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The roles of the hippocampus and amygdala in the formation of declarative memory

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By

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B.S., University of North Carolina – Chapel Hill, 2014

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Abstract

The roles of the hippocampus and amygdala in the formation of declarative memory

By Nathan S Ahlgrim

Declarative memory depends on the hippocampus. The basolateral complex of the amygdala (BLA) directly projects to the hippocampus and contributes to the modulation of declarative memories. This dissertation further characterized the independent roles and functional interaction of these two regions. In the first aim of this dissertation, the specificity with which the amygdala and hippocampus represent visual and memory-related stimulus characteristics was investigated by analyzing single-unit activity in the human hippocampus and amygdala during a recognition memory task. Specifically, evidence for sparse coding was investigated. Sparse coding could demonstrate a specificity of single-unit coding that cannot be observed by analyzing units independently or within a population. Both regions showed evidence of sparse coding, but the hippocampus and amygdala were tuned towards different stimulus characteristics. These coding patterns demonstrated that the amygdala encodes specific information relevant to declarative memory and can likely modulate hippocampal activity in a specific manner. However, these specific representations of stimuli are only relevant to memory if they are consolidated. Certain local field potential patterns, derived from coordinated neuronal activity, are known to prioritize consolidation in the hippocampus. Previous experiments that prioritized memory via BLA stimulation were thought to increase these oscillations beneficial to memory. In the second aim, the BLA was optogenetically stimulated in rats to determine what oscillatory activity in the BLA could increase pro-memory oscillatory activity in the hippocampus. Only stimulation that replicated a gamma wave (50 Hz) whose amplitude was modulated by the phase of a theta wave (8 Hz) was sufficient to increase pro-memory oscillations in the hippocampus. Taken together, the two aims of this dissertation demonstrate the distinct roles of the amygdala and hippocampus in how declarative memory is formed and consolidated. The amygdala can encode information relevant to memory with specific ensemble activity, but it also can coordinate hippocampal activity through specific modulation of ongoing oscillatory activity.

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Facts and events that are remembered long-term are the minority in a memory system that is skewed towards forgetfulness. The rare individuals with Highly Superior Episodic Memory, who can recall autobiographical events with near-perfect accuracy (LePort, Stark, McGaugh, & Stark, 2017; Parker, Cahill, & McGaugh, 2006), are the exceptions to prove the rule. Many scientists, philosophers, and other thinkers believe the propensity to forget is a blessing, whether from the peace of moving past traumatic events or from the focus of only remembering the most recent parking place instead of sorting through the thousands in the past (Nietzsche, 1997; Parker et al., 2006). As critical as forgetting may be, the phenomenon is only beneficial if the unimportant facts and events are forgotten while the important ones (biologically or personally) are prioritized to be preferentially remembered. Which facts and events are assigned to be remembered or forgotten—and how that assignment is achieved—is a question that cannot be fully answered at the level of behavior. Basic neuroscience research of specific brain regions and networks is needed to understand, and perhaps control, how memories are formed and modulated. The gaps in the current understanding of these processes motivated the current dissertation.

Many individual brain regions and networks have been found to be involved in the process of memory prioritization. Those regions that are fundamental for memory formation and the processing of affective content are logical areas of focus. Decades of research have identified the relationship between the hippocampus and the amygdala in particular as a critical component in the process of prioritizing the minority of details that persist in memory (Bergado, Lucas, & Richter-Levin, 2011; McGaugh, 2002, 2004; Richter-Levin & Akirav, 2003). The hippocampus plays a critical role in the formation of declarative memories (Manns & Eichenbaum, 2006; Squire, Stark, & Clark, 2004) and the amygdala is recruited in response to stimuli with

emotional, social, or other affective significance (Fried, MacDonald, & Wilson, 1997; Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007; Yang, Bellgowan, & Martin, 2012). Thus, the individual properties of these two regions sets up the interaction between the two as a logical circuit involved in the modulation of memory strength based on the affective salience of a stimulus. The ability to modulate memory in this way is of critical evolutionary value, and this value is observed by the conservation of this system across species. There is striking homology of the hippocampus (Butler, 2017; Manns & Eichenbaum, 2006), amygdala (Janak & Tye, 2015; McDonald, 1998), and the connections between them (Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000; Wang & Barbas, 2018) across the class Mammalia. Therefore, the same questions can be asked, and similar answers can be gained, from models in rodents, non-human primates, and humans. Research across species has been designed to analyze the information present in these two regions to better understand the relationship between the amygdala-hippocampus circuit and the prioritization of memory.

This dissertation investigated this network by analyzing it on two distinct, but related levels. Aim 1 (Chapter 2) assessed how events and facts are first represented by neuronal activity at the time of encoding. The primary question of Aim 1 was whether and how information about memory content and social salience is specifically represented in the amygdala and hippocampus. After the initial representation, that information is thought to be processed between the amygdala and hippocampus for consolidation. The conditions in which the activity beneficial to consolidation is promoted was investigated in Aim 2 (Chapter 3). The primary question of Aim 2 was what oscillatory output from the amygdala is required to increase oscillations in the hippocampus shown to be beneficial to memory. These properties of the hippocampus and amygdala—how they fire at the population level down to individual neurons—

can inform how specific memories are prioritized for long-term consolidation over others, and can offer insights into how that prioritization can be manipulated to forget the maladaptive memories, and remember the important ones. The following sections will contextualize the aims of this dissertation within the broader understanding of hippocampal function, amygdala function, and how they are currently understood to interact in memory processes.

1.1. The hippocampus is critical for declarative memory

The hippocampus is part of the broader hippocampal formation, which is comprised of the entorhinal cortex, dentate gyrus, hippocampus, subiculum, presubiculum, and parasubiculum (Witter & Amaral, 2004), and is organized on a longitudinal axis (the dorso-ventral axis in the rodent hippocampus corresponding to the posterior-anterior axis in primates; Manns and Eichenbaum, 2006; Strange et al., 2014). It has long been understood to play a critical role in declarative memory (Manns & Eichenbaum, 2006; Squire et al., 2004). Human patients with damage to the hippocampus live with profound anterograde and temporally graded retrograde amnesia (Manns, Hopkins, & Squire, 2003; Zola-Morgan, Squire, & Amaral, 1986), meaning that the ability to form declarative memories after the hippocampal resection was almost completely erased, and memories from the time immediately prior to the resection were similarly impacted. Therefore, the hippocampus has been shown to be critically involved in the formation and initial storage of declarative memory in humans. Animal studies that lesioned or inactivated the hippocampus have confirmed the necessity of hippocampal function in the formation of declarative memory (Manns & Eichenbaum, 2006; Squire et al., 2004). The hippocampus acts as a hub of many input streams, receiving strong input from regions including the entorhinal cortex, perirhinal and parahippocampal cortices, and many subcortical regions (e.g., medial septum, locus coeruleus, amygdala; Witter & Amaral, 2004). As such, the hippocampus is well situated

to process and synthesize the many types of information that are integrated into memories of events and facts.

As evidence for its multimodal responsivity, the hippocampus is responsive to many modalities of information on a single-neuron and population level. Place cells—neurons that fire in a specific location in space (O'Keefe & Dostrovsky, 1971)—are perhaps the most notable of these selective neurons. First characterized in rodents, place-selective neurons have also been observed in the human hippocampus (Ekstrom et al., 2003; Miller et al., 2013) and other species. Neurons in the human hippocampus have also been found which are selective to the memory status of an object in the same way place cells are to location (Folkerts, Rutishauser, & Howard, 2018; Halgren, Babb, & Crandall, 1978; Rutishauser, Ross, Mamelak, & Schuman, 2010; Rutishauser et al., 2015). Given the change in spatial representation in the rodent hippocampus in response to the introduction of a goal (Kobayashi, Tran, Nishijo, Ono, & Matsumoto, 2003; Mamad et al., 2017), similar learning-related processes likely exist across species as well. Generally, hippocampal activity is necessary to bind objects in time and/or place, such that memory for an environmental context, specific aspects of object recognition, and spatial navigation all suffer in the absence of a typically functioning hippocampus (Andersen, Morris, Amaral, Bliss, & O'Keefe, 2007).

Hippocampus-dependent memory, however, is not consolidated in an isolated circuit. The strength and accuracy of memories can be modulated by factors internal and external to the subject. In humans, the semantic relatedness of stimuli (Puff, 1970), the attention paid to a stimulus (Gardiner & Parkin, 1990), and the level of engagement with the stimulus (Craik & Lockhart, 1972) are known to influence how effectively the memory is encoded. Emotional or affective content is an additional factor that often covaries with the others. That said, emotional

or affective content in and of itself modulates memory through activity of the basolateral complex of the amygdala (BLA). Hormonal systems (Gold & Van Buskirk, 1975; Hui et al., 2004; Liang, Juler, & McGaugh, 1986; McGaugh & Roozendaal, 2002) and behavioral states (Roozendaal, McEwen, & Chattarji, 2009) triggered by emotional content activate the BLA, which in turn drives the resulting change in memory. The perpetual influence of the amygdala on the hippocampus is a critical component of the processes underlying declarative memory.

1.2. The amygdala: more than the emotion center of the brain

The amygdala has long been understood to be critically involved in a complex suite of behaviors, from threat assessment to social cognition to general emotional reactivity (Klüver & Bucy, 1939; LeDoux, 1998). Far from a single brain region, the term "amygdala" refers to a heterogeneous group of nuclei, which is typically divided into the BLA (comprised of the lateral, basal, and accessory basal nuclei), cortical-like nuclei, centromedial nuclei, and other nuclei (although precise divisions vary, see Sah, Faber, Lopez De Armentia, & Power, 2003; Schmitt et al., 2012; Swanson & Petrovich, 1998). These divisions largely track across rodents, non-human primates, and humans, with the primary difference being the nuclei derived from cortical progenitors (i.e., BLA) have expanded in conjunction with the neocortex of primates (Janak & Tye, 2015). The amygdala is highly interconnected with cortical and subcortical regions (Petrovich, Canteras, & Swanson, 2001; Sah et al., 2003; Swanson & Petrovich, 1998), situating it to be involved in diverse systems and behaviors.

The comprehensive role of the amygdala can be well described by documenting the lives of rare patients with selective damage to the amygdala. Patient S.M. was one such patient, who was originally characterized by Adolphs, Tranel, Damasio, and Damasio (1994). She lived with Urbach-Weithe disease resulting in complete and restricted bilateral damage to the amygdala.

She did not display any profound deficits of intelligence, motor function, language, or other domains, but the initial characterization of her emotional reactivity strongly supported the concept of the amygdala as the emotional or social center of the brain. Patient S.M. was first characterized as "fearless" to both laboratory and real-world stimuli (Feinstein, Adolphs, Damasio, & Tranel, 2011). Like other patients with damage to the amygdala (Bechara et al., 1995; LaBar, LeDoux, Spencer, & Phelps, 1995), Patient S.M. appeared unable to develop a conditioned fear response. In this way, the experience of human patients agrees with the animal literature showing fear conditioning to be dependent on amygdala activity, and specifically the BLA (Izquierdo, Furini, & Myskiw, 2016; Phillips & Ledoux, 1992). Without BLA activity, neither animals nor human patients are able to associate a cue (e.g. a tone) with an aversive stimulus (e.g. a foot shock), and never learn to express a fear response to the cue.

Deficits in the processing of social cues were also apparent in Patient S.M. and other patients with amygdala lesions. Early reports characterized them as unable to identify fearful expressions, even though their recognition of other expressions was unimpaired (Adolphs et al., 1994; Adolphs et al., 1999). In addition, they judged unfamiliar faces as more trustworthy than healthy controls (Adolphs, Tranel, & Damasio, 1998). Complete amygdala damage unequivocally altered her emotional reactivity in natural settings in addition to the laboratory. When presented with fear-inducing experiences from haunted houses to snakes and spiders (towards which she previously expressed her aversion), it was as if fear was displaced by an "overwhelming feeling of curiosity" (Feinstein et al., 2011). Initially, at least, patient S.M. appeared to demonstrate that emotional and social processing was almost wholly dependent on the amygdala.

Further tests of S.M. illuminated the nuance in the amygdala's function that was only possible to decipher in a human patient. First, her inability to identify fearful expressions was revealed to be an impairment of selective attention, not comprehension. Patient S.M. did not spontaneously focus on the eyes of a human face as healthy controls do (similar to patterns observed in autism spectrum disorders; see Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Pelphrey et al., 2002), but she was able to identify fearful expressions when explicitly instructed to focus her attention on the eyes (Adolphs et al., 2005). Also contrary to the original conclusions, she was not truly immune to fear and panic; she reacted with typical fear responses when the fear stimulus was generated "interoceptively" with an increased concentration of carbon dioxide (Feinstein et al., 2013). Her emotional repertoire was such that neither of the clinical psychologists who evaluated patient S.M. (blind to her condition) diagnosed her with any psychiatric condition as defined by the DSM-IV (Tranel, Gullickson, Koch, & Adolphs, 2006). Therefore, although the amygdala damage profoundly affected patient S.M., the studies of her condition clarified that the production and comprehension of emotional and social cues is not wholly dependent on a typically functioning amygdala. Experiments in human patients and animal models have converged to broaden the role of the amygdala to that of an indicator of affective salience.

Broadening the scope of the amygdala, and specifically the BLA, from a fear and reward center to a center responsive to affective salience better encompasses the suite of behaviors it is known to influence (Morrison & Salzman, 2010; Pessoa, 2010; Phelps & LeDoux, 2005). Affective salience includes negative (e.g. fear and pain) and positive (e.g. food and mating) characteristics. Stimuli at both ends of the spectrum are processed by the BLA (Murray, 2007), and the BLA is positioned to modulate the memorability of all stimuli with a substantial affective

component. This framework explains why the amygdala is recruited during reward learning (Murray, 2007), a function that would at first appear contradictory to the amygdala's initial characterization. It also explains why amygdala activity is involved in the preferential attention to and appraisal of, but not comprehension of social cues (Adolphs et al., 2005). Other circuitry underlies the ability to understand wide-eyed fear or an angry glare, but amygdala activity identifies it as important. Thus, it follows that facts and events can also be identified as important and tagged for memory consolidation. Multiple lines of research across species have shown memories of multiple modalities to be modulated by amygdala activity.

1.3. The amygdala and memory

BLA activity has been shown to be critically involved in learning and memory-related events that have some emotional or affective component (McGaugh, 2004). Under normal circumstances, events with emotional content are remembered more often and more strongly than those lacking emotional content, which recruits and is dependent on the amygdala (Adolphs, Tranel, & Denburg, 2000; Hamann, Ely, Grafton, & Kilts, 1999; McGaugh, 2004). The interplay between affective salience and memory takes many forms behaviorally, ranging from flashbulb-like memories of traumatic or highly charged events (Brown & Kulik, 1977), to witnesses of armed encounters maintaining a weapon-focus in their memory (Steblay, 1992), to research participants remembering positively or negatively arousing pictures better than neutral pictures (Bradley, Greenwald, Petry, & Lang, 1992; Hamann et al., 1999). This is not to say that emotional content always positively correlates with memory accuracy. Tests of eyewitness testimony, criminal justice, and laboratory studies have demonstrated how the emotional content, emotional reactivity, and even confidence of the memory, does not directly correlate with the accuracy of the memory itself (Phelps & Sharot, 2008; Talarico & Rubin, 2003). Therefore,

emotion, or affective salience more broadly, cannot be said to enhance memory accuracy. Even so, it can certainly modulate the propensity to recall emotional events.

Fear memories are known to be prioritized for consolidation; they can persist on the order of months or years even in rodents (Gale et al., 2004; Maren, Aharonov, & Fanselow, 1996). Selective lesions and other manipulations in animal models have been able to functionally dissociate the heterogeneous groups of amygdalar nuclei. In so doing, the BLA has been shown to be primarily responsible for the amygdala's effects on memory (Izquierdo, Furini, & Myskiw, 2016; Sah et al., 2003). The centromedial amygdala, in contrast to the BLA, is required for expression of the autonomic and physiological responses to emotionally salient cues as opposed to modulation of the declarative memory (Janak & Tye, 2015; Sah et al., 2003; Shackman & Fox, 2016), and will not be addressed in this review. Mapping the inputs to the BLA can help describe what originates fear conditioning, emotional memories, and even conditions like Post-Traumatic Stress disorder (PTSD), but it is the BLA's outputs that can describe how the resulting memories are modulated and expressed. Now that the amygdala is known to be involved in such a wide array of procedures and computations, focus has expanded to investigate its connections with the hippocampus. Characterizing this network can help uncover how the suite of behavioral change observed in patients and lesion models occurs mechanistically.

1.3.1. The amygdala as a hippocampal modulator

The BLA, particularly the posterior portion of the BLA, projects strongly to the hippocampus and surrounding areas. The ventral subiculum and CA1 receive the most direct input, with projections strongest at the ventral/anterior pole and thinning towards the dorsal/posterior pole. The reciprocal connections from the hippocampus travel largely along the same pathways (Pitkänen et al., 2000). Though highly interconnected, double dissociation

studies of amygdala and hippocampal damage in humans have demonstrated the differential role of the two regions in emotional memories. Whereas a patient with an amygdala lesion could not acquire an emotional response to an conditioned stimulus even though they remembered the semantic information of the training, a patient with a hippocampal lesion could not remember the semantic information but successfully acquired an emotional response to the conditioned stimulus (Bechara et al., 1995). These results in humans complement and clarify results from animal models. The search for the "engram," or the collection of neurons that represent a specific memory, has led to various interpretations of the BLA's role in affective memory. In such rodent studies, BLA neurons that were especially active during learning a new association (often fear conditioning) are selectively labelled (Butler, Wilson, Gunnersen, & Murphy, 2015; Han et al., 2009; Kitamura et al., 2017). Inhibiting those specific populations prevents the expression of fear memory, whereas activating them evokes a fear response in unconditioned contexts. These results have led to the conclusion that the BLA is the site of the fear memory. Even so, the results do not negate the possibility of additional modulatory roles.

Activity in the BLA, central amygdala, hippocampus, and other regions are involved in the experience and expression of learned fear. The connectivity between the BLA and hippocampus are such that activating a labelled ensemble of hippocampal neurons has been found to produce the same behavioral effects (i.e., conditioned fear response) as the stimulation of BLA ensembles (Liu et al., 2012; Liu, Ramirez, & Tonegawa, 2014). Neither "engram," in the BLA nor hippocampus, can be independently responsible for the expression and experience of fear memory for two reasons. Firstly, both engrams are projecting to an intact brain, so downstream connections are still functional. Secondly, the semantic and emotional content were not dissociated as they were in the human study (Bechara et al., 1995). The collection of

evidence demonstrates that select neurons are activated during fear memory, and that those neurons are sufficient to drive the final conditioned response (i.e. freezing). In the intact brain, the BLA can play an active role in memory both as an activation node and as a modulator that is removed from the actual memory trace. These complementary abilities suggest that BLA activity can modulate specific memories when its activation is restricted to specific events.

Successful learning of emotionally salient tasks like cued and contextual fear conditioning (Huff & Rudy, 2004; Johansen et al., 2010; Maren et al., 1996; Phillips & LeDoux, 1992) and inhibitory avoidance (Holloway-Erickson, McReynolds, & McIntyre, 2012; Huff, Miller, Deisseroth, Moorman, & LaLumiere, 2013; McIntyre, Hatfield, & McGaugh, 2002; McIntyre et al., 2005; McReynolds et al., 2010; McReynolds, Holloway-Erickson, Parmar, & McIntyre, 2014b) are dependent on amygdala activity. In addition, the strength of those memories and resulting behavioral expression can be increased or decreased by increasing or decreasing amygdala activity, respectively (Gold & Van Buskirk, 1975; McIntyre et al., 2005; McReynolds et al., 2010). Stress and corticosterone injections typically enhance the consolidation of memories, but those modulatory effects are erased if BLA activity is inhibited (Roozendaal, Griffith, Buranday, de Quervain, & McGaugh, 2003; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Roozendaal, Schelling, & McGaugh, 2008). In fact, stress and corticosterone, dependent on BLA activity, have been shown to bias memory systems away from retrieval and towards consolidation generally (de Quervain, Roozendaal, & McGaugh, 1998; Roozendaal et al., 2003). These results provided some of the first evidence that BLA activity can bias consolidation in a way that is in tension with retrieval. BLA activity modulates hippocampus-dependent memory, and this effect by necessity means that it manipulates the neurophysiology of the hippocampus to facilitate enduring change in those circuits.

Experimental manipulations of BLA function have shown that activity within this circuitry can modulate memory regardless of what activates it, or how it is activated. Although the amygdala would naturally be activated by events with strong affective salience, the amygdala-hippocampus circuit has been experimentally manipulated to enhance memories of non-arousing events, such as object recognition tasks (Barsegyan, McGaugh, & Roozendaal, 2014; McReynolds, Anderson, Donowho, & McIntyre, 2014a; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008; Roozendaal et al., 2003). Amygdala activity is even known to influence what may be the most characteristic of hippocampal neural correlates; place cells remap following emotionally salient events, and the remapping is dependent on BLA activity (Donzis, Rennaker, & Thompson, 2013; Kim, Kim, Park, Cho, & Kim, 2012; Kim et al., 2015; Moita, Rosis, Zhou, LeDoux, & Blair, 2004; Wang et al., 2012; Wang, Yuan, Keinath, Ramos Álvarez, & Muzzio, 2015). The collection of evidence shows the amygdala to influence hippocampal activity and resulting memory in multiple domains.

1.3.2. The amygdala and cellular analogues of memory

The phenomenology of memory modulation by affective content demonstrates the conditions in which hippocampal activity is modulated by the BLA, but behavioral outputs do not describe the mechanisms of that change. A mechanistic understanding of how the amygdala modulates the hippocampus is a prerequisite to building a more comprehensive model of the BLA-hippocampus circuit. Biological and physiological markers of memory are key output measures of such models. These correlates of memory are objective measures of memory processes that provide targets for interventions to manipulate memory (positively or negatively). Manipulations of the BLA, both artificially and behaviorally, have been shown to modulate the physiological markers of memory, highlighting the mechanisms driving the changes in memory.

Long-term potentiation (LTP) and depression (LTD) were some of the first identified neural correlates of memory (Bliss & Lomo, 1973). Activity in the BLA has been found to directly influence synaptic strength and network connectivity within the hippocampus, as measured by LTP (Akirav & Richter-Levin, 2002). In contrast, stimulation of the central amygdala has no effect on the hippocampal circuit (Frey, Bergado-Rosado, Seidenbecher, Pape, & Frey, 2001), again dissociating the subregions of the amygdala. These experiments have shown that the influence of the BLA on the hippocampus is mediated at least in part through indirect pathways. Even though there is no evidence of direct projections from the amygdala to the dentate gyrus (Pitkänen et al., 2000), BLA stimulation is sufficient to reinforce and enhance LTP at the dentate gyrus when applied close in time to the tetanic stimulus in the perforant path (Abe, 2001; Frey et al., 2001). In contrast, BLA stimulation impairs LTP when applied hours before tetanic stimulation, demonstrating a similar temporal profile to the effects of stress on encoding compared to retrieval. Indeed, BLA stimulation can shift from inducing LTP to long-term depression (LTD) in the dentate gyrus with changes to timing or intensity (Nakao, Matsuyama, Matsuki, & Ikegaya, 2004). The fact that BLA activity changes its modulatory influence over a period of hours is further evidence of a multidimensional network. Activating these diverse projections in naturalistic settings can then produce long-lasting effects with the co-occurring hormonal and stress responses that typically accompany pronounced amygdala activity.

Even when only considering the outcome of perforant path LTP, evidence points to the BLA influencing the hippocampal formation through a multitude of pathways and intermediary regions. First, Thomas, Assaf, and Iversen (1984) demonstrated that paired-pulse facilitation (PPF) at the perforant path synapse could be achieved when the first pulse was delivered to the

lateral amygdala (part of the BLA), and the second pulse was delivered to the entorhinal cortex. Since PPF is a presynaptic mechanism of synaptic strengthening (Kleschevnikov et al., 1997; Schulz, Cook, & Johnston, 1994), its induction by split amygdala/entorhinal cortex stimulation suggests that the entorhinal cortex is a critical intermediary between the amygdala and hippocampus. Still, the amygdala–entorhinal cortex–hippocampus pathway cannot cover all brain regions involved in the modulation of the perforant path. Effective consolidation from early phase to late phase LTP (e-LTP, l-LTP) has been shown to be dependent on the fimbria-fornix (Jas, Almaguer, Frey, & Bergado, 2000). In addition, BLA-modulated LTP reinforcement is dependent on brain-wide muscarinic receptor activity (Frey et al., 2001; Jas et al., 2000). The medial septum and diagonal band of Broca are the primary sources of acetylcholine in the hippocampus (Dannenberg, Young, & Hasselmo, 2017; Mesulam, Mufson, Wainer, & Levey, 1983; Solari & Hangya, 2018), so the influence of muscarinic activity on BLA-mediated changes to hippocampal LTP implicates the medial septum and diagonal band of Broca as contributors to this functional network. The differentiation between e-LTP and l-LTP also points to the multiple mechanisms at play. Lesions to the fimbria-fornix do not impair BLA-evoked potentials in the hippocampus or e-LTP, only the maintenance and consolidation into l-LTP. Therefore, multiple pathways are recruited to establish and maintain synaptic changes even with the precise and isolated stimulation of the perforant path.

The complexity of amygdala–hippocampus interactions expands when effects beyond the dentate gyrus are considered. Comparing the effects on the dentate gyrus and CA1 offer insight into how amygdala activity, such as by stress or emotional content, can have seemingly opposing effects on memory depending on the specific experimental conditions. Depending on the stimulation parameters, electrical stimulation of the BLA *in vitro* can enhance LTP in the dentate

gyrus, where the same stimulation will have no effect or impair LTP in the CA1 (Vouimba & Richter-Levin, 2005). A possible mechanism behind this difference is that, unlike the dentate gyrus, the CA1 receives direct projections from the BLA. This cellular mechanism may be one way in which identical BLA activity can enhance consolidation while impairing retrieval (as discussed in 3.1). All the evidence from *in vitro* investigations into the amygdala-hippocampus circuit underscore the fact that amygdala-hippocampus modulation encompasses a multitude of pathways and effects. The wide-ranging, and at times contrasting, effects of the BLA on the hippocampus highlight its role as a true modulatory region.

BLA activity likely modulates hippocampal plasticity through the expression of plasticity-related proteins within the hippocampus, which can stabilize or destabilize changes in synaptic strength. The immediate early gene Arc/Arg3.1 (Link et al., 1995; Lyford et al., 1995) is one such effector which is activated by exposures to new contexts (Guzowski, McNaughton, Barnes, & Worley, 1999; Huff et al., 2006) and is necessary for synaptic growth and the consolidation of LTP and long-term memory (Guzowski et al., 2000; Plath et al., 2006; Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008). Stimulation of the BLA is sufficient to increase hippocampal Arc/Arg3.1 transcription (McIntyre et al., 2005; McReynolds et al., 2014a; McReynolds et al., 2010), and inhibiting Arc/Arg3.1 blocks the memory-enhancing effects of BLA activation (Guzowski et al., 2000). Thus, the BLA can modulate LTP, and long-term memory as a result, by triggering the plasticity pathway mediated by Arc/Arg3.1.

1.4. Precisely controlling amygdala activity to modulate specific hippocampal memories

Although the amygdala is typically stimulated by intense stimuli in experimental settings, the degree of activation required to modulate memory may be much lower. Experimental stimuli can take the form of aversive painful shocks, highly arousing images like graphic gore or

pornography (Kurdi, Lozano, & Banaji, 2017; Lang, 2008), or infusions of norepinephrine or glucocorticoids that increase BLA activity for multiple hours (McIntyre et al., 2002; Pelletier, Likhtik, Filali, & Paré, 2005). Such intensity, often operationalized as arousal, may be necessary to detect changes in regional neural activity via fMRI or PET scans. However, experiments demonstrating the sufficiency of small amygdala ensembles to activate fear memories (Butler et al., 2015; Kitamura et al., 2017) show that general upregulation of activity may not be a necessary component of BLA modulation of the hippocampus. Activity in a small population of BLA neurons sufficiently represents specific memories. Such activity could be representative of sparse coding in the amygdala. Coding in this way would show a given stimulus to be represented by a small minority of neurons in the BLA. In conjunction, a given neuron would only represent a small minority of stimuli. Restricted activation of BLA neurons could then modulate specific memories based on concurrent activity in the hippocampus. The combination of these two factors would then send specific information to the hippocampus, demonstrating a modulatory mechanism that is distinct from the long-observed enhancement of memory by nonspecific increases in activity.

Brief electrical stimulation of the BLA has demonstrated the ability to modulate the BLA-hippocampus circuit with millisecond precision at much lower intensity than traditional manipulations. This type of artificial stimulation allows BLA activity to modulate hippocampal memories that are normally independent from the BLA. Both motor learning (Bergado, Rojas, Capdevila, Gonzalez, & Almaguer-Melian, 2006) and spatial memory (Almaguer-Melian et al., 2005) was improved with brief electrical stimulation to the BLA, demonstrating that memory modulation depends on BLA activity, not the stimulus characteristics themselves.

Brief stimulation of the amygdala has not only demonstrated a dissociation between the ability to prioritize memory and the affective characteristics of a stimulus, but it has demonstrated a dissociation between affective experience and amygdala activity. A series of experiments carried out in both rodents and humans has demonstrated the extreme temporal precision of prioritized memory possible with electrical BLA stimulation. By electrically stimulating the BLA immediately after exploration of a non-affective object, the long-term memory for that object is enhanced without affecting the memory of objects immediately preceding or following the manipulated object (Bass & Manns, 2015; Bass, Nizam, Partain, Wang, & Manns, 2014; Bass, Partain, & Manns, 2012; Inman et al., 2018). Not only does the stimulation fail to produce a physiological arousal response (e.g. freezing in rats, increase skin conductance and heart rate in humans), but none of the human participants who underwent this BLA stimulation could perceive the stimulation (Inman et al., 2018). Thus, BLA stimulation at low intensity (20 μA in rats, 0.5 mA in humans) prioritized nonaffective declarative memory for consolidation into long term memory without activating the physiological cascades that are typically activated during arousal. Not only was the stimulation temporally precise, but the memory was modulated in a temporally precise manner as well. Electrical BLA stimulation therefore replicates how high-arousal images are preferentially remembered even when interspersed with neutral images (Adolphs, Cahill, Schul, & Babinsky, 1997; Adolphs et al., 2000; Hamann et al., 1999). Such specificity for memory modulation offers a behavioral analog to the cellular phenomenon of plasticity being restricted to specific synapses via synaptic tagging (Bergado et al., 2011).

The ability for BLA activity to prioritize individual hippocampal memories suggests that it can be more specific than modulating hippocampal activity through general increases or

decreases of activity, but that both general and specific activity can modulate hippocampal memory. For instance, the enhancement of memories following nonspecific increases in BLA activity by electrical stimulation or infusion of norepinephrine may occur by biasing certain BLA ensembles more than others, but the general increase in BLA input to the hippocampus may also bias coincidentally active hippocampal ensembles towards consolidation regardless of the identity of BLA input. However, experimental evidence suggests that the specific ensembles in the BLA that are activated by a unique stimulus likely also drive the consolidation of their downstream hippocampal targets. For that to be the case, affective and social information would need to be encoded by ensembles in the BLA. Aim 1 (Chapter 2) was designed to assess how the firing properties of neurons in both the amygdala and hippocampus could facilitate this prioritization of specific information.

1.5. Neural states beneficial to memory

Certain patterns of neural activity in the hippocampus need to be induced by BLA activity in order to prioritize specific facts and events to be consolidated into memory. The long-lasting changes in hippocampal circuitry necessary for long-term memory are driven by spike-timing dependent plasticity, which is in turn coordinated by neuronal oscillations. Hippocampal oscillations have long been known to correlate with distinct behavioral states and memory performance. Theta, the dominant oscillation in the hippocampus (6-10 Hz in rats) (Buzsáki, 2002; Lisman, 2005), is known to fluctuate based on activity level and arousal (Montoya, Heynen, Faris, & Sainsbury, 1989; Sheremet et al., 2019), and organize neural activity for successful navigation (Buzsáki, 2005; Buzsaki & Moser, 2013). In addition, oscillations at the slow gamma range (30-55 Hz in rats) have been shown to correlate with successful encoding, to the extent that power in the slow gamma range at the time of encoding can be predictive of later

retrieval success (Jutras, Fries, & Buffalo, 2009; Sederberg, Kahana, Howard, Donner, & Madsen, 2003; Sederberg et al., 2007; Trimper, Galloway, Jones, Mandi, & Manns, 2017).

Both theta and slow gamma are critically involved in mnemonic processes, but their interaction with each other is also correlated with memory performance, specifically the modulation of gamma amplitude by phase of theta (i.e. comodulation / cross-frequency coupling; (Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009; Trimper, Stefanescu, & Manns, 2014). Comodulation is a more accurate representation of ongoing neural activity; slow gamma power is often elevated in the hippocampus for seconds at a time, but the amplitude of each gamma cycle is not constantly elevated. Rather, specific behavioral states like object investigation (Trimper et al., 2017; Zheng, Bieri, Hwaun, & Colgin, 2016) increase slow gamma power and theta-gamma comodulation, indicating that gamma amplitude is changing cycle-by-cycle.

Theta-gamma comodulation can enable successful memory processes, which has been most closely studied in the context of phase procession during navigation tasks. A single theta cycle is thought to organize individual neuron activity (i.e., ordering neurons from first to last) and gamma cycles (Buzsáki, 2002; Lisman, 2005). Therefore, the relative position of gamma cycles cannot contain actionable information in the absence of an overarching theta cycle to reference against. These oscillations are one mechanism by which the BLA could modulate memory consolidation. Increasing oscillatory activity, especially that of theta-gamma comodulation, could therefore be one mechanism by which nonspecific BLA input could prioritize hippocampus-dependent memory.

Like theta in the hippocampus, gamma is a prominent oscillation in the BLA (Bauer, Paz, & Paré, 2007; Feng et al., 2019) and fluctuates with fear (Amir, Headley, Lee, Haufler, & Pare,

2018; Seidenbecher, Laxmi, Stork, & Pape, 2003; Stujenske, Likhtik, Topiwala, & Gordon, 2014). These oscillations in the amygdala influence oscillatory activity in the regions it projects to, including the hippocampus. Oscillatory activity between the amygdala and hippocampus synchronizes during fear expression (Seidenbecher et al., 2003) and the processing of social information (Zheng et al., 2017). As such, the connections between the amygdala and hippocampus are functional and able to promote pro-memory states in the hippocampus that naturally occur. Therefore, direct manipulation of the oscillatory output of the BLA could synchronize the ongoing activity in both regions and prime the concurrently activated hippocampal ensemble for consolidation regardless of the specific BLA input.

The specific source of oscillations influenced by BLA activity may also explain its differential effects on consolidation and retrieval. Both the entorhinal cortex and CA3 are known to drive gamma rhythms in CA1, which have been shown to correlate with encoding and retrieval, respectively (Colgin et al., 2009). The bias towards consolidation over retrieval driven by BLA activity may also be transmitted through the relative strength of these two systems. Anatomical (Pitkänen et al., 2000) and functional (Thomas et al., 1984) projections are stronger from the BLA to the entorhinal cortex than to CA3, which would allow for preferential recruitment of the consolidation circuitry. Therefore, the BLA modulates the pattern and source of oscillatory activity within the hippocampus by manipulating existing pathways to achieve its long-term effects.

1.5.1. Inducing beneficial memory states by indirect modulation

The influence of ongoing hippocampal neural activity in the success or failure of encoding is precisely why manipulation of specific memory traces cannot be successfully achieved through coarse manipulations of the hippocampus itself. Memory is often impaired

when the hippocampus is directly stimulating in an open loop regardless of the stimulation frequency (Coleshill et al., 2004; Halgren, Wilson, & Stapleton, 1985; Jacobs et al., 2016; Lipponen, Woldemichael, Gurevicius, & Tanila, 2012). These impairments likely arise from the fact that direct stimulation disrupts the ongoing oscillations in the hippocampus, which effectively scrambles the information being processed in the circuits. In contrast, stimulation of input regions to the hippocampus have had much better success. Stimulation of the entorhinal cortex in humans (Suthana et al., 2012) and medial septum in rats (Izadi et al., 2019) has improved spatial learning when hippocampal stimulation did not. Generally, stimulation of input regions including regions in the medial temporal lobe and the amygdala have been more effective at enhancing hippocampal function.

An alternative strategy to stimulating input regions is directly stimulating the hippocampus in a closed loop. This strategy is used to prevent hippocampal activity from being scrambled by selectively applying stimulation at specific neural states (e.g. during a sharp-wave ripple or at a specific phase of theta). For instance, the exact same stimulation protocol produced LTP when triggered by the peak of ongoing hippocampal theta, and Long-Term Depression (LTD) when triggered by the trough of ongoing hippocampal theta (Huerta & Lisman, 1995). Behavioral effects of stimulation also exhibit this phase-dependence; encoding of spatial memory was only enhanced when hippocampal activity was selectively inhibited at the peak of theta, whereas retrieval was only enhanced when hippocampal activity was selectively inhibited at the trough of theta (Siegle & Wilson, 2014). These results suggest that, in order to successfully modulate hippocampal memory, any interventions would be most effective by being responsive to the ongoing hippocampal oscillations. Doing so typically requires stimulation on a closed

loop. Without it, disrupting native oscillations can impair memory, and the effects of blindly applying stimulation on top of those oscillations can vary with each trial.

The alternative to closed-loop stimulation would be an open-loop stimulation that resets and re-synchronizes native hippocampal oscillations without fully disrupting them. Phase reset has been shown to be triggered by natural behaviors and interaction with a stimulus (Mormann et al., 2005), and the extent to which an event triggers hippocampal theta to reset its phase has been shown to correlate with later memory performance in some instances (Jutras, Fries, & Buffalo, 2013). Therefore, an exogenous stimulation that resets the phase of hippocampal oscillations may bypass the phase-dependent effects and other scrambling signals that fail to prioritize memory. This was the motivation of Aim 2 (Chapter 3), which was designed to modulate the output from the BLA to facilitate communication between the two regions while putting the hippocampus in a neural state beneficial to memory.

1.6. Summary of experiments

The consequences of specific representations in or manipulations of the amygdala on hippocampal activity are still largely unknown. On a more basic level, it is not currently known whether neuronal representations in the amygdala are analogous to those in the hippocampus. The degree of similarity between the two regions is important because that will influence the linearity of the relationship between activity in the amygdala and hippocampus. Modulation of declarative memory is a direct downstream effect of that relationship, so there is an outstanding need to understand what is processed in the amygdala, how that processed information is transmitted to the hippocampus, and what that information does to the resulting hippocampal representation.

The ways in which the amygdala and the hippocampus are involved in the consolidation of declarative memory were assessed in two aims to determine how relevant information is first represented by neuronal activity, and how it is then coordinated for consolidation. The first aim investigated the pattern of amygdala and hippocampus activity in humans during a declarative memory task that includes social stimuli known to preferentially recruit the amygdala (Chapter 2). Here, the focus was on single-unit activity so that the neural correlates of memory and stimulus characteristics could be deciphered, as opposed to general activation patterns. The specificity of BLA-mediated enhancement of memory suggests that the specificity of individual stimulus representations plays an important role in how the resulting memory is modulated under naturalistic conditions. The extent of sparse coding in both regions was therefore analyzed to assess one possible coding scheme the amygdala could use to represent specific memories. Neuronal representations of both social and nonsocial as well as remembered and forgotten stimuli were analyzed in the hippocampus and amygdala to determine the extent to which the coding of those categories differs between the two regions. Previously, only the hippocampus was found to employ sparse coding to represent the memory status of a stimulus (Wixted et al., 2018; Wixted et al., 2014). By including socially-salient information, Aim 1 (Chapter 2) investigated if the amygdala would sparsely code information it is more strongly tuned to represent. Even with the apparent differential use of sparse coding between the regions, many studies have found memory to be similarly represented in both regions (Fried et al., 1997; Rutishauser et al., 2010; Rutishauser et al., 2015). These findings are at odds with the conceptualization of the amygdala as a modulatory region with a role distinct from the hippocampus. However, neurons in those previous studies were classified in both regions using the same criteria, perhaps biasing the results in both regions towards similarity while bypassing

the differences in firing distributions that may exist. Therefore, analyzing single-unit data in response to stimuli with characteristics preferred by the hippocampus (memory) and amygdala (social salience), their distinct roles are more likely to be uncovered.

Whereas the first aim observed neuronal activity to determine how information is naturally represented in a way to enable modulation of specific memories, the second aim precisely manipulated the circuit in rats to determine what nonspecific output from the BLA could induce a positive memory state in the hippocampus (Chapter 3). These downstream changes in oscillatory activity are necessary to consolidate the representations observed in the first aim to long-term memory. This aim built upon existing research showing brief electrical stimulation to prioritize recognition memory for later consolidation (Bass & Manns, 2015; Bass et al., 2014; Bass et al., 2012; Inman et al., 2018). Analyses in this aim focused on coordinated network activity (i.e., local field potentials) to compare the responses elicited in the hippocampus by BLA stimulation to oscillatory activity known to correlate with and predict good memory.

Taken together, these two aims addressed fundamental questions about how neural activity within and between the hippocampus and amygdala contribute to declarative memory in low arousal settings. The following results will describe the ways in which the amygdala interacts with the hippocampus in a time-restricted manner. In doing so, the roles of the two regions will be further dissociated. In addition, the parameters necessary for successful and reliable open-loop modulation of the hippocampus will be described by real-time recordings of both regions. The ability of the amygdala to prioritize memories by both natural and artificial means reaffirms its ability to act as a relatively universal modulator, not one tied to emotional experiences or general arousal.

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Chapter 2: The hippocampus and the amygdala employ sparse coding to represent distinct information during a recognition memory task

Abstract

The amygdala has long been known to be involved in the modulation of hippocampus-dependent declarative memory. Given the effect of amygdala activity on the consolidation of emotional or arousing stimuli, one possibility is that amygdala activity is a nonspecific modulator of memory processes in other brain regions. Another possibility is that affective stimuli are represented by specific ensembles of neurons that project to the hippocampus to modulate specific memories. These possibilities need not be mutually exclusive. To investigate the specificity of information content in both the amygdala and hippocampus, the firing of single units in humans were analyzed during an image-based recognition memory task. Trials were grouped by memory status (e.g., repeated/novel; remembered/forgot) and social salience (e.g., human/animal/neither) to determine how information was represented by the two regions. Activity of simultaneously recorded populations was not sufficient to differentiate memory status or social salience. However, the distribution of firing across all presentations of a trial type demonstrated that both the amygdala and hippocampus employ sparse coding to represent information, but in different ways. Only the hippocampus represented the memory status of an image with sparse coding. The amygdala was shown to employ sparse coding of socially-salient information, demonstrating the specificity of amygdala coding during a recognition memory task. These results show that the hippocampus and amygdala sparsely code information in distinct ways, and this property of information representation may facilitate the ability of the amygdala to modulate specific and isolated hippocampal memories.

2.1. Introduction

The hippocampus and the amygdala are two highly interconnected brain regions known to be critically involved in memory formation and modulation (LaLumiere, McGaugh, & McIntyre, 2017; Manns & Bass, 2016; McGaugh, 2004). Declarative memories depend on hippocampal activity (Manns & Eichenbaum, 2006; Squire, Stark, & Clark, 2004), and stimuli with emotional, social, or other motivational significance are known to be represented and processed by single unit activity in the amygdala (Fried, MacDonald, & Wilson, 1997; Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007; Yang, Bellgowan, & Martin, 2012). Recordings of single- and multi-unit activity in both regions of humans and non-human primates have identified neural correlates of socially salient and emotional stimuli (Fried et al., 1997; Minxha et al., 2017), larger concepts of stimuli (Kamiński et al., 2017; Rey et al., 2015), and memory performance (Folkerts, Rutishauser, & Howard, 2018; Halgren, Babb, & Crandall, 1978; Rutishauser, Ross, Mamelak, & Schuman, 2010; Rutishauser et al., 2015). In such experiments, the social salience of stimuli typically covaries with arousal. Correlating these two characteristics in such a way has provided key information about how a certain class of ethologically-relevant information is represented, but it has limited the characterization of how social salience in itself is represented in the hippocampus and amygdala, and how it influences the resulting memory processes. Humans and non-human primates are tuned to process socially-relevant information such as eye gaze, faces, and facial expressions, so those characteristics are likely to be represented in ongoing neural activity in a way that modulates memory consolidation. Analyses of single-unit recordings in the human hippocampus and amygdala have typically focused declarative memory (Rutishauser et al., 2015; Wixted et al., 2018; Wixted et al., 2014) on social and emotional content (Wang et al., 2014), not the intersection of the two.

The mechanisms of representing a given stimulus has been theorized to fall somewhere on a continuum of sparsity. On one extreme, a single neuron would fully and exclusively represent a stimulus feature. This was the underlying model behind the "concept cells" that would only fire in response to the picture, name, or other representation of a specific celebrity (Quiroga, Reddy, Kreiman, Koch, & Fried, 2005). More broadly, this model is the motivation behind identifying individual neurons that reliably respond to all stimuli with a certain characteristic, such as faces of conspecifics (Minxha et al., 2017) or all remembered stimuli (Faraut et al., 2018b; Halgren et al., 1978; Rutishauser et al., 2015). Under this strategy, analyzing single neurons based on their responsiveness would provide the most information because of the high selectivity of neurons. On the other extreme, representations of a stimulus would be fully distributed across a neuronal population, such that every neuron would be involved with every stimulus. Under this strategy, analyzing the entire population of recorded neurons would provide the most information because no single neuron would be selective enough to reliably correlate with a stimulus. In between these two extremes is a sparse or sparse-distributed coding scheme, in which a subpopulation of neurons represents a given stimulus, and a given neuron only responds to a minority of stimuli. Under this strategy, analyzing the firing rate distribution to a category of stimuli would provide the most information, because it could identify responses to stimuli even when not uniform across the entire category. Sparse coding of mnemonic information has been demonstrated in the hippocampus (Wixted et al., 2018; Wixted et al., 2014), but it has not been observed in the amygdala. The existing observations leave open questions about the coding strategies of the amygdala to mnemonic information, but also whether a similar coding scheme is used to represent socially salient information in the two regions.

Previously, a subset of neurons in both the hippocampus and amygdala were shown to preferentially fire during presentation of remembered/forgotten pictures or specific picture categories during such a recognition task in similar proportions (Faraut et al., 2018b; Rutishauser et al., 2015). Additionally, recorded units were found to discriminate visual information earlier than memory information, alluding to the greater degree of processing influencing the firing of neurons whose firing was correlated with memory status (Rutishauser et al., 2015). Mnemonic information has been observed at the population level as well. The activity of the population of neurons recorded together has been found to be more similar between two presentations of an image when the image was remembered than when it was forgotten (Folkerts et al., 2018). Thus, a given stimulus was represented by similar neural activity both times it was presented, but only if that stimulus was remembered. The dependence on memory for similar patterns of neural activity suggest the populations contained greater information about the representation of the stimulus, not the low-level characteristics. Therefore, single units in the human amygdala and hippocampus have already been shown to convey information relevant to recognition memory.

The current study evaluated the patterns of single-neuron activity during a recognition memory task that contained socially salient information in the human hippocampus and amygdala to build on existing information about the single-neuron correlates of recognition memory. Participants were shown a given image once during the study phase and once during the test phase, for a maximum of two presentations of a single image. Therefore, the current task was not optimized to identify image-specific representations or the consistency of image representations, but it was well-suited to identify categorical representations. Representation of both memory and social salience were assessed to determine whether and how the two domains were differentially represented. Social salience was operationalized by categorizing the images

presented to participants as containing non-human animals, humans, or neither animals nor humans. Neural activity has long shown these image characteristics to be represented especially strongly in the amygdala (Fried et al., 1997; Minxha et al., 2017; Yang et al., 2012), but the patterns of activity and the distribution of information across the neuronal populations used to differentiate those characteristics have yet to be identified. Patterns of activity within a population and across all recorded units were assessed. First, neurons were identified based on their categorical responses to a specific stimulus category. Second, all neurons recorded from a given testing session were analyzed as a single population. Third, all neurons in a given region across all recording sessions were analyzed as a single pseudopopulation to investigate the extent to which each stimulus parameter was represented through sparse coding. To investigate this possibility, the firing distribution of all recorded units across all presentations of a stimulus condition were assessed and visualized with quantile-quantile (QQ) plots, as in Wixted et al. (2014). QQ plots compare the shape of distributions by comparing the numerical values of equivalent quantiles in a distribution. This allows for a comparison of skewness and bimodality, both of which would provide evidence for sparse coding. Plotting the firing distributions in this way has previously shown mnemonic information to be sparsely coded in the hippocampus (Wixted et al., 2018; Wixted et al., 2014). Sparse coding has also been modelled as an effective coding scheme within a simulated hippocampal network (McClelland, McNaughton, & O'Reilly, 1995). The amygdala has not yet been observed to utilize a sparse coding pattern, although the existence of discrete ensembles of neurons representing specific stimuli (Butler, Wilson, Gunnersen, & Murphy, 2015; Han et al., 2009; Kitamura et al., 2017) suggest that sparse coding may be employed for certain classes of stimuli. Analyzing firing rate distributions can resolve information content that was obscured by population analyses, since only a minority of neurons

would be recruited in response to a given stimulus. Also, a given neuron would modulate its firing to only a small number of stimuli in a sparse coding pattern, and this pattern would be obscured by analyzing all trials together. In previous studies, screening neurons for selectivity to visual categories or memory would have eliminated the neurons that were a part of a sparse coding scheme from all subsequent analyzes.

Therefore, the current analyses aimed to determine what stimulus characteristics are represented in the hippocampus and amygdala during a recognition memory task, and the differences between those representations. The recorded populations did not discriminate between trials of varied social salience or memory status. The results showed that both the amygdala and hippocampus employ sparse coding, but to different stimulus characteristics. Both the hippocampus and the amygdala demonstrated sparse coding based on the social salience of a stimulus, but in opposing ways. In contrast, only the hippocampus demonstrated sparse coding based on the memory status of a stimulus. Thus, the firing distributions in the two regions demonstrated that the hippocampus and amygdala differentially encode mnemonic and social information, but that both regions maintain specific representations of individual stimuli.

2.2. Materials and Methods

All data were obtained by the dataset publication of Faraut et al. (2018b), freely available online (Faraut et al., 2018a). Full data and task descriptions are available at the original data publication and previous publications (Kamiński et al., 2017; Rutishauser et al., 2015). Methods of initial acquisition are summarized below, followed by a description of analyses performed in the current experiment.

2.2.1. Subjects

42 subjects (F = 15, M = 27) participated who were being monitored for seizure activity due to intractable localization-related epilepsy. Some subjects participated in more than one session, for a total of 65 recording sessions. Participation in the experiment was on a volunteer basis, and all participants provided written informed consent. The published data were de-identified. All protocols were approved by the Institutional Review Boards of the California Institute of Technology, the Huntington Memorial Hospital, and Cedars-Sinai Medical Center.

2.2.2. Data Collection and Processing

Recordings were obtained via macro-micro electrodes that each contained eight 40 μm diameter microwires (AdTech Medical Inc). One microwire of each bundle was used as a local reference for each of the other seven wires. Broadband (0.1-9,000 Hz) data were recorded, sampled at 32 kHz. The location of amygdala and hippocampus electrodes were verified with post-operative MRI scans that were registered to pre-operative scans, and full mapping information can be found in Faraut et al. (2018b).

The raw signal was bandpass filtered at 300-3,000 Hz. Spikes were sorted with the semiautomatic template-matching algorithm OSort (Rutishauser, Schuman, & Mamelak, 2006). Spikes were sorted into clusters and labeled as single units if they demonstrated stability of firing rate over time, no violation of the refractory period, shape of the interspike interval distribution, shape of the waveform, and separation from other clusters. Of the 1,576 originally recorded units, 96 were excluded from the current analyses because they were reported without waveform data. Therefore, the total number of units analyzed was 1,480.

2.2.3. Behavioral Task and Psychophysics

A full description of the task can be found at Rutishauser et al. (2015). The task is outlined in Figure 2.1A. Briefly, the task was an object recognition memory test, with the test phase starting approximately 30 minutes after the study phase. There were three versions of the task which differed in the identity of images presented, allowing the same subject to participate multiple times. All other parameters were identical across task variations. In the study phase, subjects were shown 100 novel images a single time. Each image was shown for 1 or 2 seconds. After a 0.5 second delay, participants were asked whether the image was of an animal to encourage active attention to the stimuli. Participants reported their answer with no time restriction. The next image appeared 1 second after the answer was given. The test phase was conducted the same way, except 50 of the images were repeated from the study phase, and 50 were novel. Answers during the test phase recorded the participants' confidence that the picture was novel or repeated (1 to 6). Subjects scored novel images as new and repeated images as old at above chance levels, and accuracy did not differ between repeated and novel images (Fig. 2.1B).

2.2.4. Data Analysis

Data were analyzed in MATLAB (The Mathworks, Inc.) using custom scripts and scripts adapted from (Faraut et al., 2018a). Code can be made available upon request.

Trials in both the study and test phases were categorized based on the image presented in the trial. The trials were first grouped by the semantic categories used by Faraut et al. (2018b) and Rutishauser et al. (2015). Trials were also grouped by socially salient characteristics into those that contained animals, humans, or neither. Humans were not labeled as animals, and the groups were mutually exclusive. This grouping was chosen because of the extensive evidence

demonstrating the tuning to animals and humans in the hippocampus and the amygdala specifically (Fried et al., 1997; Minxha et al., 2017; Yang et al., 2012). Finally, trials were categorized based on the memory status of the image. Memory status included the patient's performance on the recognition task (remembered or forgotten) and the novelty of the image (repeated or novel, independent of patient's judgment). Images that were repeated in the test phase were linked with the matching trials in the study phase to assess differential firing activity at the time of encoding.

2.2.4.1. Single-Unit Analyses

Units were identified based on preferential firing for specific classes within a category (semantic visual characteristics, socially salient visual characteristics, memory status) and grouped into subpopulations. These top-level analyses were performed to determine the extent to which the information within each of these categories was represented by single, dedicated units. The proportion of such selective units would also provide information about the extent to which the information was distributed across the population of recorded units. Classifying neurons in this way captured those whose firing was significantly modulated by stimulus category, but it did not necessarily capture all neurons that would contribute to a sparse coding scheme. Units were defined as significantly modulated by a given category based on the relative firing rate across categories from +200 ms to +1700 ms following stimulus onset. A 1x5 ANOVA on the firing rates grouped by semantic visual characteristics (e.g. houses, mobility, phones) was run to identify units modulated by semantic visual characteristics. All units with $p < 0.05$ were labelled as such. A 1x3 ANOVA on the firing rates grouped by socially salient visual characteristics (containing an animal, a human, or neither) was run for each unit to identify units that were modulated by the socially salient characteristics of animal/human/neither. All units with $p < 0.05$

were labelled as such. Units modulated by memory status were identified by firing rates to novel and familiar images during the test phase. Units significantly modulated my memory status were determined by a two-tailed bootstrap comparison of means with 1,000 runs, $p < 0.05$.

2.2.4.2. Population-Based Analyses

The neurons recorded from a single session were analyzed as a population to determine how distinctive the population activity was for a given stimulus category. Population-based analyses would provide the greatest amount of information if stimulus representations were fully distributed across the recorded units. High similarity within a stimulus category with large differences across categories would allow the population activity to identify the trial type, and would demonstrate that all recorded neurons contributed to a strong signal of memory or visual information. Only sessions in which 10 or more neurons from a given region were included for all population-based analyses. Units recorded in both hemispheres of a given region were analyzed together as a single population. Out of 65 sessions, 23 sessions had 10 or more units in the hippocampus (mean: 18.4 units), and 40 sessions had 10 or more units in the amygdala (mean: 19.6 units).

The information content present in the population of units in a single session was analyzed with a k-nearest-neighbor calculation (where $k = 1$) of population vectors. K-nearest-neighbor classification is frequently used for datasets in which the underlying distribution is unknown (Cover & Hart, 1967), and is a validated strategy of classifying information from hippocampal firing rates (Manns & Eichenbaum, 2009). The population vector of each trial was plotted in a multidimensional space in which each dimension represented the firing rate of one neuron. Each population vector was assigned a label based on the trial condition (trials with ingroup or outgroup images, e.g. images that included animals or not). For each trial, the nearest

neighbor by Euclidean distance was identified. In effect, the nearest neighbor of a population vector in Euclidean distance identified the trial during which the firing was most similar to a probe trial across the entire recorded population. The vector from an ingroup trial with a nearest neighbor also of a population vector from an ingroup trial was defined as a hit. The vector from an outgroup trial with a nearest neighbor of a population vector from an ingroup trial was defined as a false alarm. Hit rates, false alarm rates, and d' values were calculated for a given session, and those values were averaged across sessions. The same procedure was performed on the data after shuffling the labels of ingroup and outgroup 1000 times to establish the chance value for these metrics, since an unequal number of trials in ingroups and outgroups meant that chance would vary. Significant deviations from chance were determined by a paired-sample t-test between the shuffled and empirical data.

2.2.4.3. Pseudopopulation Analyses

A pseudopopulation of all recorded units from a given region was constructed to analyze the firing distribution across trial conditions. These analyses are best suited to identify sparse coding in a population. Spiking from +0 to +1000 ms relative to image onset was collected for each trial, and spikes of all trials were analyzed together for a given unit. Spike times were concatenated across all units of a subpopulation, which gave the distribution of spiking during a given category. The distributions of spiking across conditions were compared with a quantile-quantile plot to determine the degree of sparse coding in that subpopulation (Wixted et al., 2014). The distribution of normalized (Z-scored by the firing during the baseline period) firing rates were compared across image categories (animal, human, or neither) and memory performance within a brain region. Significant differences in the distributions of conditions and regions were determined with a cluster-based random permutation analysis of quantile-quantile plots. The

labels of the condition (e.g., animal/human/neither, remembered/forgotten) or region were shuffled, and the quantile-quantile plot for each random permutation was plotted. The maximum cluster of each permutation, as compared to the linear unity line of the distribution, was recorded. Only clusters of the empirical data that were larger than the 95th percentile of the random clusters were identified as statistically significant.

2.3. Results

2.3.1. Identification and Properties of Selective Units

Single units were identified whose firing was modulated by memory status, semantic visual characteristics, and/or socially salient visual characteristics. The proportions of units identified in each of these classifications was similar to previously published reports (Faraut et al., 2018b; Rutishauser et al., 2015). Out of the 889 recorded amygdala units and 591 recorded hippocampus units, units responsive to memory status (n = 128; $\chi^2 = 41.5$; $p < 0.001$), semantic visual characteristics (n = 280; $\chi^2 = 603.6$; $p < 0.001$), and socially salient visual characteristics (n = 229; $\chi^2 = 341.8$; $p < 0.001$) were all identified at greater than chance levels. Such significant representation of neurons that preferentially respond to specific memory or visual characteristics support the presence of distributed coding, such that all information is represented across multiple neurons. As with earlier reports, units responsive to both memory and visual information were largely independent, as only 32 units (2.2% of total, 8.8% of semantic visual) were modulated by both memory status and semantic visual characteristics, and only 36 units (2.4% of total, 15.7% of socially salient visual) were modulated by both memory status and socially salient visual characteristics. In contrast, 139 units (9.4% of new, 60.7% of socially salient visual) were modulated by both semantic and socially salient visual characteristics, demonstrating the commonalities in neural responsivity to visual characteristics across domains.

Units modulated by visual characteristics were more strongly selective for their preferred category than units modulated by memory status. Figure 2.2 shows the firing rates of the preferred categories (category of images with the highest mean firing rate) of units that fired preferentially to a specific visual characteristics, repeated images, or novel images relative to the firing of all other conditions. In the hippocampus, the socially salient categories of animal/human/neither more strongly modulated firing than the semantic picture characteristics qualitatively, but there was no difference between the categorizations in the amygdala. Visual characteristics were differentiated before memory status, as observed by the time at which peak differences in firing occurred across the unit classes. These results were similar to previous reports (Rutishauser et al., 2015).

2.3.2. Population activity did not provide sufficient information to decode trial types

The activity of all neurons recorded in a given session was not sufficient to decode most visual or memory information about the task, as demonstrated by a classifier that identified the first-nearest-neighbor by Euclidean distance of population vectors for a given trial. Figure 2.3 shows average d' for that classifier, in which ingroup trials with a nearest neighbor of an ingroup trial was defined as a hit, and outgroup trials with a nearest neighbor of an ingroup trial was defined as a false alarm . The only category classified by the population vector at a success significantly greater than with shuffled data was the animal category in the amygdala, although the effect size was small (d' = 0.11, p = 0.01). When hippocampal and amygdala units from a session were analyzed as a single population, the only condition that was categorized at greater than chance levels was the animal category (Supp. Fig. 2.2), which was reflected in d' scores (d' = 0.47, p = 0.02) and success rate (percent success = 0.74, p = 0.01). Therefore, the trial

condition, either of picture category or memory status, could not be readily classified by the firing rate of the population of recorded neurons.

2.3.3. Sparse firing evident in the hippocampus and amygdala for different trial types

Quantile-quantile (QQ) plots, which graphically compare the shapes of two distributions, were used to compare the distribution of unit firing within a given condition. Comparing the quantiles of two firing distributions has been previously by Wixted and colleagues (Wixted et al., 2018; Wixted et al., 2014) to quantify the extent of sparse coding of single units in the human hippocampus. These plots can identify information present in the responses of neurons that are not detectable at the resolution of population (Fig. 2.3) or single-cell (Fig. 2.2) analyses. Sparse coding refers to a population of units in which a given unit only responds to a minority of stimuli. Figure 2.4 shows exemplar units from the hippocampus and amygdala that demonstrate sparse firing, in which a small subset of trials is represented by a strong increase in firing rate. Figure 2.5 illustrates how changes in distributions are represented in QQ plots. Histograms of hypothetical data are shown against a normal curve in 2.5A, and the QQ plot of that distribution plotted against the normal distribution is shown in 2.5B. Distributions of the same shape exhibit a linear QQ relationship. Changes to standard deviation and shifts in the mean of the distribution do not deviate from that linear relationship. In contrast, changes in kurtosis and skew manifest in the QQ plots by deviations from the linear relationship at the extremes of the distribution. Bimodal distributions are represented as having a “bump” away from the linear relationship when compared to a normal distribution. This relationship, shown in the lower right panels of Figure 2.5A and 2.5B, is representative of idealized data demonstrating a sparse coding scheme. However, any deviation from a linear QQ relationship at the positive tail can be indicative of sparse coding, including differences in the skew and kurtosis of a distribution.

The firing rate of each neuron was Z-scored to its baseline firing rate because of the inclusion of both slow-firing units (putative pyramidal neurons) and fast-spiking units (putative interneurons). Without doing so, the fast-spiking units would be excessively weighted. Unlike the idealized data presented in Figure 2.5, the empirical data do not closely approximate a normal distribution. Supplementary Figure 2.3 show the histogram of Z-scored firing rates during the baseline of all periods in the study and test phase, respectively. The empirical data more closely approximate a gamma distribution than a normal distribution, largely driven by the preponderance of units that did not fire during a baseline period (Supp. Fig. 2.3). Thus, firing distributions from different conditions were directly plotted against each other to determine significant patterns in the data, not against a normal distribution.

The distribution of firing during baseline periods differed between the study and test phases in both the hippocampus and the amygdala. The QQ plots shown in Figure 6 most closely resemble the hypothetical data, which plotted a distribution with high kurtosis against a normal curve. Therefore, the distributions in both regions had stronger tails in the study phase than in the test phase. The deviations from a linear quantile-quantile relationship were significantly greater than chance in both regions. These data show that baseline activity changed over time in both regions. The QQ relationship deviated away from the unity line towards the “study” axis, indicating there was a stronger positive tail and more high-firing baseline periods in the study phase than the test phase. The implications of the longer positive tails for the study phase versus test phase baseline firing rates is considered in more detail in the Discussion.

Firing distributions changed with certain classes of memory performance. Figure 2.7A shows the distribution of firing during presentation of images that were remembered or forgotten during the study phase (x-axis) and test phase (y-axis). The distributions of both remembered and

forgotten images in both the hippocampus and amygdala included had stronger tails, and thus more high-firing trials in the study phase than the test phase. This relationship was observed by the deviation of the QQ relationship towards the "study" axis. These results are indicative of a repetition suppression effect, since the high-firing trials were suppressed during the test phase. Figure 2.7B shows the firing distributions to different memory performances within the test phase. Comparisons were made between remembered and forgotten repeated images, between correct and wrong images (including both novel and repeated images), and between repeated and novel images (regardless of memory performance). The firing distributions of remembered versus forgotten repeated images in the test phase deviated significantly greater than chance levels in the hippocampus, with the firing distribution of remembered images showing more high-firing trials. This pattern is represented by the plot deviating towards the "remembered" axis. All other distributions were statistically similar, since there were no deviations away from the unity line that were larger than chance. Firing distributions also changed with certain picture categories. Figure 2.8A shows how each picture category—animal, human, or neither—was represented in the study and test phases. In general, the Z-scored quantile values were larger in the study phase than the test phase, indicating that there were stronger tails and significantly more high-firing trials during the study phase than in the test phase. Both the hippocampus and the amygdala displayed this pattern, with the "neither" category in the amygdala being the only one to not show the same relationship. Therefore, the hippocampus showed repetition suppression for all visual categories. The amygdala only showed repetition suppression for "human" and "animal" visual categories. Figure 2.8B shows the firing distributions of two picture categories during the test phase. In the amygdala, the Z-scored quantile values of the "neither" category were larger than the "animal" or "human" categories, indicating significantly

stronger tails and more high-firing trials in the “neither” category. The opposite pattern was observed in the hippocampus between the “human” and “neither” categories; the “human” category had significantly more high-firing trials than the “neither” category. Therefore, “human” images were coded in opposite ways in the hippocampus and amygdala, and only the amygdala coded “animal” images and “neither” images with significantly different distributions. The implications of these relationships are further explored in the Discussion.

2.4. Discussion

Single unit recordings in the human hippocampus and amygdala during a recognition memory task were analyzed to determine whether the two regions coded memory and socially salient visual information, and the mechanisms underlying that coding. The amount of mnemonic and visual information present at three degrees of distributed coding was assessed with three separate analyses. Generally, population activity was not sufficient to classify mnemonic or socially salient content when trial information was classified by the Euclidean first-nearest-neighbor of population vectors constructed from firing rates during the stimulus period. The current analysis showed that the recorded units did not represent the information in a highly distributed pattern. These null results demonstrate the benefit of the analyses focusing on a single-neuron coding strategy in which a single neuron responds significantly more to a given stimulus characteristic (Faraut et al., 2018b; Rutishauser et al., 2015); doing so reduces the noise of the population being analyzed. However, neither population-based nor single-unit subpopulation-based analyses are well suited to determine the specificity of neuronal representations of a stimulus in a sparse-distributed coding scheme. Therefore, the firing distributions were analyzed between trial types with quantile-quantile (QQ) plots, being a validated approach in human single-unit analyses to detect sparse coding (Wixted et al., 2014),

which is one way in which specific stimuli can be represented by unique neuronal representations. The firing distributions of a pseudopopulation constructed from all units recorded across experimental sessions demonstrated that neurons in the amygdala and hippocampus encode distinct categories of mnemonic and visual information, even when the information is not detectable when averaging all trials of a given condition.

2.4.1. Decoding hippocampal and amygdalar information via population-based analyses may require larger populations or deeper processing

Population vectors from a given recording session were largely insufficient to classify memory performance and image information (Fig. 2.3, Supp. Fig. 2.2, Supp. Fig. 2.3). The lack of information obtained from this method could be largely due to the population size of recorded units, since the hippocampus and amygdala are known to represent information about memory and social salience (see Folkerts et al., 2018; Minxha et al., 2017). Suboptimal neuronal populations for research purposes are an unfortunate, but necessary, consequence of the recording electrodes being optimized for clinical purposes. The average size of analyzed populations was 18.4 units in the hippocampus and 19.6 units in the amygdala. Although those populations are the products of an impressive recording effort, populations of that size may not provide enough specialized information to be detectable. This is especially true given that both the hippocampus and amygdala are multimodal regions, responsive to auditory, olfactory, affective, location, and other information content. The nature of these regions may limit the amount of information obtainable in a fully distributed coding approach. Decoding activity in the primary motor cortex (Georgopoulos & Carpenter, 2015) for example, may require substantially smaller populations than decoding activity in the medial temporal lobe because of the relatively specialized inputs and outputs of the motor cortex (Guye et al., 2003; Rizzolatti & Luppino,

2001). A greater specificity would mean that a greater proportion of neurons in the region would contain information in the modality of interest. The identification of neurons selectively responsive to memory status and visual characteristics demonstrates how the multimodal nature of the hippocampus and amygdala increases the noise when seeking a specific signal. Only 8.7% of recorded units were modulated by memory status, and 15.5% were modulated by socially salient visual characteristics. Although those proportions were significantly above chance, that number of selective neurons means that, statistically, only one or two units per population would be reliably responsive to mnemonic or image content. Given the logistical challenges of recording single units in deep structures like the hippocampus and amygdala, information content may be best obtained with different analysis strategies. It is possible that other population-based classification strategies would uncover further information within population firing, but the use of a Euclidean first-nearest neighbor classifier was well-suited to analyze the current data. The primary strength of this strategy is that it makes no assumptions about the underlying distributions of the data or how the data may cluster. Other techniques, like linear discriminant analysis (LDA), assumes a Gaussian distribution of events and equal variance of within the two categories which cannot be safely assumed for the current data (see Supp. Fig. 2.3). In addition, the low average firing rates (hippocampus = 2.2 Hz; amygdala = 1.7 Hz) and limited number of trials for some categories limit the utility of techniques like mutual information, which can be heavily biased with limited observations (Timme & Lapish, 2018). The characteristics of the current dataset, as well as the validated use of k-nearest-neighbor categorization for hippocampal firing (Manns & Eichenbaum, 2009), made the current analysis a logical choice. That said, it is possible that other techniques like de-noising and dimensionality

reduction of could uncover other information present in these populations (Cunningham & Yu, 2014; Pandarinath et al., 2018).

2.4.2. Neural state at baseline is a critical factor in assessing event-related activity

The changes in baseline activity from the study to test phase give an important context to the comparisons of a given condition across the study and test phases. The baseline firing distributions of both regions had more high-firing events in the study phase than in the test phase. Although the mean baseline firing rates did not substantially change (hippocampus study = 2.2 Hz, hippocampus test = 2.3 Hz; amygdala study = 1.7 Hz, amygdala test = 1.8 Hz), the distributions were significantly different (Fig. 2.6). Therefore, the difference within a category between the study and test phases (as seen in 2.7A and 2.8A) are likely influenced by that baseline difference. The fact that all memory and image categories contained more high-firing trials in the study and test phase (with the exception of the "neither" image category in the amygdala) could be explained by the baseline periods in the study phase forming a similarly-shaped distribution.

The differently-shaped baseline distributions at study and test could be caused by a number of factors. It may be a consequence of greater novelty in the study phase—more novel images, and novelty of the task itself. This is unlikely, since the firing rate distributions of repeated and novel images did not significantly differ (Fig. 2.7). Alternatively, the behavioral task itself may modulate the baseline neural state of a subject. The behavioral task from the present analysis consisted of a control question that did not require much conscious effort in the study phase ("Is it an animal?") and a recognition question in the test phase ("Did you see this image before?"). Testing began approximately 30 minutes after the end of the study phase, so the change in baseline activity is unlikely to be solely a factor of time passing. The significant

difference between the baselines of these phases may then represent the firing distributions of the general neural state of encoding and detection (study) compared to recognition and retrieval (test). The differences between the study and test of memory performance (Fig. 2.7A) and picture categories (Fig. 2.8A) may be a reflection of different background activity, or different responses to the stimulus characteristics themselves. These two options present two distinct interpretations of the data in 2.7B and 2.8B, which are discussed below.

2.4.3. The hippocampus and amygdala differentially encode mnemonic and image information

Previous analyses of hippocampus and amygdala units have shown the two regions to similarly respond to memory-related factors like novelty at the neuronal level (Fried et al., 1997; Rutishauser et al., 2010; Rutishauser, Schuman, & Mamelak, 2008; Rutishauser et al., 2015). These similarities seem to be at odds with the hypothesis that the primary role of amygdala in this realm is as a memory modulator (Adolphs, Cahill, Schul, & Babinsky, 1997; Adolphs, Tranel, & Denburg, 2000; Anderson & Phelps, 2001; Manns & Bass, 2016; Phelps & LeDoux, 2005) and is distinct from the role of the hippocampus. However, studies of memory in patients with amygdala dysfunction have suggested that the mnemonic information provided by the amygdala is distinct from that provided by the hippocampus (Adolphs et al., 1997; Adolphs, Denburg, & Tranel, 2001). Although the hippocampus and amygdala demonstrate similar properties when their neurons were pre-selected for their change in firing from the study to the test phases (Rutishauser et al., 2010; Rutishauser et al., 2008; Rutishauser et al., 2015), the current results derived from a pseudopopulation of all recorded neurons highlight the different information present in the hippocampus and amygdala.

Hippocampal firing during the presentation of remembered images consisted of more high-firing trials than during the presentation of images that were forgotten (Fig. 2.6B, left). This pattern was not observed in the amygdala, which suggests that visual recognition memory is represented by a sparse coding scheme in the hippocampus, but not in the amygdala. The current results, however, differ from previous findings which demonstrated that repeated stimuli were coded in a sparse manner in the hippocampus regardless of their memory status (Wixted et al., 2018; Wixted et al., 2014). No such difference was found here (Fig. 2.7B, right), which is likely a result of the different stimulus modalities between the behavioral tasks. Wixted et al. (2014) found sparse coding of repeated words that were displayed visually, and only in the left hippocampus. Therefore, their task included an explicit language component that was not present here. The current results suggest that the repetition firing found by Wixted and colleagues coded repetition that was specific to language. In contrast, repeated images were only differentially coded by their memory performance. The hippocampus also differentially encoded human images over images that did not include humans or animals. The deviation of quantiles towards the "human" axis in the left panel of Fig. 2.8B suggests a greater degree of sparse coding for humans in the hippocampus.

The greater degree of sparse coding by the hippocampus of remembered and human images than forgotten and "neither" images would be similarly interpreted regardless of the effects of changing baseline activity (Fig. 2.6). In contrast, the data point towards one of two interpretations of amygdala coding that differ based on the extent to which changes in baseline distributions drove the differences between firing distributions during the presentation of a stimulus. Although dissimilar, both interpretations point to the specificity of single-unit activity in the amygdala being key to recognition memory. These interpretations are depicted in Figure

2.9 by illustrative Gaussian distributions of varying skewness and kurtosis. The interpretations are considered in detail in the following sections.

2.4.3.1. Repetition suppression interpretation

Under a repetition suppression interpretation, different degrees of repetition suppression across the visual categories from the study phase to the test phase would have produced the differences in the firing distributions of "human" versus "neither" and "animal" versus "neither" in the amygdala. The left panel of Figure 2.9 shows mock distributions of data that would reflect this interpretation. The strong positive tail from the "human" and "animal" categories in the study phase (Fig. 2.9, top row) would be minimized in the test phase by a repetition suppression of the high-firing events. The "neither" category would be the exception as the only category to not demonstrate a repetition suppression effect (Fig. 2.9, middle row). The repetition suppression of "human" and "animal" would therefore produce a distribution with even smaller tails than the "neither" distribution at test and be the root cause of the differences across the categories. As such, this interpretation would show the amygdala to specifically encode human and animal images with sparse coding upon the first presentation of those images, but that would be suppressed upon repeated viewing.

2.4.3.2. "Neither" selectivity interpretation

The alternative interpretation of amygdala function is that the differences between the firing distributions of image categories were driven by differences in the representations at test. The right panel of Figure 2.9 shows mock distributions of data that would reflect this interpretation. Since the distributions of baseline activity changed from study to test (as shown in Fig. 2.6), there is no way to conclusively say the repetition effects are due to the stimuli themselves (Fig. 2.9, top row). Under this interpretation, only comparisons between stimulus

categories at test would be informative because comparisons between phases were influenced to an unknown degree by changing baseline activity. Here, the distribution of the "neither" category would have a uniquely strong positive tail (Fig. 2.9, middle row), indicating that the "neither" category would be most sparsely coded. This result would be surprising, given the role of the amygdala in coding socially-salient information, especially faces of conspecifics and emotional expression (Adolphs et al., 1999; Wang et al., 2014). Encoding this information, however, does not necessitate a sparse distributed coding scheme. On the contrary, the fact that the amygdala is more specialized towards affectively- and socially-salient information could decrease the extent of a sparse coding scheme, because a larger proportion of neurons would be responsive to a larger number of images that include those elements. The sparse coding scheme observed with "neither" images, in contrast, could be a function of the coarse division of images. It is to be expected that a minority of images would contain affective or social information that is relevant to amygdala activity in the absence of a human or animal, which would explain the sparse coding of those trials.

Amygdala coding of humans would be opposite to that of the hippocampus under this interpretation. Contrasting the amygdala, the hippocampus is known to encode more granular information, including an individual's specific identity or larger "concepts" of identity (Gothard et al., 2007; Quiroga, Kreiman, Koch, & Fried, 2008; Rey et al., 2015). Human identity would therefore present greater diversity in the information to be coded in the hippocampus, which would be represented by a sparse coding scheme, as observed in Figure 2.8B. In sum, the "neither" selectivity interpretation would show the amygdala to encode specific characteristics of stimuli in the "neither" category as evidenced by sparse coding.

2.4.4. Evidence for a sparse-distributed network in the amygdala and hippocampus

The distributions found in the QQ plots suggest both regions utilized sparse coding to represent these stimulus categories. However, the proportion of neurons that categorically responded to memory status and visual characteristics also supports the distributed nature of this information. Similar to experimental (Wixted et al., 2018; Wixted et al., 2014) and theoretical (McClelland et al., 1995) models, the combination of pseudopopulation and single-unit analyses supports the use of a sparse-distributed coding scheme in both the hippocampus and amygdala. As such, a small population of neurons responded to each stimulus, and each neuron responded to a small proportion of stimuli.

2.4.5. Limitations and future directions

Many questions about the encoding of mnemonic and social information by the amygdala and hippocampus remain, and the characteristics of the current dataset point to future experiments that could inform these open questions. The current experiment utilized a recognition memory task with a single test. This setup did permit the comparison of a single novel and a single repeated group of images. However, presenting a given image a maximum of two times limited the ability to identify the extent to which any change in firing during the stimulus presentation was reflective of noise or a true signal. Future experiments could address how constant and reliable coding of a stimulus is by repeating stimuli multiple times. Such paradigms have been used in the past (Gothard et al., 2007; Quiroga et al., 2005), but not when determining the use of sparse coding in stimulus representation. Similarly, the waveform of each spike was not recorded, so individual spikes could not be differentiated from a movement artifact or other sources of electrical noise. The combination of true action potentials with noise could have skewed the present results especially when dealing with low firing rates, and therefore the

current findings would benefit from replication. Aside from the separation of signal from noise, the extent to which the activity of recorded units was representative of signal in the amygdala and hippocampus was difficult to determine because analysis was restricted to the recorded units. Therefore, future experiments could greatly add to the present conclusions by integrating local field potentials with single unit activity in a similar behavioral paradigm to assess how mnemonic and social information is encoded in the amygdala and hippocampus by other factors such as spike-field coherence and other spike-timing information. Although the conclusions drawn from the current experiment were limited by the nature of the data, the present results suggest the presence of a sparse coding scheme in both the human amygdala and hippocampus during a recognition memory task. Future experiments will help determine how precisely this coding scheme is implemented, further clarifying the roles of the two regions in the encoding and retrieval of social and nonsocial images.

2.5. Conclusions

The current study found mnemonic and visual information content to be differently represented in the hippocampus and amygdala when all units across recording sessions were included in the analyses. This strategy provides additional insight to analyses that focused on neurons significantly modulated by stimulus or trial type (Faraut et al., 2018b; Rutishauser et al., 2015). As noted by Wixted et al. (2018), removing the top 2.5% of the firing distribution removes the information that demonstrates a sparse-distributed coding network, which could artificially limit the information content that could be obtained from a population of recorded neurons. In the context of previous findings, the current results highlight the influence of task and stimulus parameters on the information present in the amygdala and hippocampus. Previously, memory content was found to be similar between the two regions (Rutishauser et al.,

2010; Rutishauser et al., 2008; Rutishauser et al., 2015), and the amygdala was not found to encode information in a sparse-distributed scheme (Wixted et al., 2018; Wixted et al., 2014). The present results differed, likely due to the task including images (instead of words) that were categorized by their social salience. The current results could not disambiguate the possibilities of sparse coding in the amygdala being a result of repetition suppression to select stimuli, or to specific responses within a stimulus category. Regardless of which possibility drove the observed results, these data show the amygdala to encode specific information during a recognition memory task that included images with social salience. The common use of sparse coding by both the amygdala and hippocampus suggests that amygdala ensembles that are specific to a given stimulus could drive the modulation of hippocampal activity, which expands the role of the amygdala beyond that of a nonspecific modulator. As the current results demonstrate, the specificity of information represented in either region may depend on the modality of information presented.

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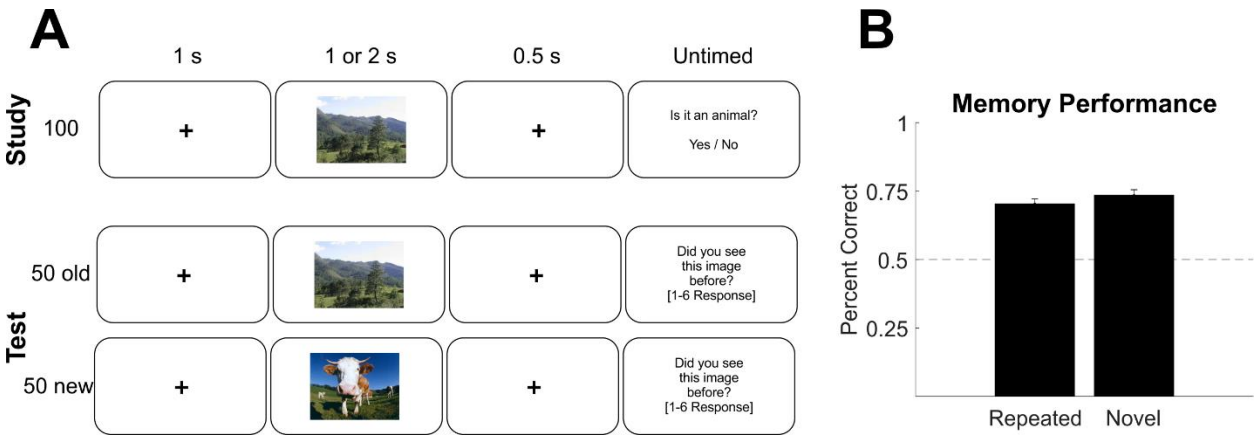


Figure 2.1. Outline of memory task. A) The study phase consisted of 100 images that were followed by a control question to promote attention to the images. The test phase consisted of 100 images, 50 novel and 50 repeated from the study phase, which were followed by a question asking the subjects' certainty the image was repeated or novel. B) Memory performance was similar for repeated and novel images. Figure adapted from Faraut et al. (2018).

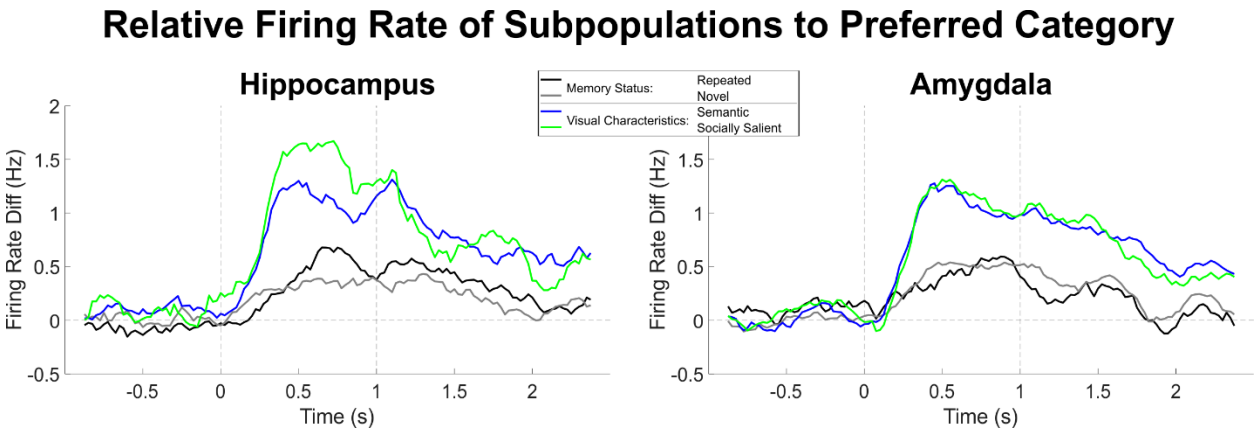


Figure 2.2. Properties of units modulated by memory status and visual characteristics. Images appeared on the screen at zero seconds and were removed after 1 or 2 seconds. Each line represents the difference in firing between the preferred category of a subpopulation of neurons and all other categories. Units modulated by semantic visual characteristics (blue) and those modulated by socially salient visual characteristics (animal/human/neither; green) fired with greater selectivity than either those preferentially responsive to repeated (black) or novel (gray) images.

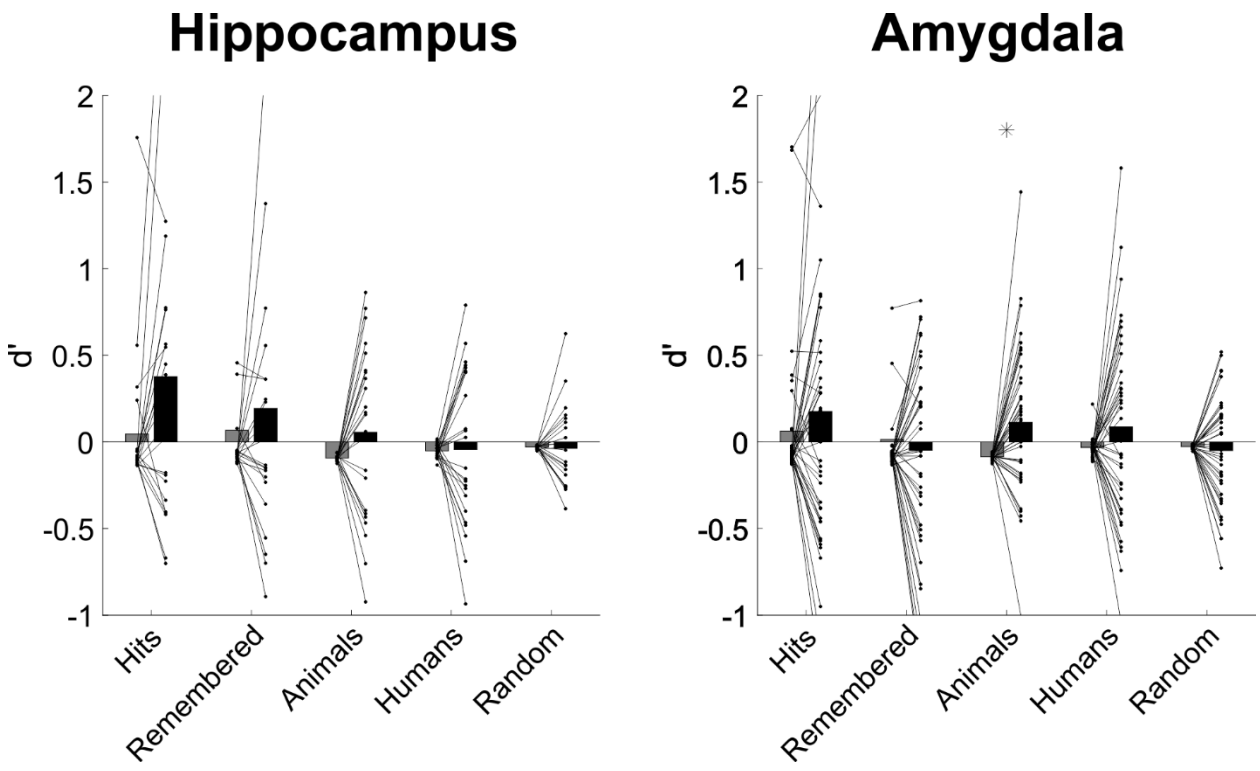


Figure 2.3. d' of a first-nearest neighbor classifier in identifying trial type by the population activity in the hippocampus and amygdala. Only populations with 10 or more neurons were included in the analyses. Grey bars represent the chance level of the metric derived from the mean of 1000 random shuffles of the data label. Black bars represent the empirical data. Asterisks denote statistical differences between the shuffled and empirical data, $p < 0.05$.

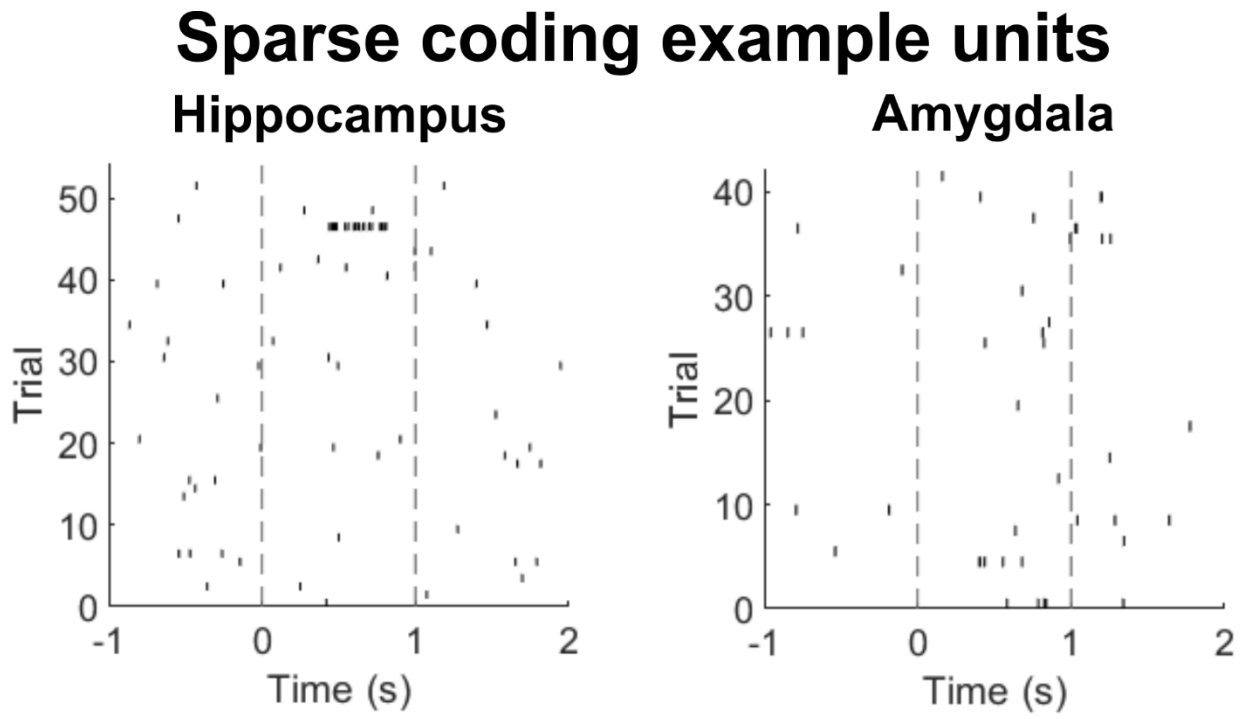


Figure 2.4. Exemplar neurons in the hippocampus and amygdala which demonstrate sparse coding. Images for the selected trials were displayed from 0 to 1 second, between the gray dashed lines. Firing is not modulated during the majority of trials, but is strongly modulated during a small subset of trials.

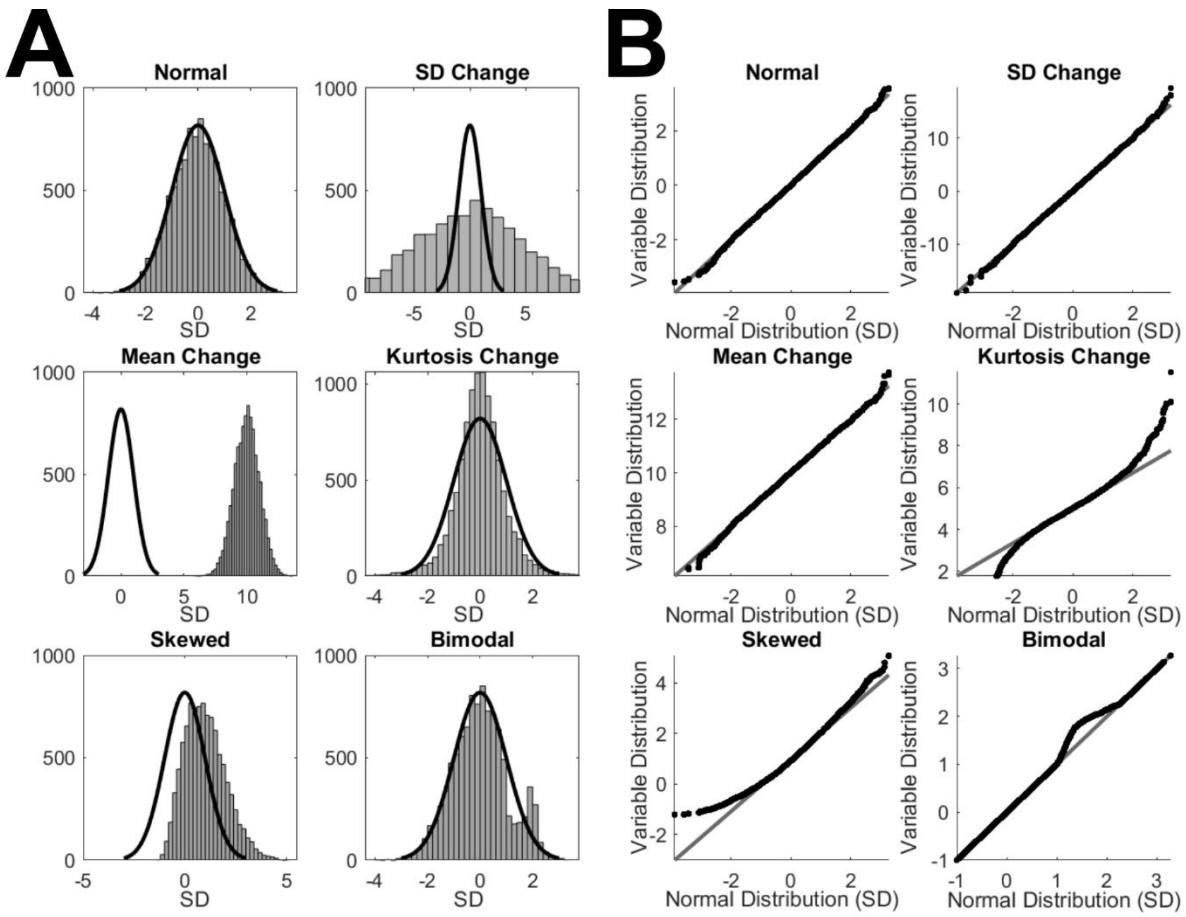


Figure 2.5. Illustration of how different distributions are represented against a normal curve in a quantile-quantile (QQ) plot. A) Histograms (gray) represent the distribution described by the title of the plot. A normal curve is plotted in black for comparison. B) QQ plots, with quantiles of the normal curve on the x-axis, and quantiles of the distribution described in the title on the y-axis. SD = standard deviation.

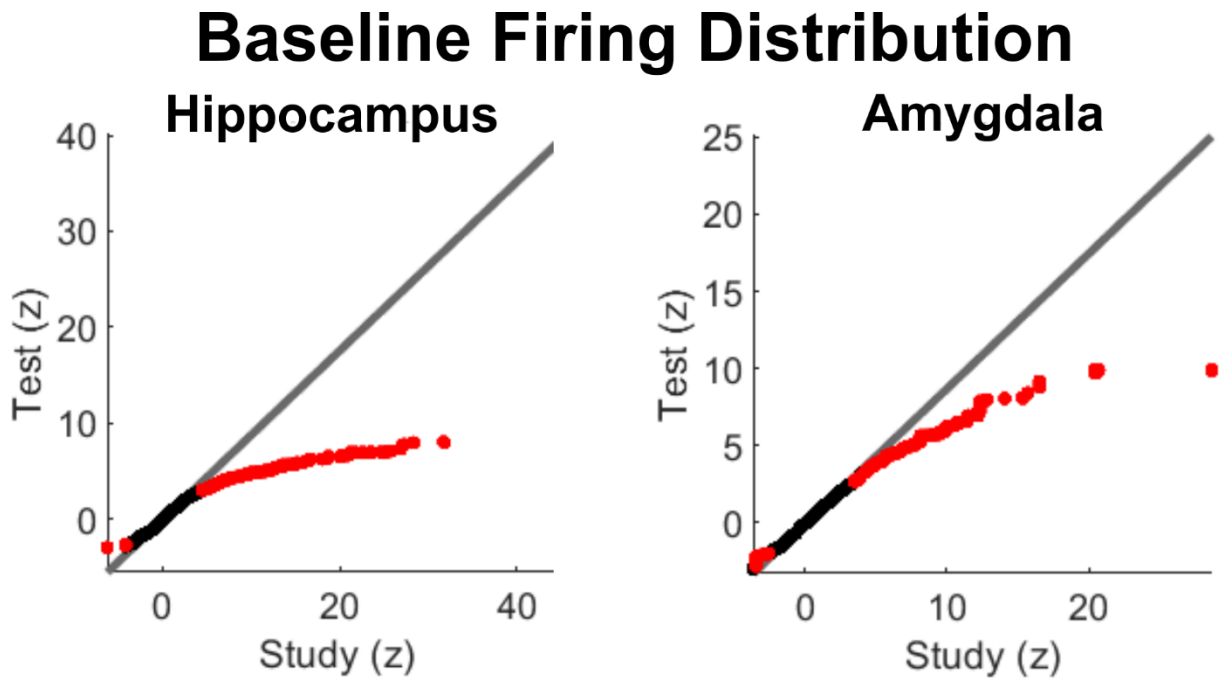


Figure 2.6. Quantile-quantile (QQ) plots of firing distributions between the baseline periods of the study and test phases in the hippocampus (left) and amygdala (right). Firing rates were Z-scored, and axes are in standard units. Red data points denote the regions of the distribution that were significantly different between the plotted conditions, as determined by a cluster-based random permutation test ($p < 0.05$, two-tailed).

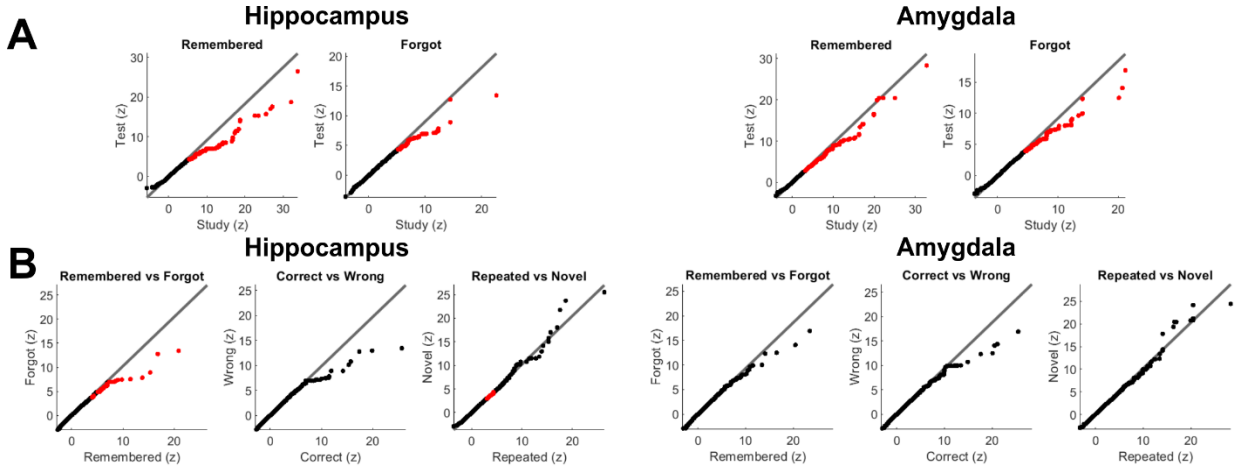


Figure 2.7. Quantile-quantile (QQ) plots of firing distributions between trials grouped by memory performance in the hippocampus (left) and amygdala (right). A) Firing to a given trial type during the study phase (x) and test phase (y). B) Firing during the test phase to different trial types. Firing rates were Z-scored, and axes are in standard units. Red data points denote the regions of the distribution that were significantly different between the plotted conditions, as determined by a cluster-based random permutation test ($p < 0.05$, two-tailed).

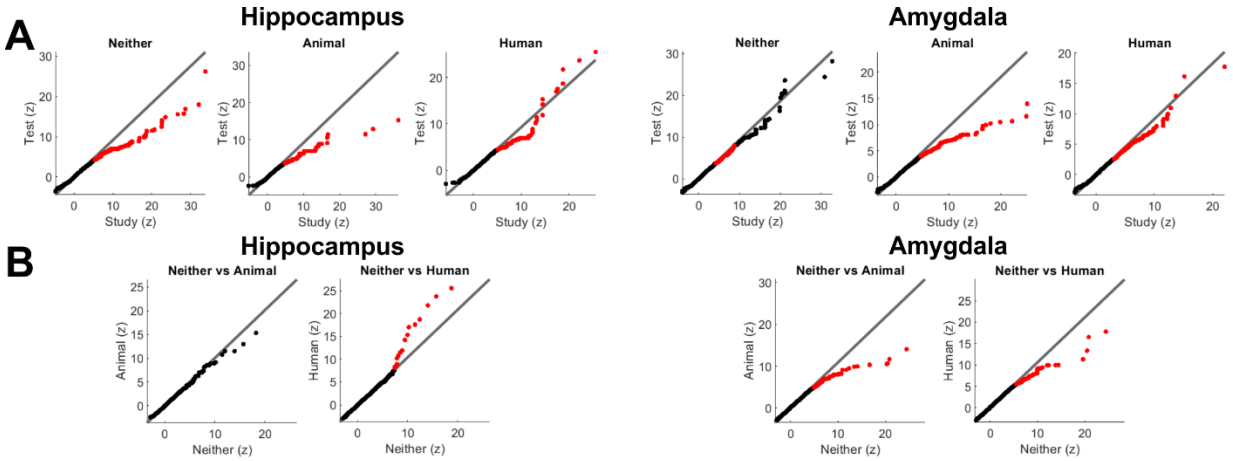


Figure 2.8. Quantile-quantile (QQ) plots of firing distributions between trials grouped by picture category in the hippocampus (left) and amygdala (right). A) Firing to a given picture category during the study phase (x) and test phase (y). B) Firing during the test phase to different picture categories. Firing rates were Z-scored, and axes are in standard units. Red data points denote the regions of the distribution that were significantly different between the plotted conditions, as determined by a cluster-based random permutation test ($p < 0.05$, two-tailed).

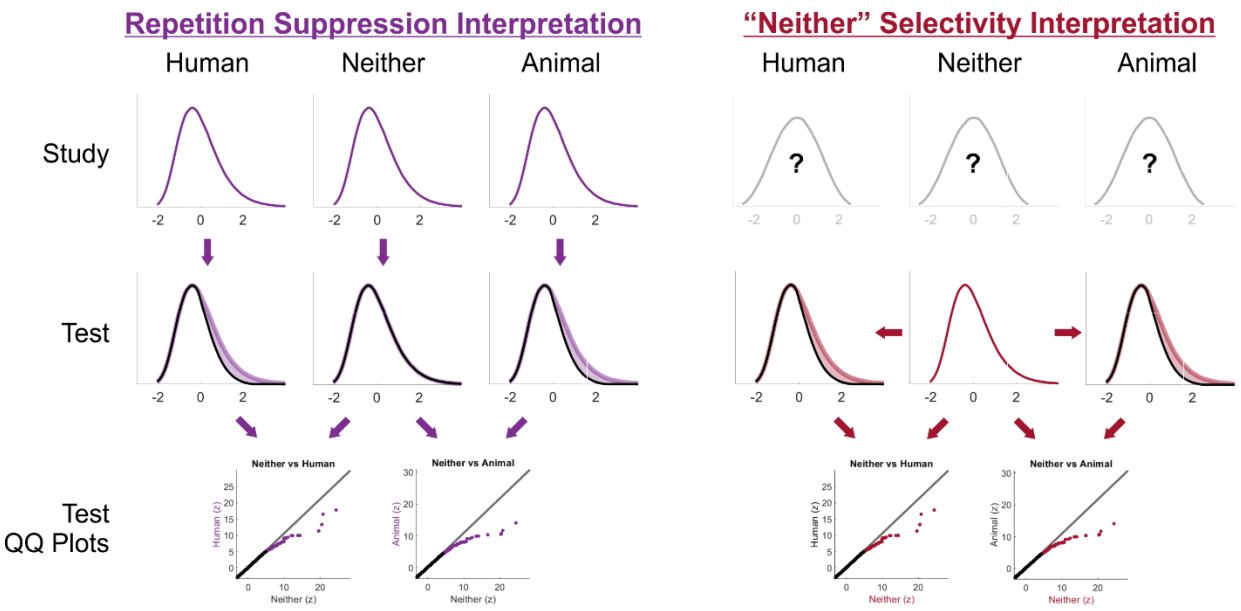
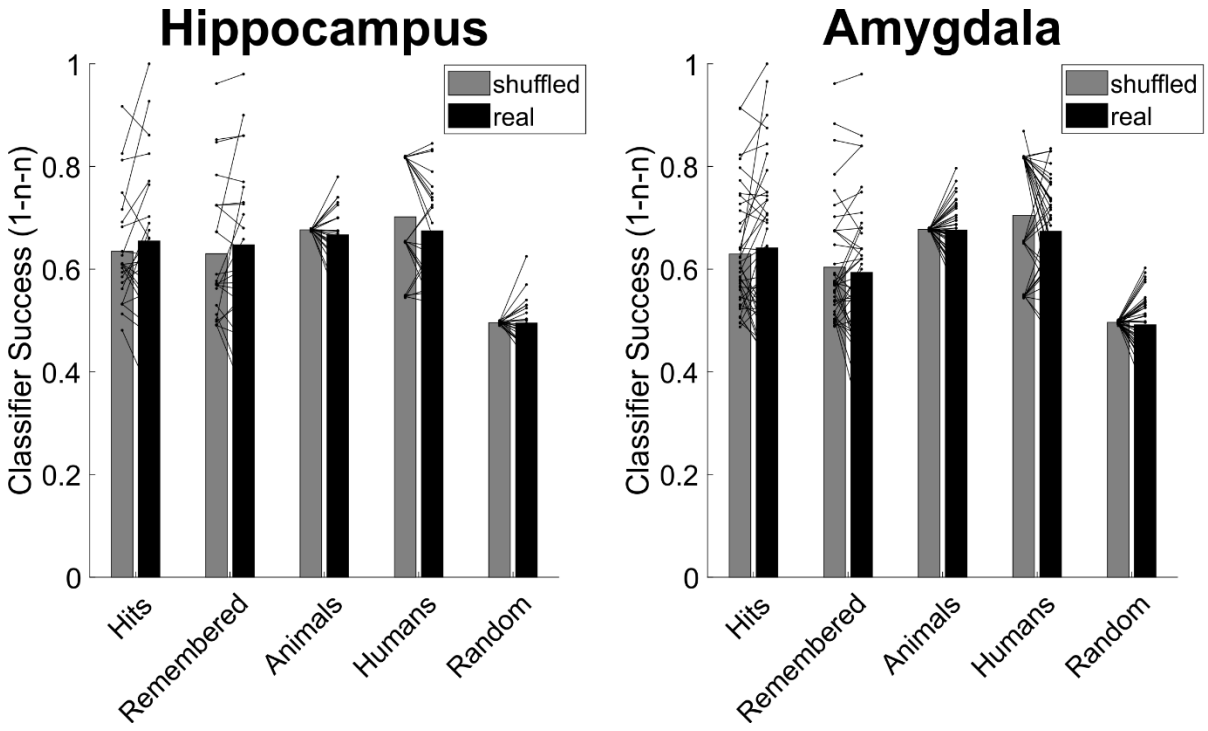
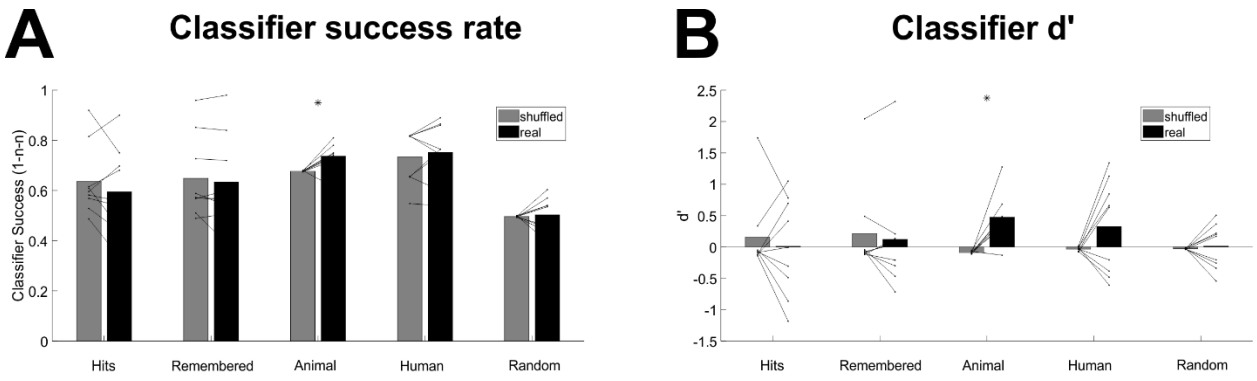


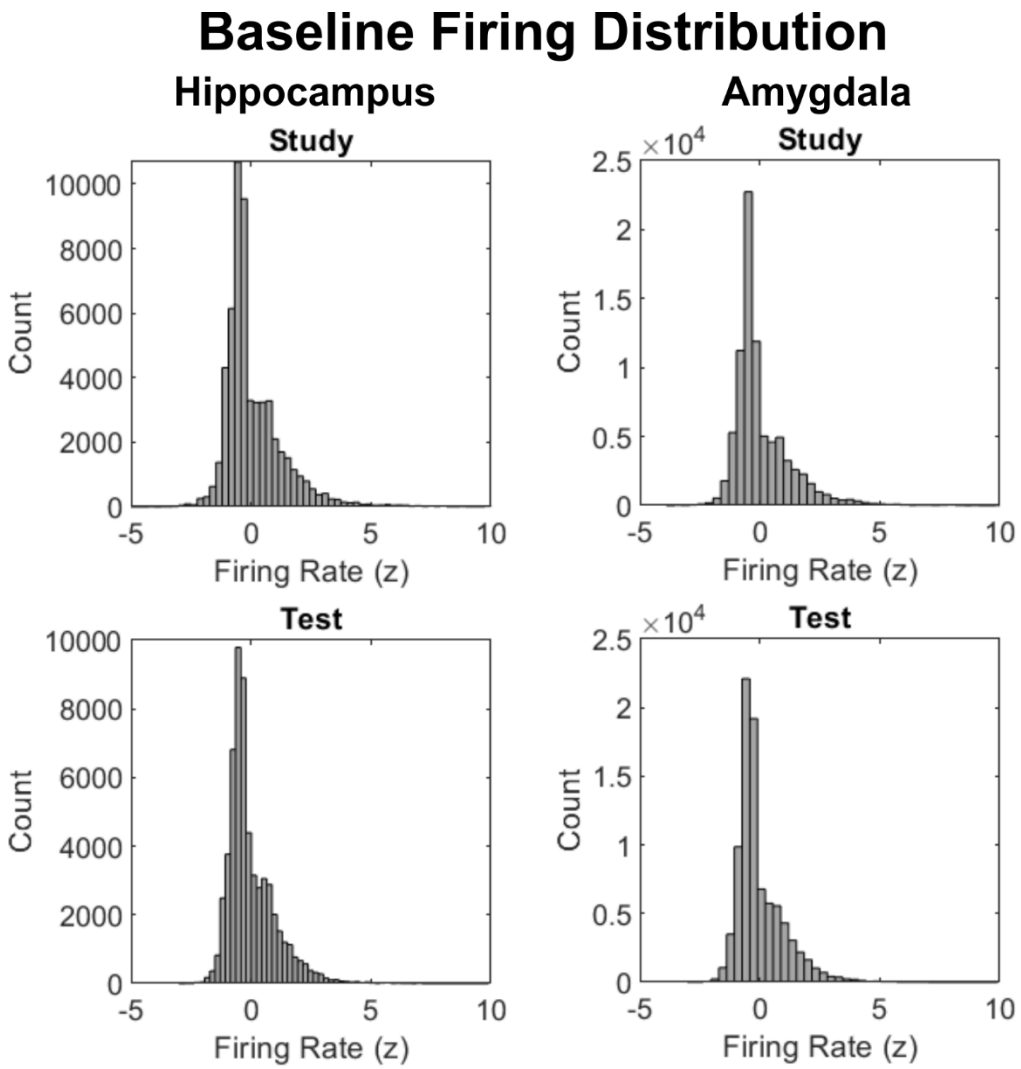
Figure 2.9. Illustrative distributions to demonstrate the repetition suppression and "neither" selectivity interpretations in the amygdala. Under the repetition suppression interpretation (left, purple), there would be strong repetition suppression for the "human" and "animal" categories, but not for the "neither" category (as observed in Fig. 8A). Therefore, the distributions of the "human" and "animal" categories would have a weaker positive tail in the test phase than the study phase (middle row). The distributions of the "neither" category would be the same in the study and test phases. When compared, the "neither" condition would therefore have the strongest tail in the test phase as a result of the suppressed tails in the "human" and "animal categories. Under the "neither" selectivity interpretation (right, maroon), the relationship between the study and test phases cannot be determined because of the changes to baseline activity between the study and test phase (as observed in Fig. 6). Here, the distribution of the "neither" category would have a uniquely strong positive tail because of the unique representation of the "neither" category. The bottom row of the left and right panels demonstrate how the QQ plots from Figure 8B would result from either interpretation.



Supplementary Figure 2.1. Success of a first-nearest neighbor classifier by percent correct in identifying trial type by the population activity in the hippocampus and amygdala. Only populations with 10 or more neurons were included in the analyses. Grey bars represent the chance level of the metric derived from the mean of 1000 random shuffles of the data label. Black bars represent the empirical data.



Supplementary Figure 2.2. Success of a first-nearest neighbor classifier by percent correct (A) and d' (B) in identifying trial type by the population activity in the hippocampus and amygdala when the populations are combined. Only populations with 10 or more neurons in each region were included in the analyses. Grey bars represent the chance level of the metric derived from the mean of 1000 random shuffles of the data label. Black bars represent the empirical data. Asterisks denote statistical differences between the shuffled and empirical data, $p < 0.05$.



Supplementary Figure 2.3. Histograms of firing rates during the baseline periods of the study and test phases in the hippocampus and amygdala. Firing rate is Z-scored to the mean and standard deviation of baseline firing during all trials in a given phase.

Chapter 3: Optogenetic stimulation of the basolateral amygdala increased theta-modulated gamma oscillations in the hippocampus

Author's contribution and acknowledgement of reproduction

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The dissertation author designed and performed the experiments, analyzed the data, and wrote the manuscript. Joseph Manns designed the experiments, analyzed the data, and wrote the manuscript.

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Abstract

The amygdala can modulate declarative memory. For example, previous research in rats and humans showed that brief electrical stimulation to the basolateral complex of the amygdala (BLA) prioritized specific objects to be consolidated into long term memory in the absence of emotional stimuli and without awareness of stimulation. The capacity of the BLA to influence memory depends on its substantial projections to many other brain regions, including the hippocampus. Nevertheless, how activation of the BLA influences ongoing neuronal activity in other regions is poorly understood. The current study used optogenetic stimulation of putative glutamatergic neurons in the BLA of freely-exploring rats to determine whether brief activation of the BLA could increase in the hippocampus gamma oscillations for which the amplitude was modulated by the phase of theta oscillations, an oscillatory state previously reported to correlate with good memory. BLA neurons were stimulated in one-second bouts with pulse frequencies that included the theta range (8 Hz), the gamma range (50 Hz), or a combination of both ranges (eight 50-Hz bursts). Local field potentials were recorded in the BLA and in the pyramidal layer of CA1 in the intermediate hippocampus. A key question was whether BLA stimulation at either theta or gamma frequencies could combine with ongoing hippocampal oscillations to result in theta-modulated gamma or whether BLA stimulation that included both theta and gamma frequencies would be necessary to increase theta-gamma comodulation in the hippocampus. All stimulation conditions elicited robust responses in BLA and CA1, but theta-modulated gamma oscillations increased in CA1 only when BLA stimulation included both theta and gamma frequencies. Longer bouts (5 seconds) of BLA stimulation resulted in hippocampal activity that evolved away from the initial oscillatory states and towards those characterized more by prominent low-frequency oscillations. The current results indicated that one mechanism by

which the amygdala might influence declarative memory is by eliciting neuronal oscillatory states in the hippocampus that benefit memory.

3.1. Introduction

The basolateral complex of the amygdala (BLA) is a key modulatory region of hippocampus-dependent memory (McGaugh, 2002). Direct activation of the BLA via pharmacological manipulations (Barsegyan, McGaugh, & Roozendaal, 2014; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008) or brief electrical stimulation (Bass & Manns, 2015; Bass, Nizam, Partain, Wang, & Manns, 2014; Bass, Partain, & Manns, 2012; Inman et al., 2018) improved performance in memory tasks not designed to be overtly emotional, such as object recognition memory tasks. Indeed, in one recent study with human participants, direct electrical stimulation targeting the BLA improved recognition memory for neutral object images despite participants reporting that they could not detect the stimulation (Inman et al., 2018). These experiments built on prior work in rodents demonstrating that the BLA mediated the influence of emotional arousal on memory performance in tasks such as inhibitory avoidance (Holloway-Erickson, McReynolds, & McIntyre, 2012; Huff, Miller, Deisseroth, Moorman, & LaLumiere, 2013; McIntyre, Hatfield, & McGaugh, 2002; McIntyre et al., 2005; McReynolds et al., 2010; McReynolds, Holloway-Erickson, Parmar, & McIntyre, 2014). Thus, existing research suggests that activation of the BLA can modulate memory for the better and can be engaged by emotional arousal or by direct intervention.

The BLA is thought to modulate memory in part by influencing memory processes in other brain regions (McGaugh, 2002; Roozendaal, Griffith, Buranday, de Quervain, & McGaugh, 2003; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). In particular, the BLA sends direct glutamatergic projections to the hippocampus and to regions such as the entorhinal and perirhinal cortices that in turn project to the hippocampus (Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). Inactivating the hippocampus via local infusion of muscimol blocked the object

recognition memory improvement triggered by electrical stimulation of the BLA (Bass et al., 2014), whereas pharmacological manipulations of the BLA such as local infusion of adrenergic agonists led to increased markers of synaptic plasticity in the hippocampus (McIntyre et al., 2005; McReynolds, Anderson, Donowho, & McIntyre, 2014). In addition, electrical stimulation of the BLA increased slow gamma oscillatory activity in the hippocampus (Bass & Manns, 2015). Many brain regions receive inputs from the BLA (Sah, Faber, Lopez De Armentia, & Power, 2003), but for modulation of hippocampus-dependent memory, the current data suggest one key region influenced by the BLA is the hippocampus itself. Understanding these mechanisms will help characterize how the brain prioritizes important memories (Manns & Bass, 2016).

One possible mechanism by which the BLA could beneficially modulate memory is by eliciting oscillatory network states that favor memory in the hippocampus and associated areas. In particular, theta (6-10 Hz in rats) oscillations are related to behavioral states (Montoya, Heynen, Faris, & Sainsbury, 1989; Sheremet et al., 2019) and memory (Buzsáki, 2005; Buzsaki & Moser, 2013; McNaughton, Ruan, & Woodnorth, 2006). In addition, hippocampal slow gamma oscillations (30-55 Hz in rats) at encoding correlated with later retrieval success (Jutras, Fries, & Buffalo, 2009; Sederberg et al., 2007; Trimper, Galloway, Jones, Mandi, & Manns, 2017). The amplitude of slow gamma oscillations in the hippocampus fluctuates and is modulated by the phase of theta, one type of phase-amplitude cross-frequency coupling (hereby referred to as theta-gamma comodulation). The degree of theta-gamma comodulation (i.e., cross-frequency coupling) is also a strong correlate of memory performance (Shirvalkar, Rapp, & Shapiro, 2010; Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009; Trimper, Stefanescu, & Manns, 2014). Indeed, recent studies using electrical stimulation to the BLA to enhance object

recognition memory have used an electrical pulse frequency meant to simulate theta-modulated gamma oscillations (bursts of 50 Hz stimulation every 1/8th of a second; Bass et al., 2012; Bass et al., 2014; Bass and Manns, 2015; Inman et al., 2018). These results indicated that stimulating the BLA with a theta-modulated gamma pulse frequency was capable of improving memory performance, but the findings did not answer whether stimulating at theta or gamma frequencies alone would suffice to elicit in the hippocampus neuronal oscillations resembling those that correlate with good memory. For example, stimulating the BLA at 50 Hz alone could in principle lead to slow gamma (i.e., 50 Hz) oscillations in the hippocampus for which the amplitude would be modulated by the phase of the endogenous hippocampal theta oscillations.

The current experiment with freely-moving rats asked if stimulating the BLA at theta and gamma frequencies could elicit in the hippocampus neuronal oscillations resembling those previously found to correlate with good memory performance. A key question was whether BLA stimulation that combined theta and gamma frequencies was needed to amplify hippocampal theta-gamma comodulation, which is known to be important for good memory. The current experiment utilized optogenetic rather than electrical stimulation of the BLA for several reasons. First, the use of a cell-type specific (CaMKII) promoter for the vector delivering the opsin (channelrhodopsin; ChR2) allowed for stimulation restricted to putative glutamatergic projection neurons in the BLA. Second, use of a blue light-sensitive opsin allowed for a control stimulation condition that used near-infrared light pulses outside the excitation spectrum of the opsin. Third, optical stimulation avoided electrophysiological recording artifacts induced by electrical stimulation. Stimulation was delivered in one-second bouts at 8 Hz to emulate theta, at 50 Hz to emulate slow gamma, and at 50 Hz bursts every 1/8th second to emulate theta-modulated gamma (50/8 Hz). Included for comparison were conditions in which one second of 20 Hz stimulation

was delivered using either blue (experimental) and near-infrared (control) light. BLA stimulation with blue light in all conditions elicited oscillatory activity in the hippocampus, but only BLA stimulation at 50/8 Hz elicited in CA1 a pattern of activity that appeared to reflect theta-gamma comodulation similar to what has been observed in studies to positively correlate with good object memory (Shirvalkar et al., 2010; Tort et al., 2009; Trimper et al., 2014).

3.2. Materials and Methods

3.2.1. Subjects

Six adult male Long-Evans rats, between 400-500g, were housed individually (12-hr light/dark cycle; stimulation during light phase). All animals were given free access to water and were food restricted, maintaining at least 90% of their free-feeding body weight. All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

3.2.2. Surgery and Drive Positioning

Rats underwent a single stereotaxic surgery for infusion of the viral vector and implantation of combined optical fiber and tetrode recording assembly. Rats were anesthetized with isoflurane (1-3% in oxygen at 1.0 L/min) and received preoperative (0.03 mg/kg buprenorphine) and postoperative (0.05 mg/kg buprenorphine, 1.0 mg/kg meloxicam) analgesia. Additional care and nutrition were given as needed. A single craniectomy was created above the BLA and intermediate third of the hippocampus (coordinate range: 2.6-5.9 mm posterior and 2.8-5.5 mm lateral to Bregma; Paxinos and Watson, 1998) for a unilateral infusion and implantation in the right hemisphere. The viral vector containing channelrhodopsin and reporter fluorophore (AAV_5-CaMKII-hChR2(H134R)-EYFP; University of North Carolina Vector Core) was infused using a stereotaxic frame (Kopf Instruments) and syringe pump (Hamilton Company). The virus

was infused through a 33-gauge needle into the BLA (coordinates: 3.5 mm posterior, 5.1 mm lateral, 8.9 mm ventral to Bregma) at 150 nL/min for a total volume of 500 nL. The needle was left in place for 10 minutes before withdrawal to allow the virus to diffuse into the surrounding tissue.

After withdrawal of the needle, the recording assembly containing a fixed optical fiber with a ceramic ferrule (200/230 nm, 0.66 NA; Plexon, Inc.) and independently-moveable nichrome tetrodes was implanted. Tetrodes were spun with 12.5 nichrome wire (California Fine Wire or Sandvik) and plated with gold to reduce the impedance to approximately 200 kΩ at 1 kHz. The optical fiber was fixed in the recording assembly so that it was positioned directly above the BLA (coordinates: 3.5 mm posterior, 5.1 mm lateral, 8.4 mm ventral to Bregma) with the base of the recording assembly at the surface of the exposed brain. Tetrodes targeting the BLA were glued to the optical fiber to target 0.25-0.75 mm below the fiber tip. Tetrodes targeting the hippocampus were each controlled by a separate driver. They were targeted at the intermediate third of the hippocampus (coordinates range: 4.5-5.9 mm posterior, 2.9-5.4 lateral mm to Bregma), since the intermediate CA1 receives direct projections from the BLA (Petrovich, Canteras, & Swanson, 2001; Pikkarainen, Rönkkö, Savander, Insausti, & Pitkänen, 1999; Pitkänen et al., 2000) and has been shown to be involved in memory enhancement by brief electrical stimulation to the BLA (Bass & Manns, 2015; Bass et al., 2014). The rat was grounded by a wire attached to a stainless-steel screw, which was implanted in the skull midline over the cerebellum. This ground screw also served as the reference for LFP recordings. After a minimum of one-week recovery, tetrodes were slowly lowered into the pyramidal cell layer of the CA1 over the following weeks (recording tetrodes in BLA were fixed to the optical fiber). No tetrodes were moved within 24 hours of stimulation and recording.

3.2.3. Optogenetic Stimulation

Testing occurred no sooner than 4 weeks post-surgery to allow sufficient time for viral transfection and opsin expression. All stimulation occurred on awake rats as they freely explored a 30-cm diameter circular recording platform bordered by an approximately 7-cm wall. Stimulation events were triggered by the experimenter no less than 10 seconds apart. Stimulation was never dependent on a particular behavioral state other than ensuring that the rat was awake throughout the experiment; the experimenter was not directly observing the animal during stimulation. Light was produced by a compact LED at 465 nm (blue) or 740 nm (near-infrared) (Plexon, Inc.). The blue LED produced light within the excitation spectrum of channelrhodopsin, and the near-infrared LED produced light outside of the excitation spectrum, a method documented to act as a reliable control (Blumberg et al., 2016; Klavir, Prigge, Sarel, Paz, & Yizhar, 2017). The LED was connected to the optical fiber's ferrule on the recording assembly by an armored patch cable (200/230 nm, 0.5 NA) and ceramic coupler (Plexon, Inc.).

Stimulation included several parameter conditions, the order of which was randomized across rats. All rats experienced a least 20 bouts of each condition. Rats received stimulation in the following conditions: 1) one second blue light at 8 Hz, 2) one second blue light at 20 Hz, 3) one second blue light at 50 Hz, 4) one second blue light in bursts of four 50 Hz pulses every $1/8^{th}$ second (50/8 Hz), 5) five seconds blue light at 50 Hz, 6) five seconds blue light at 50/8 Hz, and 2) one second near-infrared light at 20 Hz. Stimulation parameters were chosen to mimic theta (8 Hz), slow gamma (50 Hz), theta-gamma comodulation (50/8 Hz), and a frequency (20 Hz) known to reliably evoke responses from ChR2(H134R). All light pulses were of 5 ms duration. Power at the optical fiber tip was approximately 11 mW for the blue LED and 7 mW for the near-infrared LED.

3.2.4. Histology

Prior to euthanasia, the location of each tetrode was marked by passing 20-40 μA current for 10-30 seconds through a single wire of the tetrode. Rats were injected with an overdose (0.5 mL) of Euthanasia-III Solution (Med-Pharmex) after being anesthetized with isoflurane. They were then transcardially perfused with isotonic saline followed by neutral buffered formalin 10% (Harleco). Brains were extracted, post-fixed in neutral buffered formalin 10% for 24 hours, and submerged in a 30% sucrose solution until saturated. Brains were sectioned on a freezing stage microtome at 40 μM thickness and stored in 0.1 M phosphate buffer. All sections were mounted on slides coated with gelatin and chromium potassium sulfate dodecahydrate (Fisher Scientific). For verification and localization of virus expression, slides were covered with Vectashield with DAPI (Vector Laboratories), and cover slipped. Expression of channelrhodopsin was inferred by the expression of the conjugated fluorophore, observed on an epifluorescence microscope for regional expression and on a confocal microscope for cell body and fiber identification. BLA tetrodes were localized by staining for acetylcholinesterase, which robustly stains the basal nucleus of the BLA. Hippocampal tetrodes were localized under light microscopy following a Nissl stain (cresyl violet).

3.2.5 Data Acquisition and Analysis

Local field potentials (LFPs) were recorded from tetrodes in the BLA and hippocampus with a sampling rate of 1.5 kHz and were filtered from 1 to 400 Hz. The LFP from one tetrode in the pyramidal layer of CA1 and one tetrode in the BLA was used for each rat. Spiking data were not analyzed due to too few well-isolated single units. All data were obtained with the NSpike data acquisition system (nspike.sourceforge.net). Analyses were performed in MATLAB (MathWorks) using custom scripts and the Chronux toolbox (Bokil, Andrews, Kulkarni, Mehta,

& Mitra, 2010). Power of the BLA and CA1 LFPs was estimated using a multitaper fast Fourier transform similar to previous reports (Bass & Manns, 2015; Trimper et al., 2017). The modulation index (MI) for phase-amplitude cross-frequency coupling (i.e. comodulation) was calculated as previously described (Tort et al., 2009).

For all analyses, results were averaged within a rat across all trials of a given condition, and then the data from all rats were averaged. For some analyses, a rat's data from a single stimulation condition were normalized prior to averaging across rats to demonstrate more clearly the impact of stimulation. In particular, for analyses of average stimulation-evoked LFPs in the time domain, LFPs from four seconds before stimulation onset to five seconds after stimulation offset were Z-transformed based on the mean and standard deviation of each single trial sweep. For spectral analyses in the frequency domain, FFT analyses were conducted on the raw LFPs. Absolute power is shown in spectrograms. However, for plots of moving-window spectrograms, estimates of power were normalized (Z-transformed) to a pre-stimulation baseline period (from -2 to -1 seconds before the onset of stimulation) to visualize more clearly the impact of stimulation.

Statistical significance was determined using a random permutation approach in which LFP data from the stimulation and baseline periods were randomly shuffled 1000 times. All analyses were recalculated for each random shuffle, and statistical significance was defined as metrics falling outside the 95^{th} percentile of the distribution obtained from the random shuffles. More specifically, power plotted in spectrograms was analyzed with a cluster-based permutation in which clusters were defined as frequency ranges in which the power values were greater than 2.5 standard deviations above or below the mean of the data. Only clusters spanning more than 1 Hz were considered. The random cluster permutation distribution included only the largest

cluster from each random permutation. Clusters (frequency ranges) in the original data that were outside the 95th percentile of the random cluster distribution were labelled as statistically significant. This cluster-based approach was used because it preserves the overall alpha level (Maris & Oostenveld, 2007). For 5-second stimulation conditions, the same cluster-based random permutation approach was used for each second of stimulation. Power during seconds 1-5 were compared against baseline activity (-2 to -1 seconds before stimulation) independently to determine how the response to stimulation developed over time. Changes in comodulation were analyzed in a pre-determined theta-gamma range (6-10 Hz phase frequency, 30-55 Hz amplitude frequency). A random permutation analysis of variance was used to determine the significance of the effect of stimulation condition on the pre-defined theta-gamma comodulation. Specifically, the variance was defined as summed variance to the mean across stimulation conditions. The mean was the average comodulation index across conditions, and the variance was the difference between the comodulation during an individual condition and the mean. The random permutation was constructed by shuffling the condition labels for each stimulation bout. The resulting variance from the mean of the shuffled data populated the random permutation distribution. The effect of stimulation condition on comodulation was considered significant if the variance of the original data fell outside the 95th percentile of that distribution.

3.3. Results

3.3.1. Histological verification of stimulation and recording locations

Postmortem histological analysis verified placement of the optical fiber dorsal to the BLA, expression of channelrhodopsin in the BLA, and location of recording tetrodes in the BLA and CA1 of the intermediate hippocampus. Figure 3.1 shows a schematic of the stimulation and recording approach as well as example histology. The tip of all unilaterally-implanted optical

fibers was confirmed to be positioned 0.2 to 0.6 mm dorsal to the BLA. In all six rats, the viral vector transfected cell bodies in the BLA, as evidenced by punctate expression of the fluorophore conjugated to the opsin (Fig. 3.1D). Expression in the amygdala was restricted to the BLA. In two rats, the viral vector spread to a modest degree into the adjacent piriform cortex. However, the off-target neurons were largely outside the cone of light (with a 0.66 numerical aperture, light was emitted from the optical fiber at 29.0°), since the majority of labeled neurons in the piriform cortex were in the dorsal endopiriform nucleus (Paxinos & Watson, 1998). Thus, the impact of light stimulation was largely restricted to neurons in the BLA in all rats. In all rats, fluorophore-labeled fibers were visible in the temporal half of the hippocampus, particularly in the lacunosum-moleculare layer of CA1 and subiculum (Fig. 3.1E), consistent with past studies showing projections from the BLA to hippocampus terminating in this specific area (Pitkänen et al., 2000; Wang & Barbas, 2018). Analyses of neural data focused on LFPs recorded from single electrodes in the BLA and CA1 in order to align the current results with past results from humans and rats (Bass & Manns, 2015; Bass et al., 2014; Bass et al., 2012; Inman et al., 2018) and because too few well-isolated single neurons were recorded to permit spiking analyses. All six rats had at least one tetrode positioned in the basolateral nucleus, and five rats had at least one tetrode positioned in the pyramidal layer of the CA1. The pyramidal layer was selected as a target layer to allow for comparison with past studies (Bass & Manns, 2015; Trimper et al., 2014), and because it could be localized at the time of recording by the presence of putative pyramidal neuron spiking. Analyses of LFP data from the BLA thus included six rats, whereas analyses of data from CA1 included five rats.

3.3.2. Effects of one-second optogenetic BLA stimulation on BLA and CA1 LFPs

Figure 3.2 shows the mean normalized (Z-transformed) LFP in the BLA and CA1 during one-second bouts of optical stimulation of the BLA. Stimulation was delivered up to 70 times per condition (mean number of stimulations per condition = 39.9; range = 20 to 70) over the course of multiple recording sessions for each rat (mean number of recording sessions per rat = 2.83; range = 1 to 4). The optical stimulation was a blue 465-nm light delivered at 8 Hz, 20 Hz, or 50 Hz, or as bursts of four 50-Hz pulses delivered every 1/8th second ("50/8 Hz"). A control condition consisted of one second of 20-Hz near-infrared 740-nm light to the BLA, a wavelength known to be outside the excitation spectrum of channelrhodopsin (Mattis et al., 2012). Optical stimulation with blue light in the 8 Hz, 20 Hz, 50 Hz, and 50/8 Hz conditions evoked large responses of the same frequencies in the LFPs in the BLA (Z-scores ranged from about -1 to +1 across conditions) and moderate responses of the same frequencies in the LFPs in CA1 (Z scores ranged from about -0.4 to +0.4 across conditions). Evoked responses in both regions appeared to cease soon after termination of stimulation in each condition. The control 20-Hz near-infrared optical stimulation did not evoke appreciable responses in either the BLA or CA1. These results suggest that optical stimulation of the BLA with blue light was capable of evoking frequency-matched responses in both the BLA and CA1 and that the evoked responses were a direct result of activation of the opsin, not an optoelectric artifact or an artifact of the recording system.

Although the overall responses to one-second of light stimulation were similar in the BLA and CA1, a closer inspection highlighted important differences between the regions. Figure 3.3 shows mean normalized evoked responses in the BLA and CA1 to individual pulses of light delivered to the BLA. Averaged across all blue light stimulation conditions, the latency from onset of the first light pulse in each bout of stimulation to the initial peak of evoked response was 6.67 ms in the BLA, which reflects the response time of the opsin to light stimulation (Mattis et

al., 2012). The latency to the initial peak was 12.7 ms in CA1 (Fig. 3.3A), a 6.03-ms difference, suggesting that the responses recorded in CA1 were neither triggered directly by the light nor conducted passively by brain volume but instead were evoked by monosynaptic connections from the BLA. Averaging across all light pulses separately for each condition shows additional differences between the responses in the BLA and CA1 (e.g., averaging across all 8 pulses in the 8 Hz condition). BLA LFPs were characterized by evoked responses of the same width (approximately 9 ms) in the 8 Hz, 20 Hz, 50 Hz, and 50/8 Hz conditions (Fig 3.3B-F). The initial evoked responses were followed by smaller responses in the fast gamma range (60-120 Hz), which is a prominent frequency band in the amygdala (Amir, Headley, Lee, Haufler, & Pare, 2018; Feng et al., 2019). In contrast, CA1 LFPs during stimulation with blue light displayed a more continuous waveform that had a sawtooth shape for 8 Hz and 20 Hz conditions and a sinusoidal shape for 50 Hz stimulation (Fig. 3.3B-D). For the 50/8 Hz condition, LFPs in the BLA and CA1 both showed 8 Hz and 50 Hz components in the shape of the response to the four 50-Hz pulses delivered every $1/8^{th}$ second (Fig 3.3F). However, the 8-Hz response was out of phase between the BLA and CA1, and the 50-Hz response was delayed by at least a full 50-Hz cycle in CA1 compared to the BLA. Thus, the 50-Hz responses were largest on the rising slope of the 8-Hz wave in the BLA but largest on the falling slope of the 8-Hz wave in CA1. The differences in LFP responses between the BLA and CA1 suggested that stimulation of the BLA modulated activity within the hippocampus above and beyond a simple recapitulation of the stimulation effects in BLA. LFPs in both regions showed a small artifact during 20 Hz near-infrared stimulation, but the artifacts were the opposite polarity and occurred at a shorter delay as compared to those produced by blue light stimulation (Fig. 3.3D).

A final comparison of waveforms between the BLA and CA1 during BLA stimulation focused on the average normalized response following the last light pulse in each bout of stimulation (Fig. 3.3G). LFPs in the BLA following the last pulse of light were similar to the previous analyses of LFPs averaged across all light pulses in a condition (Fig. 3.3B-F), and LFPs averaged across the first light pulse of each condition (Fig. 3.3A). For example, for each condition, the delay of initial peak responses of the BLA LFP following the final BLA pulse was similar (range = 6.67-8.67 ms) to the average delay in response to the first pulse (6.67 ms) and was followed by fast, small amplitude activity in each case. In contrast, LFPs responses in CA1 following the last pulse of light differed across stimulation conditions. The times to initial peak response in CA1 following the final BLA pulse were 14.0, 19.3, 16.0, and 9.33 ms for 8 Hz, 20 Hz, 50 Hz, and 50/8 Hz conditions, respectively. In addition, a full extra cycle of slow gamma activity persisted in CA1 following the last pulse of 50/8 Hz stimulation and, to a lesser extent (and with different timing) following the last pulse of 50 Hz stimulation. The frequency-dependent persistent activity in CA1 supports the characterization of responses in the CA1 as oscillations rather than concatenated evoked responses, particularly for the 50/8 Hz stimulation condition.

3.3.3. Effects of one-second optogenetic BLA stimulation on the power spectra of the BLA and CA1

Figure 3.4 shows the power spectra for the 8 Hz, 50 Hz, and 50/8 Hz conditions following a multitaper fast Fourier transform (FFT) of the BLA and CA1 LFP traces (see Materials and Methods for analysis details, including testing for statistical significance). The results are shown as normalized (Z-transformed) moving window power spectrograms as well as standard power spectrograms to illustrate and statistically evaluate changes in the theta and

gamma frequency ranges during stimulation relative to a pre-stimulation baseline. The moving window power spectrograms were calculated using a 1-second sliding window, so power values for a given timepoint contain information from the preceding and following 0.5 seconds. For LFPs from the BLA, BLA stimulation in the 8 Hz and 50 Hz conditions resulted in increased power in the 8 Hz and 50 Hz frequency ranges (plus harmonics), respectively. The increase in 50 Hz power was statistically significant for the 50 Hz condition, and the increase in power in the 8 Hz harmonic ranges (peaks at 16, 24, 32, 40, 48, and 56 Hz) was statistically significant for the 8 Hz condition. Stimulation in the 50/8 Hz condition resulted in statistically significantly increased BLA power at 8 Hz, 40 Hz, 48 Hz, and 56 Hz. In contrast to the results from BLA LFPs, CA1 LFPs showed power with prominent peaks in the 8 Hz range during the baseline in all conditions but did not show increased power in the 8 Hz range for any stimulation condition. The lack of increase in 8 Hz CA1 power in the 8 Hz stimulation condition contrasts with the clear entrainment of CA1 LFPs at 8 Hz during 8 Hz stimulation (see Fig. 3.2 top right panel, and Fig. 3.3B). Thus, the phase but not the amplitude of ongoing hippocampal theta oscillations appeared to be modulated by 8 Hz BLA stimulation. Stimulation in both the 50 Hz and 50/8 Hz conditions resulted in significantly increased CA1 power in the slow gamma range. However, the peak frequency of CA1 power increase was 50 Hz during 50 Hz stimulation yet 48.7 Hz during 50/8 Hz stimulation, which is closer to a harmonic (48 Hz) of the underlying 8 Hz pattern than 50 Hz. Thus, the 8-Hz entrainment of CA1 LFPs during 8 Hz stimulation and the slightly shifted peak slow gamma power increase in the 50/8 Hz (48.7 Hz rather than 50 Hz) both suggest that the 8-Hz component of BLA stimulation in the 8 Hz and 50/8 Hz conditions did influence CA1 LFPs. Nevertheless, the only statistically significant power increases in CA1 during BLA stimulation in 8 Hz, 50 Hz, and 50/8 Hz conditions were in the slow gamma range (~50 Hz).

3.3.4. Theta-gamma comodulation during optogenetic stimulation

A key question was whether optogenetic stimulation of putative BLA glutamatergic projection neurons could increase gamma oscillations in the hippocampus for which the amplitude was modulated by the phase of theta oscillations—the type of phase-amplitude cross-frequency coupling (here referred to in brief as comodulation) known to be important for memory (Shirvalkar et al., 2010; Tort et al., 2009; Trimper et al., 2014). Figure 3.5 shows comodulation in CA1 during BLA stimulation relative to baseline in the 8 Hz, 50 Hz, and 50/8 Hz conditions. Only stimulation in the 50/8 Hz condition statistically significantly ($p < 0.05$ per a random permutation analysis; see Methods and Materials) increased theta-gamma comodulation relative to baseline (mean modulation index [MI] = 0.67×10^{-4}, -1.33×10^{-4}, and 1.73×10^{-4}, for 8 Hz, 50 Hz, and 50/8 Hz conditions, respectively). In addition, the stimulation condition was a statistically significant factor ($p < 0.05$ per a random permutation analysis) in theta-gamma comodulation across 8 Hz, 50 Hz, and 50/8 Hz conditions (see Materials and Methods for analysis details). Thus, theta-modulated gamma oscillations were increased in CA1 only when BLA stimulation included both theta and gamma frequencies.

3.3.5. Temporal effects of optogenetic stimulation

The final question was whether longer bouts of BLA stimulation might elicit larger or different responses as compared to one second of simulation. Figure 3.6 shows activity in the BLA and CA1 during five seconds of BLA stimulation in the 50 Hz and 50/8 Hz conditions (n=3 for these data). The BLA LFPs did not appreciably change over the five seconds of BLA stimulation in either condition. In contrast, CA1 LFPs substantially changed from the first to the fifth second of stimulation, such that prominent fast oscillatory activity at the beginning of stimulation was almost completely replaced by slow oscillatory activity by the end of

stimulation. For the 50 Hz stimulation condition, the 50 Hz CA1 oscillations in the first second returned to baseline levels and were largely replaced by slow oscillations in the 8 Hz and 16 Hz ranges by the fifth second of stimulation. For the 50/8 Hz condition, CA1 oscillations in the 48-Hz range decreased moderately and CA1 oscillations in 8 Hz and 16 Hz ranges increased markedly from the first to fifth second of BLA stimulation. In the BLA, 5-second BLA stimulation at 50 Hz stimulation evoked statistically significant increases in gamma power for each of the five seconds. In addition, 5-second BLA stimulation at 50/8 Hz stimulation evoked statistically significant increases in both theta (plus harmonics) and gamma power for each of the five seconds of stimulation. Thus, longer bouts of BLA stimulation resulted in temporally static responses in BLA LFPs but temporally dynamic responses in CA1 LFPs. It is unclear why the 5-second BLA stimulation resulted in different hippocampal activity as compared to 1-second of BLA stimulation. In any case, the present results suggest that brief (less than 2 seconds) optogenetic 50/8 Hz stimulation of the BLA would be most likely to elicit hippocampal oscillatory states thought to be beneficial to memory.

3.4. Discussion

Brief optogenetic stimulation of the BLA at 8 Hz, 20 Hz, 50 Hz, and 50/8 Hz reliably elicited responses at matching frequencies in LFPs recorded in the BLA and CA1 in freely exploring rats. However, stimulation responses in CA1 differed from responses in the BLA in several ways. As compared to the responses in the BLA, the responses in CA1 across conditions were delayed by approximately 6 ms, displayed more continuous (sinusoidal or sawtooth) waveforms, and showed dynamic oscillatory changes across longer bouts (5 seconds) of stimulation. Thus, CA1 LFPs showed responses during BLA stimulation that broadly resembled neuronal oscillations, whereas BLA LFPs showed responses that resembled concatenated evoked

responses. Moreover, the responses in CA1 LFPs to BLA stimulation differed between the 8 Hz, 50 Hz, and 50/8 Hz stimulation conditions, which were the focus of the current study. In particular, BLA stimulation in the 50 Hz and 50/8 Hz conditions led to increased power close to 50 Hz in CA1, but none of the 8 Hz, 50 Hz, and 50/8 Hz conditions led to increased CA1 power in the 8 Hz range, despite 8 Hz BLA stimulation clearly entraining the phase of the ongoing 8 Hz theta oscillation in the hippocampus. A key finding was that one second of 50/8 Hz BLA stimulation preferentially increased in CA1 LFPs 50 Hz oscillations for which the amplitude was modulated by the phase of the 8 Hz oscillations, a type of phase-amplitude cross-frequency coupling (theta-gamma comodulation) known to be important for good memory. Thus, artificial stimulation of the BLA appears to be capable of increasing in the hippocampus neuronal oscillations that resemble endogenous oscillatory states that are thought to benefit memory formation. The results are discussed in more detail below.

3.4.1. BLA projections to CA1 were among many potential BLA projections activated by stimulation

The BLA includes the basal, lateral, and accessory basal nuclei (Sah et al., 2003). Neurons in these nuclei send axons to regions essential for declarative memory, including the hippocampus, entorhinal cortex, and perirhinal cortex, as well as to many other regions of the brain and to other amygdalar nuclei (Pitkänen et al., 2000; Pitkänen et al., 1995; Sah et al., 2003; Savander, LeDoux, & Pitkänen, 1996). Thus, optogenetic stimulation of putative glutamatergic BLA projection neurons could have influenced neuronal activity in the hippocampus both directly and indirectly. One potential pathway mediating the indirect effects of BLA stimulation on the hippocampus is the pathway from perirhinal cortex to entorhinal cortex to hippocampus (Burwell & Amaral, 1998; Witter & Amaral, 2004). For example, activation of the amygdala is

thought to facilitate information transfer from the perirhinal cortex to the entorhinal cortex, which in turn would influence the input to the hippocampus (Kajiwara, Takashima, Mimura, Witter, & Iijima, 2003; Paz, Pelletier, Bauer, & Pare, 2006). In addition, stimulation of the BLA-entorhinal cortex pathway was previously found to enhance hippocampal-dependent memories (Wahlstrom et al., 2018). Additional support for the importance of this perirhinal-entorhinal pathway comes from past studies showing that BLA stimulation modulated hippocampal LTP in the dentate gyrus (Abe, 2001; Akirav & Richter-Levin, 2002; Vouimba & Richter-Levin, 2005), which receives input from the entorhinal cortex but not from the BLA (Pitkänen et al., 2000; Witter & Amaral, 2004). As such, the BLA likely normally engages indirect pathways to influence hippocampal activity and to modulate memory.

Nevertheless, the current results indicated that direct BLA-CA1 projections were an important pathway through which optogenetic stimulation of BLA neurons influenced hippocampal activity. Infusions of the viral vector specifically targeted neurons in the posterior portion of the basal nucleus in the BLA, a region previously found to have strong direct projections to CA1 (Pitkänen et al., 2000). Postmortem histology in the present study confirmed expression of the opsin and reporter fluorophore in cell bodies in this nucleus as well as in fibers in the lacunosum-moleculare layer of intermediate CA1, consistent with the laminar profile of past anatomical studies of direct basal nucleus projections to CA1 (Pitkänen et al., 2000; Wang & Barbas, 2018). Thus, CA1 recording tetrodes were positioned near the soma (in pyramidale) of pyramidal neurons likely receiving synaptic inputs on their apical dendrites (in lacunosum-moleculare) from opsin-containing BLA neurons. Further, the short delay (6.03 ms) observed between BLA and CA1 responses to initial pulses of BLA stimulation strongly supported the involvement of this monosynaptic pathway. Previous studies have also shown that manipulation

of this direct pathway was sufficient to drive behavioral changes (Huff, Emmons, Narayanan, & LaLumiere, 2016; Rei et al., 2015). Taken together, the results suggested that the direct projection from BLA to CA1—although only one of many BLA projections—was important for the hippocampal responses increased by optogenetic BLA stimulation.

3.4.2. BLA stimulation modulated neuronal oscillations in CA1

The pattern of CA1 LFP activity in response to BLA stimulation reflected more than a concatenation of depolarizing events. Instead, CA1 LFPs responded to 1-second BLA stimulation in a manner more characteristic of neuronal oscillations, evidence for synaptic transmission that included (but was not limited to) direct BLA to CA1 projections. Specifically, LFP activity in CA1 during each of the 1-second BLA stimulation conditions showed rhythmic sinusoidal or sawtooth waveforms that corresponded to the stimulation frequency. In contrast, LFP activity in the BLA showed a sharp evoked response to each light pulse that was disconnected from preceding responses and was unrelated to stimulation frequency. One possible source of the differences between responses in BLA and CA1 LFPs was that the CA1 LFP responses may have been shaped by the low-pass frequency filtering that occurs during synaptic transmission, particularly in the case of synapses on the distal portion of apical dendrites of pyramidal neurons (Buzsáki, Anastassiou, & Koch, 2012), as was likely in the present study. Indeed, it is possible that synaptic transmission between regions is generally important in translating the effects of artificial stimulation to effects more reminiscent of endogenous activity. Nevertheless, the emergence of rhythmic oscillatory activity in CA1 LFPs likely also reflected circuit dynamics in the hippocampus. Possible examples include local excitatory-inhibitory interactions between CA1 pyramidal neurons and interneurons (Buzsáki & Wang, 2012) and rhythmic inputs to the hippocampus from a number of brain regions (Buzsáki, 2002). Thus,

direct BLA to CA1 projections were likely key to initiating CA1 LFP responses to stimulation, but the emergence of oscillatory activity in CA1 also likely depended on other intra-hippocampal and extra-hippocampal influences on CA1 activity.

One of these main influences appeared to be ongoing theta oscillations in the hippocampus. Theta (~8 Hz) oscillations in the hippocampus are prominent and are thought to emerge from a number of influences, including pacemaker inputs from medial septum and entorhinal cortex, from periodic activity of local interneurons, and from resonance properties of pyramidal neurons (Buzsáki, 2002). In the present study, theta power in CA1 LFPs was high at baseline, and neither 1-second BLA stimulation at 8 Hz nor at 50/8 Hz increased theta power in CA1 despite producing a large increase in theta power in BLA LFPs. However, the phase of CA1 theta oscillations appeared to reset and become strongly entrained to the 8 Hz component of both 8 Hz and 50/8 Hz BLA stimulation. That is, hippocampal oscillations in the theta band were still modulated by BLA stimulation, even without significant increases in CA1 theta power.

3.4.3. Theta-modulated 50-Hz BLA stimulation was necessary to increase theta-modulated gamma oscillations in CA1

A main question motivating the present study was whether BLA stimulation combining theta and gamma frequencies was needed to increase in the hippocampus gamma oscillations for which the amplitude was modulated by the phase of theta, the type of theta-gamma comodulation that is normally observed in the hippocampus (Bragin et al., 1995; Buzsaki et al., 2003). An alternate possibility was that continuous 50 Hz BLA stimulation would interact with endogenous hippocampal theta oscillations to also produce theta-modulated gamma oscillations. Another possibility was that 8 Hz BLA stimulation would modulate extant hippocampal gamma oscillations. Finally, it had been possible that 50/8 Hz BLA stimulation would misalign with

existing theta and gamma oscillations in the hippocampus and not result in theta-modulated gamma oscillations in CA1. In short, it was possible that all or none of the stimulation conditions of interest would increase theta-gamma comodulation in the hippocampus. Nevertheless, the results of the current study showed that theta-gamma comodulation within CA1 was significantly increased during 50/8 Hz stimulation but not 50 Hz stimulation or 8 Hz stimulation. The results are important because hippocampal gamma oscillations are normally modulated by theta phase and because hippocampal theta-gamma comodulation is a neural state previously observed to correlate with successful encoding and retrieval of hippocampal memory (Shirvalkar et al., 2010; Tort et al., 2009; Trimper et al., 2014). Indeed, one hypothesis about amygdala-mediated declarative memory enhancement is that activation of the BLA elicits theta-modulated gamma oscillations in the hippocampus, which in turn promotes spike-timing dependent plasticity for recently active synapses (Manns & Bass, 2016).

3.4.4. Comparing the effects of optogenetic BLA stimulation to those of electrical BLA stimulation

Several previous studies in rats (Bass & Manns, 2015; Bass et al., 2014; Bass et al., 2012) and humans (Inman et al., 2018) observed improved 24-hr recognition memory performance for neutral objects when the initial presentation of the objects was immediately followed by one second of 50/8 Hz electrical stimulation of the BLA. One of the studies in rats (Bass & Manns, 2015) also recorded neuronal activity in the intermediate hippocampus at the time of stimulation and observed increased coherence (both field-field and spike-field) between CA3 and CA1 in the slow gamma range (theta-gamma comodulation was not reported). The similarity in increased hippocampal gamma oscillations between this prior study and the present study suggests that activation of the BLA via either electrical or optogenetic stimulation can elicit oscillatory states

in the hippocampus that favor memory. Nevertheless, the mechanisms of BLA activation likely differed between optogenetic and electrical stimulation. For example, optogenetic stimulation in the present study more preferentially depolarized glutamatergic cell bodies in the transfected area (though the depolarization of any neuron may have been stochastic rather than deterministic for the 50-Hz stimulation; Cardin et al., 2009; Weitz et al., 2015), whereas electrical stimulation in prior studies would have stimulated all neuron types as well as fibers of passage (Histed, Bonin, & Reid, 2009). Perhaps reflecting these differences, electrical pulses delivered to the BLA in the prior study (Bass & Manns, 2015) resulted in initial evoked responses in the hippocampus after a delay (24 ms) that suggested a polysynaptic effect of stimulation rather than the monosynaptic effect thought to be important in the present study. One possibility is that electrical stimulation of the BLA more strongly engaged the BLA-perirhinal/entorhinal-hippocampus pathway, whereas optogenetic stimulation of the BLA more strongly engaged the BLA-hippocampus pathway. If so, the results would suggest that activation of either pathway would be sufficient to produce memory-promoting oscillatory states in the hippocampus characterized by slow gamma oscillations.

3.5. Conclusions and future directions

The ability of 50/8 Hz optogenetic BLA stimulation to elicit theta-gamma comodulation in the hippocampus provides important insights into how the amygdala may modulate the hippocampus to prioritize memories with affective salience. Memory modulation should benefit some memories more than others if important moments are to be remembered better than unimportant moments. Thus, amygdala stimulation will likely need to be temporally specific to prioritize memories effectively. Indeed, consideration of temporal specificity will be important for any possible future therapeutic interventions and may be one explanation for the mixed

results of past memory studies targeting amygdala activity (Agren, 2014; Taylor & Torregrossa, 2015). In addition, previous experiments have shown that the effects of direct hippocampal stimulation can depend on the neural state immediately prior to stimulation, which is possibly why closed-loop stimulations of the hippocampus have sometimes enhanced memory (Berger et al., 2011; Ezzyat et al., 2017; Hampson et al., 2012; Hampson et al., 2018), whereas open-loop stimulations (i.e., delivered irrespective of ongoing activity) typically impair memory (Jacobs et al., 2016; Lacruz et al., 2010). In contrast to these studies of direct hippocampus stimulation, 50/8 Hz electrical stimulation of the BLA has reliably improved memory even when stimulation onset was not dependent on ongoing neuronal activity (Bass & Manns, 2015; Bass et al., 2014; Bass et al., 2012; Inman et al., 2018), perhaps because BLA stimulation was able to reset the phase of ongoing theta oscillations, as observed with optogenetic BLA stimulation in the present study. Finally, the anatomical specificity of BLA stimulation will be an additional important consideration moving forward. Future experiments using optogenetic stimulation of specific BLA projections (e.g., BLA to hippocampus) will be required to determine how projection-specific stimulation of the BLA might differentially impact hippocampal activity or memory performance. Indeed, it is an open question as to whether the glutamatergic neuron-specific optogenetic stimulation used in the present study would result in similar memory enhancement as observed in past studies using electrical stimulation of the BLA.

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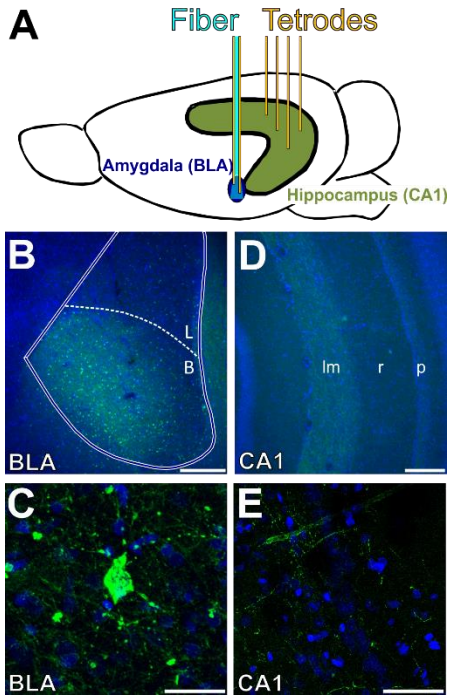

Figure 3.1. Location of opsin expression, optogenetic stimulation, and tetrode recording. (A) Tetrodes (yellow) were lowered into the CA1 subregion of the intermediate hippocampus (green) and basolateral amygdala (blue). An optical fiber (cyan) was lowered to 0.25-0.75 mm above the target depth in the BLA. (B-E) Images of coronal sections of the BLA (B,C) and intermediate hippocampus (D,E). Nuclei are shown in blue (DAPI), and eYFP expression (conjugated to the opsin) is shown in green. (B) CaMKII+ neurons were transfected in the basal nucleus of the amygdala. (C) The opsin was preferentially expressed in the cell bodies of the BLA. (D) Projections from the BLA also expressed the viral vector in the lacunosum-moleculare layer of CA1 in the intermediate hippocampus. (E) Hippocampal expression of the opsin was restricted to axonal projections; no cells were labeled in the hippocampus. Scale bars are 250 μm in B and D, and 30 μm in C and E. BLA: basolateral complex of the amygdala; B: basal nucleus; L: lateral nucleus; lm: lacunosum-moleculare; r: radiatum; p: pyramidale.

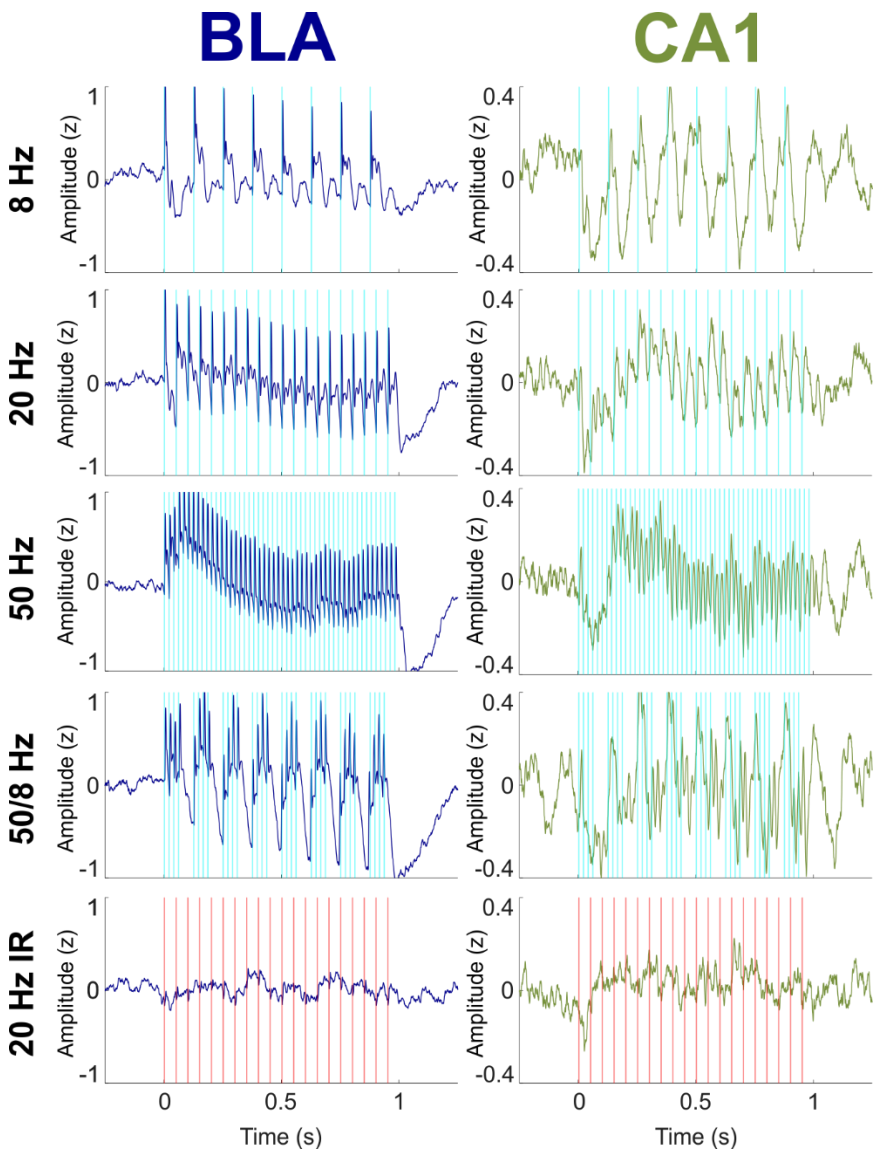

Figure 3.2. Effects on BLA and CA1 LFPs of optogenetic BLA stimulation with blue (experimental) light at 8 Hz, 20 Hz, 50 Hz, and 50/8 Hz and with near infrared (IR; control) light at 20 Hz. LFPs were Z-transformed within conditions for each rat and then averaged across all rats. Left: Evoked responses in the BLA (blue) tracked stimulation at all frequencies. Right: LFPs in the CA1 (green) tracked stimulation at all frequencies with more regular oscillatory activity than what was seen in the BLA.

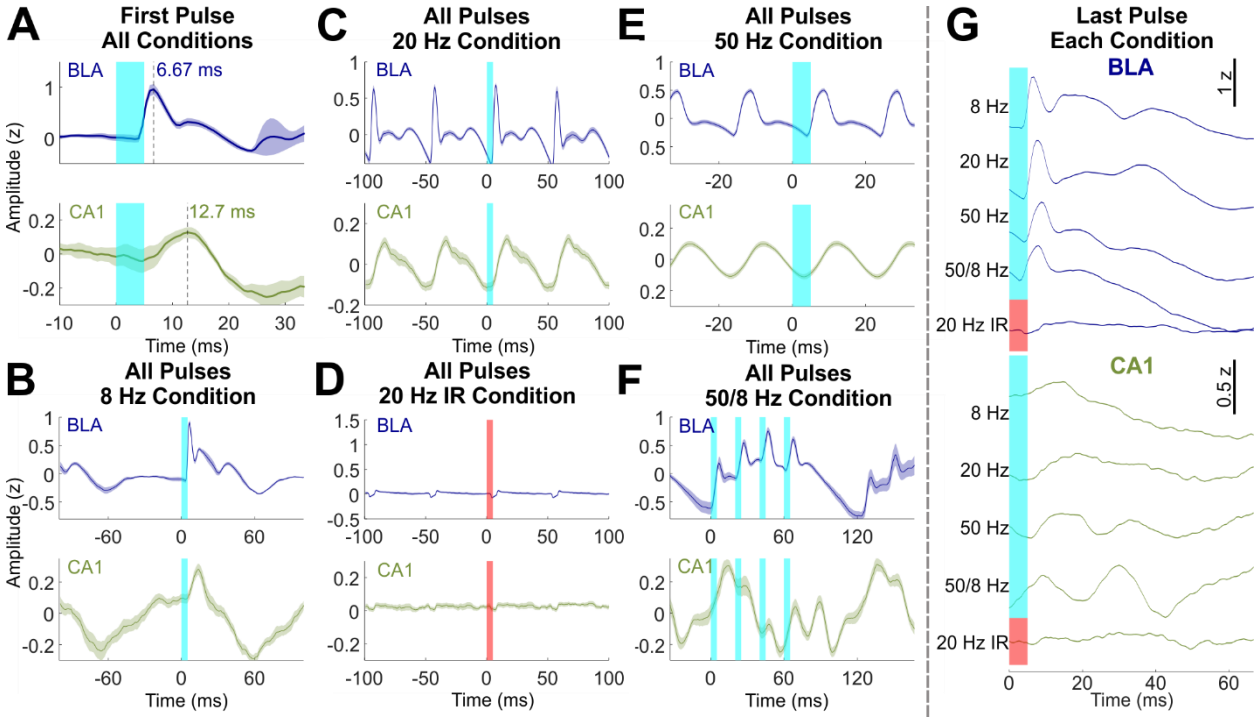

Figure 3.3. Effects of BLA stimulation pulses differed between BLA and CA1. **(A)** Response latencies to the first light pulse of all conditions in the BLA (blue, top) and CA1 (green, bottom). Time to LFP peak response was 6.67 ms in the BLA and 12.7 ms in CA1. **(B-F)** Averaged LFP across all light pulses within the 8 Hz, 20 Hz, 20 Hz near-infrared, 50 Hz, and 50/8 Hz stimulation conditions, respectively (averaged across bursts of 50 Hz pulses for F). Responses in the BLA were characterized by fast activity that was similar across conditions, whereas oscillatory activity in CA1 was dependent on stimulation frequency. Maximal gamma activity preferentially occurred at the peak of theta in the BLA and trough of theta in CA1. **(G)** Response in the BLA and CA1 after the last pulse of each condition. 50/8 Hz stimulation produced persistent gamma in CA1 after termination of stimulation.

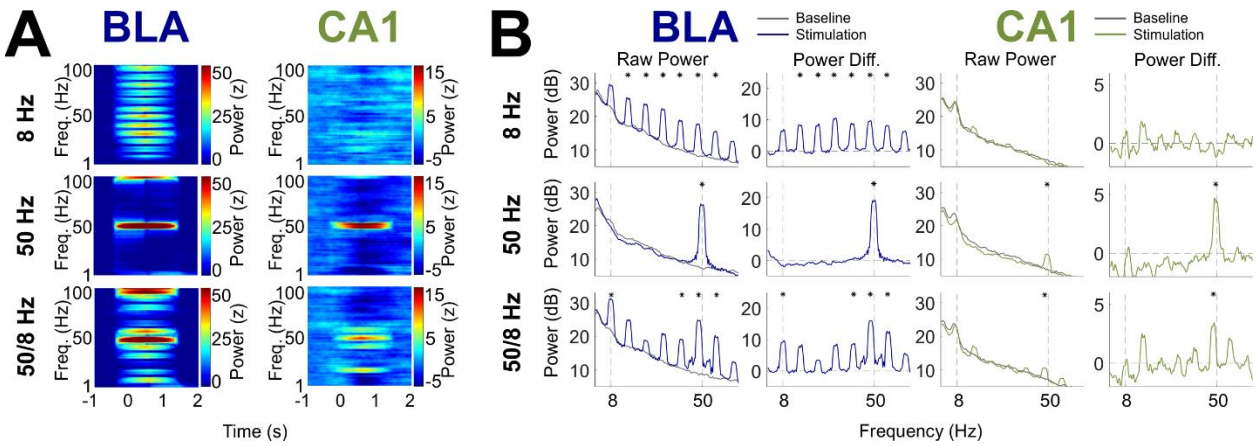

Figure 3.4. BLA and CA1 LFP Power in response to optogenetic BLA stimulation. **(A)** Moving window spectrogram of power around the stimulation event. Power was normalized to the baseline period for clarity. **(B)** Spectrogram of power during stimulation, displayed as absolute decibels (left) and with the baseline period subtracted (right). Asterisks indicate frequency ranges that differed significantly between stimulation and baseline periods.

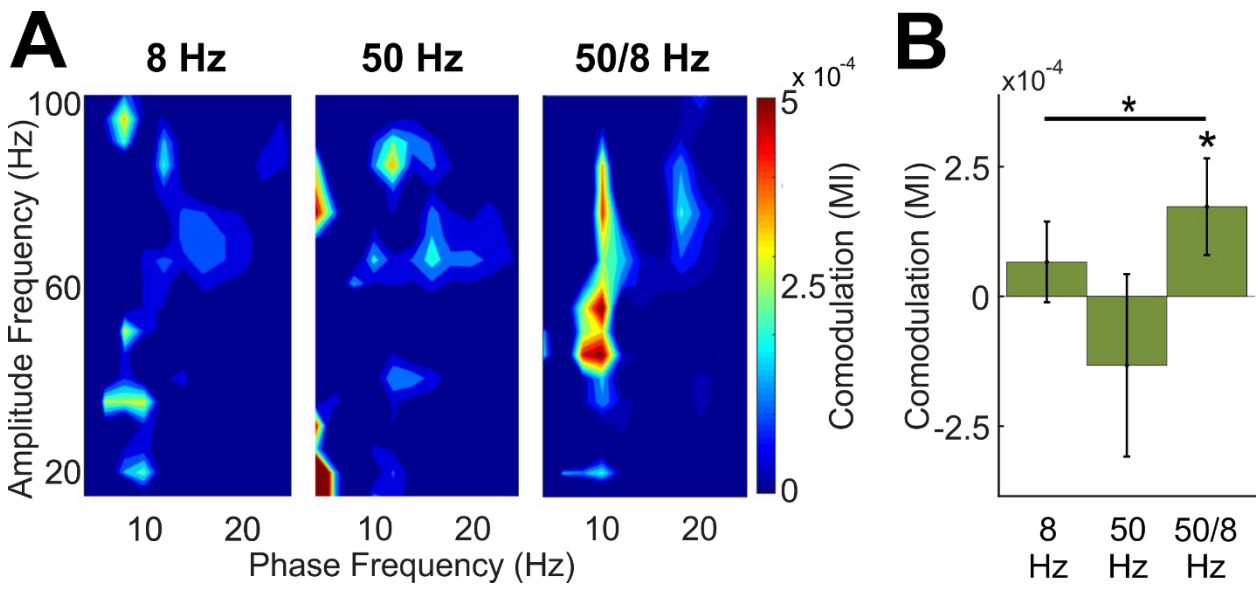

Figure 3.5. Comodulation within CA1 across BLA stimulation conditions. **(A)** Comodulogram during 1-second BLA stimulation with the baseline period subtracted for clarity. **(B)** Total theta-modulated-slow gamma during stimulation relative to baseline for each stimulation conditions. Asterisks indicate that theta-gamma comodulation was significantly increased during 50/8 Hz stimulation and that theta-gamma comodulation was significantly different across conditions.

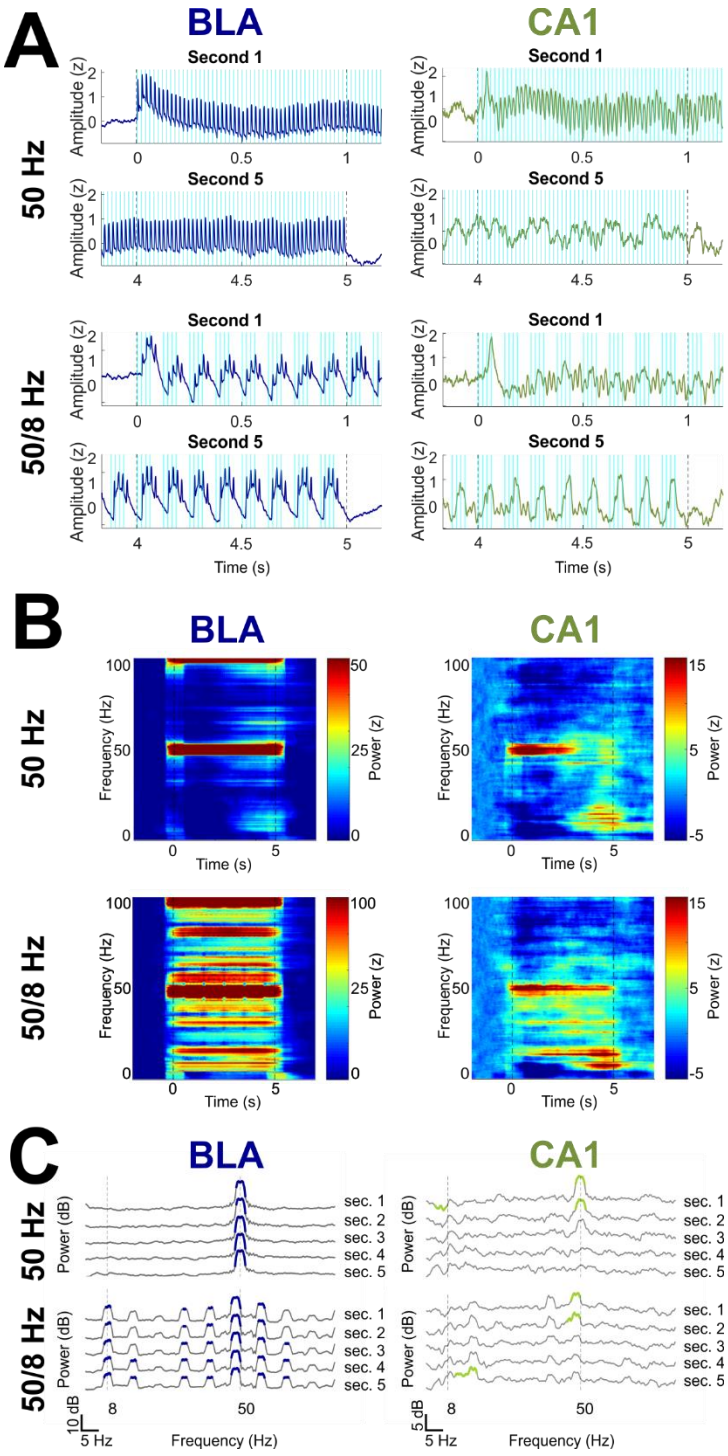

Figure 3.6. Effects of five-second 50 Hz and 50/8 Hz stimulation on the BLA (blue) and CA1 (green). **(A)** Averaged BLA and CA1 LFPs in the first and last seconds of 5-second BLA stimulation. LFPs were Z-transformed and averaged across rats. **(B)** Moving window power

spectrogram during 50 Hz and 50/8 Hz five second stimulation, normalized to the baseline period for clarity. **(C)** Power spectrogram of second 1 (top) to second 5 (bottom), normalized against the baseline period. Frequency ranges that differed significantly between stimulation and baseline are highlighted in blue (BLA) and green (CA1).

Chapter 4: General Discussion

4.1. Summary of Findings

The two aims of this dissertation demonstrated the specificity by which amygdala activity can modulate hippocampus-dependent declarative memory. This specificity was observed both in the information content coded by the amygdala and in the oscillatory dynamics necessary to increase pro-memory activity in the hippocampus. In Chapter 2, the activity of the two regions during a visual recognition memory task in humans was analyzed to determine how mnemonic and social information is naturally represented. That chapter assessed the specificity (or lack thereof) of information content in the two regions. The endogenous activity during a recognition memory task demonstrated distinct coding schemes in the amygdala and hippocampus. Information about the memory status or social content of the stimuli could not be identified by the population activity in either region. However, the firing rates of all recorded units were differently distributed based on the memory status and social salience of the stimuli. The hippocampus showed evidence of sparse coding of remembered stimuli more so than forgotten stimuli. Memory status was not similarly coded in the amygdala. Both the hippocampus and the amygdala showed distinct firing distributions for socially-salient stimuli. The data suggested two likely sources of difference between the firing distributions of stimulus categories in the amygdala. Regardless of the source, the amygdala was shown to encode specific information more granularly than would be the case if it exclusively acted as a generic modulator.

The downstream effects on the hippocampus from exogenous stimulation of the basolateral complex of the amygdala (BLA) in rats were investigated in Chapter 3. Chapter 3 extended the findings of Chapter 2 by determining the specificity of oscillatory communication between the amygdala and hippocampus by directly stimulating the BLA. The primary goal was to determine what oscillatory activity in the BLA can be translated to hippocampal activity that

would benefit memory. Coordinated oscillatory activity was of interest because although Chapter 2 demonstrated that specific amygdala activity likely participates in the modulation of hippocampal activity, nonspecific increases in BLA activity have also been shown to modulate memory. Theta-modulated gamma oscillations in the hippocampus are known to be beneficial to memory (Sederberg, Kahana, Howard, Donner, & Madsen, 2003; Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009; Trimper, Stefanescu, & Manns, 2014), and were thus the principal indicator of a pro-memory state in the hippocampus. Theta-modulated gamma oscillations were not increased during optogenetic BLA stimulation at 8 Hz or at 50 Hz. Therefore, even though both frequency bands naturally occur in the hippocampus (Lisman & Jensen, 2013), the ongoing native oscillations are not sufficient to modulate constant pulsed stimulation into theta-modulated gamma. In contrast, BLA stimulation that replicated theta-modulated gamma activity (i.e. 50/8 Hz) robustly increased theta-modulated gamma activity in the hippocampus.

Taken together, Chapter 2 shows how the amygdala neurons encode information in a specific pattern that is distinct from that of hippocampal neurons during recognition memory. Chapter 3 shows that the information encoded by the firing of those amygdala neurons most effectively modulate hippocampal activity when organized on a theta-modulated gamma oscillation. Both chapters recruited the amygdala under low-arousal settings. The amygdala is naturally recruited by affective and socially-salient stimuli to modulate hippocampal memory, but these chapters showed that memory processes can be effectively modulated when the circuit is recruited regardless of affective content. Temporally-restricted neural activity in the BLA was observed to encode and transmit information to the hippocampus that is positioned to facilitate hippocampal plasticity. The results imply that modulation of neutral memories likely utilizes pathways that are distinct from those activated under high arousal. Unlike cued fear conditioning

or context learning, initial neuronal representations, and therefore synaptic plasticity, both regions likely participate in the consolidation of the final memory trace. Detailed descriptions of these pathways from future experiments will clarify how declarative memories are specifically prioritized, and how that process can be manipulated for experimental and clinical effect.

4.2. Sparse coding underlies representations of mnemonic and social information

Hippocampal processing has long been studied to uncover how information is represented in that circuit, but less attention has been paid to BLA processing. Although the BLA shares many fundamental features with the hippocampus like multimodal input (Sah, Faber, Lopez De Armentia, & Power, 2003; Witter & Amaral, 2004), plasticity mechanisms (Abe, 2001; Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001), and local inhibitory circuits (e.g. Samson, Dumont, & Pare, 2003; Stark et al., 2014), it was unknown whether information was represented in a similar coding scheme. Previous research suggested there would be at least partial overlap in how the regions code information since stimulation of neuronal ensembles that were active during fear conditioning in either region produced similar behaviors (Kitamura et al., 2017; Liu et al., 2012; Ramirez, Tonegawa, & Liu, 2013). However, only the hippocampus had been previously shown to engage in a sparse coding scheme, at the exclusion of the amygdala (Wixted et al., 2018; Wixted et al., 2014). The present results demonstrated that the amygdala represents information with a sparse coding scheme. Sparse coding necessitates a degree of specificity in the representations of stimuli among the entire neuronal population, since that coding scheme means neurons only respond to a small minority of stimuli. The specific stimuli in Chapter 2 were categorized based on the presence of humans or animals, but sparse coding likely underlies other information relevant to the amygdala like facial expression (Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007) and emotional arousal (Phelps & LeDoux, 2005).

The existence of sparse coding in both the amygdala and hippocampus suggest that sparse coding is a general representation strategy for association cortices, just as it is common across primary cortices (Crochet, Poulet, Kremer, & Petersen, 2011; Poo & Isaacson, 2009; Vinje & Gallant, 2000). A common coding scheme would also explain how information about specific stimuli can be prioritized for consolidation in the hippocampus. Labelling social salience in a way that is not replicated by the hippocampus enables the prioritization of recognition memories that is dependent on amygdala activity (Adolphs, Cahill, Schul, & Babinsky, 1997; Adolphs, Tranel, & Denburg, 2000).

Once the information is coded in such a way to be useful to the hippocampus, the hippocampus needs to be in a neural state conducive to consolidation for amygdala modulation to have a lasting impact on memory. Chapter 3 demonstrated how open-loop optogenetic stimulation of the BLA can increase theta-modulated gamma oscillations in the hippocampus which are known to correlate with good memory (Shirvalkar, Rapp, & Shapiro, 2010; Tort et al., 2009; Trimper et al., 2014). Stimulation of the BLA with a theta-modulated gamma train (i.e. 50/8 Hz) in this way is known to prioritize object recognition memory (Bass & Manns, 2015; Bass, Nizam, Partain, Wang, & Manns, 2014; Bass, Partain, & Manns, 2012; Inman et al., 2018). Thus, stimulation of the BLA enables hippocampal memory enhancement without the need for synchronizing the stimulation to specific neural states in the hippocampus (Ezzyat et al., 2017; Hampson et al., 2018). The fact that relatively unsupervised amygdala stimulation succeeds when hippocampus stimulation requires a high degree of supervision shows the functional BLA-hippocampus circuit to encompass both a direct connection and a larger interconnected network. Being so highly interconnected, BLA stimulation affects more than the hippocampus. Previously observed effects of BLA stimulation necessitate the involvement of a wider network (Abe, 2001;

Kleschevnikov et al., 1997) even though direct BLA-hippocampus projections are sufficient to reproduce certain effects in isolation (Felix-Ortiz & Tye, 2014; Huff, Emmons, Narayanan, & LaLumiere, 2016). In fact, isolated activity in the direct pathway would likely impair some aspects of memory performance, because doing so would decouple the hippocampus from entorhinal cortex input, which has been shown to drive encoding (Colgin et al., 2009). Increased theta synchrony, decreased gamma synchrony, and increased gamma power across the brain have been shown to correlate with memory performance (Solomon et al., 2017). Therefore, part of the benefit to memory may be that BLA stimulation increases hippocampal gamma power quite strongly in a way that is not synchronous with other connected brain regions, while simultaneously synchronizing theta by inducing a phase reset (Jutras, Fries, & Buffalo, 2013). In this way, activity in the BLA prioritizes hippocampal memory by recruiting direct and indirect projections to the hippocampus.

4.3. Implications for specific memory modulation

The behavioral effects of BLA stimulation in previous studies demonstrate that its effects are specific enough to prioritize specific memories even if multiple brain regions are recruited. That said, these specific effects were shown to be a product of both specific and nonspecific activity in the amygdala. The positive memory state in the hippocampus induced by endogenous or exogenous BLA activity promotes the consolidation of whatever ensembles are activated by the current stimulus. Given both regions demonstrate sparse coding, ensembles in both the amygdala and the hippocampus are likely integrated into the organizational scheme of theta-modulated gamma oscillations. Once co-activated in that way, the final memory trace would then be consolidated through synaptic plasticity in both the BLA and the hippocampus. The existence of this phenomenon could be tested in future experiments that label restricted ensembles of

neurons in both regions activated by specific stimuli. Such results would test the involvement of BLA as a modulatory region and a locus of plasticity. If the BLA was found to be involved in both processes, its roles in fear conditioning and the prioritization of specific memories would be reconciled.

The segregation positive or negative inputs to principal neurons in the BLA offer a model for how this information may be transmitted between regions. Distinct subpopulations of principal neurons in the BLA have been found to respond to rewarding or aversive stimuli (Kim, Pignatelli, Xu, Itohara, & Tonegawa, 2016). Although the segregation is easiest to observe between rewarding and aversive stimuli, it is likely that other stimulus characteristics are similarly segregated into subpopulations within the amygdala. There may be overlap between social and valence information, but the existence of sparse coding in the amygdala suggests that socially-salient information is also represented by a distinct class of neurons. These neurons, when active concurrently with a pro-memory state in the hippocampus, would then provide the input to the hippocampus necessary to prioritize specific stimuli.

Unlike the nucleus accumbens or central amygdala, the hippocampus receives relatively equal projections from the positive and negative subpopulations of the BLA (Beyeler et al., 2016). Therefore, specific hippocampal memories of stimuli with any valence are primed to be modulated by direct input from the amygdala. Combining these direct and specific projections with the increased pro-memory oscillations puts the network in an optimal state to prioritize memory of any valence that co-occurs with amygdala activity.

4.4. Clinical and behavioral relevance to amygdala-mediated memory

Understanding the relationship between the amygdala and hippocampus, and between emotion and memory more broadly, has concrete clinical relevance. The specific effects of the

amygdala-hippocampus network in Chapters 2 and 3 were observed in non-pathological states, which are likely altered in many psychiatric and neurological conditions. Although emotional arousal (and pharmacological analogues of arousal) improves memory under many circumstances, runaway emotional enhancement of memory can produce a disordered and maladaptive state. Disordered memory, characterized by generalization, rumination, and resistance to extinction, is characteristic of anxiety disorders like specific phobias and Post-Traumatic Stress Disorder (PTSD; American Psychiatric Association, 2013) . Once disordered memories are entrenched, attempted treatments often fail to beneficially modulate the traumatic memory. Since the functional connectivity between the amygdala, hippocampus, and related brain regions has been shown to be dysregulated in patients with PTSD (Chen & Etkin, 2013; Rabinak et al., 2011; Shin, Rauch, & Pitman, 2006), the patterns observed in Chapter 2 and 3 are not likely replicated in pathological cases like these. Instead, the theta-modulated gamma communication between the two regions that likely prioritize consolidation of ensembles activated in both regions could be an adaptive state to target with therapeutic interventions.

Some manipulations of BLA activity previously shown to enhance or impair memory have been translated to models of anxiety disorders and to potential therapies to address the therapeutic gap. Experiments by Nader, Schafe, and Le Doux (2000), which inhibited the expression of a previously consolidated fear memory by inhibiting protein synthesis in the amygdala after reactivation of the memory, were some of the first to propose a clinical application for manipulations of this system. In theory, patients could have memories for traumatic events weakened after reactivation, thus freeing them from continually reliving that experience. A large body of work in humans has tried to similarly disrupt consolidation or reconsolidation of fear memories to prevent runaway enhancement of emotional memories

(Agren, 2014; Soeter & Kindt, 2015). Propranolol, a beta-adrenergic antagonist, is a commonly used tool to inhibit BLA activity incited by emotional arousal, with the goal of preventing maladaptively robust enhancement of traumatic memories (Brunet et al., 2008; Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013). Even though these therapies are likely targeting the necessary pathways, clinical success is mixed (McGhee et al., 2009; Sharp, Thomas, Rosenberg, Rosenberg, & Meyer, 2010; Stein, Kerridge, Dimsdale, & Hoyt, 2007). A more recent and promising intervention is the administration of glucocorticoids (instead of propranolol) soon after a traumatic event or during extinction therapy to reduce PTSD symptomology (Amos, Stein, & Ipser, 2014; de Quervain et al., 2011; Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015). The theory behind this intervention is to enhance the encoding of adaptive memories (i.e. extinction) while inhibiting the retrieval (i.e. rumination) of the original traumatic memory.

Current pharmacological treatments may be fundamentally limited because of the generalized and dispersed nature of systemic injections. The existing clinical interventions are essentially intervening to modulate general emotional reactivity. Still, targeting this modulatory pathway will likely improve the efficacy of therapeutic interventions when refined. Since the amygdala specifically encodes stimuli with sparse coding, modulating its overall activity may be too coarse to reliably manipulate a specific memory encoded by a sparse population of neurons. Still, exogenous manipulation of the circuit could improve clinical interventions because the older a memory is, the more resistant it is to be restructured through reconsolidation (Milekic & Alberini, 2002; Suzuki et al., 2004). Therefore, biasing the hippocampus towards encoding new information through amygdala stimulation may be necessary in the context of destabilizing an entrenched traumatic memory.

Current technology is at a state in which interventions on the BLA-hippocampus circuit like that in Chapter 3 may soon be feasible in the clinic. Even the supposed necessity of invasive procedures for intracranial stimulation may not be necessary to specifically target the amygdala and prioritize specific memories. Noninvasive ultrasound and electrical stimulation have been developed in animal models to modulate subcortical structures (Folloni et al., 2019; Grossman et al., 2017; Yuan, Yan, Ma, & Li, 2016). If the technology can be translated to human patients, the oscillatory activity in the amygdala could similarly drive the encoding of new and specific information for those working to weaken a traumatic memory or strengthen a necessary one. A foundational knowledge of how the BLA and hippocampus interact to dictate which memories are remembered and which are forgotten is the first step towards more precise control of that system. Doing so would preferentially consolidate the sparse populations of neurons active in both regions through an artificially increased theta-modulated gamma oscillation. If successful, that would have the behavioral effect of prioritizing a single specific memory for clinical use.

4.5. Future directions for the laboratory and the clinic

The aims of this dissertation have given new insights into how the BLA-hippocampus circuit processes information for declarative memory functions, but critical information needs to be added to the model. Both aims suggest that both direct and indirect pathways from the BLA to the hippocampus are recruited, but the necessity of other brain regions is unknown. As an example, understanding the involvement of the entorhinal cortex in this process is critical not only for the basic understanding of the circuit, but translatability to the clinic. With the entorhinal cortex being one of the first brain regions to be affected by the development of Alzheimer's Disease (Van Hoesen, Hyman, & Damasio, 1991), attempts to prioritize memories

in these patients with manipulations of BLA activity would be ineffectual if the entorhinal cortex is a necessary component.

The finding that the amygdala sparsely codes certain stimulus characteristics provides new opportunities for describing the functions of the BLA in declarative memory. Only socially salient information was found to be sparsely coded in the amygdala in Chapter 2, which fits with previous research showing social information to stimulate general amygdala activity (Adolphs, 2001; Minxha et al., 2017). Attempts to use amygdala activity to modulate hippocampal memory may therefore depend on what stimulus characteristics are represented, with socially-salient stimuli being differentially modulated than overtly emotional stimuli. Amygdala activity biases the hippocampus towards encoding and away from retrieval (de Quervain, Roozendaal, & McGaugh, 1998; Roozendaal, McEwen, & Chattarji, 2009), so the use of social stimuli may enable stronger and more specific encoding. As the results of the two previous chapters demonstrate, the amygdala's influence is likely derived from both the action potentials of specific amygdala ensembles and the coordinated oscillatory activity that primes the hippocampus to consolidate the hippocampal networks that coactivate with the BLA. Ideally, this ability to influence consolidation would hold true whether the modulation is targeting the formation of a new memory or deliberate extinction learning of an older memory.

Accurate and adaptive labelling of facts and events as important and worth remembering, or unimportant and able to be forgotten, is key to successfully navigating the overwhelming quantity of inputs presented by daily experience. Amygdala activity is one way in which memories are labelled and prioritized within the hippocampus. By acting as a modulatory region, the amygdala can bias a memory towards consolidation without changing the content of the memory itself. To do so, it encodes information with strategies that are common throughout the

brain. Sparse coding and the coordination of theta and gamma oscillations within the BLA produce the downstream effects necessary to modulate hippocampal activity, and the resulting hippocampal memory.

4.6. References

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