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Amygdala Stimulation Enhances Memory for Specific Events by Modulating the Hippocampus

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Amygdala Stimulation Enhances Memory for Specific Events by Modulating the Hippocampus

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B.A., Cornell University, 2007

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An abstract of
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Abstract

Amygdala Stimulation Enhances Memory for Specific Events by Modulating the Hippocampus

By David I. Bass

Prioritization of information into long-term memory is essential for survival. Emotional arousal enhances memory for these events, and this emotional enhancement of memory is mediated by the amygdala. The amygdala has projections to many regions throughout the brain, including the hippocampus, a structure that supports memory for events. The goal of the research discussed in the present dissertation is to understand how the amygdala modulates the hippocampus in the service of enhancing memory. Characterizing the interaction between these two structures is fundamental to understanding how important information is prioritized for consolidation. Furthermore, a deeper understanding of this physiological interaction will further our understanding of the pathophysiology driving affective disorders, such as post-traumatic stress disorder. To address this goal, we developed an object recognition memory task to test memory for specific events in rats and demonstrated that brief electrical stimulation to the basolateral complex of the amygdala (BLA) selectively enhanced memory in a stimulus-specific manner when memory was tested 1 day later. In a follow-up study, we demonstrated that amygdala-mediated enhancement of memory for specific events depends on the hippocampus. Finally, we obtained recordings of local field potentials and spiking activity in the hippocampus from rats that received amygdala stimulation while performing the memory task. Data analysis took advantage of the ipsilateral connectivity between the BLA and the hippocampus by contrasting ipsilateral and contralateral stimulation. The results indicate that ipsilateral BLA stimulation elicits low gamma coherence between CA3 and CA1. Stimulation did not have a substantial impact on the firing rate of pyramidal unit populations, but ipsilateral stimulation induced a strong phase preference of CA3 pyramidal spikes relative to low gamma oscillations in the local field potential of CA1. Thus, the main effect of BLA stimulation on hippocampal pyramidal units was via modulation of spike timing. Correlations between improved memory and intra-hippocampal synchronization have previously been reported, but this is study is unique in that gamma synchronization was induced to enhance memory.

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Chapter 1. Introduction: Background & Motivation

1.1 The Amygdala, the Hippocampus, and Memory

1.1.1 Prioritization of memory

Prioritizing the consolidation of important information into long-term memory is vital for survival (LeDoux, 2012). Therefore, understanding how certain memories are remembered better than others is a fundamental question in neuroscience and psychology. It is well established that emotional arousal enhances memory (McGaugh, 2013; Talmi, 2013) and that the amygdala plays a key role in modulating the this enhancement for several types of memory (LaBar & Cabeza, 2006). Thus, although the amygdala plays a central role in examples of stimulus-response conditioning, such as fear conditioning, it also plays a more general role in modulating memory for emotional material in other brain regions (McGaugh, 2004).

The goal of the research discussed in the present dissertation is to understand how the amygdala modulates the hippocampus in the service of enhancing types of everyday memory for facts and events, commonly known as declarative memory (Squire & Zola, 1996). Characterizing the interaction between these two memory structures is fundamental to understanding how important information is prioritized and preferentially consolidated. Moreover, a deeper understanding of this physiological interaction will further our understanding of the pathophysiology driving certain affective disorders, such as post-traumatic stress disorder (PTSD) (Bremner et al., 1995; Langevin, De Salles, Kosoyan, & Krahl, 2010; McEwen & Magarinos, 2001; Rodrigues, Ledoux, & Sapolsky, 2009).

The intrinsic structure and function of the amygdala and the hippocampus are well preserved across mammalian taxon, suggesting that there has been a consistent evolutionary pressure to preserve their computational processes (Manns & Eichenbaum, 2006; Paz & Pare, 2013). Contributions of the amygdala to behavior and memory have been particularly well preserved across species (Lang & Davis, 2006), while apparent divergence in the role of the hippocampus can largely be attributed to variations in the input from surrounding neocortical regions (Manns & Eichenbaum, 2006). The key point is that the rodent amygdala and hippocampus are reasonable models for investigating the basic computational mechanisms of their human counterparts.

1.1.2 Memory systems

The amygdala is important for the memory enhancement observed for emotionally arousing events (Larry Cahill et al., 1996; Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000; Dolcos, LaBar, & Cabeza, 2005; Hamann, Ely, Grafton, & Kilts, 1999), but it is not absolutely necessary for baseline memory function for neutral stimuli (Adolphs, Cahill, Schul, & Babinsky, 1997). Thus, amygdala activation can enhance many types of memory, while inactivation does not necessarily impair memory.

The amygdala can affect memory through many different mechanisms. For example, activation during an event can improve memory encoding by boosting attention and perception (Phelps & Ledoux, 2005; Talmi, 2013). The amygdala also has projections to the sympathetic nervous system that stimulate release of peripheral hormones into systemic circulation (Rodrigues et al., 2009). Peripheral release of sympathetic hormones stimulates central release of norepinephrine, which, in concert

with glucocorticoids, enhances consolidation of memory (LaBar & Cabeza, 2006; Ledoux, 2000; Liang, Bennett, & McGaugh, 1985; McGaugh, 2013). Additionally, there are direct neural projections that may play a critical role in tagging specific neural synapses for enhanced plasticity (Bergado, Lucas, & Richter-Levin, 2011; Curtis & Pare, 2004). Direct manipulation of the amygdala can theoretically engage all of these mechanisms, so in order to focus on direct contributions of the amygdala to memory consolidation, such manipulations have traditionally followed an exposure or study period (Paul E Gold, Hankins, Edwards, Chester, & Mcgaugh, 1975; Kesner, 1982; McGaugh & Roozendaal, 2009; McIntyre et al., 2005; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008).

Directly manipulating the amygdala has contributed greatly to our understanding of interactions between memory systems (LaBar & Cabeza, 2006; McGaugh, 2004). For example, intracranial stimulation of the amygdala has been shown to enhance memory by modulating consolidation in other memory systems, such as the hippocampus, the neocortex, and the striatum (Bergado, Rojas, Capdevila, Gonzalez, & Almaguer-Melian, 2006; Chavez, McGaugh, & Weinberger, 2009; Packard, Cahill, & McGaugh, 1994). Indeed, it is thought that the amygdala exerts its influence across many different memory tasks by facilitating consolidation in other memory systems, including the hippocampus. This theory is supported by the finding that the amygdala is not necessary for retrieval or maintenance of several types of memory following successful consolidation (Packard & Teather, 1998). Additionally, the memory trace does not need to contain strictly emotional information, as otherwise neutral information benefits from emotional arousal

when this material is presented immediately preceding arousal (Anderson, Wais, Gabrieli, Waisc, & Gabrielic, 2006; Roozendaal, Okuda, Zee, & McGaugh, 2006).

In order to understand how the amygdala enhances memory, investigation should focus on downstream effects of amygdala activation. Amygdala stimulation facilitates the induction of long-term potentiation (LTP) in the hippocampus following tetanic stimulation of neural projections into the hippocampus, but amygdala stimulation is insufficient for LTP induction when delivered alone (Akirav & Richter-Levin, 1999b; Frey, Bergado-Rosado, Seidenbecher, Pape, & Frey, 2001; Ikegaya, Saito, & Abe, 1995). In parallel with the electrophysiology, molecular studies have shown that activation of the amygdala during a hippocampal-dependent memory task results in upregulation of hippocampal expression of Arc (McIntyre et al., 2005), an immediate early gene that is important for maintenance of LTP in the hippocampus (Guzowski et al., 2000). Thus, the amygdala is thought to enhance consolidation of hippocampal-dependent memories by modulating synaptic plasticity in the hippocampus (Bergado et al., 2011).

The hippocampus is an important structure for supporting declarative memory (Alkire, Haiert, Fallon, & Cahill, 1998; Scoville & Milner, 1957; Squire, Stark, & Clark, 2004). Historically, one of the most common methods of characterizing hippocampal function is to study human patients with hippocampal lesions, or to induce hippocampal lesions in animal models (Broadbent, Gaskin, Squire, & Clark, 2010; Clark, Zola, & Squire, 2000; Manns & Squire, 1999; Moser & Moser, 1998; Zola et al., 2000). These studies laid the groundwork for understanding hippocampal contributions to spatial navigation and episodic memory. However, it is difficult to extrapolate from these studies how such information is represented in terms of neuronal activity. To get at these

mechanisms, investigators use a variety of imaging, cellular, and electrophysiological tools. Electrophysiological studies have been particularly influential for understanding how memory for time and space are represented (Kjelstrup et al., 2008; S. Leutgeb et al., 2005; MacDonald, Lepage, Eden, & Eichenbaum, 2011; O'keefe & Nadel, 1978) and how these representations may be consolidated in to long-term memory (Buzsáki, 1986; Carr, Jadhav, & Frank, 2011; Foster & Wilson, 2006; Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994). Furthermore, electrophysiological data has provided key insights linking spatial representation in rodents and with representational space for episodic memory in humans (Buzsáki & Moser, 2013; Manns, Howard, & Eichenbaum, 2007; Smith & Mizumori, 2006).

1.1.3 Review of amygdala and hippocampal anatomy

Understanding the anatomical connections between the hippocampus, the entorhinal cortex, the perirhinal cortex, and the amygdala provides a framework for investigating the emotional enhancement of memory. The circuitry has been extensively mapped, and pertinent findings are summarized below. There are, perhaps, two main points to take from the anatomy. The first is that there are many neural pathways through which the BLA could modulate activity in the hippocampus on a millisecond time scale. These anatomical connections, combined with the well established role of the BLA in modulating memory, make the BLA an ideal target to stimulate for enhancing hippocampal-dependent memory. The second point is that hippocampal anatomy suggests that a key step in hippocampal computations is the transmission of information from CA3 to CA1 (blue projection in Fig. 1.1). Therefore, recording electrophysiological activity in

these 2 regions simultaneously should yield insight into the physiology of amygdala-mediated enhancement of memory for events.

Fine grained anatomical studies in rodents have indentified many subnuclei of the amygdala, with much research on learning and memory focusing on the basolateral complex of the amygdala (BLA). The BLA consists of three nuclei: the lateral nucleus, the basal (or basolateral) nucleus, and the accessory basal (or basomedial) nucleus (Pape & Pare, 2010). All three nuclei have projections to the hippocampus, with the majority of projections arising from the basal and accessory basal nuclei (Petrovich, Canteras, & Swanson, 2001; Pikkarainen, Ronkko, Savander, Ricardo, & Pitkanen, 1999). Hippocampal projections from the BLA are exclusively ipsilateral and target the temporal (ventral in rodents, anterior in humans and non-human primates) and intermediate regions of the hippocampus.

The major cortical inputs to the hippocampus come from the superficial layers of the entorhinal cortex (layers II and III) via the perforant path (van Strien, Cappaert, & Witter, 2009). The traditional model of hippocampal circuitry poses that information flows unidirectionally along the transverse axis (proximal to distal) from the dentate gyrus to CA3 to CA1 and subiculum, and then back out to the deep layers of the entorhinal cortex (layers V and VI). Consistent with this model, the dentate gyrus sends projections to CA3 via the granule cell mossy fibers, and CA3 pyramidal cells project to CA1 via the Schaffer collaterals. The only rout out of the hippocampus from CA3 is via CA1, which suggests the Schaffer collaterals bridge a critical bottleneck for hippocampal function. However, several anatomical features underscore that the trisynaptic model of hippocampal anatomy is an over simplification. Specifically, there are prominent

recurrent collaterals in CA3, back projections from CA3 to dentate gyrus, and direct projections from the entorhinal cortex to CA1 and CA3. The contributions of each of these pathways remain unclear, but there is growing evidence that each hippocampal region performs unique computations contributing to memory encoding, consolidation, and retrieval (J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Neunuebel & Knierim, 2014). For example, the recurrent collaterals in CA3 may create a positive-feedback circuit for an autoassociative network (Guzowski, Knierim, & Moser, 2004). That is, projections between CA3 cells facilitate complete retrieval of a memory trace from only a partial activation by a cue associated with the memory. In contrast, the dentate gyrus is characterized by sparse coding and neurogenesis, 2 features thought to facilitate separation of distinct autoassociative networks in CA3 (Deng, Aimone, & Gage, 2010).

The presence of robust projections from the amygdala to the hippocampus and adjacent cortical structures suggests that the amygdala has wide spread influence over the hippocampus (Fig. 1.1). Direct projections to CA3 and CA1 could modulate synaptic transmission and synaptic plasticity at the CA3-CA1 synapse (Petrovich et al., 2001; Pikkarainen et al., 1999), a key connection in the hippocampus and thus a key connection in the extended hippocampal memory system. Multi-synaptic pathways via the perirhinal and entorhinal cortex may allow the amygdala to exert its influences over the flow of information in and out of the hippocampus thereby prioritizing some memories over others (Furtak, Wei, Agster, & Burwell, 2007; Kajiwara, Takashima, Mimura, Witter, & Iijima, 2003; Kerr, Agster, Furtak, & Burwell, 2007; Paz, Pelletier, Bauer, & Pare, 2006; Petrovich et al., 2001; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000). These indirect

connections may also explain how BLA stimulation facilitates LTP induction in the septal region of the dentate gyrus, (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995), which receives no direct BLA input. Although the precise contributions of each pathway are unknown, it is clear that the amygdala predominantly modulates synaptic plasticity in the ipsilateral hippocampus (Ikegaya et al., 1995). Therefore, studies that investigate how the amygdala modulates hippocampal function should focus on the ipsilateral hippocampus (Fig 1.2).

1.1.4 Amygdala and hippocampal dysfunction in affective disorders

Amygdalo-hippocampal abnormalities in structure and function have been implicated in depression, schizophrenia, Alzheimer's disease, autism, and anxiety (Adhikari, Topiwala, & Gordon, 2010; Felix-Ortiz et al., 2013; Phelps & Ledoux, 2005; Sheline, Wang, Gado, Csernansky, & Vannier, 1996; Small, Schobel, Buxton, Witter, & Barnes, 2011), but perhaps no more so than in PTSD (Bremner et al., 1995; Langevin et al., 2010; McEwen & Magarinos, 2001; Rodrigues et al., 2009). Much research has shown that chronic stress induces hippocampal atrophy and impairs hippocampal LTP in concert with dendritic arborization and enhanced neural plasticity in amygdala (J. J. Kim, Ja, Lee, & Han, 2005; J. J. Kim, Lee, Han, & Packard, 2001; Vyas, Mitra, Rao, & Chattarji, 2002). The relationship between these two structures and PTSD seems readily apparent on the surface. The amygdala enhances memory consolidation during emotional arousal, and patients who suffer from PTSD have extremely vivid memories of traumatic events. Thus, PTSD may be an extreme manifestation of the physiological mechanisms for enhanced memory consolidation. The underlying pathophysiology of PTSD is likely

to contain many more intricacies, but this point only furthers the importance of understanding the normal, physiological interaction between the amygdala and the hippocampus. Simply, the baseline physiology must be clearly defined in order to understand the pathophysiology.

1.2 The Novel Object Recognition Memory Task

1.2.1 Initial conception

The novel object recognition memory task tests a rat's memory from a single trial (Ennaceur & Delacour, 1988) and is thought to be analogous to visual paired comparison tasks used in humans and nonhuman primates. Typically, a rat is placed in a square box with 2 identical objects. The rat is allowed to freely explore these objects for a set amount of time, perhaps 5 min, and then is removed from the box. The objects are replaced by a novel object and a duplicate of the original objects, and the rat is put back into the box and allowed to freely explore the objects again. In general, retention intervals are less than 1 day, but the duration between the study and the test can be increased or decreased to make the task more or less demanding.

The novel object recognition memory task relies on the fact that rats, like humans and nonhuman primates, have an innate preference for novelty. Thus, when the rat is put back in the box, the degree to which it explores the novel object over the repeated object can be used to infer memory for the repeated object. Memory performance is quantified in terms of a discrimination index, or the ratio of novel object exploration to total object exploration during the test:

$$\text{Discrimination Index} = \frac{\text{Novel}}{\text{Novel + Familiar}}$$

When the rat spends an equal amount of time exploring both objects, the discrimination index is 0.50, indicating that the rat has no memory for the repeated object. Very good memory performance is 0.67, indicating that the rat explored the novel object twice as much as the familiar object. The key advantage of the discrimination index is that it normalizes exploration times, minimizing the impact of more curious rats on the final result.

1.2.2 A novel paradigm for testing memory for specific events in rats

Multiple trials with good and poor memory performance are necessary to elucidate the electrophysiological mechanisms of amygdala-mediated enhancement of memory because the differences between any 2 trials can vary for a number of reasons. Thus, for studies in the present dissertation, the novel object recognition memory task was modified to test each rat's memory for multiple specific events.

There are three aspects of this task that make it well suited for testing amygdala-mediated enhancement of memory for specific events. The first is that, at baseline, rats do not remember objects when memory is tested 1 day later, but they remember objects when memory is tested soon after the initial encounter. The second is that BLA stimulation improves memory performance. Specifically, rats remember objects 1 day later only if the initial encounter was immediately followed by BLA stimulation. Finally, this amygdala-mediated enhancement of memory depends on the hippocampus. BLA stimulation does not enhance memory when hippocampal function has been disrupted.

1.3 Neural Oscillations and Plasticity

1.3.1 Local field potentials

Local field potentials (LFPs) are neural oscillations obtained from extracellular electrodes within the brain. They can fluctuate between 0.05 – 500 Hz and are thought to reflect rhythmic synchronization of neuronal populations (Buzsáki & Draguhn, 2004). Frequencies below 100 Hz can be conceptualized as the summed local input into a region because lower frequency oscillations predominately originate from current fluctuations in the local, post-synaptic dendritic arbor (Buzsáki, Anastassiou, & Koch, 2012).

The power spectrum in the LFP falls of at $1/f$, where f is the frequency, but changes in behavioral state can elicit distinct peaks in certain frequency bands in rodents, namely, theta (4 – 12 Hz) and gamma (30 – 100 Hz). Much research has shown that rhythmic synchronization of neural activity in these frequency bands supports memory processes in the hippocampus and that multiple mechanisms contribute to oscillations in both bands (Buzsáki, 2002; Colgin & Moser, 2010; Hasselmo, 2005; Womelsdorf et al., 2007). For example, theta oscillations are generated by input from the medial septum and the entorhinal cortex, but hippocampal pyramidal cells also have intrinsic membrane oscillations in the theta range (Buzsáki, 2002). Gamma frequency oscillations reflect the activity from a combination of fast-spiking interneurons and the CA3 network of recurrent collaterals, and synchronization of activity in CA3 into a gamma frequency rhythm can entrain neural activity in CA1 (Csicsvari, Jamieson, Wise, & Buzsáki, 2003; Hájos & Paulsen, 2009).

Synchrony between LFPs from different regions is considered a hallmark of neural interaction and is often measured in terms of coherence (Siegel, Donner, & Engel, 2012). Coherence reflects two components: power comodulation and phase synchronization. Power comodulation quantifies how well the amplitude of a frequency band in one region corresponds to the amplitude in a second region and is measured in terms of power in the cross spectrum. Phase synchronization quantifies how well the phase of an oscillation in one region predicts the phase in another region and is measured by phase coherence. High phase coherence does not mean that the phases of 2 oscillations are completely aligned, but rather that the phases are consistently aligned. Indeed, two regions with high phase coherence commonly have a slight phase offset. Phase offset between regions can adjust for conduction delays, ensuring that spikes from the one region reach the second during the preferred oscillatory phase, or it can prevent noise in a third region from contaminating the signal (Juergen Fell & Axmacher, 2011; Fries, 2005).

The complex stimulation pattern used to study amygdala-mediated enhancement of memory in this dissertation was chosen to mimic theta-gamma cross-frequency coupling, which is commonly observed in the hippocampus (Belluscio, Mizuseki, Schmidt, Kempter, & Buzsáki, 2012; Buzsáki & Wang, 2012). Specifically, in theta-gamma phase-amplitude coupling, the amplitude of gamma oscillations increases during specific phases of the theta cycle. The strength of theta-gamma phase-amplitude coupling has been linked to memory performance on spatial tasks and on object recognition memory tests in multiple studies (Shirvalkar, Rapp, & Shapiro, 2010; Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009; Trimper, Stefanescu, & Manns, 2014).

1.3.2 Gamma oscillations in memory

Neurons tend to fire during specific phases of the gamma cycle (Juergen Fell & Axmacher, 2011), suggesting that pyramidal cell spiking is only permitted during brief temporal windows (Fries, Nikolić, & Singer, 2007). These windows may be imposed by the rhythmic inhibition of fast-spiking interneurons (Buzsáki & Wang, 2012). As a result, pyramidal outputs and inputs are clustered into discrete time bins (less than 20 ms), and clustering of spikes into these time bins may facilitate spike-timing dependent plasticity (Bi & Poo, 1998; Jutras & Buffalo, 2010). Spike-timing dependent plasticity is a form of activity-dependent synapse modification that contributes to LTP and is thought to underlie hippocampal-dependent learning (Abbott & Nelson, 2000; Caporale & Dan, 2008). In essence, spike-timing dependent plasticity occurs when a neuronal spike reaches a second neuron and the second neuron fires shortly thereafter. Under such conditions, the second neuron will be more likely to fire in response to the future signals from the first neuron. An important implication of spike-timing dependent plasticity is that spikes clustered together in close temporal proximity will be more likely to elicit a response from a post-synaptic neuron than spikes that are more temporally dispersed (Abbott & Nelson, 2000). Thus, increases in power and coherence in gamma frequency bands may correspond to epochs of enhanced neural plasticity. The relationship between memory for items and gamma synchrony within the hippocampus has been demonstrated in rodents (Trimper et al., 2014), nonhuman primates (Jutras, Fries, & Buffalo, 2009), and humans (J Fell et al., 2001; Sederberg, Kahana, Howard, Donner, & Madsen, 2003).

Neural activity across different regions can synchronize within different gamma frequency sub-bands to support learning and memory. For example, the BLA may modulate plasticity in the rhinal cortices and the striatum by synchronizing neural activity in the low gamma band, below 60Hz (Paz et al., 2006; Popescu, Popa, & Pare, 2009). Rhythmic synchronization of activity in the entorhinal cortex and CA1 at high gamma frequencies, above 60 Hz, is thought to reflect transfer of information from the neocortex into the hippocampus (Colgin et al., 2009), while low gamma synchronization between CA3 and CA1 corresponds to greater intra-hippocampal communication (Colgin et al., 2009; Trimper et al., 2014). The dichotomy between low and high gamma in the hippocampus may correspond to the encoding of new information in the hippocampus and to the retrieval of old information from previously formed neural ensembles within the hippocampus, respectively. Thus, one hypothesis is that memory encoding occurs during bouts of high gamma, while bouts of low gamma correspond to memory retrieval (Carr, Karlsson, & Frank, 2012; Colgin & Moser, 2010). However, this argument is challenged by the finding that memory performance in a novel object recognition memory task improves with increased low gamma coherence between CA3 and CA1 during item encoding (Trimper et al., 2014). Either way, synchronization of neuronal spiking within the gamma frequency band likely plays a fundamental role in memory formation (Jutras et al., 2009; Paz et al., 2006; Popescu et al., 2009).

1.4 Rationale for Electrophysiology

Electrophysiological methods of data collection can accurately and reliably detect neural computations that occur on a millisecond and sub-millisecond timescale. Therefore, this method was chosen to investigate how the amygdala modulates hippocampal function to enhance memory. All electrophysiological data were obtained via electrodes that were stereotaxically implanted and lowered down slowly into a thin layer of pyramidal cells in the hippocampus over the course of 1-3 months. In general, the extracellular signal can be split into two types of data streams: continuous LFP data and discrete spike data. A low frequency band pass filter (1 – 400 Hz) is used to collect the LFP data, and a high pass filter (600 – 6000 Hz) is used to collect spike data. Single-contact electrodes are adequate for recording LFPs, but electrodes with 4 adjacent contacts, known as tetrodes, are necessary to maximize the yield of spike data from each recording location

The key advantage of recording with tetrodes comes from the close spatial proximity of the contacts. The relative position of individual neurons within 50 μm of the tip can be triangulated in up to 4 dimensions because the recorded amplitude of any spike, or action potential, decreases with distance between the neuronal soma and the recording contact (Buzsáki, 2004). Spikes cluster off into single units, and the activity of each unit can be analyzed in relation to the activity of other single units or in relation to the LFP. In theory, an individual tetrode can record up to 140 single units (Buzsáki, 2004), but in practice this number is much lower.

1.5 Specific Aims

The overall goal of the present body of research is to gain insight into the neurobiological underpinnings of memory enhancement through manipulation of endogenous mechanisms within the brain. The following chapters discuss the relevant findings from 3 specific aims that were designed to address this goal. Each subsequent aim builds on the findings of the previous, constructing a cohesive body of work.

The objective of Specific Aim 1 is to demonstrate amygdala-mediated enhancement of memory only for specific events in a rodent model. To achieve this goal, rats were trained to perform a list-based novel object recognition memory task while receiving brief electrical stimulation to the amygdala. In Specific Aim 2, the objective is to determine whether the hippocampus plays a critical role in amygdala-mediated enhancement of memory for specific events. Finally, the objective of Specific Aim 3 is to characterize how the amygdala modulates hippocampal activity in the service of enhancing memory by recording electrophysiological signals from the hippocampus of rats performing the same memory task as in the previous studies. Amygdala-mediated enhancement of memory was successfully demonstrated in each separate cohort.

The outcomes of this research will have a broad significance relevant to memory enhancement and may further understanding of the abnormal amygdalo-hippocampal interactions that underlie dysfunction in affective disorders, such as PTSD.

Figure 1.1 Basic anatomical model of the extended hippocampal formation with amygdala inputs

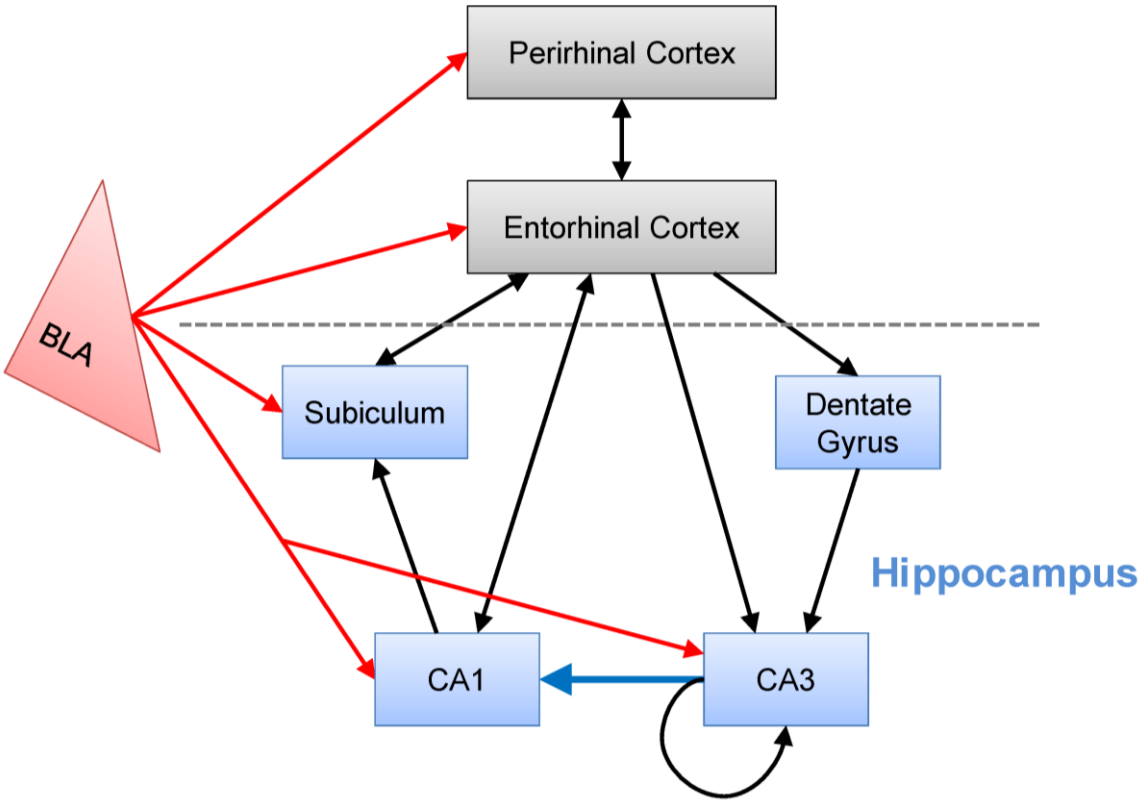

Figure 1.1 Basic anatomical model of the extended hippocampal formation with amygdala inputs. The basolateral complex of the amygdala (BLA, red) sends projections to hippocampus (blue), the entorhinal cortex, and the perirhinal cortex, suggesting that it modulates information processing across the hippocampal formation. One of many routs for information to enter the hippocampus is from the perirhinal cortex via the entorhinal cortex. Information processing in the hippocampus is traditionally modeled as a unidirectional flow along the transverse axis of the hippocampus from dentate gyrus to CA3 to CA1 and back out to the entorhinal cortex via the subiculum, but there are 'shortcuts' that allow information to flow directly from the entorhinal cortex to CA3 and CA1 (Manns & Eichenbaum, 2005). Transmission of information from CA3 to CA1 (blue projection) may represent a key step in memory-related computations as this is the only rout for information to exit the hippocampus from CA3 and dentate gyrus. The recurrent collaterals, or projections from CA3 back to CA3, may generate oscillations that contribute to the gamma power observed in the LFP spectrum of CA3.

Figure 1.2 Functional connectivity between the amygdala and the hippocampus

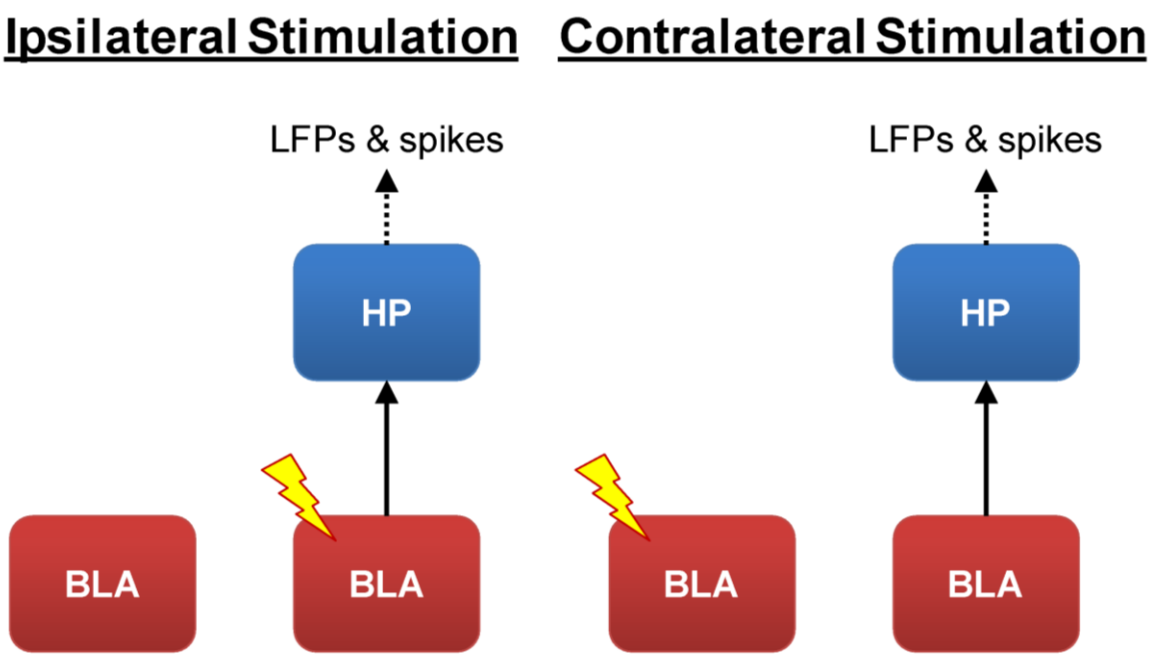

Figure 1.2 Functional connectivity between the amygdala and the hippocampus. The basolateral complex of amygdala (BLA) is ipsilaterally connected with the hippocampus (HP). Thus, electrophysiological recordings can detect the effects of stimulation in the ipsilateral BLA, but the effects of contralateral stimulation will be indiscernible. Stimulation artifact will appear in the signal during stimulation of either amygdala. Lightning bolt indicates stimulation; LFPs = local field potentials.

Figure 1.3 Object recognition memory task: general design

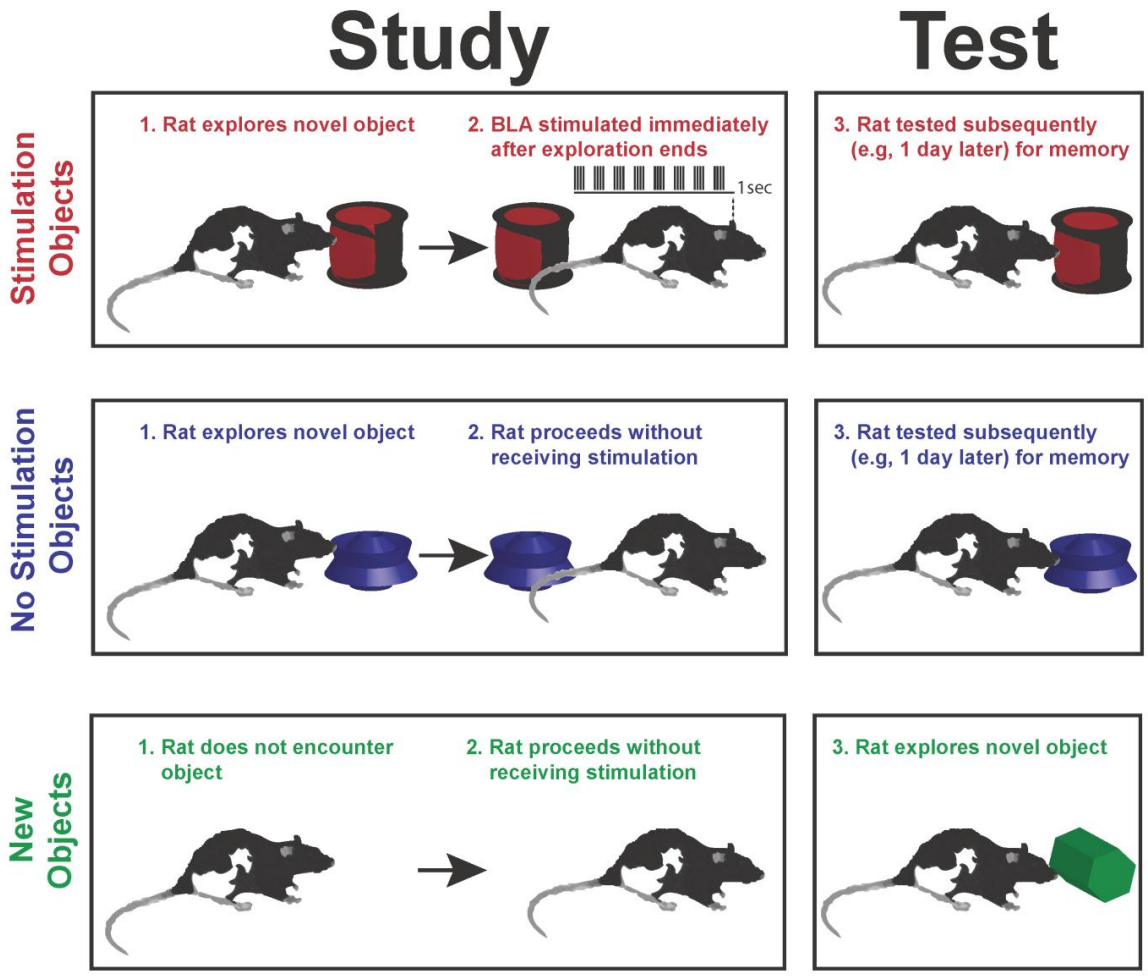

Figure 1.3 Object recognition memory task: general design. Rats encounter a series of objects that they are allowed to freely explore. Immediately following some of these initial encounters (Stimulation objects), rats received brief electrical stimulation to the BLA. For control objects (No Stimulation objects), no stimulation is delivered. Memory for objects is subsequently tested by comparing exploration of repeated objects to exploration of novel objects (New objects).

Chapter 2. Event-Specific Enhancement of Memory via Brief Electrical Stimulation to the Basolateral Complex of the Amygdala in Rats[1]

2.1 Abstract

The basolateral complex of the amygdala (BLA) modulates memory for emotional events, and direct activation of the BLA following a learning session can enhance subsequent memory. Yet optimal enhancement of episodic memory during emotional events would likely require that BLA activation occur close in time to the event and to be brief enough to target specific memories if some events are to be remembered better than others. In the present study, rats were given a novel object recognition memory task in which initial encounters with some of the objects were immediately followed by brief electrical stimulation of the BLA, and these objects were remembered better one day later as compared to objects for which the initial encounter was not followed by stimulation. The results indicated that BLA stimulation can enhance memory for individual events, a necessary ability for the BLA to modulate episodic memory effectively.

2.2 Introduction

The basolateral complex of the amygdala (BLA) has been shown to mediate enhancement of memory for emotional events (McGaugh, 2004). During these events, activation of the BLA can indirectly benefit memory by boosting arousal, attention, or perception (Phelps & Ledoux, 2005), and studies with experimental animals aimed at understanding its direct influence on memory have therefore followed a tradition of manipulating activity in the BLA only after the completion of a learning session so as to set aside the contributions of these other factors (e.g., Paul E Gold et al., 1975; Kesner, 1982; McGaugh & Roozendaal, 2009). This approach has been particularly productive, and these studies have shown that sustained activation of the BLA following the completion of a learning session can enhance subsequent memory (Pare, 2003), even in instances such as object recognition memory in which emotional arousal ordinarily does not make an essential contribution (Roozendaal et al., 2008). These effects on behavior are paralleled by findings indicating that BLA activation can alter synaptic plasticity in the hippocampus, both in studies of rats performing memory tasks (McIntyre et al., 2005), and in studies of long-term potentiation (LTP) induction in anesthetized rats (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995). The efficacy of post-session manipulations, along with evidence of altered plasticity in other brain structures, has suggested that the BLA can enhance memory by modulating cellular processes related to memory consolidation in other brain regions (McGaugh, 2004).

The approach of manipulating activity in the BLA after a learning session has been important for distinguishing the contribution of the BLA to memory from its

contribution to emotional arousal. However, the approach departs from the activation of the BLA that ordinarily occurs in emotional events in that, in experimental post-session manipulations, the BLA activation is removed from the time of the event and is typically rather long in duration due to the frequent use of pharmacological agents to activate the structure. Optimal enhancement of episodic memory during emotional events would likely require that BLA activation occur close in time to the event and to be brief enough to target specific memories if some events are to be remembered better than others. That is, it is difficult to understand how post-session manipulations of the BLA could prioritize memories of specific events in a way that would correspond to what is thought to occur in emotional encounters. Specifically, previous studies using post-session BLA manipulations have shown that the time window for modulating memory can stretch from several minutes up to an hour after the session (P E Gold, Hankins, & Rose, 1977; Vafaei, Jezek, Bures, Fenton, & Rashidy-Pour, 2007), but it remains unclear whether the amygdala can achieve the temporal specificity necessary to modulate memory over the course of seconds to minutes. In addition, it is unclear whether BLA stimulation facilitates plasticity in target structures generally or if the BLA can facilitate plasticity in a way that benefits some memories more than others.

Taken together, these considerations indicate that an important question is whether a briefer and more temporally-targeted activation of the BLA can modulate plasticity with a selectivity that allows for event-specific enhancement of memory. To address this question, rats in the present study were given a novel object recognition memory task in which initial encounters with some of the to-be-remembered objects were immediately followed by brief electrical stimulation of the BLA. When rats were tested

one day later, memory for these objects was enhanced as compared to memory for objects not initially followed by stimulation.

2.3 Method

2.3.1 Subjects

Thirteen adult male Long-Evans rats were tested. Four of these rats were excluded from data analysis after histological inspection revealed that the stimulating electrodes missed their target locations in the basolateral complex of the amygdala. Rats were individually housed (12h light/dark cycle; testing during light phase) with free access to water and were placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

2.3.2 Surgery

Stereotaxic surgery was performed after rats were deeply anesthetized with isoflurane and given buprenorphine as an analgesic. Twisted, bipolar stimulating electrodes (platinum, 0.075 mm diameter, teflon insulation; Plastics One, Roanoke, VA) were aimed bilaterally at the basolateral complex of the amygdala (3.7 mm posterior, 5.2 mm lateral, and 9.2 mm ventral to bregma; Paxinos & Watson, 2007). For 24 hours following surgery, rats were given additional subcutaneous injections of buprenorphine every 8-12 hours as an analgesic and then were allowed an additional 4-6 days to recover.

2.3.3 Electrical Stimulation

During testing, unilateral (half left, half right) electrical stimulation of the BLA was generated by a current generator (S88X Dual Output Square Pulse Stimulator; Grass Technologies, West Warwick, RI) and a stimulus isolator (SIU-BI Stimulus Isolation Unit; Grass Technologies), which sent a constant current of 20 µA in 8 trains of 4 pulses (each pulse = 500 µs biphasic square wave; pulse frequency = 50 Hz; train frequency = 8 Hz) for 1 sec with each press of a hand-held trigger. The electrical stimulation was intended to resemble 50 Hz gamma bursts superimposed on an 8 Hz theta wave.

2.3.4 Novel Object Recognition Memory Task

Rats performed a recognition memory task that was based on rats' spontaneous preference for exploring novel objects more so than repeated objects (Ennaceur & Delacour, 1988). In general, rats encountered new and repeated objects as they completed clockwise laps on a circular track (outside diameter = 91.5 cm; track width = 7 cm). Objects were attached to the outside edge of the track on small platforms. The objects were randomly selected from a collection of plastic, wood, metal, or ceramic junk objects that were typically larger than 10 cm^3 but smaller than 2000 cm^3. Rats were rewarded with a small piece of chocolate on a central runway for completing each lap, irrespective of object exploration. Rats were trained to complete laps for two weeks prior to surgery and were retrained for two weeks following recovery, during which time they were exposed to objects that were not used during testing.

Figure 2.1 shows a schematic of the task design. After completing all 24 trials of the Study Phase, rats immediately began testing for half of the repeated objects on the Immediate Test (mean delay between the initial encounter with an object during study and its repetition during test was 72 mins; range = 40-110 mins). The next day, rats were tested on the remaining half on the 1-Day Test. On each trial of the study phase, rats were presented with three objects, for one of which exploration was immediately (1-3 sec) followed by stimulation of the BLA. On each trial of both tests, rats were presented with 3 objects: a repeated object whose exploration during the Study Phase was followed by BLA stimulation ("Stimulation"), a repeated object whose exploration during the Study Phase was not followed by BLA stimulation ("No Stimulation"), and a novel object ("New"). No stimulation was delivered on either test. Duplicates were used for repeated objects. Objects were randomly assigned to the conditions, and the locations of objects were counterbalanced across trials. During the Study Phase only, two experimenters participated in testing each rat, one who tested the rat and was unaware of group assignment of objects and one who observed the Study Phase from behind a curtain and pressed the trigger to deliver electrical stimulation. An additional session was run in which the procedure was identical to the standard procedure except that the current generator did not deliver electrical stimulation following trigger presses.

2.3.5 Histology

At the end of testing, small marking lesions were made at the tips of the electrodes before rats were euthanized. Brain sections were stained for

acetylcholinesterase to facilitate identification of structures in the BLA. Localization of stimulating electrodes was verified by a second rater who was unaware of rat identity.

2.3.6 Data Analysis

Frame-by-frame (30 frames/sec) analysis of digital video was used to record times when a rat initiated or terminated exploration of an object. A rat was considered to be exploring an object only if the rat was within 2 cm of the object and was showing evidence of active investigation (e.g., sniffing and directed attention). If a rat did not examine all 3 objects of a trial during the Study Phase, the trial was discarded and those objects were not included on either of the tests. Five videos of the 1-Day Test were rescored by a blinded observer, and the median exploration times were well correlated between observers (correlation coefficients of 0.989, 0.992, and 0.996, for Stimulation, No Stimulation, and New objects, respectively).

To assess memory for Stimulation and No Stimulation objects during the Immediate Test and 1-Day Test, a discrimination index was calculated to evaluate the extent to which a rat explored New objects more so than either the repeated Stimulation objects or repeated No Stimulation objects on each test. Specifically, a discrimination index was obtained for each rat by dividing the median New object exploration time by the sum of the median New object and median repeated object exploration time [for Stimulation objects: New/(New + Stimulation); for No Stimulation objects: New/(New + No Stimulation)]. This discrimination index resulted in a number for which 0.50 represented no memory and higher numbers represented better memory for repeated objects. Discrimination index scores were averaged across rats and the means were

compared to a baseline of 0.50 with a one-sample t-test. Discrimination index scores for Stimulation and No Stimulation objects were compared with paired-samples t-tests. Additionally, a stimulation by test interaction was evaluated with a 2X2 repeated measures ANOVA.

2.4 Results

Histology revealed that the tips of the both the left and right stimulating electrodes were located in the BLA (3 in the lateral nucleus, 12 in the basal nucleus, and 3 in the accessory basal nucleus, all between 3.3 mm and 4.4 mm posterior to bregma, Paxinos & Watson, 2007) for the 9 rats included in the data analysis. None of the rats showed signs of stress (vocalizations, defecation, or freezing) or seizures in response to electrical stimulation.

During the Study Phase, all objects were novel, and electrical stimulation was delivered to the BLA only after rats disengaged from exploration. As a result, rats spent a similar amount of time exploring the Stimulation (mean sec ± SEM = 3.39 ± 0.62), No Stimulation (3.27 ± 0.67), and New objects (2.95 ± 0.48).

Figure 2.2A shows the discrimination index scores for the Immediate Test and the 1-Day Test. For the Immediate Test, the scores for Stimulation and No Stimulation objects were both significantly above baseline (mean ± SEM: Stimulation = 0.64 ± 0.03, $t(8) = 4.52$, $p < 0.01$; No Stimulation = 0.63 ± 0.03, $t(8) = 3.98$, $p < 0.01$) and were similar (Stimulation vs. No Stimulation: $t(8) = 0.29$, $p > 0.1$). In contrast, for the 1-Day Test, the discrimination index was significantly above baseline for only the Stimulation

objects (mean ± SEM; Stimulation = 0.67 ± 0.04, $t(8) = 3.83$, $p < 0.01$; No Stimulation = 0.51 ± 0.05; $t(8) = 0.26$, $p > 0.1$), and the discrimination index differed significantly between the groups of repeated objects (Stimulation vs. No Stimulation: $t(8) = 2.85$, $p < 0.05$). Further, a 2X2 repeated measures ANOVA showed a significant stimulation by test interaction ($F(1,8) = 5.06$, $p = .05$). These results suggested that objects were remembered well on the Immediate Test regardless of BLA stimulation but that objects were remembered on the 1-Day Test only when initial encounters with those objects during the Study Phase had been followed by BLA stimulation.

To address the possibility that factors other than the BLA stimulation (e.g., experimenter's expectations) might have influenced the results, a session was conducted in which, unbeknownst to the experimenter placing the objects and handling the rats, the current generator was disconnected from the cable leading to the rat. Figure 2.2B shows that there was no benefit of sham stimulation on the 1-Day Test (mean sec ± SEM: sham Stimulation = 0.40 ± 0.07 vs. sham No Stimulation = 0.51 ± 0.09, $t(8) = 1.89$, $p = 0.1$), suggesting that non-specific factors did not account for the memory-enhancing effect of BLA stimulation. Indeed, memory for sham Stimulation objects was significantly less than for actual Stimulation objects on the 1-Day Test (sham Stimulation vs. Stimulation: $t(8) = 3.02$, $p < 0.05$).

2.5 Discussion

The results of the present study indicated that electrical stimulation of the BLA led to enhanced memory for individual objects when memory was tested one day later.

Objects for which the initial encounter was followed by brief BLA stimulation were remembered well on a test given one day after the study phase, but objects for which the initial encounter was not followed by BLA stimulation appeared to be forgotten by that point. On a test given immediately after the study phase (for which the study-test delay for each object averaged 72 minutes), memory was unaffected by BLA stimulation. The observation that the benefit of BLA stimulation emerged only after a protracted period of time suggests that the stimulation exerted its influence on memory for individual objects by modulating memory processes related to cellular consolidation, a suggestion consistent with a large body of work on the role of the amygdala in emotional memory in both humans and experimental animals (LaBar & Cabeza, 2006; McGaugh & Roozendaal, 2009; McGaugh, 2004) and with studies specifically examining the role of the BLA in enhancing object recognition memory in rats (Roozendaal et al., 2008, 2006).

The present study adds to that body of work by indicating that direct activation of the BLA can target memory for individual events. Previous studies in experimental animals established that sustained activation of the BLA following a learning session could enhance subsequent memory, but this enhancement was thought to have encompassed all trials during the learning session. In some scenarios, a blanket enhancement in memory that extends to all information acquired in the past hour or so would be advantageous (e.g., training on a procedural task). However, in the case of episodic memory, modulation by the BLA would be most effective if the activation could selectively target only the important memories (i.e., those of events that immediately trigger amygdala activation in real-world encounters). The present results obtained with interleaved Stimulation and No Stimulation object encounters indicate that BLA

activation, when brief and temporally-targeted, can indeed prioritize some memories over others. These results correspond well with studies in humans in which indirect activation of the amygdala using emotional images led to item-specific memory enhancement (Anderson et al., 2006) and trial-specific modulation of amygdala activity (Canli et al., 2000).

The observation that BLA stimulation did not affect performance on the immediate test highlights a role for protracted processes related to memory consolidation but also indicates that stimulation did not induce positive or negative dispositions towards objects. Rats explored repeated objects similarly on the immediate test irrespective of whether or not the initial encounter with the object was followed by stimulation of the BLA (mean sec ± SEM; Stimulation = 0.89 ± 0.32; No Stimulation = 0.86 ± 0.27). In a previous study that used a similar task but did not include BLA stimulation (Manns & Eichenbaum, 2009), objects encountered for the sixth time were explored for a much shorter time (their Figure 2.2; mean sec ± SEM = 0.33 ± 0.16). When median exploration times were used to calculate exploration times for each rat in the previous study (in the same manner used in the present study), the exploration time for the sixth encounter averaged across rats was even lower (0.03 ± 0.03 sec), which corresponded to a discrimination score of 0.99 when calculated using median exploration times for novel objects from the previous study (mean of medians = 1.78 ± 0.01). This comparison indicates that the similar exploration of the Stimulation and No Stimulation objects in the present study was not due to a floor effect on exploration times (or to a corresponding ceiling effect on discrimination scores).

It remains unclear exactly what the neuronal effects of BLA stimulation were, and thus the mechanisms of event-specific memory enhancement in the present study are unknown. However, structures in the hippocampal memory system are important for object recognition memory (Clark et al., 2000; Ennaceur, Neave, & Aggleton, 1996), and BLA activation can alter synaptic plasticity in the hippocampus (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995; McIntyre et al., 2005) as well as other cortical regions (Chavez et al., 2009). Thus, one possibility is that BLA stimulation engaged projections from the BLA to the entorhinal cortex, perirhinal cortex, and/or hippocampus (Petrovich et al., 2001; Pitkanen et al., 2000) and that triggering of these projections selectively benefited plasticity in at least one of these targets.

The slow emergence of the memory enhancement (> 1 hour) in the present study would suggest that the BLA stimulation influenced molecular cascades related to late-phase LTP at these synapses, as these cascades have been observed to take hours to unfold (Alberini, 2009). In particular, it has been suggested that BLA activation may not directly impact early-phase LTP in target structures but instead may encourage the transition of synapses in early-phase LTP to late-phase LTP (Akirav & Richter-Levin, 1999b; Ikegaya et al., 1995). For example, a previous study in anesthetized rats found that BLA stimulation following LTP induction had minimal impact on LTP at 1 hour but reinforced maintenance of LTP beyond 1 hour (Frey et al., 2001), findings that parallel the current behavioral results. Thus, BLA stimulation may be able to facilitate late-phase LTP at specific synapses by impacting only synapses at which early-phase LTP was most recently initiated by an object encounter event. Regardless of the mechanism, the finding in the present study of event-specific enhancement of object recognition memory via

brief electrical stimulation of the BLA is an important finding in that it demonstrates the ability of the BLA to target individual events within the stream of incoming information, a necessary ability for the BLA to modulate episodic memory effectively.

Figure 2.1 Schematic of the novel object recognition memory task.

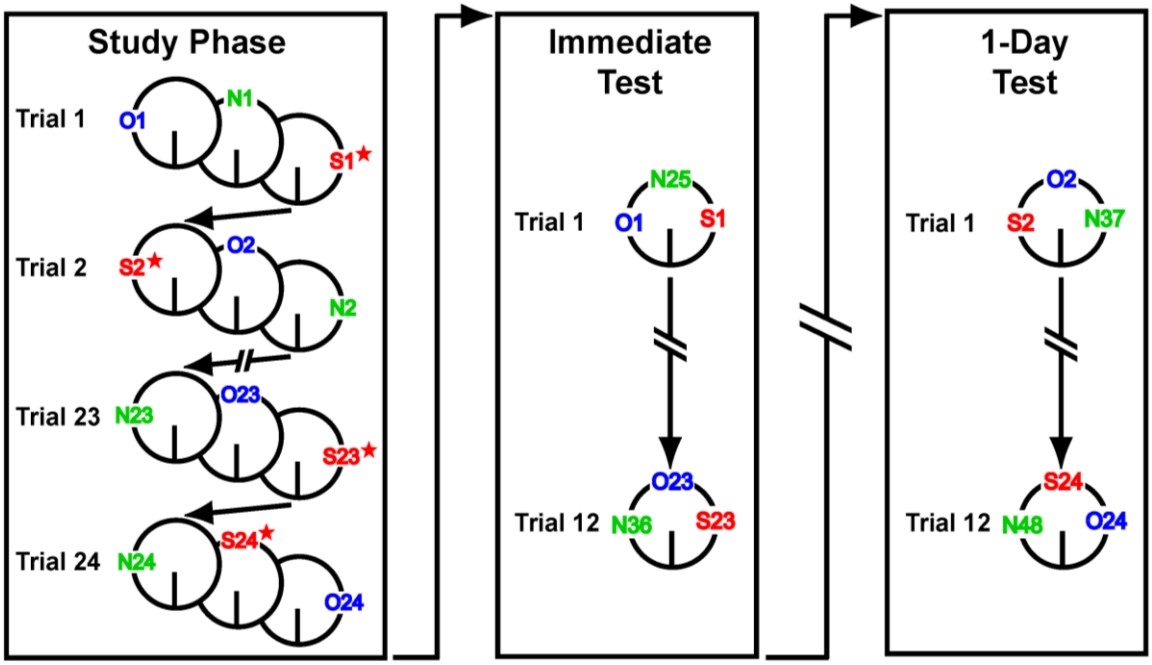

Figure 2.1 Schematic of the novel object recognition memory task. Rats encountered three groups of objects in each phase: Stimulation objects (denoted by an "S"), for which brief electrical stimulation was delivered to the BLA immediately after a rat disengaged from exploration during the Study Phase only and which were repeated during one test, "No Stimulation" objects (denoted by an "O"), which were also repeated during one test, and "New" objects (denoted by an "N"), which were not repeated. Objects presented on the Immediate Test were not included on the 1-Day Test. Objects within a trial were presented on the same lap during the Immediate Test and during the 1-Day Test but were presented on separate laps during the Study Phase in order to better isolate the influence of amygdala stimulation to a particular object. Rats completed a lap on an empty track between each trial on all three phases (see Method for details).

Figure 2.2 Performance on recognition memory tests shown as a discrimination index

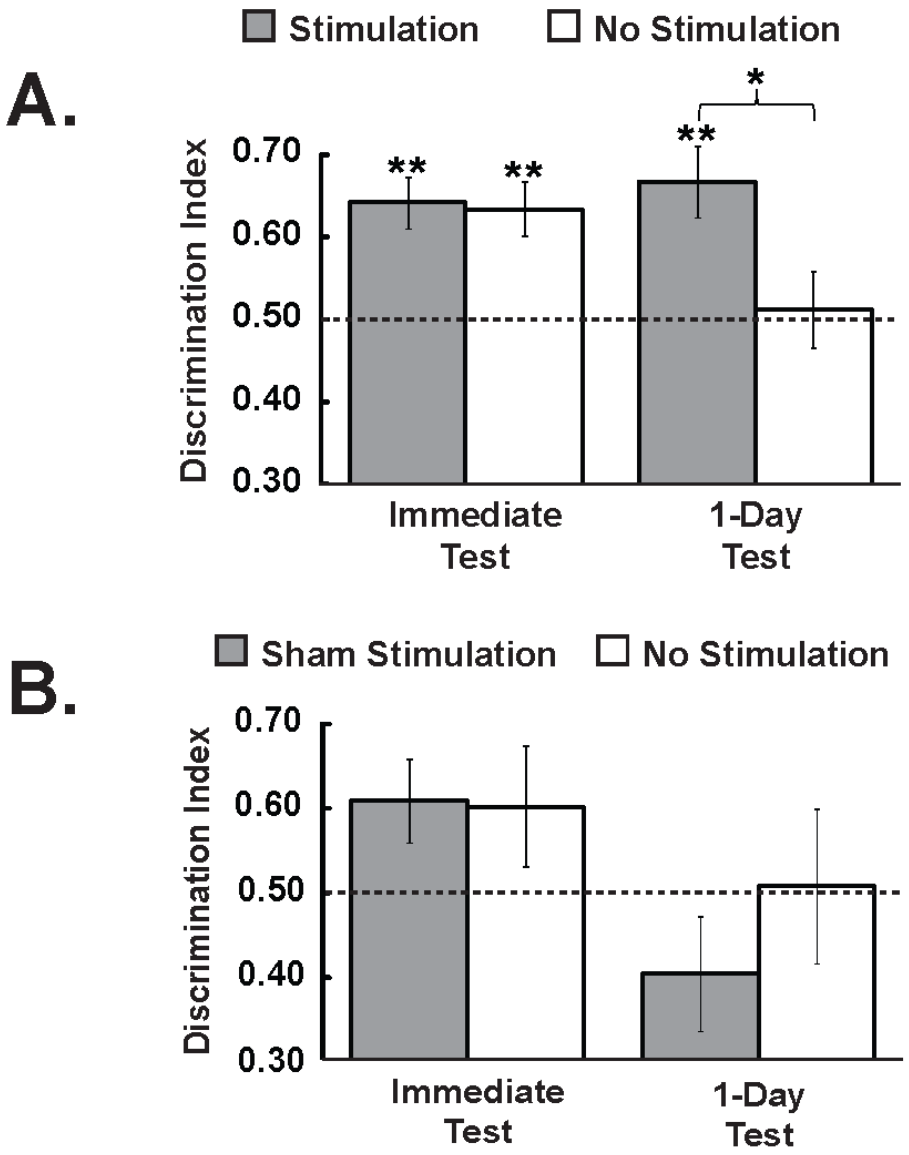

Figure 2.2 Performance on recognition memory tests shown as a discrimination index (n=9). A. Rats remembered repeated objects well during the Immediate Test, but remembered repeated objects during the 1-Day Test only if exploration of those objects during the Study Phase had been followed by brief electrical stimulation of the BLA. **B.** Rats' memory for objects paired with sham stimulation during the Study Phase was significantly worse on the 1-Day Test than rats' memory for objects paired with electrical stimulation during the Study Phase. The dashed line indicates chance performance. Error bars show SEM. * = $p < 0.05$, ** = $p < 0.01$.

Chapter 3. Amygdala-Mediated Enhancement of Memory for Specific Events Depends on the Hippocampus[2]

[2] Adapted from Bass DI, Nizam ZG, Partain KN, Wang A, & Manns JR (2014). Amygdala-mediated enhancement of memory for specific events depends on the hippocampus. *Neurobiology of Learning and Memory, 107, 37-41.*

3.1 Abstract

Emotional events are often remembered better than neutral events, a type of memory prioritization by affective salience that depends on the amygdala. Studies with rats have indicated that direct activation of the basolateral complex of the amygdala (BLA) can enhance memory for neutral events, and if the activation is brief and temporally targeted, can do so in a way that benefits memories for specific events. The essential targets of BLA activation in the case of event-specific memory enhancement were unknown, but the hippocampus was known to receive direct projections from the BLA and to support memory for events. In the present study, rats received counterbalanced infusions of either muscimol, a $GABA_A$ receptor agonist, or saline into the hippocampus prior to performing a novel object recognition memory task during which initial encounters with some of the objects were immediately followed by brief electrical stimulation to the BLA. When memory was tested 1 day later in the saline condition, rats remembered these objects well but showed no memory for objects for which the initial encounter had not been followed by BLA stimulation. In contrast, no benefit to memory of BLA stimulation was observed in the muscimol condition. The results indicated that brief activation of the BLA can prioritize memories for events by enhancing memory for some object encounters but not others and that this benefit to memory depends on interactions between the amygdala and the hippocampus.

3.2 Introduction

Research with humans and experimental animals has shown that moderate emotional arousal tends to improve memory and that this improvement depends on the basolateral complex of the amygdala (BLA; Cahill & McGaugh, 1998; LaBar & Cabeza, 2006; McGaugh, 2004; Paré, 2003). One important implication is that memories for emotional events are often remembered better than neutral events, thereby prioritizing memory by affective salience (L Cahill & McGaugh, 1995; McGaugh, 2013). Numerous studies in rats have demonstrated that post-training activation of the BLA enhances memory consolidation when the BLA is activated shortly after the end of the session (Pare, 2003), including memory not typically considered to contain emotional content, such as memory for novel objects (Roozendaal et al., 2008). However, an important unanswered question had been whether brief activation of the BLA could enhance memory for only some events within a learning session, leaving others unaffected so as to maintain the prioritization of the targeted memories.

To address this question, a recent novel object recognition memory study in rats used brief (1 s) electrical stimulation of the BLA immediately following the offset of object exploration for some objects and found that those objects were remembered well one day later (Bass, Partain, & Manns, 2012). In contrast, control objects encountered in the same session were forgotten by that time. The results indicated that activation of the BLA could indeed prioritize memories for specific events, but the essential targets of the BLA activation were unclear as the BLA has broad connections throughout the brain (Krettek & Price, 1977). One primary candidate was the hippocampus, a structure known

to receive direct projections from the BLA (Petrovich et al., 2001; Pitkanen et al., 2000), to show evidence of upregulated synaptic plasticity following BLA activation (Akirav & Richter-levin, 1999; Frey, Bergado-Rosado, Seidenbecher, Pape, & Frey, 2001; Ikegaya, Saito, & Abe, 1995; McIntyre et al., 2005), and to be important for declarative memory (Squire et al., 2004).

The goal of the present study was to ask whether this BLA-mediated enhancement of memories for specific object encounters depended on the hippocampus. Similar to the previous study (Bass et al., 2012), rats performed a novel object recognition memory task during which brief electrical stimulation of the BLA followed offset of object exploration during the study phase. In addition, during counterbalanced sessions, rats received bilateral infusions of either fluorophore-conjugated muscimol (FCM) or vehicle solution (phosphate-buffered saline, PBS) into the hippocampus prior to the study phase. Muscimol is a $GABA_A$ receptor agonist that inhibits pyramidal neuron activity, and by conjugating the drug with fluorophore, the extent of drug diffusion can be estimated under fluorescent microscopy (Allen et al., 2008; Jacobs, Allen, Nguyen, & Fortin, 2013). The results of the present study replicated the previous findings (Bass et al., 2012), and extended the prior work by showing that the BLA-mediated memory enhancement depended on the hippocampus.

3.3 Method

3.3.1 Subjects

Seventeen adult male Long-Evans rats were individually housed with free access to water (12-hr light-dark cycle; testing during light phase) and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All procedures were approved by the Institutional Animal Care and Use Committee at Emory University.

3.3.2 Surgery

All rats underwent stereotaxic surgery under isoflurane anesthesia to implant twisted, bipolar stimulating electrodes (platinum, 0.075 mm diameter, teflon insulation; Plastics One, Roanoke, VA) bilaterally in the BLA (3.5 mm posterior, 5.2 mm lateral, and 8.9 mm ventral to bregma) and to implant stainless steel guide cannulae (26 gauge; Plastics One, Roanoke, VA) bilaterally just dorsal to the intermediate hippocampus (5.6 mm posterior, 5.2 mm lateral, and 2.0 mm ventral to bregma). Implants were affixed to the skull with dental acrylic, and stylets were then placed into each cannula and projected 1.5-2.0 mm ventrally past the tip in order to maintain patency of the tube. Rats were allowed to recover for at least 1 week prior to training.

3.3.3 Electrical stimulation and muscimol infusions

The parameters for electrical stimulation were the same as those previously used by Bass et al. (2012). A hand-held device was used to trigger a 20 µA current from a current generator (S88X Dual Output Square Pulse Stimulator; Grass Technologies, West Warwick, RI) that was delivered unilaterally to the BLA (half left, half right) through a stimulus isolator (SIU-BI Stimulus Isolation Unit; Grass Technologies). The 1-sec stimulation consisted of 8 trains, each with 4 pulses (biphasic square wave pulse width =

500 µs; pulse frequency = 50 Hz; train frequency = 8 Hz). None of the rats showed signs of acute stress (vocalizations, defecation, or freezing) or seizures in response to electrical stimulation.

All infusions were performed under light anesthesia (0.5-2% isoflurane). A volume of 1 µL was infused bilaterally and simultaneously at a rate of 0.25 µL/min (UltraMicroPump; World Precision Instruments, Sarasota FL) through a 33 gauge injection needle that projected 2.0 mm ventral to the tip of the guide cannula. Injection needles remained in place for an additional 2 min following the infusion. Rats were given 30 min to recover from anesthesia prior to testing. Each rat was tested in a counterbalanced manner, once following an infusion of FCM (0.5 µg/µL) and once following an infusion of vehicle (PBS). No infusions were conducted prior to the 1-Day Test Phase.

3.3.4 Object recognition memory testing

Figure 3.1 shows the procedure for object recognition memory testing, which was similar to the procedure reported in Bass et al. (2012), with the exception that FCM or PBS was infused bilaterally into the hippocampus prior to the Study Phase in the present study. Rats were trained for 2-3 weeks prior to surgery and for 2 weeks following recovery to complete clockwise laps around a circular track (diameter = 91.5 cm, width = 7 cm) for a small food reward. Objects made of plastic, ceramic, metal, or wood were placed on small platforms on the outside of the track at three possible positions. Rats were permitted to explore objects voluntarily, and a reward was given at the end of the lap irrespective of exploration. Immediately following exploration (within 1 sec) of some

objects during the Study Phase, rats received brief electrical stimulation to the BLA (Stimulation objects). Stimulation followed exploration in order to minimize any impact of stimulation on exploration behavior and to align the present experiment with previous studies that targeted memory consolidation. Encounters with Stimulation objects were interleaved with encounters of objects that were not followed by stimulation (No Stimulation objects). Duplicates of both Stimulation objects and No Stimulation objects were then presented along with novel objects (New objects) during the two test phases during which no stimulation was delivered. Memory was tested for half of the objects during the Immediate Test, which occurred immediately following the Study Phase (approximately 45 min after the start of the Study Phase). Memory for the other half of the objects was tested 1 day later on the 1-Day Test.

3.3.5 Histology

In order to provide an estimate of the extent of diffusion, FCM was again infused into hippocampus several days following the final test session using the same procedure as before. In addition, small electrolytic marking lesions were made at the tips of the BLA stimulating electrodes. Rats were then euthanized with an i.p. injection of euthanasia solution (Euthasol) 75 min after the infusion. Rats were perfused transcardially with saline and formalin, and their brains were extracted and subsequently sliced into 3 interleaved series for staining: cresyl violate for general anatomical landmarks, acetylcholinesterase for highlighting the basal nucleus in the amygdala, and no stain for visualization of FCM diffusion using a 529 nm – 576 nm light source (peak FCM absorption, 543 nm; Eclipse TE300 Inverted Microscope; Nikon, Melville, NY).

3.3.6 Video scoring and behavioral data analysis

Using frame-by-frame inspection of video, a rat was judged to be exploring an object if its nose was within 2 cm of the object and the rat was showing evidence of whisking and/or sniffing. Trials on which a rat did not explore the Stimulation object during the Study Phase were excluded as BLA stimulation was to be triggered only at the offset of exploration. This procedure had the potential to artificially increase the exploration times of Stimulation objects relative to other object types. Therefore, only trials in which a rat explored all three object types during the Study Phase were included. Object recognition memory performance during the test phases was calculated as a standard discrimination index that quantified the tendency of rats to explore repeated objects for less time than new objects. Specifically, the mean exploration time for new objects was divided by the sum of the mean exploration time for new and repeated objects [for Stimulation objects: New/(New + Stimulation); for No Stimulation objects: New/(New + No Stimulation)].

3.4 Results

3.4.1 Histology and subject exclusion

Figure 3.2 shows an example of a stimulating electrode marking lesion in the BLA and an example of an FCM infusion in the hippocampus. Five rats were excluded from analyses after histological inspection revealed that either the tip of a stimulating

electrode was outside the BLA or the infusion site extended outside the hippocampus. One rat was euthanized prior to completing the experiment due to an infection near the implant site. Testing was discontinued for two more rats after those rats failed to complete all test sessions after several attempts. All rats were excluded or included before analyzing their behavioral data. For the 9 rats included in the final analysis, the stimulating electrodes were all in the BLA in both hemispheres and were positioned near the basal nucleus specifically. For those nine rats, the FCM diffusion appeared to be approximately 1-2 mm in diameter and contained in the hippocampus in both hemispheres, centered near CA2 in the transverse axis (extending to adjacent portions of CA3, CA1, and dentate gyrus) and at an intermediate region of the septal-temporal axis.

3.4.2 Object recognition memory performance

Figure 3.3 shows performance on the recognition memory task calculated as a discrimination index. A 2x2x2 (stimulation condition by infusion condition by test phase) repeated measures ANOVA revealed a statistically significant 3-way interaction ($F(1,8) = 5.83$, $p<0.05$), a stimulation condition by infusion condition interaction ($F(1,8) = 22.07$, $p<0.01$), and a partial effect of infusion ($F(1,8) = 7.25$, $p < 0.05$). Closer analysis of the data suggested that in the PBS condition, Stimulation and No Stimulation objects were remembered similarly on the Immediate Test but that only the Stimulation objects were remembered on the 1-Day Test. Specifically, on the Immediate Test, the discrimination index scores for Stimulation and No Stimulation objects following infusions of PBS were similar and both significantly above baseline chance levels (mean ± SEM: Stimulation = 0.57 ± 0.03, $t(8) = 2.20$, $p < 0.05$; No Stimulation = 0.57 ± 0.02,

$t(8) = 3.44$, $p < 0.01$). On the 1-Day Test in the PBS condition, discrimination index scores for only the Stimulation objects were significantly above baseline (mean ± SEM: Stimulation = 0.61 ± 0.03, $t(8) = 3.65$, $p < 0.01$; No Stimulation = 0.53 ± 0.03, $t(8) = 0.95$, $p > 0.1$), and the discrimination index scores for Stimulation objects were significantly greater than those for No Stimulation objects ($t(8) = 2.75$, $p < 0.05$). In comparison to the data from the PBS condition, discrimination index scores for Stimulation and No Stimulation objects following infusions of FCM were near chance levels on the Immediate Test (mean ± SEM: Stimulation = 0.47 ± 0.03, $t(8) = 1.03$, $p > 0.1$; No Stimulation = 0.46 ± 0.04, $t(8) = 0.81$ $p > 0.1$). On the 1-Day Test in the FCM condition, the No Stimulation performance was numerically above chance (mean ± SEM = 0.56 ± 0.04), although this difference did not approach statistical significance ($t(8) = 1.46$ $p > 0.1$). It is unclear why the performance for this condition was not closer to chance levels, as was the case for both FCM conditions on the Immediate Test. Nevertheless, performance for Stimulation objects on the 1-Day Test in the FCM condition was very clearly at chance levels (mean ± SEM = 0.50 ± 0.03, $t(8) = 0.00$, $p > 0.1$). Further, on the 1-Day Test, the discrimination index scores for Stimulation objects under PBS were significantly greater than the scores for Stimulation objects under FCM ($t(8) = 2.55$, $p < 0.05$). Thus, the data from the FCM condition indicated that any benefit to memory of BLA stimulation was blocked by inactivation of the hippocampus.

One potential concern was that, during the Study Phase, rats unexpectedly explored Stimulation objects slightly yet significantly more than No Stimulation objects during the PBS (mean ± SEM: Stimulation = 2.43 sec ± 0.21; No Stimulation = 2.09 sec ± 0.24; $t(8) = 2.99$, $p < 0.05$) and FCM conditions (mean ± SEM: Stimulation = 2.89 sec

± 0.34; No Stimulation = 2.11 sec ± 0.27; $t(8) = 3.67$, $p < 0.01$) and that this extra time might have influenced subsequent memory performance. The cause of these differences was not entirely clear, and the similar memory performance between Stimulation and No Stimulation objects on the Immediate Test would suggest that these slight exploration time differences during the Study Phase were not sufficient to account for the large differences observed between Stimulation and No Stimulation objects on the 1-Day Test in the PBS condition. Nevertheless, to ask whether increased object exploration times during the Study Phase could relate to better memory performance (i.e., decreased exploration times) during the Immediate Test or 1-Day Test, exploration times from the Study Phase were correlated with exploration times from each test for each rat. The resulting Pearson's r values were converted to z scores to permit averaging across rats and to permit t-tests against chance (z score of 0). No significant negative correlations were observed that would have suggested that the improved test performance generally related to slight increases in Study Phase exploration times. Indeed, the only correlations that were statistically significant were positive correlations, which would strongly suggest that any differences in exploration times during the Study Phase could not account for improved memory for the Stimulation/PBS objects on the 1-Day test (mean z ± SEM: Stimulation/PBS/Immediate = -0.06 ± 0.29, $t(8) = 0.65$, $p > 0.1$; No Stimulation/PBS/Immediate = 0.32 ± 0.35, $t(8) = 2.71$, $p < 0.05$; Stimulation/FCM/Immediate = 0.29 ± 0.46, $t(8) = 1.85$, $p = 0.1$; No Stimulation/FCM/Immediate = 0.55 ± 0.90, $t(8) = 1.85$, $p = 0.1$; Stimulation/PBS/1-Day = 0.24 ± 0.33; $t(8) = 2.11$, $p < 0.1$; No Stimulation/PBS/1-Day = 0.31 ± 0.54; $t(8) = 1.71$,

$p > 0.1$; Stimulation/FCM/1-Day = 0.49 ± 0.32; $t(8) = 4.56$, $p < 0.01$; No Stimulation/FCM/1-Day = 0.15 ± 0.35; $t(8) = 1.26$, $p > 0.1$).

3.5 Discussion

The present results replicated and extended prior results from a related study (Bass et al., 2012). In the PBS condition of the present study, brief electrical stimulation to the BLA following offset of exploration of novel objects resulted in those objects being remembered well one day later. In contrast, object encounters not followed by BLA stimulation appeared to be forgotten by that time. Consistent with the idea that BLA activation enhances memory consolidation specifically, BLA stimulation did not appear to influence performance when memory was tested immediately. The results extended the previous results by indicating that the BLA-mediated memory enhancement depended on the hippocampus. Taken together, the results indicated that brief activation of the BLA can prioritize memories for events by enhancing memory for some object encounters but not others and that this benefit to memory depends on interactions between the amygdala and the hippocampus.

There are direct and indirect connections between the BLA and the hippocampus (Petrovich et al., 2001; Pitkanen et al., 2000), and BLA stimulation can modulate plasticity in cortical areas outside the hippocampus (Chavez et al., 2009; Paz et al., 2006) as well as in the hippocampus (Akirav & Richter-Levin, 1999a; Frey et al., 2001; Ikegaya et al., 1995; E. J. Kim, Kim, Park, Cho, & Kim, 2012; McIntyre et al., 2005). A common view is that BLA activation affects signaling cascades related to late-phase LTP in

numerous brain regions in support of memory consolidation (Bergado et al., 2011; McGaugh, 2004; Pare, 2003). For example, one previous study found that BLA stimulation following LTP induction in the hippocampus in anesthetized rats had minimal impact on LTP at 1 hour but reinforced maintenance of LTP beyond 1 hour (Frey et al., 2001). In two studies of rats performing an inhibitory avoidance task (McIntyre et al., 2005; Holloway-Erickson et al., 2012), activation of noradrenergic receptors in the BLA led to improved memory performance and increased expression of proteins associated with synaptic plasticity (CaMKIIα and/or Arc) in the hippocampus and rostral anterior cingulate cortex. Thus, although the current data suggested that BLA stimulation in the PBS control condition directly or indirectly led to essential changes in hippocampal plasticity-related signaling pathways, the hippocampus may represent one of several potential targets of BLA activation for object recognition memory. In any case, as muscimol is biologically active for only a few hours (Arikan et al., 2002), the impact of BLA stimulation on the hippocampus must have occurred within a few hours of encountering the objects. Indeed, given that Stimulation and No Stimulation objects were often encountered less than a minute apart in the Study Phase, it is likely that these signaling cascades in the hippocampus were triggered by BLA stimulation almost immediately and were rather specific to the particular object encounter.

The similar performance for Stimulation and No Stimulation objects during the Immediate Test in the PBS condition indicates that, although the signaling cascades for upregulated plasticity in the hippocampus were likely triggered rapidly, the benefit to memory was not visible until much later, presumably when the extended process of cellular consolidation (Alberini, 2009; Dudai, 2004) had more fully unfolded. This

similar performance also suggests that the BLA stimulation did not lead to any fear-related associations with the objects, as one would have expected rats to avoid these objects on the Immediate Test if that were the case. Indeed, electrical stimulation of the BLA has been shown to be ineffective for inducing conditioned fear in rats (E. J. Kim et al., 2013). A second finding from the Immediate Test is that rats did not demonstrate memory for any objects following infusions of FCM. Thus, the role of the hippocampus in the present study was not specific to memory consolidation. Indeed, the present results suggest that, although the hippocampus-dependent enhancement of memory consolidation by activation of the BLA emerges up to a day later, the hippocampus may also play a more general role in memory for object encounters at earlier time points. Future research will be needed to understand how BLA activation can intervene in these hippocampal processes to tag some memories for priority consolidation.

Figure 3.1 Schematic of infusion and object recognition memory procedures

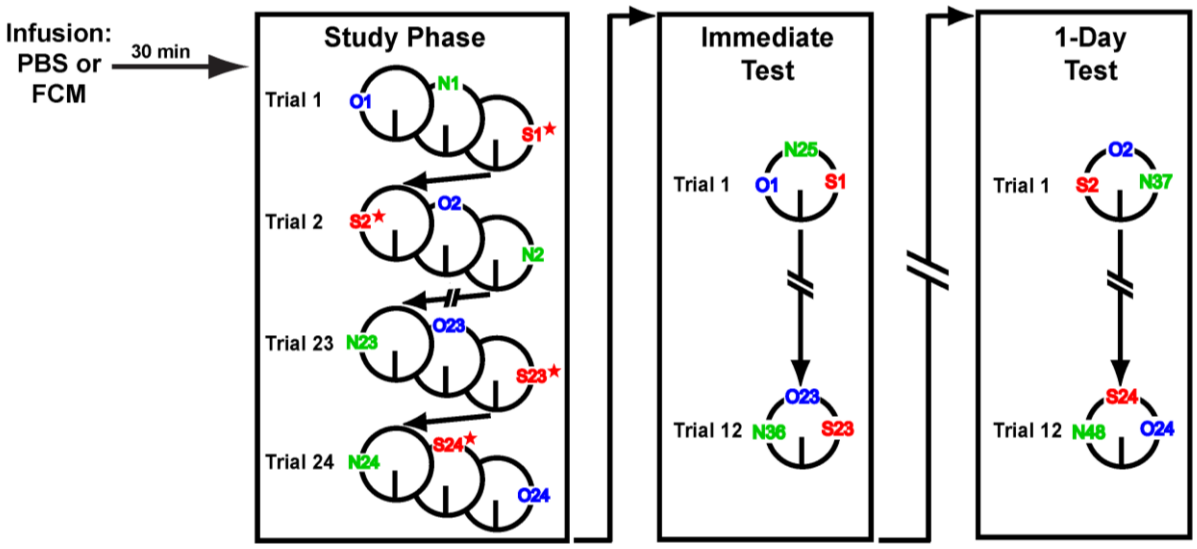

Figure 3.1 Schematic of infusion and object recognition memory procedures. Rats were infused with FCM or control vehicle (PBS) 30 minutes prior to testing. In each trial of the Study Phase, rats encountered 3 novel objects such that there was one object from each group of objects (Stimulation objects denoted by an "S", No Stimulation objects denoted by an "O", New objects denoted by an "N"). All objects were presented on individual laps to better isolate the influence of electrical stimulation to the amygdala. Electrical stimulation (denoted by a star) was delivered immediately following encounters with Stimulation objects. Memory for objects from half of the trials was tested during the Immediate Test, and memory for objects from the other half was tested during the 1-Day Test. Corresponding test trials contained duplicates of Stimulation and No Stimulation objects, but New objects were replaced by additional novel objects. All 3 objects were presented together on one lap. No stimulation was delivered during either test phase.

Figure 3.2 Example photomicrographs of histology

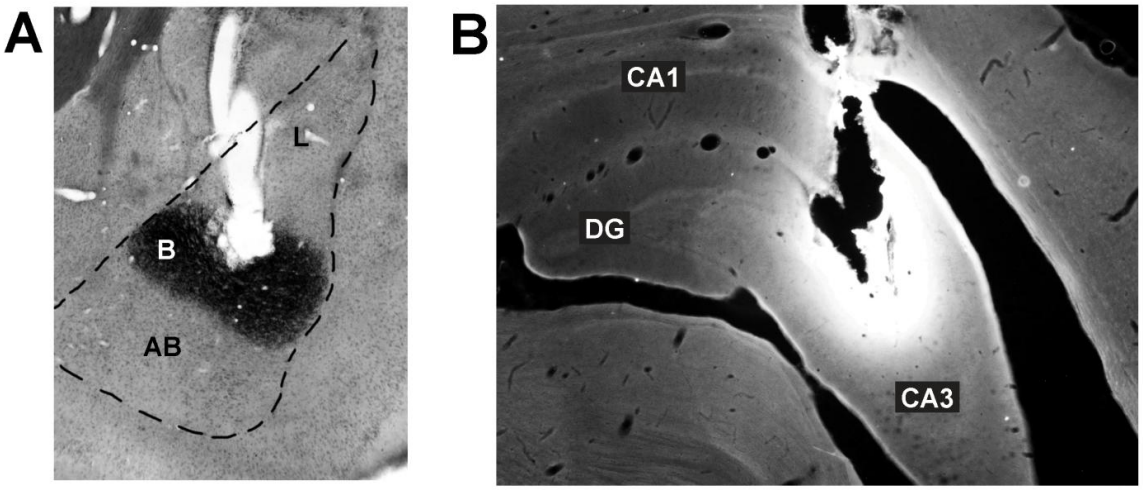

Figure 3.2 Example photomicrographs of histology. **A.** Stimulating electrode localization in the BLA (dashed outline) as shown on tissue stained for acetylcholinesterase. The tips of all electrodes for all rats included in the analyses were contained in the BLA (lateral nucleus, L; basal nucleus, B; accessory basal nucleus, AB). **B.** Fluorescent image of an infusion of FCM into the hippocampus. Infusions of FCM for all rats included in the analyses appeared to be contained within the hippocampus. The tips of all injection cannulae were localized near CA2 (between CA1 and CA3 fields) in the transverse axis and at an intermediate region of the septal-temporal axis of the hippocampus.

Figure 3.3 Performance on recognition memory tests following infusions of FCM or control vehicle (PBS) represented as a discrimination index.

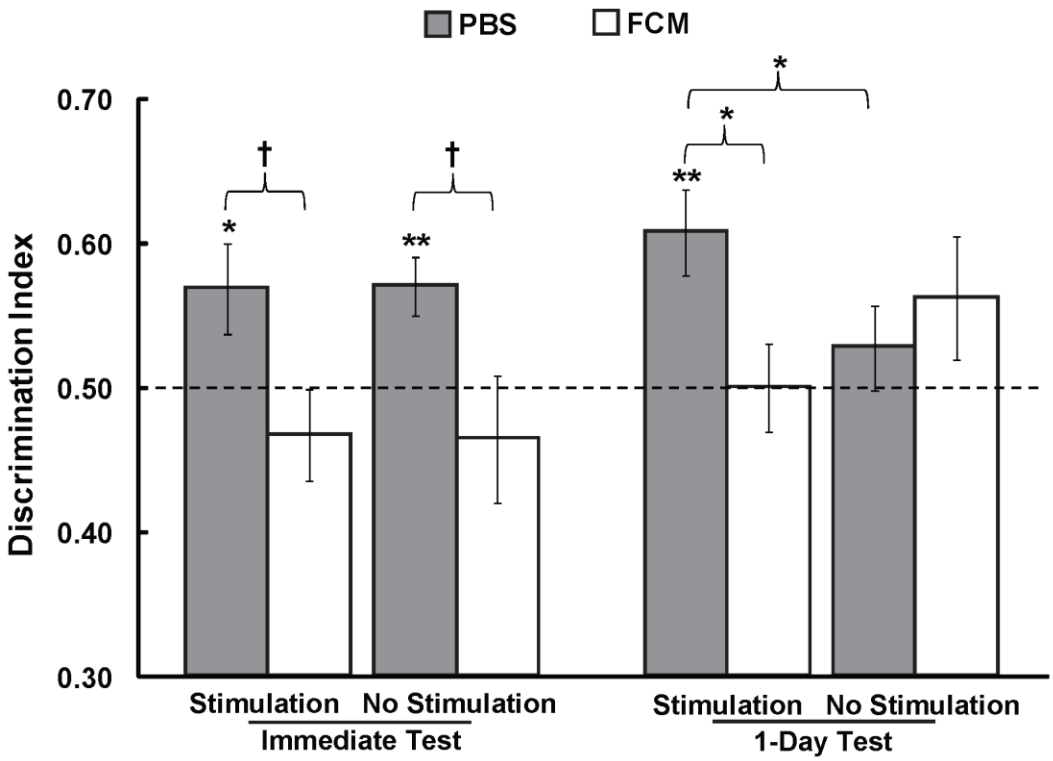

Figure 3.3 Performance on recognition memory tests following infusions of FCM or control vehicle (PBS) represented as a discrimination index. Following infusions of PBS into the hippocampus, rats demonstrated memory for both groups of repeated objects during the Immediate Test but showed memory for only Stimulation objects on the 1-Day Test. Following infusions of FCM into the hippocampus, rats did not demonstrate memory for objects in either test phase. The dashed line indicates chance performance. Error bars show SEM. * = $p < 0.05$, ** = $p < 0.01$, † = $p < 0.1$.

Chapter 4. Memory-Enhancing Electrical Stimulation of the Amygdala Elicits Gamma Band Synchronization between CA3 and CA1 in the Hippocampus

4.1 Abstract

Prioritizing consolidation of important information is key to survival. Emotional arousal can activate the basolateral complex of the amygdala (BLA), which can enhance consolidation of memory for these events. Research in rats has shown that directly manipulating the BLA can enhance memory for non-arousing, emotionally-neutral events, as well. We previously reported that brief electrical stimulation of the BLA in rats enhanced object recognition memory in a stimulus-specific manner (Bass et al., 2012) and that this amygdala-mediated enhancement of memory depends on the hippocampus (Bass, Nizam, Partain, Wang, & Manns, 2014). The goal of the present study was to determine how activation of the amygdala modulates the hippocampus in the service of enhancing memory. To address this question, local field potentials and spiking data were recorded in the hippocampus of rats that performed the novel object recognition memory task used in previous work. Electrical stimulation was delivered to the BLA immediately following some of the initial object encounters and enhanced memory for these objects when memory was tested 1-day later. Analyses of the electrophysiological data indicated that BLA stimulation elicited coherence in the low gamma band (less than 60 Hz) between CA3 and CA1 ipsilateral to the side of stimulation, and further, that CA3 pyramidal spikes had a strong phase preference relative to low gamma oscillations in CA1. These findings are consistent with a large body of literature on gamma oscillations and memory, and they provide evidence of a causal link between gamma synchronization and memory consolidation.

4.2 Introduction

Important information can be prioritized for long-term consolidation via emotional activation of the amygdala (Cahill et al., 1996; McGaugh, 2004; Roozendaal et al., 2006). The mechanisms underlying amygdala-mediated enhancement of memory have been extensively investigated by directly manipulating the amygdala shortly following a training session (Gold et al., 1975; McGaugh & Roozendaal, 2009; McIntyre et al., 2005; Packard et al., 1994). Results from these studies and studies on hippocampal plasticity in anesthetized rats (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995) indicate that the amygdala modulates the hippocampus and that modulation of the hippocampus is important for memory enhancement in tasks that normally engage the hippocampus. Previous studies have shown that the amygdala can enhance memory in a stimulus-specific manner and that this amygdala-mediated enhancement of memory depends on the hippocampus (Bass et al., 2014, 2012). However, it remains unclear precisely how the amygdala prioritizes specific memories for enhanced long-term consolidation.

Different brain regions can communicate through rhythmic synchronization of neural activity (Buzsáki & Draguhn, 2004; Juergen Fell & Axmacher, 2011; Fries, 2005; Womelsdorf et al., 2007). In particular, gamma frequency (30 – 90 Hz) synchronization is thought to be important for learning and memory as a strong spike-phase preference in the gamma range clusters spikes into narrow temporal windows (~20 ms), which can facilitate spike-timing dependent plasticity (Bi & Poo, 1998; Buzsáki & Wang, 2012; Jutras & Buffalo, 2010). Indeed, numerous studies have shown that synchronization of

hippocampal activity within the gamma frequency band corresponds with good memory (Colgin et al., 2009; Jutras et al., 2009). Recently, low gamma (less than 60 Hz) coherence between CA3 and CA1 was shown to correlate with improved object recognition memory (Trimper et al., 2014). Thus, one hypothesis is that amygdala activation promotes processes related to late-phase LTP in the hippocampus, and one mechanism is increasing spike-timing dependent plasticity via oscillatory-mediated spike timing.

To test this hypothesis, we recorded local field potentials (LFPs) and spiking activity from rats that received amygdala stimulation while performing a novel object recognition task that depends on the hippocampus. Brief electrical stimulation to the amygdala immediately following some of the initial object encounters enhanced memory for these objects when memory was tested 1-day later. Electrophysiological analyses took advantage of the ipsilateral BLA-hippocampus connectivity and contrasted hippocampal responses during ipsilateral and contralateral stimulation. The results indicated that hippocampal synchrony between CA3 and CA1 markedly increased in the low gamma range during ipsilateral stimulation. The results also indicated that CA3 pyramidal spikes had a strong phase preference relative to low gamma oscillations in CA1 during ipsilateral stimulation. Overall, the results strongly suggest that amygdala stimulation enhances memory by augmenting mechanisms of spike-timing dependent plasticity in the hippocampus.

4.3 Method

4.3.1 Subjects

Nine adult male Long-Evan rats were housed individually (12-hr 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 body weight (~400 g). All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

4.3.2 Novel object recognition memory task

4.3.2.1 Behavioral testing

Rats were trained for 2-3 weeks before surgery to complete clockwise laps around a circular track (diameter = 91.5 cm, width = 7 cm), and training was resumed following a 1-2 week recovery period following surgery. Figure 4.1 shows a schematic of the novel object recognition memory task. The procedure was the same as that used in Bass et al. (2012) and Bass et al. (2014). Briefly, rats were allowed to freely explore a series of objects as they completed laps around the track for a small food reward. Objects between 10 cm^3 and 2000 cm3 in volume were placed on one of 3 small flaps around the outside perimeter of the track. During the Study Phase, some of the object encounters were followed by brief electrical stimulation to the BLA (Stimulation objects), while other objects were not (No Stimulation objects). Memory for half of these objects was tested immediately after the end of the Study Phase (Immediate Test). Memory for the other half was tested approximately 24 hours later (1-Day Test). On both tests, objects from the Study Phase were repeated in the same location using identical duplicates to avoid scent marking. Memory was assessed for repeated objects on both tests by comparing

exploration of repeated objects to exploration of novel objects (New objects). Stimulation was always unilateral, though rats received stimulation to both the left and right BLA across trials throughout the Study Phase.

4.3.2.2 Video scoring and behavioral analysis

All behavioral sessions were recorded on digital video (30 frames/sec) for frame-by-frame analysis of exploration. A rat was considered to be exploring an object if the rat was within 2 cm of the object and showing signs of active investigation (e.g. whisking and directed attention), but exploration was not scored if the rat climbed over the object to sniff the air. This method of analyzing behavior has been shown to have high inter-rater reliability (Bass et al., 2012; Galloway, Lebois, Shagarabi, Hernandez, & Manns, 2014). A trial was not included for analysis if the rat skipped any of the objects during the Study Phase. Exploration times were averaged for each group of objects and used to calculate a discrimination index for each rat. The discrimination index is a standard means of quantifying memory on an object recognition memory task. It is calculated by dividing the exploration of novel objects on the test by the sum of the exploration for novel objects and repeated objects [for Stimulation objects: New/(New + Stimulation); for No Stimulation objects: New/(New + No Stimulation)]. Discrimination scores were compared with paired-samples *t*-tests to determine whether there was a significant difference between memory for Stimulation objects and No Stimulation objects.

4.3.3 Surgery and tetrode positioning

Stereotaxic surgery was performed under isoflurane (1-3% in oxygen) on all rats to implant twisted bipolar stimulating electrodes (platinum, 0.0075 mm diameter, Teflon

insulation, Plastics One, Roanoke, VA) bilaterally into the BLA (3.5 mm posterior, 5.2 mm lateral, and 8.9 mm ventral to Bregma) and a chronic recording assembly with independently movable tetrodes over the hippocampus. The intermediate third of the longitudinal axis of the hippocampus was targeted because it receives projections from the BLA (Petrovich et al., 2001; Pitkanen et al., 2000), plays a critical role for rapid learning in the hippocampus (Bast, Wilson, Witter, & Morris, 2009) , and is necessary for the brief electrical stimulation of the BLA to be effective in enhancing memory (Bass et al., 2014). Four nichrome wires were twisted together to make tetrodes, and each contact was plated with gold to reduce the impedance to ~200 kΩ at 1 kHz. A ground wire was attached to a stainless steel screw implanted midline over the cerebellum. Buprenorphine (0.05 mg/kg) was administered as an analgesic immediately before and after surgery, and again 12 hrs later. Oral meloxicam (1 mg/kg) was administered when rats awoke following surgery, and again at 24 and 48 hrs post-surgery. Rats were allowed to recover over the next 1-2 weeks, and tetrodes were slowly lowered into the pyramidal cell layer of CA3 and CA1 over the following 1-2 months in ~60 μm increments. Tetrode localization was assisted by the electrophysiological hallmarks of CA3 and CA1. Testing occurred at least 24 hrs after the last tetrode adjustment, and tetrodes were not adjusted between the Study and the 1-Day test.

4.3.4 Electrical stimulation

The electrical stimulation parameters were the same settings as those used in previous studies (Bass et al., 2014, 2012). A hand held device was used to manually trigger a 20 μA current from a current generator (S88X Dual Output Square Pulse

Stimulator, Grass Technologies, West Warwick, RI). Stimulation was delivered unilaterally for 1 sec in 8 trains of 4 pulses (train frequency = 8 Hz, pulse frequency = 50 Hz, biphasic pulse width = 500 μs). None of the rats showed signs of acute stress (e.g., vocalizations, defecation, urination, or freezing) or developed seizures. For most electrophysiological analyses, the biological effects of stimulation were disambiguated from the stimulation artifact by contrasting the results of ipsilateral and contralateral stimulation. It was reasoned that the impact of the stimulation artifact should be present during both ipsilateral and contralateral stimulation, but because BLA-hippocampal connectivity is ipsilateral, the biological effects of BLA stimulation should be much more prominent during ipsilateral stimulation. As an additional control, a subset of rats were stimulated under general anesthesia (2% isoflurane in oxygen) and using an 80 Hz pulse frequency (5 pulses per train, train frequency = 8 Hz) as they completed laps around the circular track.

4.3.5 Histology

Rats were anesthetized prior to euthanasia, and a 20 μA current was passed through each stimulating electrode and recording tetrode for 20 sec creating an electrolytic lesion at the tips of the electrodes for post mortem localization. Brain sections containing tracts from the stimulating electrodes were stained for acetylcholinesterase to facilitate identification of structures in the BLA. The remaining sections were stained with cresyl violate to facilitate visualization of general anatomical landmarks for localization of the recording tetrodes.

4.3.6 Data acquisition and analysis

4.3.6.1 Acquisition of electrophysiological data and spike sorting

Local field potentials (LFPs; sampling rate = 1.5 kHz; bandpass filter = 1-400Hz) and spike data (sampling rate = 30 kHz, bandpass filter = 600-6000 Hz) were obtained with the NSpike data acquisition system (nspike.sourceforge.net), and spike sorting was performed offline using software (Offline Sorter, Plexon Inc., Dallas, TX). Clusters of spikes were manually defined based on waveform shape and amplitude across all 4 wires. Only clusters with clear boundaries were included in the data set. Putative pyramidal units were distinguished from interneurons based on firing rates and autocorrelograms, as these measures have been well characterized for pyramidal units and interneurons in the hippocampus (Csicsvari, Hirase, Czurko, & Buzsáki, 1998; Fox & Ranck, 1981). The remaining analysis were all performed in Excel (Microsoft, Seattle, WA) and in MATLAB (MathWorks, Ntick MA) with custom scripts and Chronux, an open-source toolbox for analysis of LFPs and spike data (Bokil, Andrews, Kulkarni, Mehta, & Mitra, 2010).

4.3.6.2 Artifact reduction

Each 0.5 ms pulse of electrical sitmulation in both the ipsilateral and contralateral BLA produced a large electrical artifact of approximately 3-4 ms in the local field potentials recorded by tetrodes in the hippocampus. To address this issue, a template was calculated for each pulse within a train (4 templates) as the median artifact from all ipsilateral or contralateral trials within a session and subtracted from the LFP. Similar algorithms have been used in previous studies (e.g., Montgomery, Gale, & Huang, 2005; Wichmann, 2000) to compensate for the stimulation artifact. This approach was chosen to

minimize the artifact while preserving as much of the signal as possible. All further analyses of the LFP during stimulation were performed following artifact reduction.

4.3.6.3 Hippocampal LFP synchrony

LFPs for each rat were obtained from a tetrode localized to the pyramidal layer in the distal half of CA1 and from a tetrode in the pyramidal layer in the proximal half of CA3 to minimize rat-to-rat variability and because proximal CA3 and the BLA project directly to distal CA1 (Petrovich et al., 2001; Pikkarainen et al., 1999; van Strien et al., 2009). Multi-taper fast Fourier transforms were performed similar to previous work (Trimper et al., 2014). The multi-taper fast Fourier transform was used to quantify coherence, phase offset, cross spectral power, CA1 power, and CA3 power. Moving time window coherograms and spectrograms were calculated with a sliding time window of 0.5 sec and 0.05 sec steps. A frequency bandwidth ± 6 Hz was used. Phase coherence was derived from moving time window estimates of the phase offset to determine whether increased phase consistency contributed to increases in coherence. Specifically, phase offset (Φ) was represented as a vector ($e^{i\Phi}$) for each trial, and then the phase coherence was calculated as the squared complex magnitude of the mean resultant vector, resulting in a number between 0 and 1 (Hurtado, Rubchinsky, & Sigvardt, 2004). Line graphs for the other measures were derived by averaging the moving window data from 0.25 sec after the onset of an event to 0.25 before the offset of an event because the 0.5 sec sliding window resulted in a loss or contamination of data within 0.25 sec of the event boundaries. Coherence and phase coherence estimates were Fisher transformed. The cross spectrum power, CA3 power, and CA1 power were $\log_{10}$ transformed and multiplied by 10 to convert to decibels. Estimates were calculated for each rat, bias

corrected for differences in the number of trials (Bokil, Purpura, Schoffelen, Thomson, & Mitra, 2007), and then averaged together.

Statistical significance was assessed using a random permutation approach that adjusted for family-wise error rate (Maris & Oostenveld, 2007). Briefly, the data for 5 rats can be partitioned into 2 groups (e.g. ipsilateral vs. contralateral) 2^5 different ways. The difference of each permutation was compared to the difference of the original data sets and clusters 2 standard deviations above or below the original difference were identified. The maximum value for each permutation was taken as the maximum value of the absolute with-in cluster sum. Only clusters in the original data set exceeding the 95^{th} percentile of the maximum cluster values were identified as statistically significant differences between groups. Thus, with 5 rats contributing data to two groups (e.g. ipsilateral vs. contralateral stimulation), the actual value must be greater than all random permutations to reach statistical significance ($p = 1/2^5$, or 0.03).

Analyses of the LFPs during stimulation focused on comparisons between ipsilateral and contralateral stimulation. It was reasoned that contralateral stimulation would serve as the best control for several reasons. A prominent stimulation artifact was present under both stimulation conditions, but evoked responses were only observed during ipsilateral stimulation. Studies of long-term potentiation (LTP) in the hippocampus have shown facilitated induction of LTP specifically with ipsilateral BLA stimulation (Ikegaya et al., 1995). Finally, a comparison of ipsilateral and contralateral stimulation controls for the potential impact of stimulation on event behavior that could impact the power spectra, coherence, and spike patterns.

4.3.6.4 Spike-field relationship

The relationship between spikes and the phase of oscillations in the LFP was characterized in 3 separate frequency bands (theta: 4-12 Hz, low gamma: 30-55 Hz, and high gamma: 65-90 Hz). The preferred phase of spiking was estimated by calculating the mean phase angle (mu), such that 0 degrees indicates the peak of the cycle. The phase for each spike was calculated as the ratio of time from the first peak over the inter-peak time and then multiplied by $2*\pi$:

$$\text{phase} = \frac{\text{spike} - \text{peak}_n}{\text{peak}_{n+1} - \text{peak}_n} * 2 * \pi$$

The strength of spike-phase preference was estimated as the mean resultant length (MRL) of the mean resultant vector. As the MRL can be biased, the phase preference was also calculated with pairwise-phase consistency (PPC), which has been shown to be unbiased (Vinck, van Wingerden, Womelsdorf, Fries, & Pennartz, 2010). PPC is calculated as the average cosine value of the absolute angular pairwise distance between each observation. Although the parametric space for this measurement can range from -1 to 1, only very small data sets (less than 10 or 20 observations) and the most extreme, large data sets have PPC values greater than 0.1. In comparison, the MRL has a broader parametric space with larger data sets, but it is a biased estimate of phase precision. Thus, both methods were used.

Interneurons have relatively high firing rates, typically over 5 Hz (Csicsvari et al., 1998; Fox & Ranck, 1981), so estimates of the phase relationship were obtained separately for each unit. However, most pyramidal units fire at very low rates, less than 1 Hz. Thus, in order to obtain a more accurate estimate of the spike-phase relationship for

pyramidal spikes, data from all CA1 pyramidal units across rats were compiled into 1 aggregate bin and data from all septal CA3 units were compiled into a second bin.

Statistical significance was determined through random shuffling of the data 2000 times. Only trials with at least 1 spike were included. Ipsilateral and contralateral trials were randomly shuffled within subject, keeping the number of ipsilateral and contralateral trials from each rat consistent with the original data set. Phase consistency estimates were obtained for each shuffle of the data, and the contralateral values were subtracted from the ipsilateral values, yielding a distribution of randomly shuffled differences. A spike-field phase preference was considered to be significantly different between stimulation conditions if the actual difference lay outside the middle 95% of the data set. This 95% confidence interval was Bonferroni corrected to control for multiple comparisons between the three anatomically meaningful spike-field combinations (CA1-CA1, CA3-CA3, and CA3-CA1).

4.3.6.5 Spike pattern analyses

Firing rates of individual pyramidal units were calculated for 2 epochs of interest, the duration of object exploration and the 1 sec of stimulation. For multi-neuron analyses, the firing rate of each unit during the epoch of interest was used to construct a multivariate data point representing each epoch. A K nearest neighbor approach (KNN; k = 1) was performed to compare stimulation-induced spiking patterns to those observed during exploration of objects in the corresponding trial. Classification was considered accurate if the stimulation spike pattern most closely resembled the pattern of activity observed during exploration of the object immediately preceding stimulation. A similar

strategy was used in a previous study to demonstrate encoding of item-related information in the hippocampus (Manns & Eichenbaum, 2009). KNN classification accuracy was calculated separately for ipsilateral and contralateral stimulation and then averaged across rats. Only rats with at least 20 active, putative pyramidal units were included in the analyses. Active units were defined as units with at least 1 spike during object exploration or stimulation.

A subsequent question was whether KNN classification accuracy correlated with memory for objects on the following day. Previous work has shown that the accuracy of reactivations of spatial representation correlates with improved learning (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010). It was reasoned that recording more units, and thereby obtaining a better estimate of the neural population activity, would results in better accuracy. To adjust for this confound, the classification accuracy for each rat was divided by the number of active units. The discrimination index on the 1-Day Test was plotted as a function of the adjusted accuracy to determine whether there was a correlation, that is, to determine whether spiking patterns during stimulation predict memory for objects 1 day later.

4.4 Results

4.4.1 Histology and Subject Exclusion

Nine subjects completed behavioral testing, but histology revealed that 2 subjects had stimulating electrodes outside the BLA. These subjects were excluded from further analyses. Figure 4.2A shows an example of recording tetrode lesions in distal CA1 and

proximal CA3, and Figure 4.2B shows an example of a stimulating electrode marking lesion in the BLA. The behavioral data from the 7 remaining subjects (Fig. 4.2C) replicated the behavioral results from 2 previous studies in which brief electrical stimulation of the BLA immediately following object encounters enhanced memory for those objects 1 day later (Bass et al., 2014; 2012). Specifically, on the Immediate Test, memory for both Stimulation and No Stimulation objects was above chance (mean ± SEM: Stimulation = 0.61 ± 0.05, $t(6) = 2.14$, $p = 0.05$; No Stimulation = 0.61 ± 0.05, $t(6) = 2.37$, $p < 0.05$) with no difference between groups (Stimulation vs. No Stimulation: $t(6) = 0.08$, $p > 0.1$). However, on the 1-Day Test memory for Stimulation objects was significantly greater than memory for No Stimulation objects (Stimulation vs. No Stimulation: $t(6) = 2.69$, $p < 0.05$). There was a trend for the discrimination score for Stimulation objects to be above chance (mean ± SEM: 0.57 ± 0.04, $t(6) = 1.74$, $p = 0.1$) while the same trend was not observed for No Stimulation objects (mean ± SEM: 0.51 ± 0.04, $t(6) = 0.72$, $p > 0.1$). It is unclear why the discrimination score for Stimulation objects on the 1-Day Test was not significantly above baseline as in the previous 2 studies, but the most likely explanation is that the present study lacked sufficient power. Previous studies included 9 rats each, while the current study had only 7 subjects.

Table 4.1 specifies the data rats contributed to each analysis. Six subjects had LFP recordings from both CA1 and CA3, but prominent 60 Hz line noise prevented the use of the LFP data from one of these subjects. Therefore, LFP analyses included 5 rats, unless stated otherwise. A total of 385 hippocampal units were recorded, of which 371 were putative pyramidal cells (190 from CA1, 162 from CA3, and 19 from the temporal third of CA3). Of these, only units that were active during exploration or stimulation were used

in further analyses (119 CA1 units, 95 CA3 units, and 11 units from temporal CA3). The 14 putative interneurons were considered separately.

4.4.2 Robust increase in low gamma coherence during exploration

Figure 4.3A shows a moving time window coherogram of coherence between CA3 and CA1 during novel object exploration averaged across 5 rats and time locked to the onset of object exploration. Similar to a previous study (Trimper et al., 2014), analyses focused on encounters when rats explored objects for at least 2 seconds. CA3-CA1 coherence increased markedly from baseline levels in the low gamma (30-55 Hz) range following the onset of exploration. Clusters of significantly increased coherence during exploration (Fig. 4.3B) were identified using a random permutation approach that highlights 2-dimensional clusters of increased coherence while controlling for multiple comparisons (Maris & Oostenveld, 2007). Figure 4.3C shows means for the baseline and exploration coherence, phase coherence, cross spectrum power, CA1 power, and CA3 power. Significant increases in low gamma coherence, low gamma phase coherence, low gamma cross spectrum power, and gamma CA3 power were observed during exploration. Results replicate the findings of previous work (Trimper et al., 2014).

4.4.3 Evoked responses in the LFP are visible as oscillations in the low gamma range

Figure 4.4 shows examples of the LFP in CA1 and CA3 during 1 train of ipsilateral stimulation and 1 train of contralateral stimulation. The red lines indicate the original artifact, and the black trace indicates the final LFP data with the artifact greatly reduced following mean artifact template subtraction. An example template of the third

artifact in each train is shown to the right. All subsequent LFPs analyses were performed following this method of artifact reduction. Prominent evoked potentials were observed in the LFP from CA3 during ipsilateral stimulation ~21 ms after each pulse, and smaller magnitude evoked potentials were visible in the LFP from CA1 (most prominent evoked potential at ~84 ms after the first pulse in each train of 4 pulses). The evoked potentials in CA1 were difficult to visualize as they had the same polarity as the stimulation artifact. However, the polarity inversion suggests the CA1 responses were not merely artifacts of volume conduction from CA3. Evoked responses were not observed during contralateral stimulation. Autocorrelograms of the LFPs in CA1 and CA3 and the crosscorrelogram show a ~48 Hz oscillation (Figure 4.5), consistent with evoked potentials every ~21 ms. The signal was preserved when the residual artifact was reduced further by subtracting out the contralateral estimates. In summary, the results indicate that mean template subtraction adequately reduced the impact of the stimulation artifact, and that the impact of the residual artifact was reduced further by contrasting ipsilateral and contralateral stimulation.

4.4.4 Ipsilateral BLA stimulation elicits low gamma synchronization

It was reasoned that the stimulation artifact would arrive simultaneously in CA3 and CA1, and therefore the residual artifact should be readily identified in a moving time window of the phase offset between regions as a 0 degree difference. That is, clusters of pixels without a 0 degree phase offset should be more likely to correspond to a biological signal. Figure 4.6A shows a ~0 degree offset above 45 Hz during contralateral stimulation, but CA3 and CA1 are nearly 180 degrees out of phase throughout the gamma

frequency band during ipsilateral stimulation. A 180-degree phase offset is more consistent with the offset in CA3 and CA1 low gamma oscillations during baseline (before and after stimulation) and exploration (Trimper et al., 2014). The moving time window of coherence in Figure 4.6B shows a significant increase in low gamma coherence during ipsilateral stimulation relative to contralateral stimulation. Specifically, increased coherence between CA3 and CA1 was observed in the same band that increased during object exploration. Template subtraction substantially decreased coherence estimates at the pulse frequency, 50 Hz, during contralateral stimulation, which suggests that the high coherence during ipsilateral stimulation is indeed a product of biological activity. Clusters of significantly increased coherence were identified using the same random permutation techniques that were used to identify increased coherence during exploration. One concern was that the evoked potentials in CA3 were driving substantial increases in CA3 low gamma power, and that increased low gamma power in CA3 could account for the increase in low gamma coherence between regions. To address this possibility, phase coherence and cross spectrum power were independently assessed for the 1 second of stimulation, and 1-dimensional clusters of significance were identified using a random permutation approach. Figure 4.6C shows that ipsilateral stimulation significantly increased phase coherence and cross spectrum power in the low gamma range. In summary, the results indicate that ipsilateral stimulation increased CA3-CA1 coherence in the low gamma range (30-55 Hz) and that this increase in coherence reflects increases in phase coherence and cross spectrum power in the low gamma range.

One question was whether the marked increase in coherence was observed in the low gamma range because the stimulation frequency was 50 Hz or because BLA input

facilitates oscillatory activity in the hippocampus and that CA3 and CA1 tend to synchronize in the low gamma range (Colgin & Moser, 2010). That is, it was unclear whether BLA stimulation entrained the hippocampus at the stimulation frequency or facilitated endogenous mechanisms within the hippocampus. To address this question, a subset of rats received 80 Hz stimulation as they completed laps around the circular track. Figure 4.7A shows that ipsilateral stimulation at 80 Hz induced a prominent increase in CA3-CA1 coherence in the low gamma band (~30-50 Hz), nearly the same frequency band as before. Moreover, no effect of contralateral stimulation was observed in the low gamma range. Thus, the results support the hypothesis that BLA input facilitates processes that are endogenous to the hippocampus.

Another question was whether the rats' behavioral state impacted low gamma coherence during amygdala stimulation. To address this question, rats received 50 Hz stimulation under deep anesthesia. Figure 4.7B shows coherence under all 3 stimulation conditions. Coherence for the 50 Hz, awake condition was re-plotted with just the 4 rats that were included in the 2 other conditions. A random permutation approach was not performed with this data set as it does not permit sufficient resolution at the extremes. There are only 2^4 permutations possible permutations ($p = 1/ 2^4$, or 0.06). Thus, to determine statistical significance, low gamma was defined *a priori* as 30-55 Hz, and the area under the curve was calculated for each condition. A 2 X 3 (stimulation side X stimulation condition) repeated measures ANOVA revealed a trend towards an interaction between stimulation side and condition ($F(2,2) = 2.74$, $p = 0.091$) and a significant main effect of stimulation side ($F(1,3) = 57.71$, $p < 0.001$). Bonferroni corrected post-hoc analyses revealed a significant increase in low gamma during 50 Hz

ipsilateral stimulation while awake (mean ± SEM: Ipsilateral vs. Contralateral, 1.12 ± 0.09 vs. 0.37 ± 0.08, $t(3) = 5.67$, $p < 0.05$) and under anesthesia (mean ± SEM: Ipsilateral vs. Contralateral, 0.81 ± 0.11 vs. 0.48 ± 0.07, $t(3) = 7.28$, $p < 0.05$). However, anesthesia significantly reduced low gamma coherence during ipsilateral stimulation (Awake vs. Anesthesia, $t(3) = 13.856$, $p < 0.005$). There was a trend towards increased low gamma coherence during ipsilateral stimulation with 80 Hz (mean ± SEM: Ipsilateral vs. Contralateral, 0.98 ± 0.11 vs. 0.34 ± 0.10, $t(3) = 3.626$, $p = 0.1$). Low gamma coherence with 80 Hz ipsilateral stimulation was not significantly different from 50 Hz (50 Hz vs. 80 Hz, $t(3) = 1.96$, $p > 0.1$), and low gamma coherence during contralateral stimulation was not significantly impacted under either condition (Awake vs. Anesthesia, $t(3) = 1.02$, $p > 0.1$; 50 Hz vs. 80 Hz, $t(3) = 0.43$, $p > 0.1$). Thus, the results suggest that behavioral state modulates induction of low gamma coherence via BLA stimulation, while stimulation frequency within the range that was tested does not impact induction low gamma coherence.

4.4.5 BLA stimulation does not substantially impact firing rate

The mean firing rates of individual pyramidal units in CA3 and CA1 were obtained for epochs of object exploration and stimulation and then averaged across rats as shown in Figure 4.8A. Firing rates were also obtained for 1 sec epochs following exploration of non-stimulation objects during which stimulation would have been delivered on that trial. That is, if stimulation were delivered 0.4 sec after the offset of exploration of the Stimulation object, the firing rate was calculated for 0.4 to 1.4 seconds following the offset of exploration of No Stimulation and New objects on the same trial.

The mean firing rates were similar across groups of objects during exploration (mean ± SEM: Ipsilateral = 0.022 Hz ± 0.011; Contralateral = 0.031 ± Hz 0.018; No Stimulation and New = 0.027 Hz ± 0.015) and during stimulation (mean ± SEM: Ipsilateral = 0.033 Hz ± 0.022; Contralateral = 0.056 ± Hz 0.046; No Stimulation and New = 0.035 Hz ± 0.020). A 2 X 3 (epoch X stimulation condition) repeated measures ANOVA did not reveal a significant interaction on pyramidal unit firing rate ($F(2,5) = 0.06$, $p > 0.1$). Further, there was no main effect of epoch ($F(1,6) = 0.53$, $p > 0.1$) or stimulation ($F(2,5) = 0.23$, $p > 0.1$). Tests for statistical significance were the same when units from CA1 and CA3 were analyzed separately. The fact that were no significant differences in firing rate as a result of stimulation is an important finding because it strongly suggests that few, if any, pyramidal spikes were lost to the stimulation artifact. Indeed the numerical value for firing rate during contralateral stimulation was greater than the numerical value for no stimulation, and the means for ipsilateral stimulation and no stimulation were nearly equivalent. Moreover, there was no significant difference between firing rates during ipsilateral and contralateral stimulation ($t(6) = 0.73$, $p > 0.1$). This null result is an important finding because it suggests that estimates of the spike-phase relationship for pyramidal units were not confounded by differences in samples sizes.

Interneurons have high firing rates, typically much greater than 5 Hz, and therefore they were each analyzed as individual units (Fig. 4.8C; 9 units from CA1 and 5 units from CA3). A 2 X 3 (epoch X stimulation condition) repeated measures ANOVA did not reveal a significant interaction on interneuron firing rates ($F(2,12) = 0.38$, $p > 0.1$), a main effect of epoch ($F(1,13) = 0.75$, $p > 0.1$), or a main effect of stimulation ($F(2,12) = 0.69$, $p > 0.1$). However, unit by unit analysis of interneuron firing rate

revealed that 7 units responded to ipsilateral stimulation with a significant reduction in firing rate ($p < 0.005$) and 1 with a significant increase in firing rate ($p < 0.005$). In response to contralateral stimulation, 4 units had a significant reduction in firing rate ($p < 0.005$), and 2 significantly increased firing rate ($p < 0.005$). Only 4 units did not show a response to stimulation. It is unlikely that many interneuron spikes were lost to the stimulation artifact as only 3 units out of 14 had a significant reduction in firing rate to both ipsilateral and contralateral stimulation, and 2 units responded with increased firing rates.

In summary, the results indicate that BLA stimulation does not result in substantial increases or decreases on the firing rate of hippocampal neuronal populations.

4.4.6 Ipsilateral BLA stimulation elicits spike-phase preferences in low gamma range

Figure 4.9A shows an example of the raw LFP following artifact reduction (black; same example stimulation as shown in Fig. 4.4) and the LFP filtered for low gamma (green, 30-55 Hz). The phase of each spike during stimulation was calculated based on the filtered LFP, and spikes from putative pyramidal units were aggregated across rats to estimate phase preference. Ipsilateral stimulation resulted in a phase relationship between spikes and low gamma oscillations in the LFPs (Fig. 4.9B). The MRL and PPC were obtained for CA3 spike – CA3 field and CA1 spike – CA1 field to estimate the strength of within-region phase preferences. The strength of phase preference between fields was estimated by calculating the MRL and PPC for CA3 spike – CA1 field because projections between regions are unidirectional from CA3 to CA1. Figure 4.10A shows

that CA3 spikes had a robust phase preference relative to low gamma oscillations in CA3 during ipsilateral stimulation (MRL = 0.240, PPC = 0.052, mu = 136 degrees) but not contralateral stimulation (MRL = 0.054, PPC = -0.002, mu = 66 degrees). CA1 spikes did not have a strong phase preference for low gamma in CA1 (Ipsilateral: MRL = 0.082, PPC = 0.004, mu = 345 degrees; Contralateral: MRL = 0.136, PPC = 0.016, mu = 297 degrees). However, there was robust phase relationship of CA3 spikes relative to low gamma oscillations in CA1 during ipsilateral stimulation (Ipsilateral: MRL = 0.215, PPC = 0.040, mu = 320 degrees; Contralateral: MRL = 0.051, PPC = -0.003, mu = 110 degrees), consistent with enhanced intra-hippocampal communication. Figure 4.10B shows a significant difference between MRLs for ipsilateral and contralateral stimulation for CA3 spike – CA3 field (MRL difference = 0.186, $p < 0.05$) and CA3 spike – CA1 field (MRL difference = 0.165, $p < 0.05$). There was no significant difference for CA1 spike – CA1 field (MRL difference = -0.054, $p > 0.1$). Results of significance testing were the same when analyses were performed with PPC values.

The same methods were used to assess phase preferences of pyramidal spikes to theta (4-12 Hz) and high gamma (65-90 Hz). In general, there was greater a phase preference of pyramidal spikes to theta in both regions during contralateral stimulation than during ipsilateral stimulation, but this did not approach statistical significance for CA1 spikes – CA1 field phase preference (Ipsilateral: MRL = 0.073, PPC = 0.003, mu = 153 degrees; Contralateral: MRL = 0.185, PPC = 0.032, mu = 117 degrees; MRL difference = -0.112, $p > 0.1$) or CA3 spikes – CA3 field phase preference (Ipsilateral: MRL = 0.067, PPC = -0.002, mu = 103 degrees; Contralateral: MRL = 0.140, PPC = 0.014, mu = 269 degrees; MRL difference = -0.073, $p > 0.1$). There was a trend for a

greater CA3 spike – CA1 field theta phase relationship during contralateral stimulation (Ipsilateral: MRL = 0.026, PPC = -0.006, mu = 208 degrees; Contralateral: MRL = 0.164, PPC = 0.022, mu = 204 degrees; MRL difference = -0.138, $p < 0.1$, Bonferroni corrected). Phase preference of pyramidal spikes relative to high gamma did not approach significance for any comparison (CA1 – CA1; Ipsilateral: MRL = 0.057, PPC = 0.001, mu = 338 degrees; Contralateral: MRL = 0.027, PPC = -0.001, mu = 332 degrees; MRL difference = 0.030, $p > 0.1$; CA3 – CA3; Ipsilateral: MRL = 0.045, PPC = -0.004, mu = 223 degrees; Contralateral: MRL = 0.063, PPC = -0.001, mu = 256 degrees; MRL difference = -0.019, $p > 0.1$; CA3 – CA1; Ipsilateral: MRL = 0.045, PPC = -0.004, mu = 250 degrees; Contralateral: MRL = 0.015, PPC = -0.005, mu = 218 degrees; MRL difference = 0.031, $p > 0.1$).

Phases consistency estimates were obtained separately for each interneuron and then averaged together to compare the effects of ipsilateral stimulation and contralateral stimulation (Fig. 4.10C). Results indicated that the phase relationship of interneurons relative to low gamma oscillations in CA3 was significantly stronger during ipsilateral stimulation than during contralateral stimulation (mean ± SEM: Ipsilateral = 0.187 ± 0.022; Contralateral = 0.074 ± 0.020; $t(8) = 3.25$, $p < 0.05$). Notably, there was not a significant negative correlation between the MRL and the firing rate during stimulation that could account for the increase in phase consistency estimates ($r(8) = -0.167$, $p > 0.1$). A similar effect on phase preferences was observed for interneurons relative to oscillations in the low gamma range of CA1, but it is unclear why this effect did not reach statistical significance (mean ± SEM: Ipsilateral = 0.149 ± 0.029; Contralateral = 0.089 ± 0.014; $t(12) = 1.54$, $p > 0.1$).

The same analyses were performed for phase preferences in the theta range and high gamma range. No effect was observed relative to theta range oscillations in either region (mean ± SEM: CA1 Ipsilateral = 0.231 ± 0.059; Contralateral = 0.239 ± 0.027; CA3 Ipsilateral = 0.301 ± 0.082; Contralateral = 0.146 ± 0.027). There was a trend towards greater phase preferences relative to high gamma range oscillations in CA3 during ipsilateral stimulation (mean ± SEM: Ipsilateral = 0.106 ± 0.024; Contralateral = 0.074 ± 0.020; $t(8) = 2.18$, $p < 0.1$), but this may have been explained in part by changes in firing rates as there was a significant negative correlation between firing rate and MRL ($r(8) = 0.688$, $p < 0.05$). No difference in phase preferences was observed relative to high gamma oscillations in CA1 (mean ± SEM: Ipsilateral = 0.117 ± 0.058; Contralateral = 0.058 ± 0.008; $t(12) = 1.303$, $p > 0.1$).

4.4.7 Ipsilateral stimulation may induce recent exploration-related patterns of spiking activity

Multi-dimensional analyses were used to compare the spiking activity of pyramidal units during exploration and stimulation. The firing rates of each neuron represented 1 dimension, such that the closer two points were in multi-dimensional space, the more similar the patterns of spiking. Only rats with more than 20 active units were included (n = 5 rats, mean ± SEM: units = 42.6 ± 9.0). A trial was considered an accurate match if the multi-dimensional representation of the spiking patterns during stimulation were nearest the representation of the Stimulation object. Figure 4.11A shows the average KNN classification accuracy for ipsilateral and contralateral stimulation. The findings suggested a trend towards greater classification accuracy during ipsilateral stimulation

than contralateral stimulation (mean ± SEM: Ipsilateral = 53% ± 12%; Contralateral = 36% ± 8%; $t(4) = 2.38$, $p < 0.1$), but the accuracy was not significantly above baseline (baseline = 33%, $t(4) = 1.69$, $p > 0.1$).

Trials on the 1-Day Test were split based on ipsilateral or contralateral stimulation condition during the Study, and memory for Stimulation objects was calculated separately for each condition as a discrimination index. Scores were compared to the adjusted KNN classification accuracy. There was a significant, positive correlation between memory for objects and the classification accuracy for the ipsilateral stimulation condition (Fig. 4.11B; $r(5) = 0.91$, $p < 0.05$), but this correlation was not observed for the contralateral condition ($r(5) = -0.18$, $p > 0.1$). A Fisher transform was performed on the rho values to compare the strength of the correlations in each condition, and the result suggested a trend towards a greater correlation between the adjusted classification accuracy and memory for ipsilateral stimulation than for contralateral stimulation ($z = 1.72$, $p < 0.1$). Overall, the results suggest that ipsilateral BLA stimulation induces hippocampal spiking patterns similar to those observed during recent object exploration and that these reactivations are important for amygdala-mediated enhancement of memory. However, the low power of the study limits the strength of the interpretation insofar as the difference in correlation values was not significant between ipsilateral and contralateral conditions.

4.5 Discussion

The present study built on several previous studies to show the amygdala enhances hippocampal-dependent memory. Behavioral results were replicated from 2 previous studies in which rats remembered objects 1day later only if exploration were immediately followed by BLA stimulation (Bass et al., 2014, 2012), and the link between low gamma synchrony in the hippocampus and good memory is consistent with previous work (Trimper et al., 2014). Now, it appears that BLA stimulation enhances object recognition memory in part by synchronizing activity between CA3 and CA1.

The effects of amygdala stimulation were predominantly ipsilateral, as predicted by the ipsilateral anatomical connectivity between structures. Thus, we were able to disambiguate the effects of amygdala stimulation from the artifact by contrasting ipsilateral and contralateral trials, and by doing so, we found that memory-enhancing amygdala stimulation elicited low gamma coherence between CA3 and CA1. This phenomenon was replicated across various stimulation conditions, suggesting that the hippocampus responds to stimulation by synchronizing neural activity in a preferred frequency domain. Analysis of the spike data indicated that the most pronounced effect of amygdala stimulation on pyramidal units was through modulation of spike timing, as opposed to broad increases or decreases in firing rate. Specifically, ipsilateral stimulation induced a strong spike-phase relationship between CA3 spikes and low gamma oscillations in the LFPs from CA3 and CA1. Comparison of spiking patterns during stimulation and recent object exploration suggested that spike patterns during stimulation were similar to patterns of activity observed during recent object exploration, and further, that the extent to which exploration spiking patterns were observed during stimulation correlated with memory for objects 1 day later.

Ipsilateral stimulation evoked a prominent response in CA3, but direct visualization of the response in CA1 was often obscured by the artifact. Indeed, distinguishing the biological effect of stimulation from the artifact was a complicating issue across many of the analyses. Thus, many steps were taken to carefully reduce the artifact while preserving as much of the signal as possible. Mean template subtraction resulted in near complete reduction of spectral power and coherence at the pulse frequency for contralateral stimulation, indicating that a true biological signal was driving the increased power and coherence estimates observed during ipsilateral stimulation.

Another possibility was that neuronal spikes were lost to the pulse artifact. Systematic loss of spike data can result in artificially lower estimates of spike rate and increased estimates of spike-phase preference during stimulation. However, comparison of data from stimulation and no stimulation trials suggests that any loss of spiking activity had a negligible impact on the results. Specifically, firing rates of pyramidal units in both stimulation conditions were comparable to firings rates during no stimulation. Moreover, robust spike-phase relationships relative to low gamma were only observed during ipsilateral stimulation. If systematic loss of spikes from the artifact had inflated estimates of spike-phase preferences during stimulation, preferences should have been equally prominent in both stimulation conditions. Finally, analysis of interneuron spiking activity revealed that few interneurons decreased firing rate in response to stimulation on both sides, and a couple interneurons actually increased firing rate during stimulation. Thus, the overall pattern of results indicates that the low gamma range coherence and phase preferences elicited by ipsilateral stimulation of the BLA cannot simply be attributed to the stimulation artifact.

The finding that amygdala stimulation enhances memory and elicits low gamma coherence between CA3 and CA1 aligns well with a large body of literature linking good memory and gamma synchronization of neural activity in the hippocampus (Fries et al., 2007). Specifically, a study with macaques performing a visual recognition memory task found that a strong phase relationship between hippocampal spikes and gamma oscillations in the LFP correlated with subsequent recognition memory performance (Jutras et al., 2009). Other research has shown that low gamma coherence between CA3 and CA1 (Colgin et al., 2009) and between the amygdala and other memory structures (Bauer, Paz, & Pare, 2007; Popescu et al., 2009) facilitates the transfer of information in manner that benefits memory. Overall, there is a growing body of evidence linking neural activity in the low gamma range to good memory encoding and consolidation. The present study contributes to this body of knowledge by showing that mnemonic processes in the hippocampus can be manipulated by BLA stimulation to improve memory.

Recent studies on spatial memory in rodents have shown that reactivation of spatial representations correlates with improved memory (Dupret et al., 2010; Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009; Jadhav, Kemere, German, & Frank, 2012) and that these reactivations are commonly observed during bouts of increased low gamma coherence between CA3 and CA1 (Carr et al., 2012). These findings would seem to parallel the effects of amygdala stimulation insofar as ipsilateral BLA stimulation elicited low gamma coherence between CA3 and CA1 and appeared to induce spiking patterns that were similar to neural activity observed during recent object exploration. Similar to the correlation between spatial memory and reactivation of spatial representation, the quality of object-related spike pattern reactivations correlated with

memory for objects. Thus, it would appear that the mechanisms for forming memories for events are similar to the processes that support spatial navigation.

Perhaps, one of the more important contributions of the present study is that it provides strong evidence of causation between hippocampal gamma synchrony and memory. That is, direct stimulation of the BLA induced gamma coherence between CA3 and CA1, and then memory was enhanced 1 day later. At the crux of this argument is that inactivation of the hippocampus during BLA stimulation completely attenuates the memory enhancing effects of amygdala stimulation (Bass et al., 2014). Taken together, these data strongly suggest a causal link between gamma synchronization and memory consolidation.

Gamma synchronization facilitates communication and plasticity through coordination of neuronal spiking (Juergen Fell & Axmacher, 2011; Fries, 2005). Compelling evidence stems from the finding that the fast spiking-interneurons that drive gamma oscillations also impose rhythmic inhibition on pyramidal cells and that this rhythmic inhibition results in brief temporal windows (less than 20 ms) of spiking activity that benefit spike-timing dependent plasticity (Bi & Poo, 1998; Buzsáki & Wang, 2012; Fries et al., 2007). Spike-timing dependent plasticity is one of several forms of activity-dependent synapse modification that predominantly depends on post-synaptic activation in order to induce LTP (Abbott & Nelson, 2000; Caporale & Dan, 2008) . The implication is that multiple pre-synaptic spikes in close temporal proximity will be more likely to elicit a response from a post-synaptic neuron. In addition, a pre-synaptic spike will be more likely to induce LTP. Thus, rhythmic synchronization of neural activity to gamma range oscillations is thought to function as an input gain (Jutras & Buffalo, 2010).

One hypothesis to explain how the amygdala can selectively enhance memory is that increases in low gamma coherence between CA3 and CA1 during object exploration result in transient increases in LTP in the neuronal ensemble representing item-related information. The initial induction of LTP may be sub-threshold for inducing late-phase LTP, but subsequent amygdala activation could be sufficient to reactivate these neural representations and benefit late-phase LTP at specific synapses via gamma-modulated spiking. Support for this hypothesis comes from the fact that amygdala stimulation can facilitate late-phase LTP induction following stimulation of hippocampal inputs, but it is not sufficient to induce LTP (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995).

In summary, the present body of work represents an important contribution to our understanding of memory system interactions and emotional-mediated enhancement of memory. The experiments and analyses combined findings from multiple lines of research into a coherent framework for understanding amygdala-mediated enhancement of memory for specific events in the hippocampus. Sleep studies should be incorporated into further electrophysiological experiments as much work has shown that reactivation of neural representations during slow wave sleep can benefit long-term memory (Diekelmann & Born, 2010; Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994). Moreover, insights gained from sleep studies on amygdala-mediated enhancement of memory may explain why the benefits of amygdala stimulation are not observed until 1 day later. Finally, the fact that memory enhancement is a protracted process suggests that activation of the amygdala modulates late-phase LTP via cell processes that converge on gene transcription (Alberini, 2009).

Figure 4.1 Schematic of the novel object recognition memory task

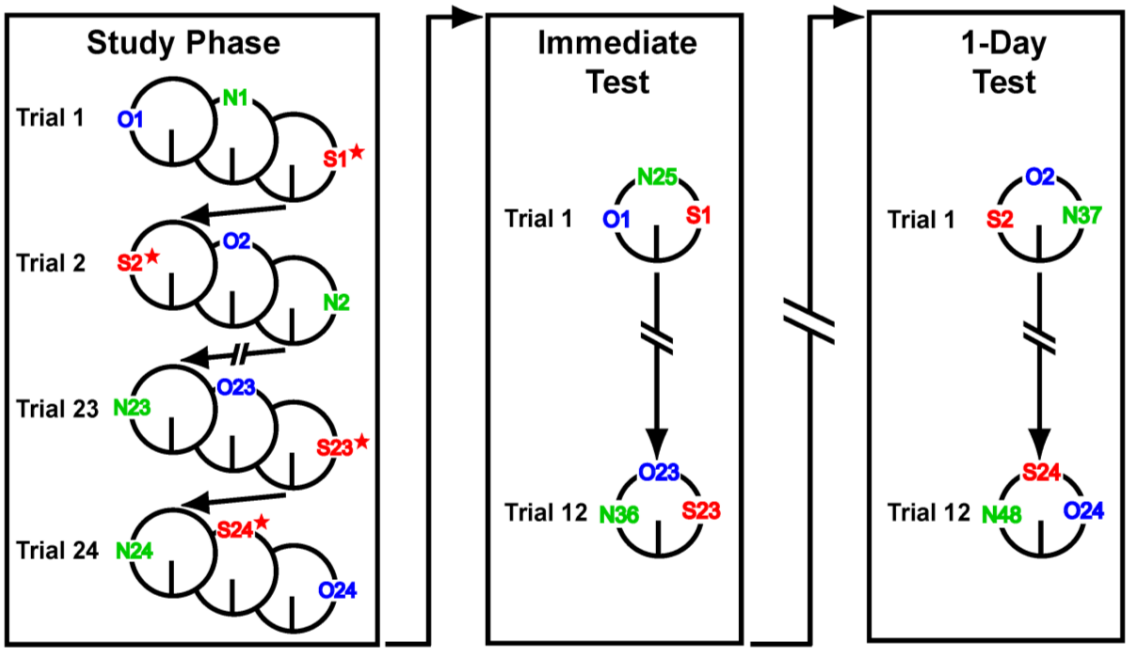

Figure 4.1 Schematic of the novel object recognition memory task. Rats encountered a series of objects as they completed laps around a circular track. Each trial contained 3 unique objects, one from each object group. Stimulation objects ("S") were followed by brief electrical stimulation to the BLA (denoted by a star) immediately following the offset of exploration during the Study. No Simulation objects ("O") were not followed by stimulation. New objects ("N") were replaced by novel objects on the test. Memory for Stimulation and No Stimulation objects was assessed either on the Immediate Test (~1 hr after the start of the Study) or the 1-Day test (on the following day) by placing replicates in the same position and comparing exploration of these objects to the exploration of the novel objects that had replaced the New objects.

Figure 4.2 Histology and behavioral results

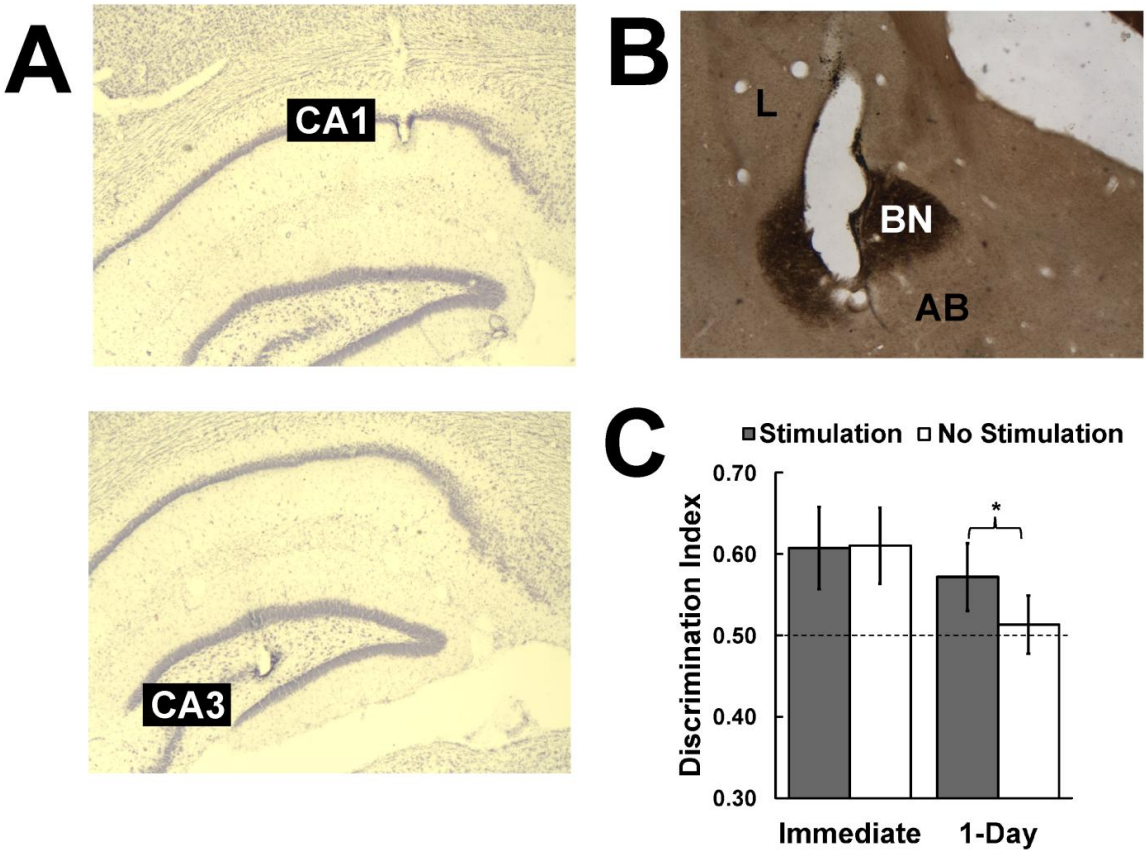

Figure 4.2 Histology and behavioral results (n = 7). A. Tetrode localization in the pyramidal layer of CA1 and CA3 in the hippocampus on sections with cresyl violate staining. LFPs were obtained only from tetrodes with tips in the pyramidal layers. **B.** Electrode localization in the BLA on a section stained for acetylcholinesterase. Both electrode tips were localized to the BLA (lateral nucleus, L; basal nucleus, BN; accessory basal nucleus, AB) for all rats included in analyses. **C.** Performance on object recognition memory test presented as a discrimination index. The results from the present study replicate the results from 2 previous studies (Bass et al., 2014; 2012) using the same behavioral task and amygdala stimulation parameters. The dashed line indicates baseline performance. Gray bars indicate memory for Stimulation objects, and white bars indicate memory for No Stimulation objects. Rats remembered Stimulation objects better than No Stimulation objects on the 1-Day Test. Error bars show the SEM.* $p < 0.05$.

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Table 4.1 Subject inclusion

Rat	Behavior	CA3 - CA1 Coherence	80 Hz & Anesthesia	Spike-Field Coherence	Spike Pattern Analysis	Comments
1	X			X	X	no CA3 recordings
2	X	X		X		< 20 active units
3						missed right stimulating electrode
4	X	X	X	X	X	
5	X	X	X	X	X	
6						missed left stimulating electrode
7	X				X	60 Hz line noise
8	X	X	X	X		< 20 active units
9	X	X	X	X	X	
Total	7	5	4	6	5	

Figure 4.3 Low gamma coherence during object exploration

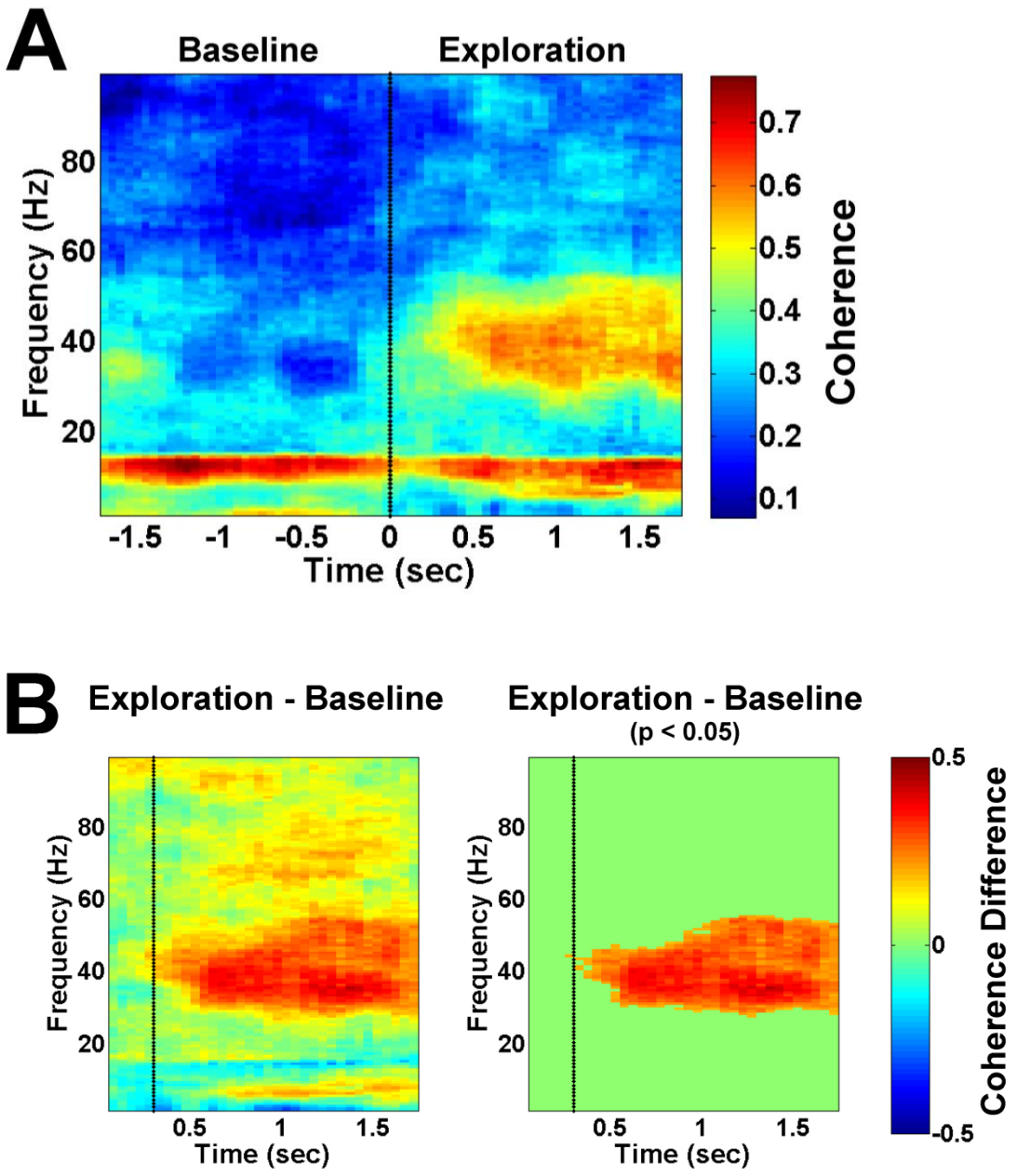

Figure 4.3 Low gamma coherence during object exploration

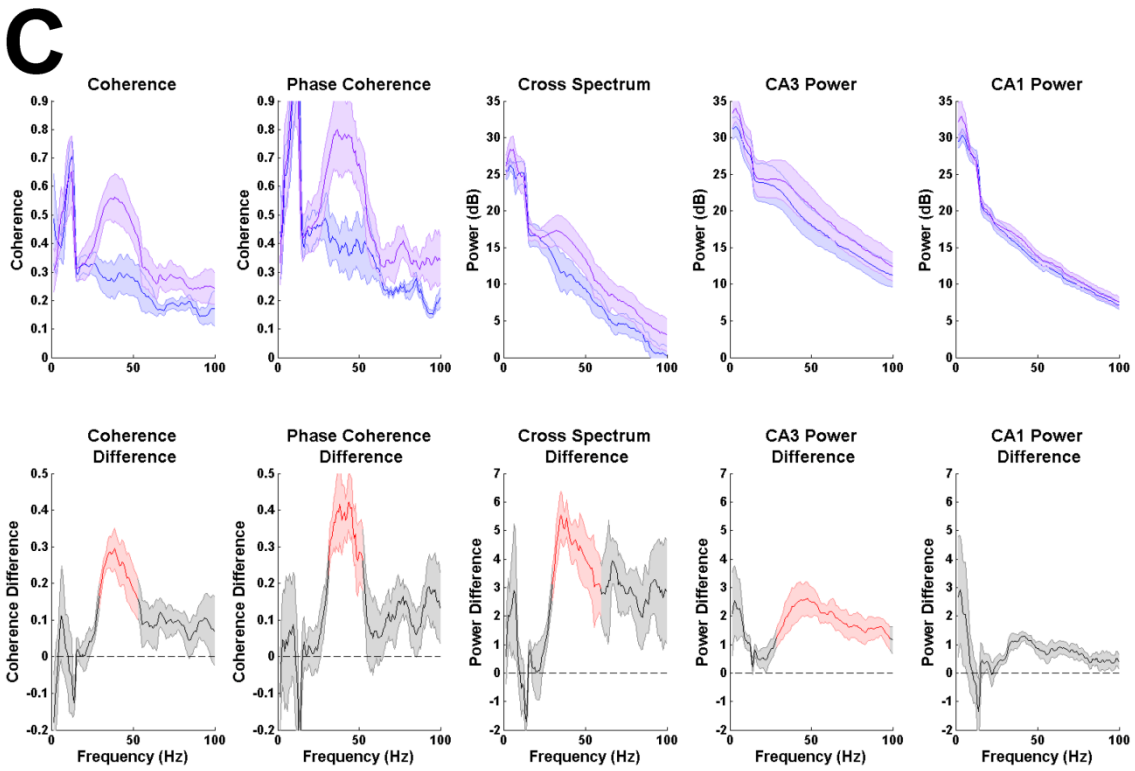

Figure 4.3 Low gamma coherence during object exploration (n = 5). A. Moving time window of coherence between CA3 and CA1 time-locked to onset of exploration. Low gamma coherence increased during exploration relative to baseline (2 sec prior to exploration). Only encounters with exploration times of at least 2 sec were included for analysis. Color indicates coherence values, with red indicating peak coherence and blue indicating low coherence. **B.** The difference in coherence (Exploration – Baseline) was plotted in the left panel, and the panel on the right shows only pixels that exceeded statistical significance ($p < 0.05$) as determined by a random permutation approach (see Method). The results indicate a significant increase in low gamma coherence between CA3 and CA1 during exploration. Color indicates difference in coherence. **C.** Estimates for coherence, phase coherence, cross spectrum power, and power in CA3 and CA1 during baseline (blue line) and exploration (purple line). Below, the difference (black line) between exploration and baseline is plotted highlighting clusters that exceeded statistical significance (red line, $p < 0.05$) as determined by a random permutation approach (see Method). Results indicate that increases in low gamma phase coherence and the cross spectrum power contribute to increased coherence during exploration. Coherence estimates were z-transformed and biased corrected. Power estimates were $\log_{10}$ transformed, multiplied by 10 to convert to decibels, and bias corrected. Shading shows SEM.

Figure 4.4 Average LFPs during stimulation.

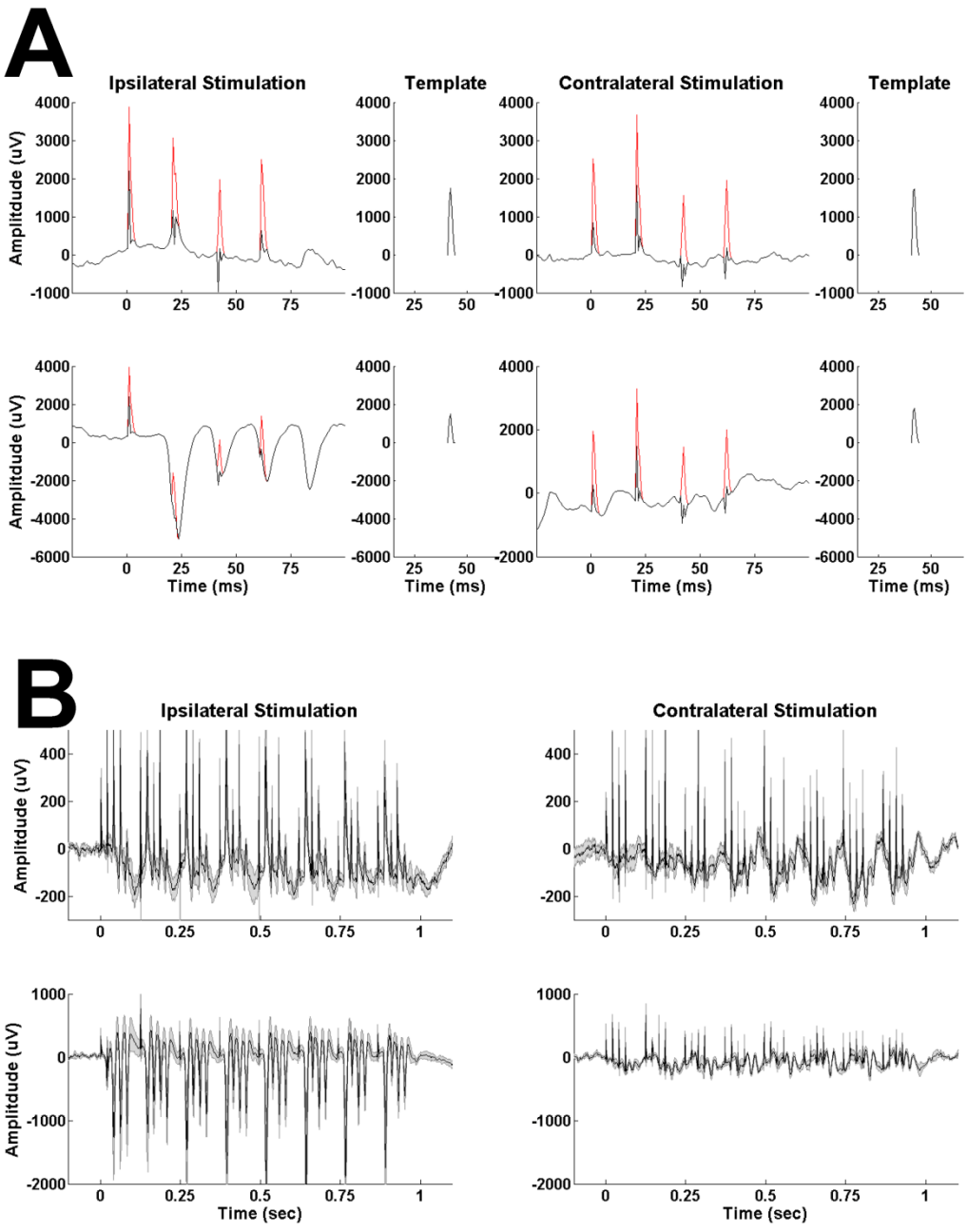

Figure 4.4 Average LFPs during stimulation. A. Raw traces of the LFP following artifact reduction. One train of 4 pulses (black line) from ipsilateral or contralateral stimulation is plotted for CA1 and CA3. Red lines indicate stimulation artifact prior to reduction (see Method). The panel to the right of each trace shows the template used to reduce the 3rd stimulation artifact in each train. Ipsilateral stimulation induces prominent evoked potentials in CA3 ~21 ms after each pulse. Smaller evoked responses of opposite polarity can be simultaneously observed in CA1. **B.** Average LFP trace during stimulation. An average was obtained for each rat, and then averaged across rats. Template subtraction greatly reduced the artifact. Ipsilateral stimulation induced a characteristic wave form, particularly in CA3. Shading shows SEM, n = 5.

Figure 4.5 Auto and cross correlograms of the LFP during stimulation

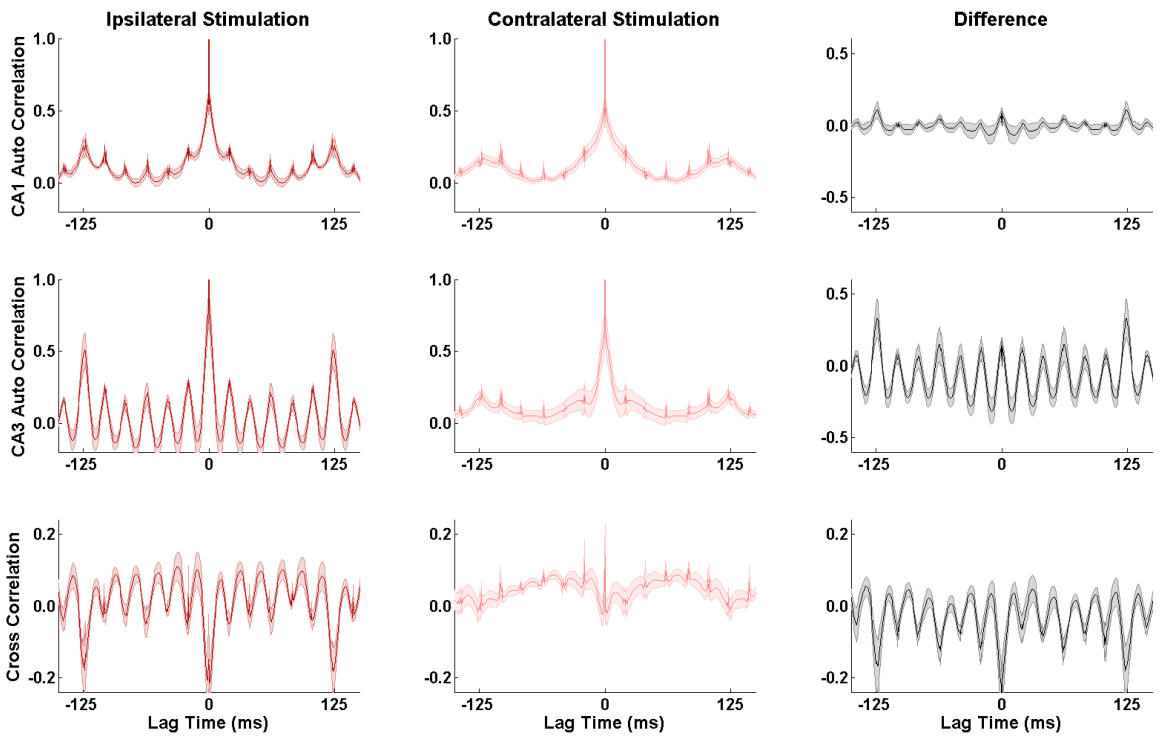

Figure 4.5 Auto and cross correlograms of the LFP during stimulation (n = 5). The top rows show autocorrelograms for CA1 and CA3, respectively. The bottom row shows the crosscorrelogram between CA3 and CA1. Low gamma oscillations (~48 Hz) are visible in both CA3 and CA1 autocorrelograms in the ipsilateral stimulation condition, but they are not visible in contralateral condition. The impact of the residual artifact from ipsilateral stimulation is further reduced by subtracting out the data for contralateral stimulation. The difference is plotted in the far right column. Shading shows SEM.

Figure 4.6 Low gamma coherence during ipsilateral stimulation

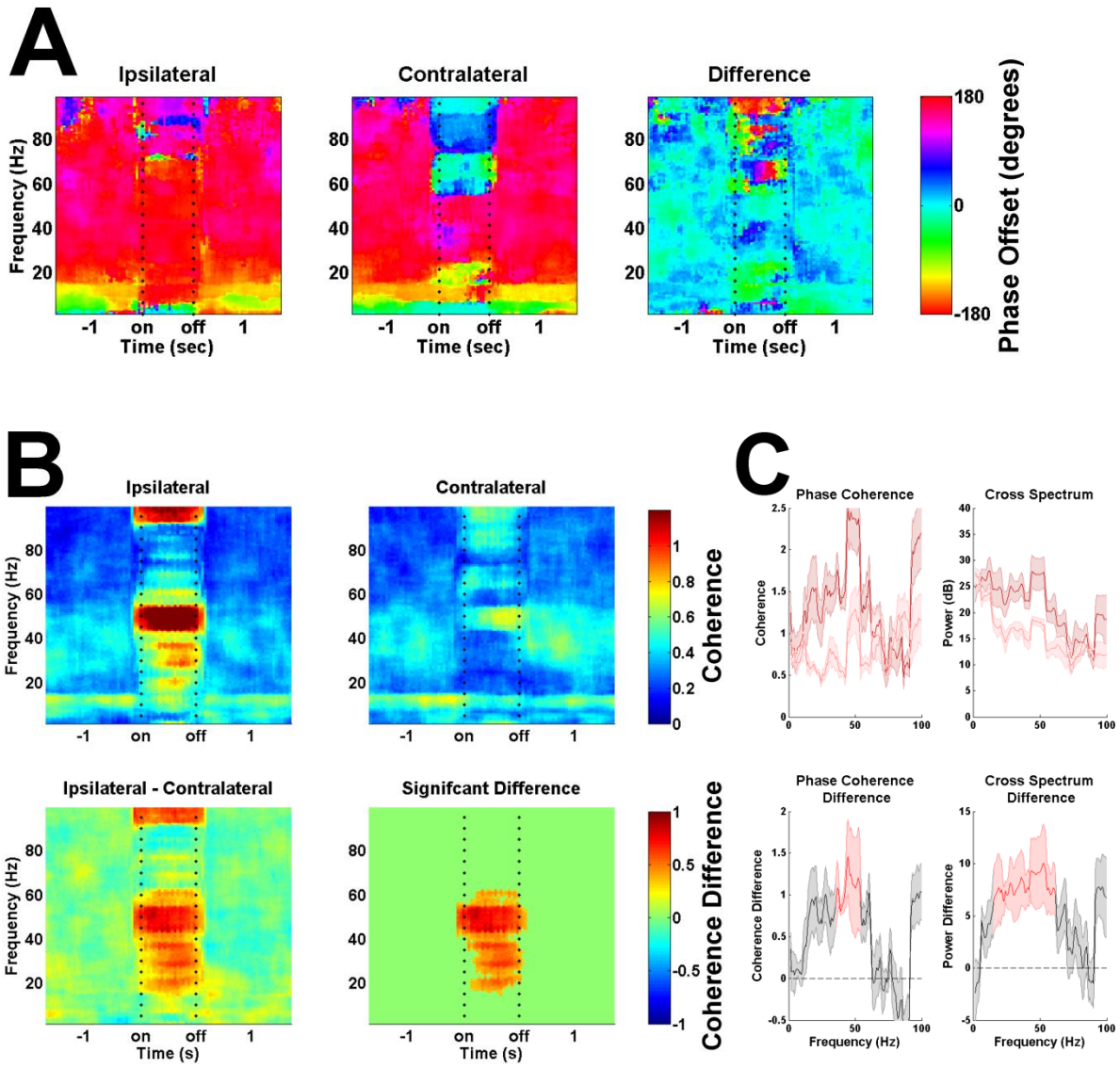

Figure 4.6 Low gamma coherence during ipsilateral stimulation (n = 5). A. Moving time windows of the phase offset between CA3 and CA1 during stimulation. There was a 180 degree phase offset between regions at the pulse frequency (50 Hz) during ipsilateral stimulation, consistent with successful reduction of the artifact. Contralateral stimulation resulted in a ~0 degree phase offset at the pulse frequency. Color indicates circular distance (-180^{o} to 180^{o}). **B.** Moving time windows of CA3 – CA1 coherence time-locked to the onset of stimulation. Increased low gamma coherence was observed during ipsilateral stimulation (upper left) but not during contralateral stimulation (upper right). There was minimal coherence at the pulse frequency during contralateral stimulation, suggesting that the effects of the artifact on coherence were successfully attenuated via mean template subtraction. In the bottom left panel, the data are plotted as the difference between ipsilateral and contralateral stimulation. Only pixels that exceeding statistical significance as determined by a random permutation method ($p < 0.05$; see Method) are plotted in the bottom right. Coherence estimates were z-transformed and biased corrected. Color in the top row indicates coherence. Color in the bottom row indicates difference in coherence (Ipsilateral – Contralateral). **C.** Phase coherence and cross spectrum power for ipsilateral stimulation (dark red) and contralateral stimulation (pink). The bottom row indicates the difference (ipsilateral – contralateral) with significant clusters highlighted in red ($p < 0.05$). Significant clusters were identified using a random permutation method (see Method). The results indicate that there were significant increases in low gamma phase coherence and cross spectrum power during ipsilateral stimulation. Coherence estimates were z-transformed and biased corrected. Shading shows SEM.

Figure 4.7 Low gamma coherence under alternate stimulation conditions

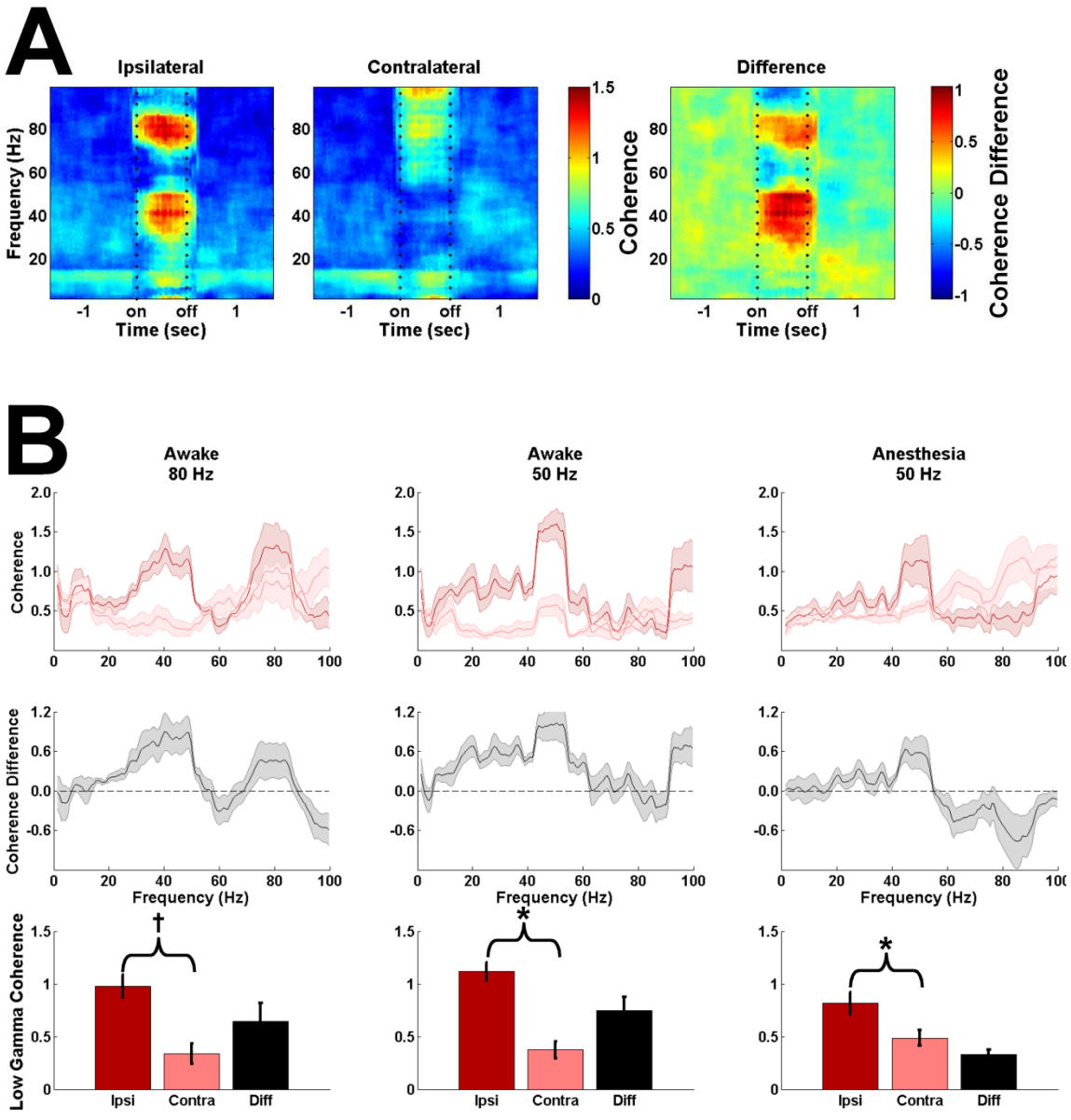

Figure 4.7 Low gamma coherence under alternate stimulation conditions (n = 4). A. Moving time window of CA3 – CA1 coherence during 80 Hz stimulation time-locked to the onset of stimulation. Low gamma coherence was increased during ipsilateral stimulation (left panel) but not during contralateral stimulation (middle panel). The results are re-plotted as the difference between conditions (ipsilateral – contralateral) in the panel on the right. Color in the 2 left panels indicates coherence estimates. Color in the right panel indicates the difference in coherence. **B.** Coherence across stimulation conditions. The middle column indicates coherence for the subset of rats that received 80 Hz stimulation and stimulation under anesthesia. The top row shows coherence estimates during ipsilateral (dark red) and contralateral (pink) stimulation. The second row shows the difference (ipsilateral – contralateral). Significance was determined by taking the area under the curve in the low gamma range (bottom row; see Method). The results indicate that there was a significant reduction in low gamma coherence during ipsilateral stimulation under anesthesia, but there was no difference in coherence between 50 Hz and 80 Hz ipsilateral stimulation. Coherence estimates were z-transformed and biased corrected. Shading and error bars show SEM. * $p < 0.05$, Bonferroni corrected; † $p = 0.1$, Bonferroni corrected.

Figure 4.8 Effect of stimulation on neuronal firing rate

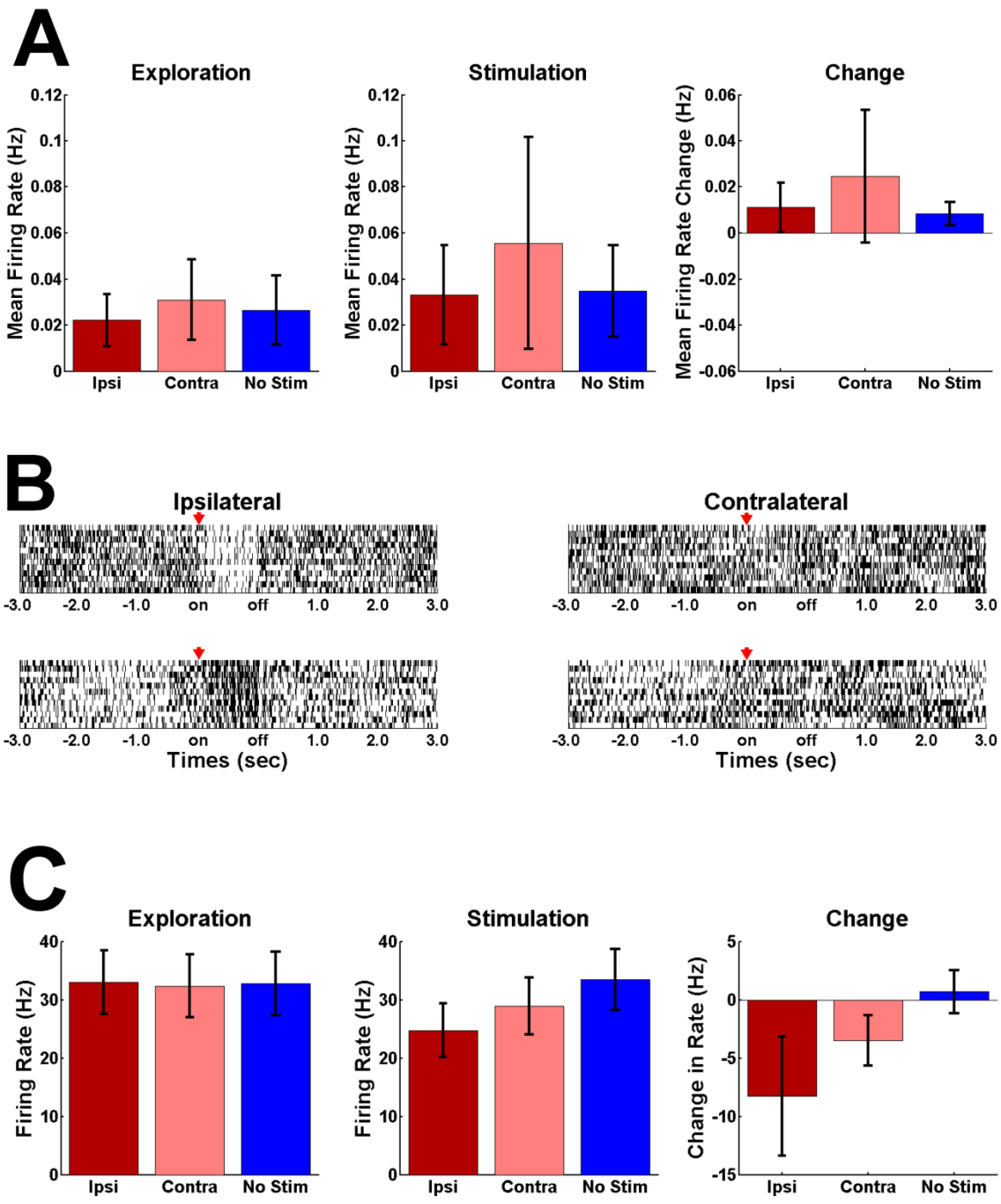

Figure 4.8 Effect of stimulation on neuronal firing rate. A. Mean firing rate of active pyramidal units during object exploration and subsequent stimulation averaged across rats (n = 7). The data suggest that stimulation did not impact the firing rate of pyramidal units. Further, it is unlikely that many pyramidal spikes were lost to artifact during stimulation. **B.** Example perievent rasters for 2 CA1 interneurons. Some interneurons decreased firing rate during stimulation while others increased firing rate. Responses were more common during ipsilateral stimulation than contralateral stimulation. **C.** Mean interneuron firing rate during object exploration and subsequent stimulation. The results indicate that stimulation did not have a common impact on firing rate across interneurons (n=14). Dark red = ipsilateral stimulation, pink = contralateral stimulation, blue = no stimulation. Error bars show SEM.

Figure 4.9 Spike – phase in low gamma

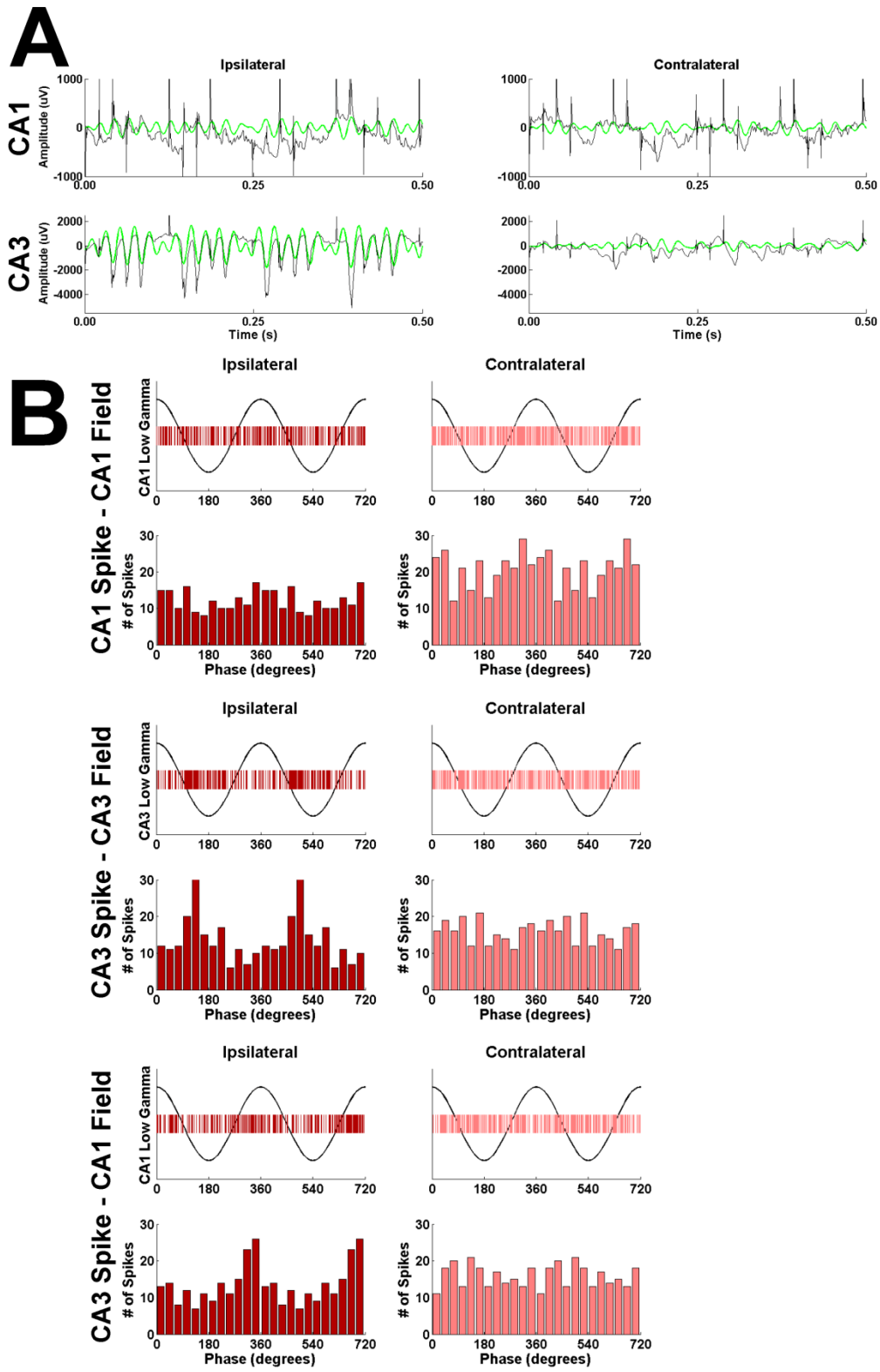

Figure 4.9 Spike – phase in low gamma. A. LFP filtered for low gamma (30 – 55 Hz). The black lines indicate raw LFP traces following artifact reduction. The green lines indicate the LFP filtered for low gamma. LFPs were recorded such that the extreme negative values were the peaks of the cycle and the extreme positive values were the troughs. **B.** Pyramidal spikes plotted by phase of low gamma oscillation. The black sine waves indicate an idealized gamma cycle, with peaks at the top. Each raster between 0 and 360 degrees represents 1 spike (CA1 spikes: Ipsilateral n = 363, Contralateral n = 466; CA3spikes: Ipsilateral n = 163, Contralateral n = 191). Spikes were plotted again between 360 and 720 degrees. Histograms below each raster plot indicate the number of spikes in 30 degree bins. CA3 pyramidal spikes had a prominent phase preference relative to low gamma oscillations in both fields during ipsilateral stimulation but not during contralateral stimulation.

Figure 4.10 Spike – field phase relationship in low gamma range

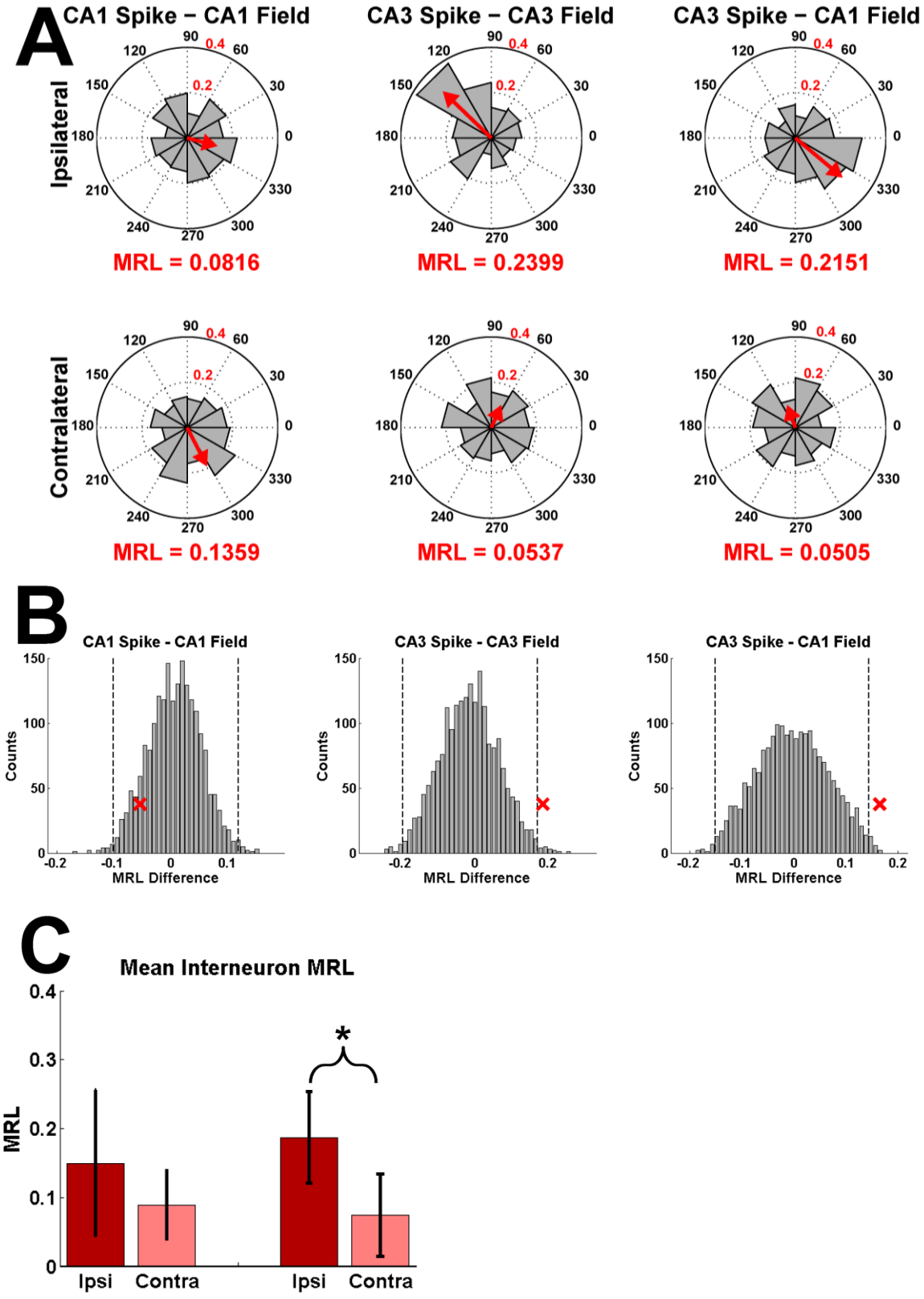

Figure 4.10 Spike – field phase relationship in low gamma range. A. Circular histograms of spike phases relative to low gamma (30 – 55 Hz). Pyramidal spike phase was plotted in circular histograms (30 degree bins) to demonstrate strength of the spike phase relationship. The mean resultant length (MRL) was calculated from the mean resultant vector for each spike-field comparison. Red arrows indicate preferred spike phase (mean phase angle) relative to low gamma oscillations and magnitude of the MRL. **B.** Distribution of MRL differences (ipsilateral – contralateral) from 1000 shuffles of the data. Results indicate that the strength of CA3 spike-phase preference exceeded statistical significance relative to low gamma oscillations in the LFPs from CA3 and CA1 during ipsilateral stimulation. Red 'X' indicates actual difference. Dashed lines indicate statistical significance ($p < 0.05$, Bonferroni corrected for multiple comparisons). **C.** Mean MRL across interneurons relative to low gamma oscillations in CA1 ($n = 13$) and CA3 ($n = 9$). Interneurons showed a significantly stronger spike-phase preference relative to low gamma oscillations in CA3 during ipsilateral stimulation (dark red) than during contralateral stimulation (pink). Error bars indicate SEM; * indicates $p < 0.05$, Bonferroni corrected.

Figure 4.11 Spike patterns during stimulation and recent object exploration

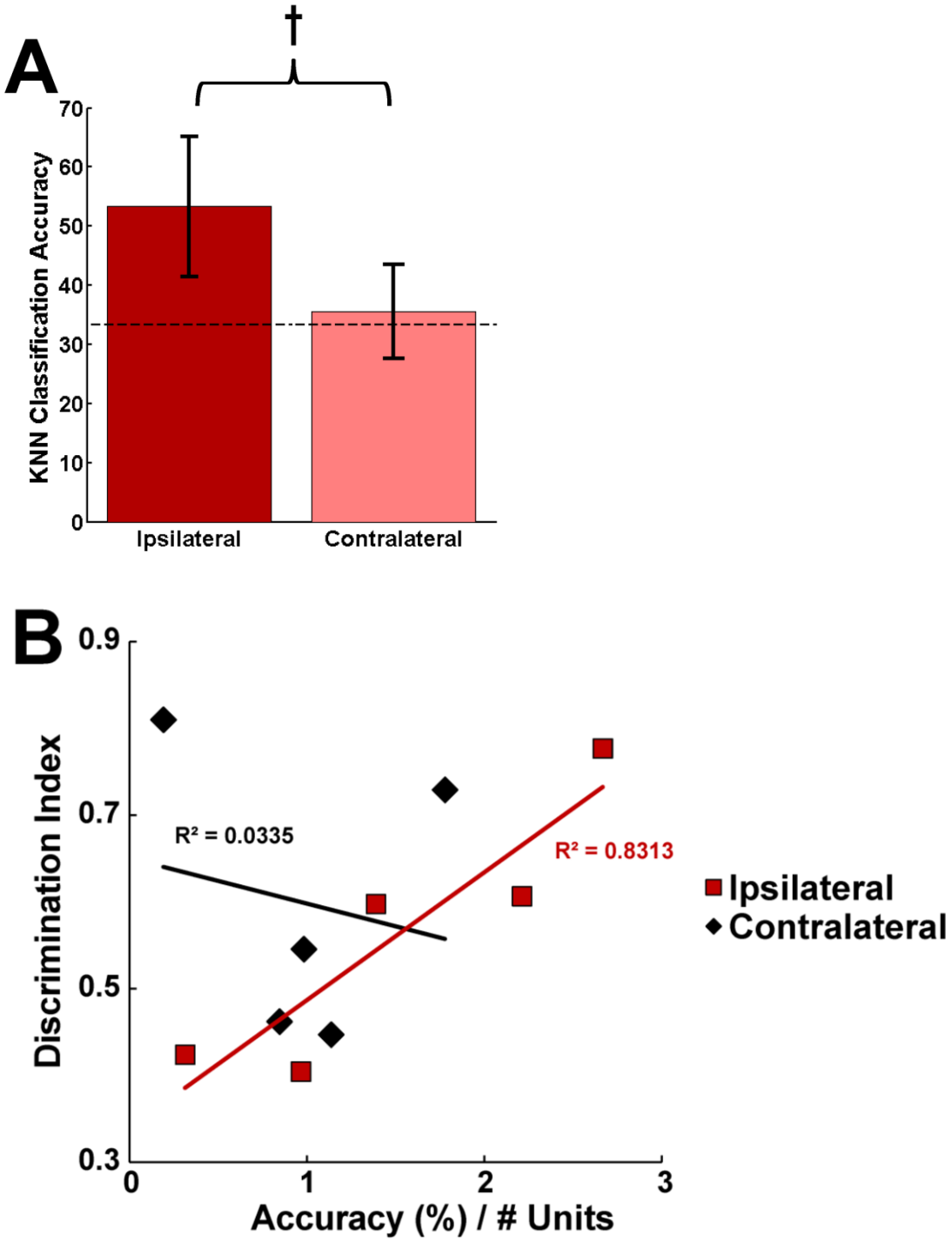

Figure 4.11 Spike patterns during stimulation and recent object exploration (n = 5). **A.** K-nearest neighbor (k = 1) classification accuracy averaged across rats. Spike patterns during stimulation were compared to spike patterns observed during exploration of objects within each trial. An accurate trial indicates that the spike pattern during stimulation was nearest to the spike pattern during exploration of the object immediately preceding stimulation, the Stimulation object. Results suggest that KNN classification accuracy was greater during ipsilateral stimulation than during contralateral stimulation. Error bars show SEM. † $p < 0.1$. **B.** Adjusted KNN classification accuracy compared to memory performance on the 1-Day test for ipsilateral and contralateral stimulation. Accuracy was adjusted for the number of active units that were recorded (see Method). The adjusted accuracy for ipsilateral stimulation correlated with memory for ipsilateral Stimulation objects on the following day ($p < 0.05$), but the adjusted accuracy for contralateral stimulation did not predict memory ($p > 0.1$).

Chapter 5. Discussion

5.1 Conclusions

5.1.1 Summary of Results and Interpretations

The goal of the research discussed in the present dissertation was to understand how the amygdala, specifically, the BLA, can modulate hippocampal function in the service of enhancing memory. This specific question was chosen because it addresses fundamental questions in basic science and has potential clinical implications. It is important to know how vital information is prioritized for consolidation into long-term memory. The amygdala functions as an intrinsic mediator of memory enhancement and, thus, became a focus in our investigation. Knowledge gained specifically regarding amygdalo-hippocampal interactions has the potential to advance the understanding and treatment of a plethora of psychiatric disorders, particularly PTSD. In Experiment 1, we demonstrate that brief electrical stimulation of the BLA can enhance memory in a stimulus-specific manner. In Experiment 2, we demonstrate that this amygdala-mediated enhancement of memory depends on the hippocampus. Finally, in Experiment 3, we show that amygdala stimulation rhythmically synchronizes neural activity in the hippocampus.

Previous studies had shown that the amygdala can enhance memory for many types of information, regardless of emotional content (Bergado et al., 2006; Chavez et al., 2009; Paul E Gold et al., 1975; Packard et al., 1994; Roozendaal et al., 2008), but the temporal specificity of amygdala-mediated memory enhancement had not been thoroughly investigated. Results from Experiment 1 demonstrate that the amygdala is indeed capable of targeting specific events within a series of object encounters for

selective memory enhancement. Consistent with predictions, the benefits of amygdala stimulation emerged 1 day later, but stimulation did not appear to have an impact when memory was tested approximately1 hr later. One interpretation is that amygdala stimulation preserved memory from decay over time, similar to the effects of amygdala stimulation on LTP in anesthetized rats. In those studies, the impact of BLA stimulation was not observed in the hour immediately following induction of LTP in the hippocampus, but LTP was preserved many hours later when LTP induction had been paired with BLA stimulation (Akirav & Richter-Levin, 1999b; Frey et al., 2001; Ikegaya et al., 1995).

Object encounters were brief events (typically no more than 3 sec), and the hippocampus supports memory for events (Squire et al., 2004). Thus, the hippocampus seemed a likely target of amygdala modulation for memory enhancement. Results from Experiment 2 demonstrate that the hippocampus is necessary for amygdala-mediated enhancement of memory in this task. Rats remembered objects 1 day later only if the object encounters were immediately followed by amygdala stimulation *and* the hippocampus had not been inactivated. Moreover, rats could not discriminate between repeated and novel objects on the Immediate Test if a small segment of the hippocampus had been inactivated with muscimol. This finding suggests that the role of the hippocampus is not limited to long-term consolidation, but rather it contributes to object recognition memory at earlier time points. Indeed, several studies haves shown that the development of item-related representations in the hippocampus parallels learning over intervals much shorter than 1 day (Komorowski, Manns, & Eichenbaum, 2009; Manns & Eichenbaum, 2009). Thus, it is likely that amygdala stimulation enhances consolidation

of object-related representations in the hippocampus rather than inducing hippocampal representations.

The objective of Experiment 3 was to determine how amygdala stimulation modulates LFPs and spiking activity in the hippocampus. Rats performed the same memory task as in the first 2 experiments, and once again, rats showed stimulus-specific enhancement of memory following brief electrical stimulation to the amygdala. Results from the LFP and spike data analyses suggest that amygdala stimulation enhanced memory by augmenting mnemonic processes that are endogenous to the hippocampus. Specifically, stimulation elicited low gamma coherence between CA3 and CA1 and induced a strong phase preference of pyramidal spikes relative to LFP oscillations in the low gamma range. These mechanisms are likely endogenous to the hippocampus because both gamma coherence and spike-phase relationship to gamma frequency oscillations in the hippocampus have previously been correlated with improved recognition memory (Jutras et al., 2009; Trimper et al., 2014). Furthermore, low gamma coherence was induced at 2 different stimulation frequencies, suggesting that amygdala input facilitates oscillations in the hippocampus and that neural activity in the hippocampus tends to synchronize in the low gamma range. Notably, stimulation did not significantly impact the firing rate of pyramidal unit populations. Rather, modulation of spiking activity was predominantly via spike timing. Thus, one interpretation of the results is that the amygdala facilitates spike-timing dependent plasticity in the hippocampus to benefit by inducing oscillations that synchronize in the gamma range.

Spiking patterns during ipsilateral stimulation were similar to those observed during recent object exploration, suggesting that amygdala stimulation elicits reactivation

of neural representations for events. The degree of similarity between spike patterns during ipsilateral stimulation and recent exploration correlated with memory for objects 1 day later. These findings parallel previous reports indicating that reactivation of spatial representations during brief epochs of increased gamma synchrony between CA3 and CA1 is required for successful consolidation of spatial memory (Carr et al., 2012; Girardeau et al., 2009; Jadhav et al., 2012). However, in the present study it is unclear why reactivation was observed in some instances of induced gamma coherence but not others. The explanation may lie within the variations of low gamma coherence observed during object exploration. That is, object encounters with high coherence in the low gamma band between CA3 and CA1 may have resulted in relatively stable memory traces that could be reactivated by amygdala stimulation. In contrast, low gamma coherence during other object encounters may not have permitted memory traces to stabilize and subsequently be reactivated during stimulation. This hypothesis would be supported by the finding that spike patterns during stimulation were more similar to those observed during object exploration with greater gamma coherence than with lesser gamma coherence.

5.1.2 Alternate Interpretations

At the focus of the present dissertation is the question, how could 1 second of amygdala stimulation have such a prominent impact on memory? The interpretations submitted above argue that the key mechanism is the instantaneous modulation of endogenous processes within the hippocampus that benefit spike-timing dependent plasticity. This conclusion is based on the following lines of reasoning:

1. The hippocampus is important for memory enhancement in this task
2. Low gamma coherence between CA3 and CA1 of the hippocampus correlates with memory for objects
3. Amygdala stimulation elicits low gamma coherence between CA3 and CA1, regardless of stimulation frequency, and enhances memory for objects

However, hippocampal function was disrupted throughout the Study Phase, not just during amygdala stimulation. Thus, it is not entirely clear that gamma coherence between CA3 and CA1 specifically during amygdala stimulation is the key factor.

An alternate interpretation is that amygdala stimulation modulated memory processes in the entorhinal cortex and the perirhinal cortex and that subsequent information processing was required by the hippocampus. Recordings were not obtained from these regions, but both are important for memory and receive projections from the amygdala (Furtak et al., 2007; Petrovich et al., 2001; Pitkanen et al., 2000; Winters, Saksida, & Bussey, 2008). Specifically, the BLA sends robust projections to the lateral entorhinal cortex, the region of the entorhinal cortex that is heavily interconnected with the perirhinal cortex (Kerr et al., 2007), and the perirhinal cortex is a critical structure for object recognition memory (Ennaceur et al., 1996; Winters et al., 2008). The amygdala has been shown to facilitate the transmission of information from the perirhinal cortex to the entorhinal cortex (Kajiwara et al., 2003; Paz et al., 2006), leading to the idea that these parahippocampal structures form a ‘wall of inhibition’ between the neocortex and the hippocampus and that amygdala input is necessary to facilitate transmission of

information through this wall (Curtis & Pare, 2004). This interpretation, however, is not entirely at odds with the view that modulation of the hippocampus by the amygdala is important for memory enhancement. It is likely that amygdala stimulation enhances memory by modulating mnemonic processes throughout the extended hippocampal formation, thus the need for projections to all 3 structures (Fig. 1.1).

Another interpretation is that BLA stimulation elicits mnemonic processes that are supra-physiological or not endogenous to the hippocampus. No assays were run to compare the effects of object exploration and BLA stimulation on molecular signaling pathways in the hippocampus, and no analyses were performed specifically to compare the low gamma coherence observed during object exploration with the low gamma coherence elicited during BLA stimulation. On the surface, there were some differences. Specifically, ipsilateral stimulation induced high magnitude evoked potentials in CA3, and the estimates for coherence were substantially larger during ipsilateral stimulation (~1.0) than during exploration (~0.5). However, LTP in the hippocampus can be saturated, such that BLA stimulation has no further effect on LTP (Frey et al., 2001). This would suggest that hippocampal function cannot be enhanced beyond a certain point, consistent with the interpretation that BLA stimulation modulates processes endogenous to the hippocampus.

5.2 Methodological Considerations

Historically, intracranial electrical stimulation and drug administration are two of the most common methods of perturbing the central nervous system to study memory (Kesner, 1982; McGaugh & Roozendaal, 2009). A third, increasingly common method is optogenetic stimulation, in which microbial opsins are inserted into the genes of neurons with cell-type specific promoters (Yizhar, Fenno, Davidson, Mogri, & Deisseroth, 2011). Expression of microbial opsins allows for optic stimulation of specific cell populations within a region. All three methods have their advantages and disadvantages, and the best choice depends on the demands of the study. For example, electrical stimulation is well-suited for studying event-specific enhancement of memory because the onset and offset of stimulation can be precisely controlled. Indeed, this level of temporal control is the reason electrical stimulation was chosen as the method for manipulating the amygdala in the present body of work rather than pharmacological infusions. However, there is much debate regarding how electrical stimulation modulates neural activity.

It is unclear which neural elements are affected by electrical stimulation, whether the tissue is excited or inhibited, and how the effect on the neural elements determines cell recruitment. The answers likely depend on the exact stimulation parameters, including pulse frequency, width, and shape, as well as duration of stimulation, current amplitude, current density, electrode configuration, and distance between the electrode and the neural element (Kringelbach, Jenkinson, Owen, & Aziz, 2007; Ranck, 1975; Tehovnik, 1996). A recent study using electrical stimulation and calcium imaging found that high frequency stimulation with narrow pulses excites neurons with axonal projections immediately adjacent to the tip of the stimulating electrode, regardless of the electrode's distance from the soma (Histed, Bonin, & Reid, 2009). Critics of electrical

stimulation point to such heterogeneous cell recruitment as one of the main pitfalls of electrical stimulation. However, blanketed cell recruitment can be advantageous under certain circumstances.

Prior to the work in the present research program, it was unclear whether amygdala stimulation could selectively enhance memory in a stimulus-specific manner, and thus targeting specific anatomical projections would have been impractical. Electrical stimulation allows for simultaneous activation of many different neuronal pathways, thereby increasing the probability of exciting the right projection, or combination of projections. In comparison, optogenetics targets very specific projections within a neural circuit (Tye & Deisseroth, 2012). This unprecedented level of specificity makes optogenetics a uniquely powerful tool for elucidating the underlying neural circuitry of well established phenomenon, such as appetitive conditioning (Witten et al., 2010), fear conditioning (Johansen et al., 2010), and anxiety-related thigmotaxis (Felix-Ortiz et al., 2013; Tye et al., 2011), for which there are enough data to formulate strong hypotheses regarding the underlying neural circuitry.

The effects of pharmacological agents can last much longer than those of electrical stimulation, and pharmacological mechanisms of action are thought to be better understood. For example, muscimol is a commonly-used $GABA_A$-receptor agonist with a 2 hour half-life, such that a single infusion remains biologically active for several hours (Arikan et al., 2002; Matthews, Intoccia, Osborne, & McCafferty, 1981). Muscimol temporarily inactivates local pyramidal neurons when it is infused directly into cerebral tissue, thereby inducing an effect analogous to a temporary chemical lesion. This compound works well in studies that require temporary inactivation of a brain region for

the duration of a training session, and it allows for within-subject test designs. That is, each subject can be tested in both the treatment (muscimol) and the control (saline) condition. Fluorophore-conjugated muscimol is a recently developed compound that allows for visualization of the spread of muscimol via fluorescent microscopy (Allen et al., 2008). Subsequent work has shown that the visualized spread of fluorophore-conjugated muscimol in the hippocampus correlates with local inactivation and inhibition of synaptic plasticity as determined by a decrease in expression of Arc protein (Jacobs et al., 2013). Thus, the volume of affected tissue can be visually approximated.

5.3 Future Directions

BLA stimulation immediately modulated activity in the hippocampus. Therefore, efforts to characterize the effects of BLA had to disambiguate the biological signal from the noise of the artifact. These analyses were largely possible due to the fact that the amygdala is anatomically and functionally connected with the ipsilateral hippocampus and not the contralateral hippocampus. Ipsilateral stimulation evoked prominent responses in the raw LFP, induced coherence between CA3 and CA1, and resulted in a strong spike-phase relationship relative to low gamma oscillations in the LFP. None of these effects were observed during contralateral stimulation. This begs the question of whether the contralateral hippocampus is necessary for amygdala-mediated memory enhancement. Future studies should determine whether preserving the functional connectivity between the BLA and the hippocampus is sufficient by unilaterally inactivating the hippocampus and having rats perform the object recognition memory

task. Results from the present body of work would suggest that rats will remember objects paired with BLA stimulation contralateral to side of hippocampal inactivation, but not when the ipsilateral hippocampus is inactivated (Fig. 5.1). That is, BLA stimulation should enhance memory if the functional connectivity between the stimulated amygdala and the hippocampus is preserved.

Hippocampal inactivation attenuated amygdala-mediated memory enhancement. Much research has shown that activation of the amygdala enhances memory by modulating consolidation (McGaugh, 2004) and that mnemonic processes in the hippocampus support consolidation (Squire et al., 2004). Thus, it would appear that hippocampal inactivation blocked amygdala-mediated enhancement of consolidation in the hippocampus, but the hippocampus also supports memory encoding and retrieval. Since muscimol was infused prior to the Study (encoding), it is not possible to determine whether the hippocampus was necessary for memory encoding, consolidation, or both. In order to address this question, future studies should inactivate the hippocampus following the Study to more precisely target consolidation. If hippocampal inactivation immediately following the Study attenuates memory enhancement, then the hippocampus is necessary for amygdala-mediated enhancement of memory consolidation. However, this result would not rule out a role for the hippocampus in memory encoding. In order to determine whether the hippocampus is necessary specifically for encoding memory, future studies would need to employ a method that transiently inactivates the hippocampus, allowing function to recover prior to encoding of the next object encounter.

A single electrical pulse delivered to the ventral hippocampal commissure (vHC) briefly inactivates the hippocampus, allowing normal activity to resume within 1 sec

(Girardeau et al., 2009; Jadhav et al., 2012). This method is ideally suited for determining whether the hippocampus must be functional during encoding because hippocampal activity would be otherwise unaffected during the consolidation period. A similar question is whether the hippocampus must be active specifically during stimulation. Muscimol infusions inactivate the hippocampus for the duration of the Study Phase. Thus, it is unclear whether the hippocampus needed to be actively engaged during stimulation. An alternate possibility is that BLA stimulation modulates activity in other regions, which subsequently require hippocampal support. Future studies should use vHC stimulation to precisely time hippocampal inactivation with BLA stimulation in order to determine whether modulation of hippocampal function specifically during amygdala stimulation is necessary for memory enhancement.

Stimulation of the vHC was initially designed to disrupt bursts of hippocampal pyramidal activity during sharp-wave ripples (Girardeau et al., 2009; Jadhav et al., 2012). The current theory is that recently formed neural representations are reactivated in bursts of pyramidal spikes during sharp-wave ripples (Foster & Wilson, 2006; Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994), and that sharp-wave ripples represent transient epochs of heightened plasticity that allow for consolidation of memory traces into the neocortex (Buzsáki, 1986; Carr et al., 2011). Therefore, if bursts were important for memory consolidation, then blocking sharp-wave ripples should impair memory performance. Indeed, recent research indicates that hippocampal inactivation specifically during bursts impairs spatial memory (Girardeau et al., 2009; Jadhav et al., 2012). Future research should determine whether similar mechanisms are at play for enhancement and consolidation of memory for events.

There is good reason to believe that episodic memory emerged from the representational space forged by spatial representations in the hippocampus (Buzsáki & Moser, 2013; Smith & Mizumori, 2006), and therefore bursts may also contribute to processing of memories for specific events. Pyramidal unit activity during sharp-wave ripples should be analyzed for similarity to spiking patterns during object exploration. Presumably, the spike patterns during bursts will be more similar to the spike patterns observed during exploration of Stimulation objects than those observed during exploration of other objects. In a follow-up study, stimulation of vHC should be used to disrupt hippocampal bursts following the Study Phase. The prediction is that disruption of hippocampal bursts shortly after memory encoding will attenuate amygdala-mediated enhancement of memory for events just as vHC stimulation has been used to impair spatial memory.

Finally, in order for these result to have any clinical significance, it will be important to determine how well they correspond with human physiology. Thus far, there have been a number of findings to suggest that similar computational processes occur in the human brain. Research in epilepsy patients with electrodes implanted for clinical monitoring of siezures suggests that gamma synchronization underlies human memory encoding and consolidation (J Fell et al., 2001; Sederberg et al., 2003). Others have found reactivation of spatial representations during retrieval of episodic memories (Miller et al., 2013). However, it remains to be determined whether direct stimulation of the amygdala can enhance memory for specific events in humans as it can in rats. If so, then the mechanisms that support amygdala-mediated enhancement of memory likely share common computational processes across species.

5.4 Clinical Translation

5.4.1 Implications

Results from the present body of research contribute to our understanding of PTSD by identifying and characterizing physiological mechanisms of memory enhancement that parallel the enhancement of memories for traumatic. Results suggest that hyper-activation of the amygdala during a traumatic event may prolong gamma synchronization within the hippocampus resulting in a much stronger memory of the inciting event, and that re-experiencing the memory may actually enhance consolidation of memory.

However, patients with PTSD experience a constellation of symptoms that go far beyond the memory for the traumatic event. In particular, patients suffer from an 'over-consolidation' of fear resulting in chronic hyper-vigilance and a general withdrawal from their environment (Mahan & Ressler, 2012). Indeed, this key difference is precisely why this model of memory enhancement may not be a good model of PTSD. There are no elements of fear conditioning, or any kind of stimulus conditioning, as a result of amygdala stimulation in this paradigm. Furthermore, patients with PTSD tend to have underlying genetic variations that can result in greater glucocorticoid sensitivity (Binder et al., 2010). Enhanced glucocorticoid sensitivity may further augment amygdala-mediated enhancement of memory consolidation (McGaugh, 2004). Thus, the results of this work must be interpreted with discretion in the context of PTSD.

5.4.2 Applications

Recent evidence has emerged suggesting that memories enter a labile state when they are reactivated and that they must re-stabilize through a process known as reconsolidation (Nader & Hardt, 2009). With the improved ability to detect reactivation of memories for specific events, it may be feasible to selectively disrupt traumatic memories when they are re-experienced. However, this strategy is fraught with many complications, not the least of which is that disruption of hippocampal function in the context of PTSD may actually result in the generalization of fear responses (Maren, 2011).

An alternative approach is to coordinate inhibition of the amygdala-mediated stress response with retrieval of traumatic information, thereby attenuating the visceral response to the memory without chronically impairing responses to emotional situations that arise in daily life. This strategy would play to the strengths of the present work in identifying particularly strong memories that resulted from amygdala activation. Furthermore, inhibition of the central nucleus of the amygdala via deep brain stimulation has been shown to mitigate symptoms of traumatic stress in a rodent model of PTSD (Langevin et al., 2010). The knowledge gained from this line of research could contribute to the development of a closed-loop circuit for deep brain stimulation for the treatment of PTSD.

Figure 5.1 Unilateral inactivation of the hippocampus during amygdala stimulation

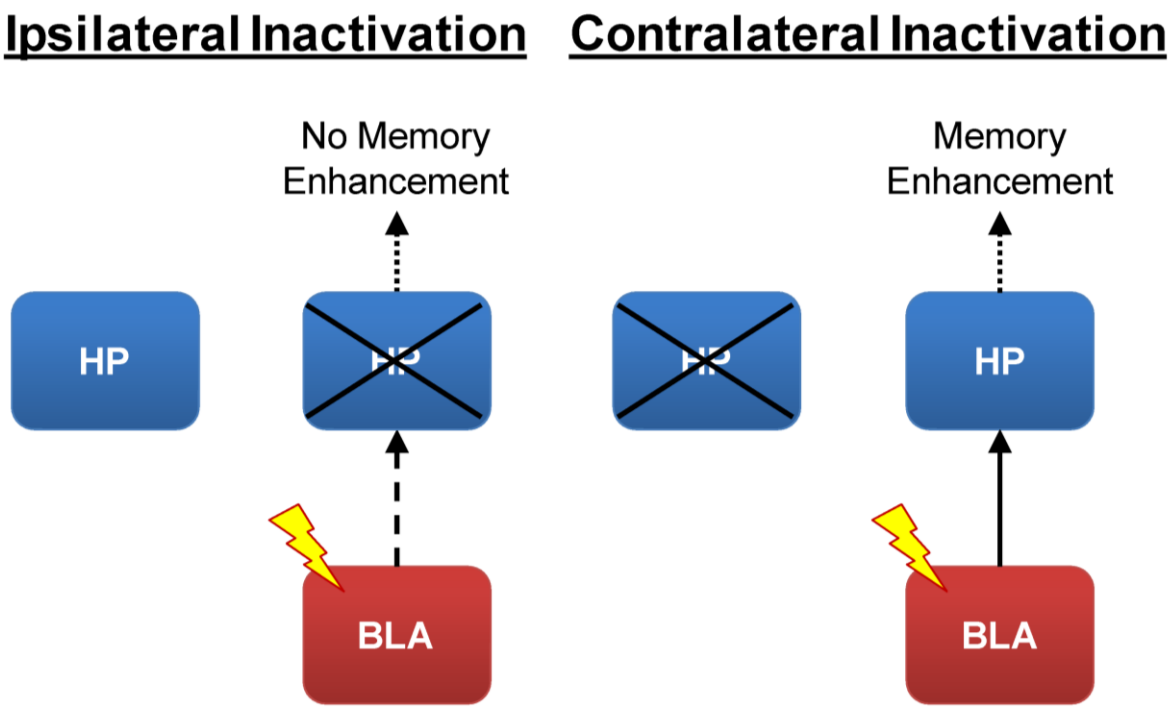

Figure 5.1 Unilateral inactivation of the hippocampus during amygdala stimulation. Inactivation of the hippocampus (HP) ipsilateral to the side of amygdala (BLA) stimulation should block the memory enhancing effects of stimulation by disrupting the functional connectivity between structures. Inactivation contralateral to stimulation should not impact the functional connectivity between the amygdala and the hippocampus, and therefore amygdala stimulation should enhance memory. Black crosses indicate inactivation. Lightning bolt indicates stimulation.

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