temporal context) cued memory for the third object previously presented with it, as the increased exploration time for the novel object after a repeated temporal context may reflect rats resolving a discord between the cued memory representation and the object actually physically present.

An interesting remaining question, then, is what neural mechanisms might underlie this behavioral and cognitive effect. The hippocampus is one brain structure heavily implicated in associative memory processing (Mayes et al., 2007), or binding of multiple memory representations into a unified representation, and in representing temporal information

(Eichenbaum, 2014). The brain region is composed of multiple physiologically distinct subregions, including dentate gyrus, CA3, CA1, and subiculum. Ample research suggests that these subregions may differentially relate to aspects of memory processing important for representing temporal relationships between distinct events, and for the subsequent retrieval of memory cued by encountering the temporal context for a second time (Kesner & Rolls, 2015). For example, dentate gyrus is one of only two sites in the adult brain continually producing new neurons (Kempermann et al., 2004). It has been suggested that one function of neurogenesis is to facilitate temporal coding by adding a sort-of "time stamp" to newly encoded memories via the transient hyper-excitability of new born neurons (Aimone et al., 2006; Aimone & Gage, 2011). Likewise, CA3 has been especially implicated in the rapid formation of associative memories, particularly when a spatial component is involved, and for the retrieval of complete memory representations given partial or degraded input (Kesner & Rolls, 2015). Additionally, a subset of neurons in CA1, deemed "time cells" (MacDonald et al., 2011) fire in a discrete temporal sequence over delay periods in such a way as to contain information about the tasks to be completed following the delay (Eichenbaum, 2014; MacDonald et al., 2011; Pastalkova et al., 2008).

In the present experiment, we recorded in vivo electrophysiological activity, both action potentials and local field potentials, simultaneously from dentate gyrus, CA3, CA1, and subiculum in rats as the animals performed the task originally implemented by Manns et al. (2015). Similar to Manns et al. (2015), we report behavioral evidence of encountering a repeated temporal context cuing memory for the object previously associated with it. We expand upon the results of Manns et al. (2015) by further demonstrating that hippocampal oscillatory power in the slow gamma range (30 – 55 Hz), particularly in dentate gyrus and CA3, is elevated immediately following exposure to the repeated temporal context relative to after novel context exposure, a time point in which rats may be retrieving the cued memory. The results suggest neural

computations performed by these two subregions, dentate gyrus and CA3, may underlie the retrieval of a cued memory by a repeated temporal context, and that the slow gamma oscillation, in particular, may relate to this neural process.

Method

Subjects

Subjects were male Long Evans rats cared for as described in General Methods (Chapter 2). In total, fourteen rats performed our behavioral task. Six of these rats had chronic electrophysiological recording devices implanted and were included in neural data analyses.

Behavioral Task

Figure 5.1, panel A, presents an illustrated schematic for the experimental task. In each of up to five experimental sessions, rats performed up to 24 trials, with each trial consisting of a single lap around the track with no objects present (blank lap) followed by two laps around the track with three objects present. Objects were adhered with Velcro to the outside perimeter of the track in the 8, 11, and 2 o'clock positions, relative to the center stem at 6 o'clock (laps 1 and 2). We employed four experimental conditions, with an equal number of trials from each condition in each session. Condition type was randomized within each four trial block. On lap 1, rats always encountered three novel objects. On lap 2, rats either encountered: (1) duplicates of the same three objects presented on lap 1 [repeat context, repeat item (RCRI)], (2) duplicates of the first two objects from lap 1 followed by a novel object [repeat context, novel item (RCNI)], (3) three novel objects [novel context, novel item (NCNI)], or (4) two novel objects followed by a duplicate of the third object on lap 1 (novel context, repeat item [NCRI]). Duplicates were employed to avoid scent marking. Toys were washed in a 50/50 alcohol/water mixture after each experimental session. Herein, the first two objects in a three-object sequence will be referred to as "context," while the third object in the sequence will be referred to as "item."

As with the task described in Chapter 4, the current task exploits rats' preference for

novelty. Rats explore novel objects to a greater degree than familiar objects, and thus, the degree of familiarity rats have with an object negatively correlates with exploration time.

Two behavioral results are hypothesized. First, we hypothesize that rats will explore repeated objects following a repeated context for a shorter duration than repeated objects following a novel context. If observed, this pattern of results will be interpreted as evidence that encountering the repeated context cued rats' memory for the third item previously bound to that context, thus enhancing rats' familiarity with the third item prior to physically encountering it for the second time.

Second, we hypothesize that rats will explore novel items following a repeated context to a greater degree than novel items following a novel context. If observed, this pattern of results will be interpreted as evidence for the repeated context cuing memory for the third item previously bound to that context on lap 1, with the elevated exploration time perhaps reflecting rats resolving a discord between the cued memory representation and the object physically present.

Neural Analyses

Neural analyses were conducted as described in General Method (Chapter 2) and focused on the 1 second window of time immediately following rats encounter with repeated versus novel contexts (i.e., the first two objects) on the second object lap, as this window is hypothesized to be a time at which the context exposure cued memory for the previously presented third object.

Results

Exploration Times

Exploration time differences for the two key comparisons are presented in figure 5.1, panel B, along with exploration time differences for the same comparisons from Manns et al. (2015). In the current data set, rats (n = 14) explored repeated objects following a repeated context (0.32 +/- 0.052 s) for a significantly shorter duration than repeated items following a

novel context (0.49 +/- 0.092 s) [t(13) = -2, p = 0.034]. Rats trended towards exploring novel items following a repeated context (1.10 + 0.19 s) more than novel items following a novel context (0.83 +/0 0.17s), though the difference failed to reach statistical significance [t(13) = 1.66, p = 0.060]. Notably, the pattern of behavioral results is similar between the current data set and Manns et al. (2015), with both providing behavioral evidence for a repeated temporal context cuing memory for the object originally paired with it.

Neural Activity

Figure 5.2, Panel A, shows the difference in spectral power by subregion during the 1s following offset of the encounter with object 2 on lap 2, for both Repeat Context conditions minus both Novel Context conditions. Exploration times on lap 1 were required to be non-zero to ensure that rats were adequately exposed to the initially presented sequence of objects. A significant subregion by condition interaction is present in the beta range, from 13.18 to 23.44 Hz, reflected in, for Repeat Context relative to Novel Context, nonsignificantly lower dentate gyrus and CA3 power and nonsignificantly higher CA1 power. As indicated by Figure 5.2, Panel B, a main effect is present across subregions at all frequency bins under consideration (5-90 Hz), again reflecting the substantially different spectral profiles present across subregions. Moreover, a main effect is present across conditions in the slow gamma range, as shown in Figure 5.2, Panel C, from 30.72 - 36.62 Hz, such that slow gamma power is greater while departing a Repeat Context relative to while departing a Novel Context.

Locomotion

To address the possibility of the neural results being influenced by rats speed of locomotion, we asked if speed of locomotion differed after departing a repeated temporal context versus a novel temporal context, within the same 1s window employed for neural data analyses. Figure 5.3 presents the results of this analysis. Speed of locomotion did not differ significantly following a repeated temporal context (48.280 +/- 8.125 cm/s) relative to following exposure to a

novel temporal context (44.850 +/- 7.479 cm/s) [t(5) = 1.33, p = 0.240]. Thus, the data suggests that spectral differences did not result from locomotive differences.

Discussion

Behavioral analyses revealed evidence of rats' memory for the third object in trial-unique three-object sequences being reactivated following re-exposure to the initially presented temporal context (i.e., the first two objects), similar to previous reports in rats and humans (Manns et al., 2015; Smith et al., 2013). Figure 5.4 illustrates the differences in hippocampal oscillatory state accompanying this cued memory retrieval. Average hippocampal power in the slow gamma range, reflected particularly in dentate gyrus and CA3, was elevated following exposure to the repeated temporal context, but not exposure to a novel temporal context. Importantly, speed of locomotion did not differ significantly across the conditions, thus mitigating concerns that neural differences were attributable to differences in average speed of locomotion.

Slow Gamma Relates to Memory for Objects in Spatiotemporal Context

Hippocampal slow gamma was elevated in the present task in a way that related to the retrieval of memory cued by a repeated temporal context. The results of the previous chapter demonstrated that the degree of hippocampal slow gamma during encoding related specifically to rats remembering items within their spatial context (Chapter 4). Thus, the present results in conjunction with those presented previously speak broadly to a role for hippocampal slow gamma in remembering items in spatiotemporal context, as well as for a role of hippocampal slow gamma in both encoding and retrieval.

In both experimental conditions being compared in the present task, rats were ambulating between object locations on the circular track. Thus, in both conditions, activity associated with locomotion, including increased theta activity in CA1 and subiculum was present. However, when the behavioral task also led to the cuing of memory retrieval, additional activity was superimposed upon the subregional network associated with locomotion. This finding furthers the

necessity of considering memory demands while evaluating oscillatory activity within the hippocampus, demonstrating that network states associated with memory can be overlaid upon network states associated with overt behavioral patterns.

Hippocampal Subregions and Memory for Temporal Context

Elevated slow gamma following exposure to a repeated temporal context was most apparent in dentate gyrus and CA3. One idea to emerge from studies of the hippocampal contribution to memory for temporal order is that multiple hippocampal subregions are involved in memory for temporal order, but that CA3 is especially important when the order memory involves a spatial component (Kesner & Rolls, 2015). For example, temporal order memory for objects in rats is impaired following CA1, but not CA3, lesions, when the sequence lacks a spatial component (Hoge & Kesner, 2007). However, lesions to either CA3 *or* CA1 disrupt temporal order memory for spatial locations (Hunsaker and Kesner, 2008). Likewise, it has been proposed that newborn cells in the dentate gyrus, one of only two locations in the adult brain continually producing new neurons (Kempermann et al., 2004), may contribute to temporal memory by offering a temporal tag to newly formed memories (Aimone et al., 2006; Aimone & Gage, 2011), though the time-course of this process may not be well suited to practically contribute to the current task. Thus, while one may have predicted that activity in CA1 be differentially involved in the cuing of memories following presentation of a repeated versus novel temporal context based on evidence for its role in temporal sequence memory (Kesner & Rolls, 2015), our present findings of numerically enhanced slow gamma in dentate gyrus and CA3 after encountering a repeated versus novel context are in line with the current literature.

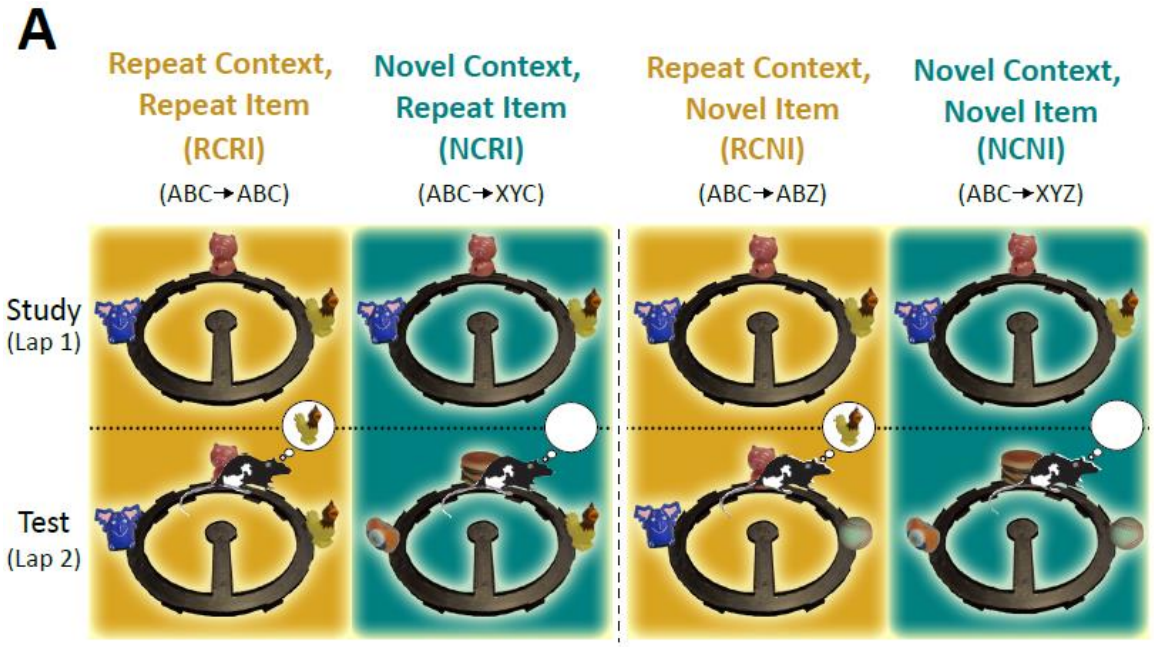
Dentate gyrus and CA3 are thought to play an essential role in completing memory representations given exposure to partial memory cues (Kesner & Rolls, 2015; Neunuebel & Knierim, 2014; Rolls, 1996; Rolls, 2015). Therefore, one interpretation for the observed pattern of neural results is that increased slow gamma activity in dentate gyrus and CA3 after exposure to

a repeated context reflects the dentate gyrus to CA3 network functioning to complete the temporal sequence memory, retrieving the memory for the third object cued by exposure to the initial two. Evidence supports the suggestions that slow gamma in CA3 is important for the retrieval of hippocampal dependent memories (Colgin et al., 2009; Colgin & Moser, 2010; Colgin, 2016). Likewise, CA3's recurrent collaterals, a system of axonal projections from CA3 that backproject to other CA3 neurons, has been extensively modeled as playing an integral role in memory retrieval (Kesner & Rolls, 2015; Rolls, 1996; Rolls 2015), a suggestion backed by experimental work supporting CA3's contributions to memory retrieval (Kesner et al., 2008; Lee & Kesner, 2004).

Conclusion

The current experiment provides behavioral evidence in rats for the cuing of an object encounter memory via re-exposure to a repeated temporal context. The findings here support the idea that hippocampal subregions are differentially involved in the memory retrieval process, with the pattern of results in CA3 and dentate gyrus differing from that present in CA1 and subiculum during the memory cuing window. Additional work will be necessary to understand more precisely the nature of the retrieved memory, particularly what aspects of the object encounter are being remembered, and, further, how hippocampal interactions with other cortical structures relates to this retrieval process.

Figure 5.1. Task schematic for temporal context repetition experiment and exploration time differences. Panel A shows a task schematic. On lap 1, in all conditions, rats saw three novel objects. On lap 2, rats encountered one of four different object conformations. Either all three objects were repeated [Repeat Context, Repeat Object (RCRI)], the first two items were novel but the third was repeated [Novel Context, Repeat Item (NCRI)] the first two objects were repeated but the third was novel (Repeat Context, Novel Item (RCNI)], or all three objects were novel [Novel Context, Novel Item (NCNI)]. In both repeat context conditions (RCRI and RCNI), a memory for the initially presented third object may be cued by the repeated temporal context. Panel B shows exploration time results for object 3 on lap 2 for the two key comparisons for both the current data set and for the data from Manns et al., 2014. On the left is the difference between exploration times for the repeated item after either a repeated context (RCRI) or a novel context (NCRI). Both data sets reveal numerical differences that suggest rats anticipated the repeated third item in the repeat context condition, though the difference is significant only for the current data set ($p = .034$). On the right is the difference between exploration times for a novel item after either a repeated context (RCNI) or a novel context (NCNI). Again, both data sets reveal numerical difference that suggest rats anticipated the repeated third item after a repeated context. In this case the difference was only statistically significant for the Manns et al. (2014) data set ($p = 0.014$), but the difference for the current data set approached significance ($p = 0.060$).


A
Repeat Context, Repeat Item (RCRI)
(ABC→ABC)
Novel Context, Repeat Item (NCRI)
(ABC→XYC)
Repeat Context, Novel Item (RCNI)
(ABC→ABZ)
Novel Context, Novel Item (NCNI)
(ABC→XYZ)
Study (Lap 1)
Test (Lap 2)

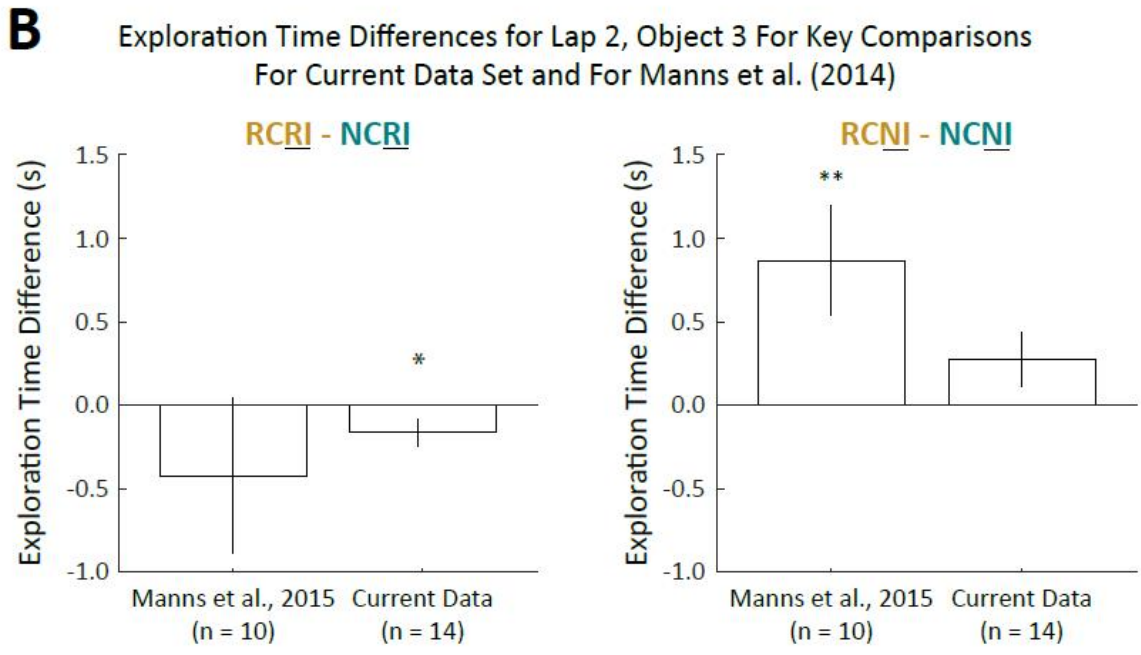
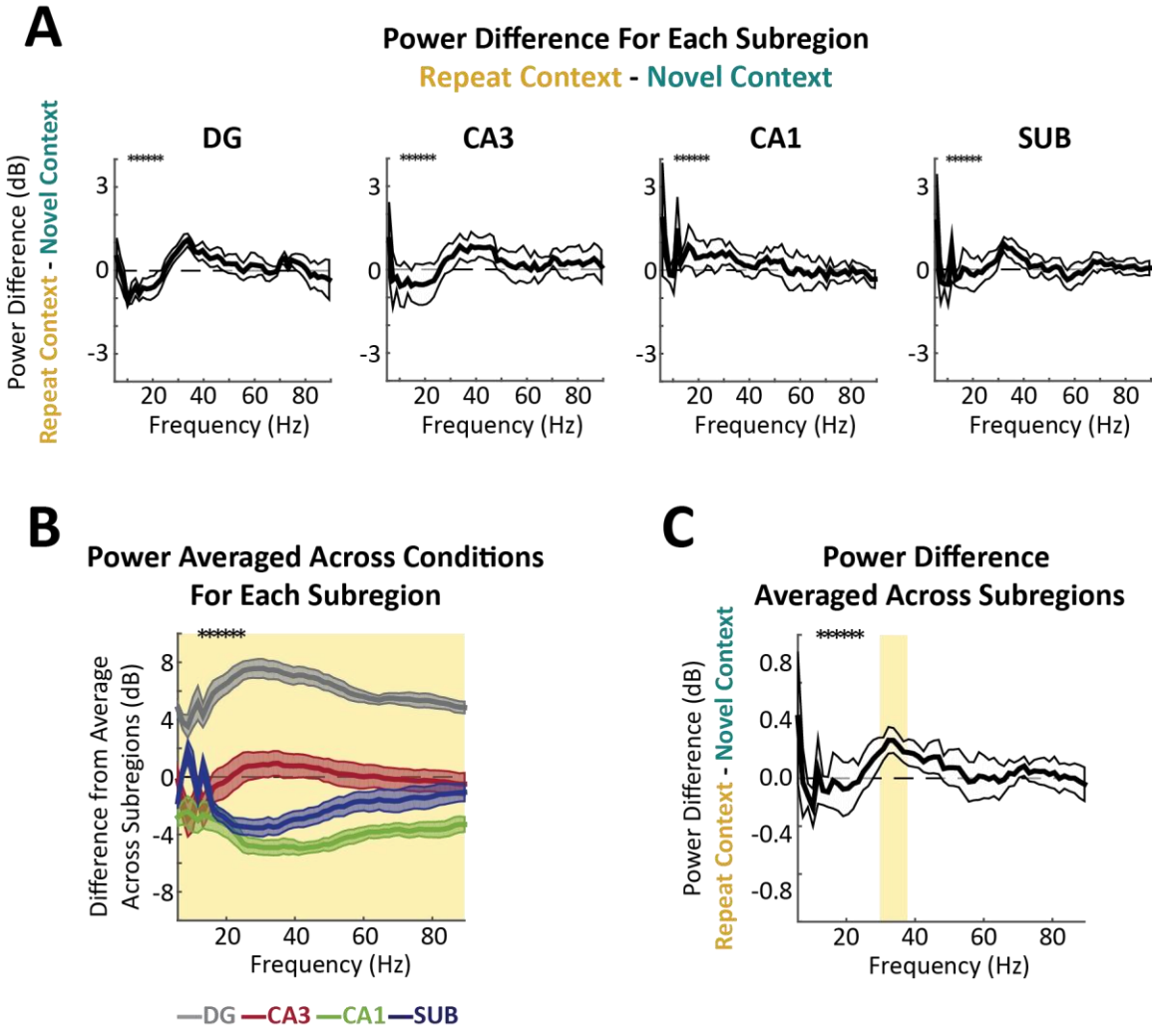

B
Exploration Time Differences for Lap 2, Object 3 For Key Comparisons For Current Data Set and For Manns et al. (2014)
RCRI - NCRI
RCNI - NCNI
Exploration Time Difference (s)
1.5
1.0
0.5
0.0
-0.5
-1.0
*
**
Manns et al., 2015 (n = 10)
Current Data (n = 14)

Figure 5.2. Spectral power differs during the 1 second post-exploration of either a repeated context or a novel context. Panel A shows spectral power differences by subregion plotted as the difference between conditions (Repeat Context – Novel Context). Dentate gyrus (DG) and CA3 both show numerical, though non-significant, differences in the slow gamma range. Asterisks denote a significant interaction from 13 to 23 Hz, reflected by numerically but modestly greater beta power in dentate gyrus and, possibly CA3, following Novel Context exposure and the reverse pattern for CA1. Panel B shows power averaged across conditions for each subregion. Again, significant differences are present at all frequency bins considered, as indicated by the yellow rectangle. Panel C shows the difference in average hippocampal power between conditions (Repeat Context – Novel Context) and reveals a main effect in the slow gamma range (31-37 Hz), such that slow gamma is greater following Repeat Context exposure relative to Novel Context exposure.


A
Power Difference For Each Subregion
Repeat Context - Novel Context
Power Difference (dB)
Repeat Context - Novel Context
DG
CA3
CA1
SUB
******
3
0
-3
20 40 60 80
Frequency (Hz)
B
Power Averaged Across Conditions
For Each Subregion
Difference from Average
Across Subregions (dB)
8
4
0
-4
-8
DG CA3 CA1 SUB
C
Power Difference
Averaged Across Subregions
Power Difference (dB)
Repeat Context - Novel Context
0.8
0.4
0.0
-0.4
-0.8

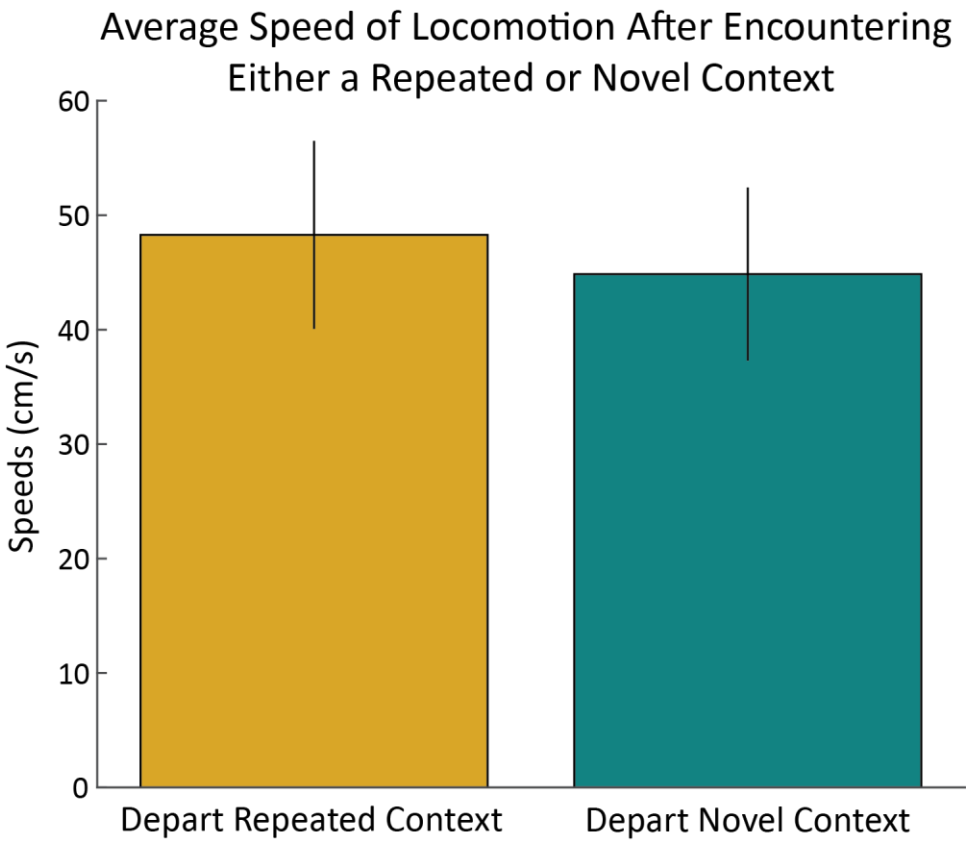


Figure 5.3. Speed of locomotion does not differ during neural analyses window. Speed of locomotion while departing object 2 on lap 2, during the same 1 second window used for neural analyses, does not differ across conditions, thus mitigating potential concerns for locomotion speed influencing oscillatory activity.

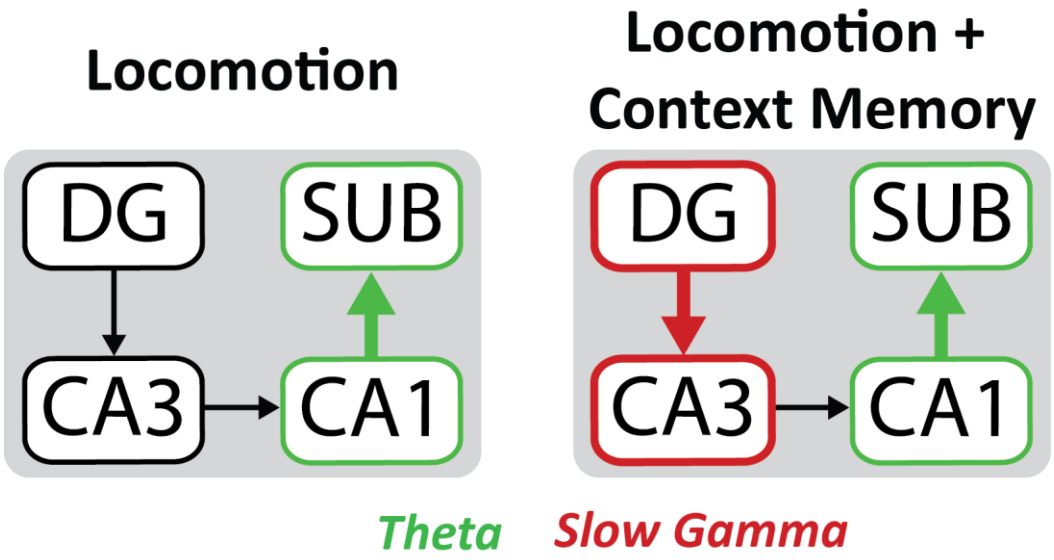


Figure 5.4. Schematic illustration of network state differences revealed by the temporal context repetition experiment. As rats depart a repeated context and can anticipate encountering the third item previously bound to that context, greater slow gamma is observed in dentate gyrus (DG) and CA3, relative to when rats depart a novel context and have no basis for anticipating the previously present third object. Importantly, neural data for both conditions was assessed as rats locomoted between objects, and, thus, theta is strongly present in CA1 and subiculum in both conditions.

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Chapter 6

General Discussion

The preceding three chapters provide, to our knowledge, the first account of simultaneously recorded local field potentials and action potentials from the four primary hippocampal subregions. Moreover, this dissertation provides the first report of how the functional network state of these four subregions, as measured through oscillatory activity, differs by behavioral state (Chapter 3), the quality and content of memories for objects in spatial context (Chapter 4), and the retrieval of a memory cued by a repeated temporal context (Chapter 5). A recurring theme throughout these experiments is that slow gamma within the hippocampus relates to remembering items in their spatiotemporal context.

Summary of Results

Chapter 3 reports that the spectral network state of the hippocampus during novel object exploration varies strikingly from that associated with other behavioral states. In particular, gamma oscillations are strongest while rats engage in novel object exploration, likely as a reflection of memory encoding. Beta oscillations in dentate gyrus and CA3 appear to dominate the spectral profile while rats remain stationary, whereas theta oscillations are associated strongly with locomotive states. In Chapter 4, the results expanded upon the findings from Chapter 3 by further indicating that the degree of slow gamma activity in the hippocampus relates specifically to the degree to which objects are remembered with their spatial locations. Chapter 5 extended these results further by demonstrating that the cuing of an object encounter memory by a repeated temporal context was associated with elevated slow gamma activity in the hippocampus, particularly in dentate gyrus and CA3.

Integrating the Present Work within the Field

The majority of the findings reported in the current document regarding hippocampal oscillatory activity and memory concern effects in the slow gamma range, with hippocampal slow gamma relating to both memory encoding and memory retrieval. One well-known hypothesis in

the field (Colgin et al., 2009; Colgin & Moser, 2010) is that slow gamma may function in the hippocampus to facilitate memory retrieval while hippocampal fast gamma may underlie memory encoding. This proposal is based on the finding that slow gamma oscillations in CA1 arise from CA3 (Bragin et al., 1995; Schomburg et al., 2014), a subregion some have associated with memory retrieval (Kesner & Rolls, 2015), whereas fast gamma oscillations in CA1 arise from entorhinal projections (Bragin et al., 1995; Schomburg et al., 2014), which are the primary afferent pathway for sensory information to reach the hippocampus (Witter, 1993). This hypothesis is supported by several experimental findings (e.g., Bieri et al., 2014; Montgomery & Buzsaki, 2007), as well as the finding in the current work of fast gamma power in CA1 during initial object encounters increasing with the amount of information being encoded (Chapter 4) and the finding that hippocampal slow gamma is elevated while rats may be retrieving memories cued by a repeated temporal context (Chapter 5).

The present findings, however, as well as those offered by previous studies from our laboratory (Bass & Manns, 2015; Trimper et al., 2014), also support a broader role for slow gamma within the hippocampus. Hippocampal slow gamma may function more generally as a mediator of intrahippocampal computation, regardless of the nature of the process underway. Rather than a one-to-one relationship between oscillatory frequency bands and memory processes, it appears that hippocampal slow gamma is a more generic neuronal processing tool. Modulation of its activity in the current context appears to relate more to memory load than to which computations in particular are being performed.

Attributing hippocampal oscillatory activity to either memory encoding or retrieval is difficult, as the processes are likely to frequently co-occur. These memory states are not mutually exclusive. Additional work will be required to further probe the hypothesized neural division of memory processes, ideally, while organisms are in states that may more directly bias the neural system towards one process or the other, such as during sleep. The results presented here,

however, speak to a broader role for slow gamma within the hippocampus, serving the purpose of facilitating communication in whatever task may be underway.

Technical Advancement

Much has been learned about memory from studies employing modern neural imaging techniques, such as fMRI, in humans. For example, understanding the lateralization of hippocampal dependent memory in relation to language (Banks et al., 2012) could not have been approached without such techniques, nor could we advance our understanding of how human diseases such as Alzheimer's disease impact hippocampal volume (Small et al., 2000). However, in vivo electrophysiological approaches, at least at present, remain the only technique available with precise enough temporal resolution to examine single-unit activity at the single-spike level and with enough spatial precision to confidently characterize interactions in deep brain structures. Likewise, rodents remain the most suitable organisms available for this work, given their trainability, advanced cognitive capacity, cost of care, and long history of behavioral and biological characterization.

Recent advances in data handling capacity allow for the recording of dozens to hundreds of neurons simultaneously, and plans are currently being enacted to advance this capacity to simultaneously recording thousands of neurons. As directly measuring synaptic activity at the level of action potentials and oscillatory activity is currently the most readily interpretable metric we have for understanding neuronal activity, in vivo electrophysiological investigations remain critical for advancing our understanding of brain function.

The approach employed in the experiments described here is a significant technical advancement within the field of hippocampal physiology. Though the hippocampus has been a hotbed for in vivo electrophysiological investigations for many decades, the results here include the first descriptions of simultaneously recorded in vivo electrophysiological activity, both spikes and local field potentials, from dentate gyrus, CA3, CA1, and subiculum. Moreover, these results

include the first description of how this activity varies by overt behavioral state and recognition memory processing, laying the foundation for future work further characterizing how this activity varies by other factors, such as information content or memory load.

Understanding the brain is one of the great goals of our modern era. To move towards this goal, progress must be made in understanding the tools employed the brain to dynamically coordinate information exchange throughout massively interconnected networks. The results offered here take one (very) small step towards this sub-goal by demonstrating that oscillatory activity within select subcomponents of the hippocampal network is differentially modulated by memory and, moreover, that this modulation in service of memory can be overlaid upon activity associated with various overt behavioral states in hitherto unknown ways.

Future Research Questions

The technical approach utilized, combined with the findings reported, offer forward an interesting and reliable test-bed for studying hippocampal oscillations and their causal relationship with memory processing. By branching forward from the characterizations offered here for when distinct oscillatory frequency bands are most apparent in the hippocampal power spectrum, future studies will be better able to causally dissect the circuitry and probe the precise mechanisms underlying oscillatory activity in different bands, as well as their functional contributions. For example, given that slow gamma oscillations in the hippocampus relate to memory encoding and retrieval, future studies might ask how optogenetic disruption of these oscillations influences these processes, and, moreover, how disruption of these oscillations within distinct hippocampal subregions differentially alters behavioral evidence of memory.

A further interesting avenue of future study may be to ask how hippocampal oscillatory activity during memory processing relates to extra-hippocampal input and output. For example, as noted above, evidence supports the suggestion that fast gamma oscillations offer a mechanism for the functional routing of information from the entorhinal cortex to the hippocampus (e.g., Bragin

et al., 1995; Colgin et al., 2009; Colgin & Moser, 2010; Colgin, 2015). Entorhinal projections differentially arrive at hippocampal subregions, with entorhinal layer 2 projecting to dentate gyrus and CA3, and layer 3 projecting to CA1 (Witter, 1993). Likewise, lateral and medial entorhinal efferents project to anatomically segregated portions of CA1 along the proximal-distal axis, with respect to dentate gyrus (Witter, 1993). Unanswered questions to be addressed include: how is incoming sensory information routed and integrated throughout these pathways in the service of associative memory formation? What unique contributions might the input to each subregion be offering to memory processing? Do the hippocampal subregions act in parallel or in series when processing input?

Conclusion

A common theme throughout this dissertation involves the layering of neuronal activity within the hippocampus relating to memory processing upon the activity in the hippocampus relating to overt behavioral state. For example, in Chapter 3, we reported that novel object exploration was associated with an oscillatory profile distinct from that associated with the cessation of locomotion. In Chapter 4, we reported that slow gamma activity during novel object exploration related to the degree to which rats remembered objects with their spatial locations. Chapter 5 provided evidence that slow gamma during locomotion could be augmented by the retrieval of memory cued by a repeated temporal context. Thus, though investigations of hippocampal activity during overt behavioral states such as running and sleeping (Ahmed & Mehta, 2012; Belluscio et al., 2012; Bieri et al., 2014; Colgin et al., 2009; Diba & Buzsaki, 2007; Zheng et al., 2015) have offered forward invaluable advances regarding the mechanisms underlying oscillatory activity, an additionally fruitful avenue worthy of consideration is to ask how these oscillatory mechanism vary with memory for events in spatiotemporal context, a function known to critically depend upon the hippocampus.

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