5-1-2019

Changes in Hippocampal-Anterior Cingulate Cortex Interactions During Remote Memory Recall

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CHANGES IN HIPPOCAMPAL-ANTERIOR CINGULATE CORTEX INTERACTIONS DURING REMOTE MEMORY RECALL

By

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Bachelor of Arts – Psychology
California State University, Northridge
2015

A thesis submitted in partial fulfillment
of the requirement for the

Master of Arts – Psychology

Department of Psychology
College of Liberal Arts
The Graduate College

University of Nevada, Las Vegas
December 2018

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

November 29, 2018

This thesis prepared by

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entitled

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is approved in partial fulfillment of the requirements for the degree of

Master of Arts
Psychology

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ABSTRACT

Changes in Hippocampal-Anterior Cingulate Cortex Interactions

During Remote Memory Recall

by

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Spatial memory is an important cognitive process that relies on extensive neural networks throughout the brain. The hippocampus (HC) is important for the formation of these memories but over time, in a process referred to as consolidation, recall becomes increasingly reliant on other brain areas. The anterior cingulate cortex (ACC), a region within the medial prefrontal cortex, is important for spatial learning, spatial working memory, and remote memory recall, but the mechanisms underlying recall processes are still unknown. To better understand the role of the ACC and HC during memory recall, we introduced rodents into a series of spatially and texturally unique environments at differing delay periods (day 1 (learning), day 11 (recent), and day 18 (remote)) while simultaneously recording local field potentials (LFPs) from both areas. We found significant increases in theta band coherence between ipsilateral ACC and HC LFPs during remote memory recall but not recent memory recall. In addition to these changes, directional analysis revealed a reversal in signal initiation, such that during the learning and recent recall condition, hippocampal theta oscillations led ACC theta oscillations. However, during the remote recall condition, the direction changed, and ACC theta led hippocampal theta activity. This experiment

provides evidence of time-dependent changes in ACC – hippocampal network interactions, and illustrates a possible mechanism that describes how the ACC mediates recall of remote spatial memories.

Keywords: Hippocampus; Anterior cingulate; Theta; Spatial memory; Remote memory

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Spatial memory is defined as the ability to store and retrieve information about our environment. For humans and other animals, quickly encoding contextually relevant information such as reliable food sources, shelter, or the locations of potential harm is critical to their survival; moreover, recalling that information at a later date is equally important, but there is still much more to be learned about the mechanisms associated with these processes. Decades of research has suggested that the hippocampus (HC) is fundamental for forming new memories in humans (Opitz, 2014), non-human primates (Templer & Hampton, 2013), and rodents (Moser & Moser, 1999), but over time, as memories become more remote, the continued role of the HC in memory processes remains unknown (Nadel & Moscovitch, 1997; Frankland & Bontempi, 2005). The anterior cingulate cortex (ACC), an area within the medial prefrontal cortex is important for a number of complex cognitive processes including: predicting outcomes (Hyman et al., 2017), evaluation of context (Hyman et al., 2012); rule learning (Fincham & Anderson, 2006), and an array of spatial working memory tasks (Ragozzino et al., 1998). Additionally, rodent studies have identified the ACC as an important brain for recall of older, or more remote, memories (Bontempi et al., 1999; Teixeira et al., 2006; Ding et al., 2008). Interestingly, the ACC and HC are linked via coherent theta oscillations during spatial learning (Benchenane et al., 2010) and tasks involving spatial working memory (Siapas et al., 2005; Hyman et al., 2010). Thus, it has become increasingly apparent that both areas are linked and further investigation into this network may help to illuminate the process of remote memory recall.

A classic example that illustrates the importance of the HC for learning new spatial information comes from Morris and colleagues (1982), where researchers lesioned the HC in their

rodent subjects and measured time spent looking for an escape platform in a water maze. Initial exposures showed no difference in latencies between the HC lesion group and controls, but on subsequent trials, the control group repeatedly located the escape platform significantly faster than the experimental group. The experimental group did eventually learn the location of the escape platform, but clearly hippocampal ablations impaired spatial learning. More recently, a modified version of this experiment was used to test the continued involvement of the HC in spatial memory recall. Using the sodium ion channel blocker lidocaine, it was revealed that the temporally inactivation of the HC impairs performance on the Morris water maze even after a thirty-day delay period after acquisition (Broadbent et al., 2006). Considering hippocampal inactivation following a delay period does not impair remote recall performance on other spatial memory tasks, such as the five-arm maze (Maviel et al., 2004), more research is needed to identify the role of the HC in this process. The rodent HC is extremely important for spatial learning, but whether or not it has a continued role in memory processes is still unknown.

While it has been firmly established that the HC is important for learning new information, whether the HC is needed for retrieval of older, or more remotely formed, information is still a topic of considerable debate (Eichenbaum, 2004). When we are awake and learning about the surrounding world, the HC is receiving input from sensory areas through the entorhinal cortex (Yoder et al., 2015), and then during sleep, the HC sends signals to other brain areas which coordinate memory formation (Buzsáki, 2015). This process of reorganization, or consolidation, may allow some memories to become dependent on other areas throughout the brain. These areas include cortical association areas like the parietal cortex (Berryhill et al., 2007), perirhinal cortex (Wiig & Bilkey, 1994), auditory cortex (Rothschild et al., 2016), and visual association areas (Mishkin, 1982), along with non-cortical structures including the cerebellum (Takehara et al.,

2003), locus coeruleus (Takeuchi et al., 2016), and ventral tegmental area (Tomapry et al., 2015.

There are two leading theories to explain this standard consolidation theory (SCT) and multiple trace theory (MTT) states that over time integrated pathways between the HC and cortical association areas weaken, and cortico-cortical pathways strengthen, eventually leading to hippocampally independent memories (Squire & Alvarez, 1995; McClelland et al, 1995). But, as described above, some types of information, like the location of an escape platform in a Morris water maze, seem to always rely on an intact HC. MTT attempted to explain this discrepancy, in short, memory traces occur in both the HC and cortical circuits and both continue to be important for memory recall (Nadel & Moscovitch, 1997). and while some types of memories can still be recalled without the HC. For example, an animal may exhibit some memory recall after complete hippocampal ablation, but the memory would be incomplete and lack substance regarding contextual aspects of that memory (Moscovitch et al, 2005) indication that some trace remains in the HC.

Outside of the HC, other areas are important for memory recall. Recently, the ACC has gained attention for its role in memory recall. In human imaging studies prefrontal areas have been shown to become more activated during effortful recall of previously learned words (Schater et al., 1998). In rodents, postmortem imaging has revealed that the ACC was more activated after recalling remotely learned contextual information (Bontempi et al., 1999), and pharmacological inactivation impaired remote recall of fear memories (Frankland et al., 2004) and spatial navigation (Teixeira et al., 2006). Taken together, these experiments indicate that the ACC is important for recall of remotely formed memories, and may serve as a link between the limbic system and cortical memory networks. However, memory impairments in human cingulotomy patients are

rare and short-lived (Cohen et al., 1999; Sharim & Pouratian, 2016), suggesting that more research is needed to confirm the ACCs involvement in memory recall.

The process of bringing contextual information into conscious thought requires multiple brain areas to work in unison. Gray (1994) theorized that physically separate brain areas can communicate via synchronous neural oscillations, or local field potentials (LFPs). LFPs are generated as the result of the summed electrical signals in a brain area, and analysis of different frequency patterns can offer unique windows into brain function. Recordings of LFPs in the rodents have revealed theta rhythm (5-12 Hz) to occur in multiple brain areas, including the amygdala (Pare & Gaudrequ, 1996), mPFC (Jones & Wilson, 2005), and HC (Buzsáki, 1986) during a variety of exploratory behaviors such as running and sniffing (Vanderwolf, 1969; Vanderwolf, et al., 1973). Moreover, theta represents the online state of hippocampal function (Buzsáki, 2002) and acts as an important mechanism for learning (Hyman et al., 2003), and disruption of hippocampal theta activity via inactivation of the medial septum significantly impairs spatial learning (Andersen et al., 1979). Theta rhythm is not specific to rodents, in humans, cortical and hippocampal theta (4-7 Hz) are associated with improved spatial navigation (Kahana et al., 1999; Zhang et al., 2015) and working memory (Gevins et al., 1997).

In recent years, data from ACC - HC research has led to the hypothesis that the HC shares information with other brain areas via theta band oscillations (Siapas et al., 2005, Jones and Wilson 2005, Hyman et al., 2005 Buzsáki & Moser, 2013; Colgin, 2015; Backus et al., 2016). Indeed, hippocampal theta entrains the amygdala when retrieving fear memories (Seidenbecher, 2003) and the ACC during decision making (Jones & Wilson, 2005a) and spatial working memory tasks (Siapas et al, 2005; Jones & Wilson, 2005; Hyman et al., 2010). In some cases, ACC theta can modulate other brain areas including the VTA during effortful tasks completion (Elston & Bilkey,

2017) and the HC during contextually guided object sampling (Place et al., 2016). Theta interactions between the HC and ACC are highly correlated with rodent behavior, and mounting evidence suggests these interactions might allow the two areas to share contextual representations (Wirt & Hyman, 2017).

In this study, we aim to better understand the role of ACC – hippocampal interactions. Previous research on this network has suggested that these areas are important for processing spatial information; because of this, we have chosen to focus on how these two areas interact during acquisition and recall after different delay periods of spatial information. To test this, we allowed animals to freely explore a series of spatially and texturally unique environments while we recorded LFPs from dorsal CA1 in the HC and the ACC. Given the hypothesis that theta oscillations are a means for the HC to communicate with other brain areas, and the ACC and HC have been previously shown to interact while learning new spatial information (Benchenane et al., 2010) we predicted that interactions between these areas will occur during the learning phase of this experiment. Moreover, if the HC has a continued role in memory recall, those interactions should continue during the recent and remote recall conditions. Lastly, if the ACC is responsible for storing or mediating memory recall theta oscillations are a means for brain areas to communicate, then ACC theta should lead hippocampal theta during remote recall conditions.

CHAPTER 2

REVIEW OF RELATED LITERATURE

The Hippocampus and Memory

Within the limbic system, the HC, located in the medial temporal lobes of the brain, is highly attuned to processing spatial information, temporal sequencing of events, and forming new memories. Decades of research has improved our understating of this brain area, few reports have been as influential as the seminal case studies of Henry Molaison (Scoville & Milner, 1957; Milner et al., 1968; Sagar et al., 1988; Smith et al., 1988; Squire, 2009). In the years since the initial report on H.M (Scoville & Milner, 1957) numerous investigations of human and non-human animal models have significantly added to our understanding of the HC, specifically its role in learning and memory. This section will review this evidence for the role of the HC in memory formation and identify some of the limitations of the current literature.

Henry Molaison (1926 – 2008), more commonly referred to as patient H.M. is perhaps the most famous case study in of all memory research. H.M. suffered from severe epilepsy that resulted from a bicycle accident in his youth. At the age of 27, he was referred to neurosurgeon William Scoville who was able to identify and remove the location of H.M.'s seizure generation. After the bilateral removal of the medial temporal lobes, H.M. the symptomology of his epilepsy improved, but unfortunately, he suffered from partial retrograde and severe anterograde amnesia, meaning he was unable to form new autobiographical memories for the rest of his life. The symptoms caused by the removal of these brain areas suggest that the HC is important for the formation of new memories. Additionally, the partial retrograde amnesia that H.M. displayed suggest that these brain areas only temporally hold information. It is true that H.M.s surgery removed much more than the bilateral hippocampal formation, and that more tissue was removed than originally thought.

However, other case studies including that of patient E.P, who suffered similar memory impairments resulting from encephalitis, have been well documented (Baxendale, 1998; Insausti et al, 2013). In addition to human case studies much of our knowledge relating to hippocampal function has come from research using animal models.

Animal models have been an invaluable tool in understanding how the brain works. Despite the fact that there are a number of neuroanatomical differences between the primate and rodent hippocampal formation, evidence from human case studies, non-human primates, and rodents suggest that memory systems between mammals are comparable (Clark & Squire, 2013; Strange et al., 2014). Using an animal model allows research to utilize experimental manipulations that would be impractical to implement on human participants. For example: direct infusions of lidocaine to the rodent HC revealed its importance for remembering the locations of a foot shock in a spatial working memory task (Farr et al., 2000); ablation to the rodent HC impairs social memory (Stevenson & Caldwell, 2014) and spatial navigation (Kosaki et al., 2014); and depth electrodes in the monkey HC revealed changes to hippocampal activity after learning new associations (Wirth et al., 2000). These studies and many others provide clear evidence for the role of the HC in learning and memory, and with the advent of new technologies our understanding of memory systems is continually improving. The use of post-mortem imaging revealed that the HC becomes highly active when learning reward locations on a radial arm maze which is indicative of this area's role in spatial discrimination. However, this experimental technique also showed that the increases were only temporary, suggesting that hippocampal involvement is limited to recently learned information (Bontempi et al., 1999). Additionally, the use of transgenic mice in neuroscience has given researcher the opportunity to examine and manipulate memory systems at the synaptic level. This has allowed researchers to identify specific brain areas of memory

formation and to effectively erase those memories (Hitt & Siegelbaum, 2014). Taken together these findings show that the HC is important for learning new information, and, at least for a short time, storing those memories.

Neural representations of memory might exist as sparse neural traces referred to as engrams which are groups of neurons that exhibit synchronized firing patterns during memory retrieval (Martin & Morris, 2002). Numerous theoretical models have pointed to the existence of such engrams (Govindarajan et al., 2006; Gelbard-Sagiv et al., 2008; Fritz et al., 2005; Hübener & Bonhoeffer, 2010; Josselyn, 2010), but conclusive evidence did not exist until researchers injected an adeno-associated virus containing channelrhodopsin (ChR2) into CA1 of the rodent HC to optogenetically stimulate memory traces and initiate memory recall. To do this, a virus along with transgenic mice were used so researchers could tag hippocampal neurons during very specific time windows while animals were contextually fear conditioned. Then at a later time in a new environment the tagged cells were optically stimulated, and the subject exhibited the same freezing behavior as observed in the fear conditioning environment (Liu et al., 2012). Using this genetic manipulation, researchers were able to describe the mechanism for how memories in the HC were recalled. The reactivation of neurons that were active in an environment during a fear conditioning task caused the subjects to recall a false memory. A follow up study took this idea further by manipulating this process and induce false memory recall. Ramirez and colleagues (2013) used the same genetic manipulations as described above to tag and control neural representations of a fear memory. Here, subjects were placed in a contextual fear conditioning chamber and the previously tagged neurons were activated via optogenetic stimulation. Then in a neutral environment, those neurons were re-stimulated, the next day subjects were reintroduced a day later into a neutral environment and they exhibited fear responses. The identification of memory traces

within the HC established this area's role in storing and retrieving new memories (Tonegawa et al., 2015) but less in known about the involvement of the HC in remote memory recall.

The HC has previously been shown to have a role in new memory formation and the above studies have revealed that, at least for a short period, some memories are dependent on this area. To test if memory dependency in the HC is temporary Takehara et al. (2003) lesioned the HC, they hypothesized that if memory transfer occurs, then hippocampal ablation would not affect recall of more remote memories. Using the trace eye blink conditioning protocol, researchers found hippocampal lesion to affect recent recall (1 day) but not remote recall (greater than 14 days), an indication that some memories do in fact become hippocampally independent after consolidation. However, this is not always the case, using different experimental techniques, some researchers have found conflicting results. Hattori et al. (2015) conditioned subjects on a similar same trace eyeblink conditioning paradigm and found that electrophysiological activity from the hippocampus continued to show learned responses, even after a 30-day delay period. While it is true that previous reports found successful recall of these same memories following a complete hippocampectomy (Kim et al., 1995; Takehara et al., 2003), Hattori's work provided a more robust representation by showing hippocampal neurons continuing to process information about conditioned behavior even after a 30-day delay.

The experiments above illustrate the importance of the HC for learning new information. Moreover, the discovery and manipulation of hippocampal engrams suggests that this area is important for storing newly formed contextual memories. Furthermore, it does seem to be true that the intact HC has continued involvement in memory recall, but evidence is less definitive. Henry Molaison's partial retrograde amnesia implied that over time, it is possible to efficiently recall memories even after complete removal of the medial temporal lobes, including the HC, which

clearly indicates that the HC has limited involvement in memory dependency. To this end, other brain areas, notably cortical structures become increasingly important for recall of previously learned information.

Information Sharing

Many cognitive processes require multiple brain areas to work together. For example: human EEG studies have reported elevated synchronization between brain areas during auditory perception (Steinmann et al., 2014), visual perception (Kottlow et al., 2012), problem solving (Cao et al., 2015) , executive function (Mizuhara & Yamaguchi, 2007), spatial navigation (Lega et al., 2012), and memory recall (Wang et al., 2018). This also occurs in rodents, during recall of fear memories, the HC and amygdala are highly synchronized via theta band oscillations (Seidenbecher et al., 2003), and the HC and cerebellum has been shown to exhibit elevated theta coherence during conditioned reflexive responses (Hoffmann & Berry, 2009). These data indicate that some the certain brain areas seem become functionally linked during task execution, including during different types of memory recall, the HC is connected with specific brain areas related to the sensory aspects of those memories (i.e. when recalling visual memories visual association memories are synchronized with the HC.

The HC has functional connections with a vast number of brain areas but anatomical studies suggest there is a special relationship with the ACC. There are direct projects from the HC to prefrontal areas (Swanson, 1981), and reciprocal connections between the ACC and the HC through the nucleus reuniens, medial dorsal thalamic nucleus, and entorhinal cortex (Vertes, 2006; Vertes et al., 2007) and direct connections from the ACC to dorsal CA1 in the HC (Rajasethupathy et al., 2015) which allow these two areas to communicate via multiple pathways. And while it is true that we are still unsure of how this occurs, a growing body of evidence suggests that

information is shared between brain areas via synchronous neural oscillations (Gray, 1994; Buzsáki, 1996; Fries, 2005; Buzsaki, 2006), during behaviors that require animals to process spatial information, the HC and prefrontal areas exhibit synchronized neuronal activity (Ekstrom et al., 2005; Hyman et al., 2005; Buzsáki, 2005). While the functional relevance of this interplay is still unclear, numerous studies have attempted to understand how synchronous interactions between these areas affect learning. When investigating oscillatory activity there are two primary measures of interest: coherence and frequency phase. Coherence is a measure of power spectra; it analyzes the rate at which two separate waveforms change frequency and amplitude in coordination with each other. High coherence between brain areas is indicative of communication (Fries, 2005) and states of high theta coherence between the HC and ACC are predictive of successful place learning (Kim et al., 2012). Separately, frequency phase can be used to assess the direction of information flow between brain areas by analyzing the synchronization between the two independent signals (Adhikari et al., 2010; Lachaux et al., 1999). Analysis of synchronicity and coherence has allowed researchers to infer a mechanism for neural communication.

Coherent and synchronous interactions between the HC and ACC are important for a number of high level processing and when the connections between the HC and ACC are severed, there are severe deficits in spatial working memory (Wang & Cai, 2006) and spatial learning (Wang & Cai, 2008). Coordination of theta range activity is associated with working memory (Hyman et al., 2010; Jones & Wilson, 2005a; Jones & Wilson, 2005b), memory retrieval (Kaplan et al., 2014), spatial decision making (Belchior et al., 2014), learning (Benchenane et al., 2010), and context exploration (Hyman et al., 2005) indicating that interactions between the HC and ACC have an important role in learning. While it is true that the research on theta band communication between these areas is extensive, more research is needed to fully understand why this relationship

is so important, how communication is generated, and the extent of the information that is being shared.

The Anterior Cingulate Cortex and Remote Memory

The ACC, a region within the medial prefrontal cortex, is important for a number of high-level cognitive processes including: movement planning and initiation (Devinsky et al., 1995); attention to self-generated action (Barbas & Mesulam, 1981); choice evaluation (Kernnerley & Wallis, 2009); monitoring conflict (Botvinick et al., 2001), pain, negative affect, depression (Onda et al., 2009), and reward history (Holroyd & coles, 2008); cognitive and attentional control (Paus, 2001); error detection (Holroyd & Coles, 2002); integration of autonomic nervous system into cognition (Critchley et al., 2003); remote memory (Frankland & Silva, 2004); and possibly remote memory recall (Bontempi et al., 1999; Frankland & Silva, 2004). However, the vast array of functions that the ACC is involved in has made pinpointing its role in long-term memory difficult.

Memory is the capacity to retain and access information and ACC ablation disrupts this process. For example, inactivating the ACC disrupts the ability to retrieve information from the short-term memory store (Seamans et al., 1995), which suggested that this area is necessary for either retaining or recalling that type of information. The ACC is also important for some types of remote memory recall. The role of the ACC in this type of memory recall was first identified by Bontempi and colleagues (1999). Using post-mortem imaging, this group revealed that the ACC had become extremely active when animals were recalling remotely formed contextual information. Such increases were not observed during learning and recent recall conditions suggesting that this area either stores or helps mediate the recall of remote spatial memories. Similar findings have been reported in a number of other behavioral paradigms including taste aversion (Ding et al., 2008), spatial navigation (Teixeira et al., 2006), and conditioned fear

responses (Frankland et al., 2004). The collection of studies above were instrumental in identifying that the ACC is involved in remote recall but because the deficits in memory recall resulted from ablation, making it difficult to draw conclusions on what role the ACC has in memory processes. However, electrophysiological methods allow researchers to monitor ACC activity in awake and behaving animals. For example, ACC neuronal recordings have shown that ACC cells exhibit unique firing patterns between contexts (Hyman et al., 2012; Ma et al., 2016), and different aspects of working memory tasks (Fuster, 1973; Jung et al., 1998; Fuster, 2001; Goldman-Rakic, 1996; Bissonette et al., 2013; Rushworth et al., 2003). However, the role of the ACC in recalling information after a long delay period, referred to here as remote recall was unclear until Takehara & McNaughton (2008) showed that neural networks in the ACC are reorganized following consolidation of fear memories putting forward a putative mechanism for the role of the ACC in remote memory recall. The experiments above offer evidence for how the ACC is involved in memory recall, but additional research is needed to further explain how the ACC is involved in this process.

In recent years, the use of novel experimental techniques has helped provide additional support for the how the ACC is involved in memory processes. Postmortem imaging has revealed that the ACC exhibits an increase in activity around the time of remote memory recall (Bontempi et al., 1999) but fails to identify any mechanisms associated with memory recall. Optogenetic stimulation has proven to be an exciting technique for understanding how the brain functions but the procedures require genetically invasive manipulations. While each technique offers a compelling view of neural function, additional evidence will help explain how the ACC and other brain areas work together to produce memory recall.

Conclusion

This review examined how the HC and ACC are involved in encoding and recalling spatial information. Indeed, the HC is important for learning and retrieving recently acquired spatial information, but its continued involvement in long-term memory is less understood. Other studies have identified that the ACC is important in the recall of remote memories, but the mechanisms involved are still unclear. Multisite electrophysiological studies have reported robust unidirectional interactions between the HC and ACC during multiple forms of spatial working memory and spatial learning and it has been inferred that these interactions are indicative of the HC sharing information with the ACC. If this is the case, when memories become dependent on the ACC, we should observe a reverse in the direction of information flow such that the ACC leads the HC during recall. To build upon current knowledge of how information is shared between brain areas and to understand how the ACC is involved in memory, we will examine how hippocampal-ACC interactions change throughout learning, recent memory recall, and remote memory recall.

HYPOTHESES & IMPLICATIONS

Hypothesis 1

We hypothesized that the interactions between the ACC and HC will increase as a result of recalling remote memories. To test this, we measured changes in theta coherence between the HC an ACC while rats completed a spatial memory recall task. We expected to find significant increases in theta coherence between the HC and ACC during the remote recall conditions but not during the learning or recent recall conditions. Theta coherence is indicative of how effective two brain areas are linked and the increases suggest that as time passes, and memories become more remote, there may be a need for more robust interactions between the HC and ACC.

Hypothesis 2

We also hypothesized that the ACC mediates remote memory recall. To test this, we performed directional analysis of LFPs generated in the ACC and HC using the lead-lag correlation method. During learning and recent retrieval of spatial information we expected that hippocampal theta would lead ACC activity as has been previously shown. However, during the remote retrieval conditions, when contextual memories are dependent on the ACC, we predicted a shift in directionality, such that the ACC would lead HC theta activity. This finding provides functional evidence in support of the ACC as an important brain area in remote memory recall.

CHAPTER 3

MATERIALS & METHODS

Subjects

A total of six male Long-Evans rats (450-600 grams) obtained from Charles River Laboratories, Inc. (Wilmington, MA) were used in this experiment. Subjects were individually housed on a twelve-hour light-dark cycle with food and water available ad libitum. Rats were handled by experimenters for a minimum of two weeks before and experimental procedures took place, and surgical procedures were not performed until subjects reached a minimum of 450 grams. All experimental procedures were approved by the University of Nevada Las Vegas Institutional Animal Care and Use Committee.

Surgery

Subjects were deeply anesthetized using isoflurane gas (1 – 3%) before being placed into a stereotaxic frame. The scalp fur was shaved, and the area washed with betadine solution before an incision was made. The incision was ~thirteen mm in length which revealed an area of the skull extending five mm anterior to bregma, 4 mm beyond lambda and laterally into the cranial ridge on both sides of the skull. When the skull was fully exposed we drilled screw holes with a number 14 burr in the following locations: +4.5 mm AP and 1.5 mm ML; +1 mm AP and 3.5 mm ML; -4.0 mm AP and 4.5 mm ML; -6.5 mm AP and 1.0 mm AP. When the holes were drilled small screws were placed in the respective areas to help affix the recording device to the skull (see Figure 1). Two posterior screws placed just above the cerebellum were connected to a grounding wire and soldered into the electric interface board (EIB) (Plexon, Dallas) as is typically done in rodent in vivo recordings (Buzsáki, 1986) (see Figure 1). Next, we performed three

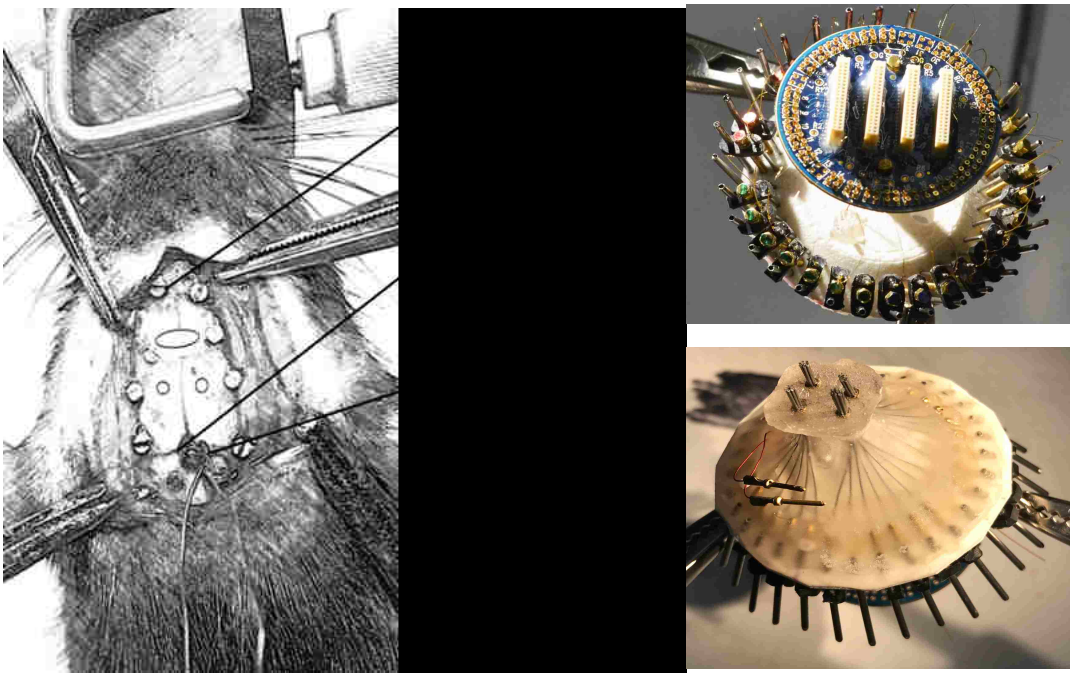


Figure 1. Sketch of surgical procedures and hyperdrive recording device. (A) The location of skull screws, ground screws, and craniotomies. Note the ground screws are placed ~ 1 and ~ 2 mm to the right of Lambda targeting the cavity between the cerebrum and cerebellum. (B) Top, view EIB and drivers. EIB is used to transfer signal to computer work station. Drivers are used to lower wires into the brain. Bottom, view of the bottom and canula bundles. Canula bundles are used to house tetrodes.

separate craniotomies, one over the frontal cortex and two over dorsal CA1 in the HC (see Figure 1). After the meninges were removed the bottom of four bundles, comprised of thirty-gauge cannula, that housed the tetrodes were placed over the desired brain area. For the ACC, two bundles containing 8 tetrodes each (bottom radius ~ 1.0 mm) were angled at ten degrees and placed touching the cortical surface + 3.0 mm AP and $\pm$ 0.5 mm ML from bregma. For the HC, two bundles containing 8 tetrodes each (bottom radius ~ 1.0 mm) were place on top of the cortical surface – 3.5 mm AP and $\pm$ 2.5 ML from bregma. When the hyperdrive bundles were positioned over the targeted brain areas. we affixed it to the skull using dental acrylic. When the dental acrylic had fully hardened, the tetrodes were lowered approximately 400 μm into the cortex. Chromic gut absorbable sutures were applied if necessary to close incision around dental acrylic and the rats were given a minimum of seven days post-surgery to recover before experimental procedures took place. During the seven-day recovery period, subjects received sub-cutaneous injections of Baytril to decrease the risk of post-surgical infection, and Anafen to reduce inflammation resulting from surgical procedures. After this time, a tether was used to connect dual headstage adapters and the EIB to the Intan acquisition board. We then slowly lowered tetrodes ventrally into the ACC (DV ~ 1.5 mm), and dorsal CA1 (DV ~ 2.0 mm) before behavioral testing began.

LFP Recordings

LFPs were obtained using a 128 channel hyperdrive recording device (see figure 1B) and an RDH evaluation board (Intan Technologies, Los Angeles, CA). The RDH board plugs into the EIB and converts analog signals into digital data then transfers that data to the RDH 2000 USB interface board (Intan Technologies, Los Angeles, CA) which feeds the digital signal into a computer workstation. Continuous data was acquired at a sampling rate of 30 KHz, during acquisition, data were bandpass filtered between 1-6000 Hz using Open Ephys open source data

acquisition software (Cambridge, MA). To identify wire placement within the HC we used stereotaxic coordinates (Paxinos & Watson 6th ed.) and tracked wire depth. For both the ACC and HC, we lowered wires into areas with the most robust activity and

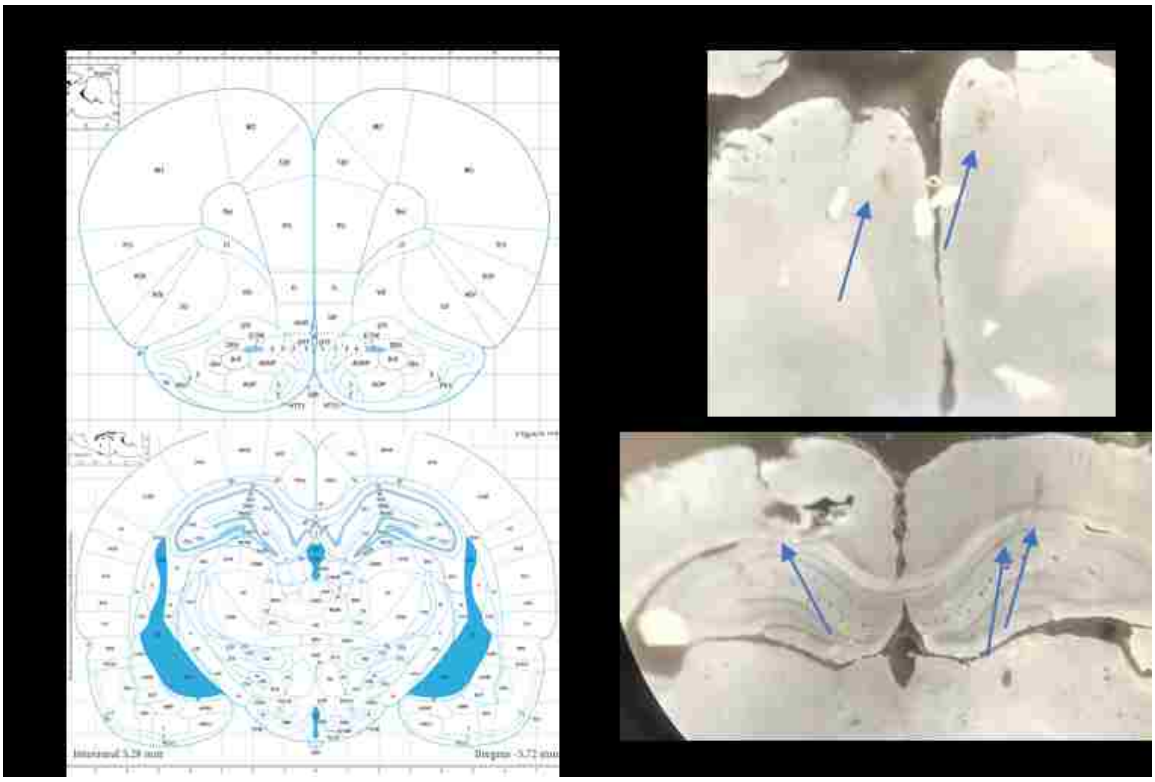


Figure 2. Histological verification of wire tracts in the dorsal CA1 in the HC and the ACC. (A) Coordinates of ACC and HC adapted from *The Rat Brain 6th ed.* (Paxinos & Watson, 2007). (B) After behavioral protocols were complete animals were deeply anesthetized under isoflurane and 100 μA current was passed through all electrodes for a minimum of 25 seconds to mark placement of tetrodes. After brains were removed they were sliced at 40 μm, placed on a glass slide and viewed under a microscope to verify placement of recording wires. These brain slices were taken from a representative subject, data from other animals were similar.

tracked wire depth. After the experimental procedures were completed, the location of recording wires was then confirmed with histology (see Figure 2A & B).

Behavioral Task

To test the hypothesis that interactions between the ACC and HC change as memories become more remote, we introduced subjects into six visually and texturally unique environments at differing delay periods, up to 18 days. For each session, animals were brought to the recording room and allowed to habituate for a minimum of thirty minutes. After that time, subjects were placed on a pedestal and fed Fruit Loops (Kellogg's, Battle Creek, MI) while the headstage and headstage adapter were plugged into the EIB. When the connection was secured, animals were placed in each environment, and allowed to freely explore for exactly ten minutes. After this time, subjects were removed from the environment and placed upon the pedestal for one minute before being placed into the next environment. For the full schedule of environment exposures on each per day and delay period see Figure 3A. On day one, subjects were introduced to environment A and B. On day two, subjects re-explored environment A and then introduced into environments C and D. On day three, subjects were re-exposed to environments B and D and then introduced to environment E. On day four, subjects were re-exposed to environments A, B, C, D, E and introduced into environment F. Seven days after the fourth day of training (day 11), subjects were reintroduced into environments A, C, and E. Fourteen days after the fourth day of training (day 18), subjects were reintroduced into environments B, D, and F (see Figures 3A & B). Because neural data from the first four days was not significantly different ($p > 0.05$), and that the current study was interested in how interactions change between brain

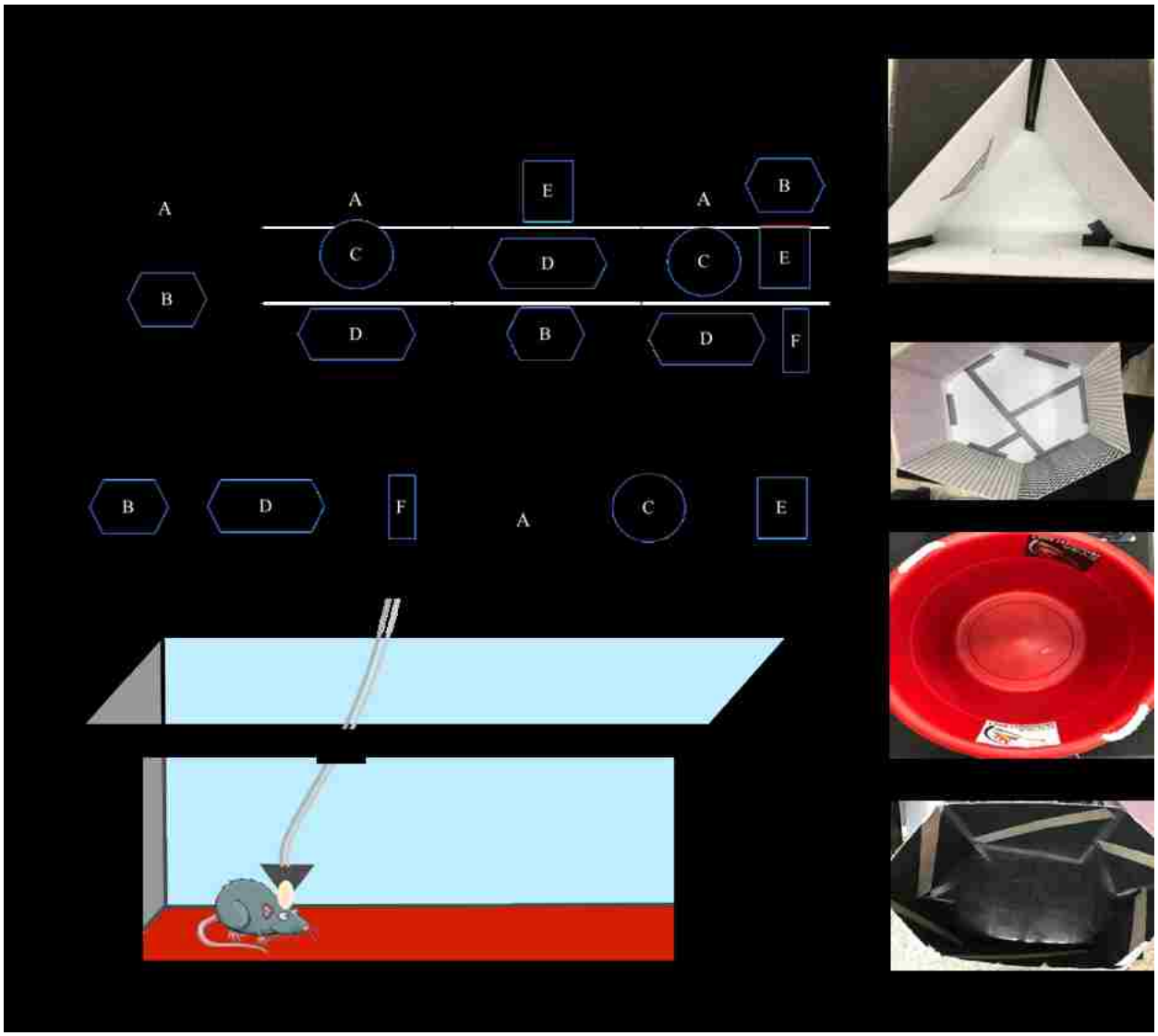


Figure 3. Behavioral task and environment examples. (A) Schematic of the behavioral protocol for the memory task. Note that subjects were exposed to environment A and B and equal amount of times throughout the experiment, and that subjects were exposed to environment A and B on day 1. environment A on day 11, and environment B on day 18. (B) Example of the subjects exposure to an environment. (C) Actual photos of the environments that the subjects were exposed to. Note each environment is made of a unique material and has a unique color and shape.

areas as a function of time, we only report data from day 1 (learning), day 11 (recent), and day 18 (remote). Each environment was a unique shape and constructed out of different building materials such that the design of each environment would provide unique visual cues and textural cues which allowed subjects to distinguish between them. Environment A was triangular shaped with walls 45 cm tall and 45 cm long made from textured white plastic. Environment B was hexagonally shaped with walls 45 cm tall and each side 30 cm long. The floor and the walls will be made from textured vinyl poster board. Environment C was circular shaped with walls 45 cm tall and a diameter of 63 cm and made from smooth red plastic. Environment D was the shape of a pentagon with sides 45 cm tall and 45 cm long constructed from poster board covered in vinyl (see Figure 3C). The disparities in shape and size of each environment was meant to give rodents enough information to be able to quickly differentiate between each context.

Data Analysis and Pre-Processing

Behavior

Rodents are naturally inquisitive to new surroundings, but after the surroundings become more familiar they will develop more comfortable tendencies and become less likely to explore their surroundings. To measure if subjects recall previous experiences within each environment, we tracked movement related to exploration using Bonsai open source software (Cambridge, MA). X-Y position data gave us an exact reading of animal location. Tracking data was then converted into Matlab (Mathworks, Natick, MA, USA) using custom-written Matlab code. Each environment was separated into standardized squares. Next, the total amount of time spent in each quadrant during the recording session to form the percent time spent value was calculated as

$$P = \frac{T_n}{(\Sigma(\Sigma T_n))}$$

where P is the percent time spent, T overall time spent, and n is each quadrant. The cumulative

sum calculation identifies the values that are different than the average. Using the Matlab function *cumsum* we calculated the cumulative sum of the values in the percent time spent value. These values create a shape similar to a logarithmic curve. we then calculated the area under the curve using the Matlab function *trapz* and all behavioral statistics were calculated using these values. To control for behavioral differences between day 1, day 11, and day, 18, we analyzed the first 200 seconds of behavioral data to ensure that changes in neural activity were not due to changes in behavior. We analyzed the last 200 seconds of behavior to test repeated exposure would affect exploratory behavior in each environment. If the subjects explored less than that would be an indication of some type of memory contextual recall.

Local Field Potentials

To analyze continuous data from LFP recordings, Open Ephys data was read into a computer workstation and down sampled to 1000 Hz using custom written Matlab code. To remove the 60 Hz noise signal, we notch filtered data between 58 and 61 Hz using Matlab function *butter*. Outliers were identified as any value more than 3 standard deviations about the mean. These values were removed and replaced with the preadjusted mean. To minimize redundancy and remove signal artifacts, we identified good recording wires in each recording location using visual inspection. This was achieved by plotting five-second time windows of activity on each tetrode. Then, we identified 1 good wire from each recording location in each of our subjects by analyzing levels of theta power using the *FFT* function in Matlab. We also analyzed the values in the FFT to ensure changes in all future analysis were not due to changes in frequency power.

Coherence

To better understand the functional relationship between the ACC and HC we computed power spectra and measured coherence between ipsilateral AC and HC for the first 200 seconds in

each environment. Data was filtered between 5-12 Hz using the high/low Butterworth filter in Matlab. Power spectra was then computed using a fast Fourier transform (FFT), then coherence values were computed between the ACC and HC using the Matlab function *mscohere*. Coherence is a measure of how signal X corresponds to signal Y by comparing power spectral densities of both signals for each frequency. The coherence value is computed as

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

where C is the coherence value between 0 and 1, P is power spectral densities, x and y are respective signals, and f is frequency (Kay, 1988). To test if coherence values between the ACC and HC change over time, we computed a one-way ANOVA on the coherence values of ipsilateral HC- ACC LFPs by delay period.

Lead-Lag Correlations

To better understand the directional effects of information flow we computed lead-lag correlations on the instantaneous waveforms generated from ACC and hippocampal LFPs. ACC and HC signals were window filtered between 5 and 12 Hz using the window filter function *fir1* in Matlab. Cross correlations measure similarities between two signals as a function of time shifts, using the function *xcorr* in Matlab, we computed the cross correlation for sliding ACC signals for every millisecond up to 100 milliseconds in each direction compared to HC signals for the for first 200 seconds of LFPs of each environment exposure. This created a distribution of all correlation coefficients which allowed us to identify when the time-series signals from the ACC and HC were most correlated. Next, we computed the Wilcoxon rank sum test to identify if the max lags between days were significantly different from zero (methods for lead-lag correlation adapted from Adhikari et al., 2010).

CHAPTER 4

RESULTS

Behavior & Fast Fourier Transform

To test for memory recall and control for behavioral changes we analyzed the behavior and frequency power. Coherence measures can be influenced by a number of behavioral factors. In rodents, hippocampal theta rhythm is highly correlated with movement speed and grooming behavior (Penley et al., 2012)**,** changes in behavior could influence theta band frequency power and possibly cause us to make inaccurate conclusions about these findings. To account for this, we identified a time period of environment exposure (the first 200 seconds of each environment) where movement was not found to be statistically different between day 1, day 11, and day 18. Moreover, we also compared FFT values during the first 200 seconds of each experimental day and found no significant differences in theta frequency power

To test the subject's recollection of each environment, we analyzed the amount of movement related to exploratory activity on day 1, day 11, and day 18. We assumed that after initial exposure the exploratory behavior would decrease, suggesting that the animal no longer viewed the environments as novel, thus clearly indicating that the subject was recalling information about the environment. Each environment was a different shape and size, so they needed to be analyzed independently. The subjects visited environment A and environment B on day 1 (see Figure 4A), note that subjects spent a large amount exploring the entire environment, and revisited environment A on day 11 (see Figure 4C) and then revisited environment B on day 18 (see Figure 4C), This can be seen that subjects only visited a limited number of locations . We analyzed the first and last 200 seconds of

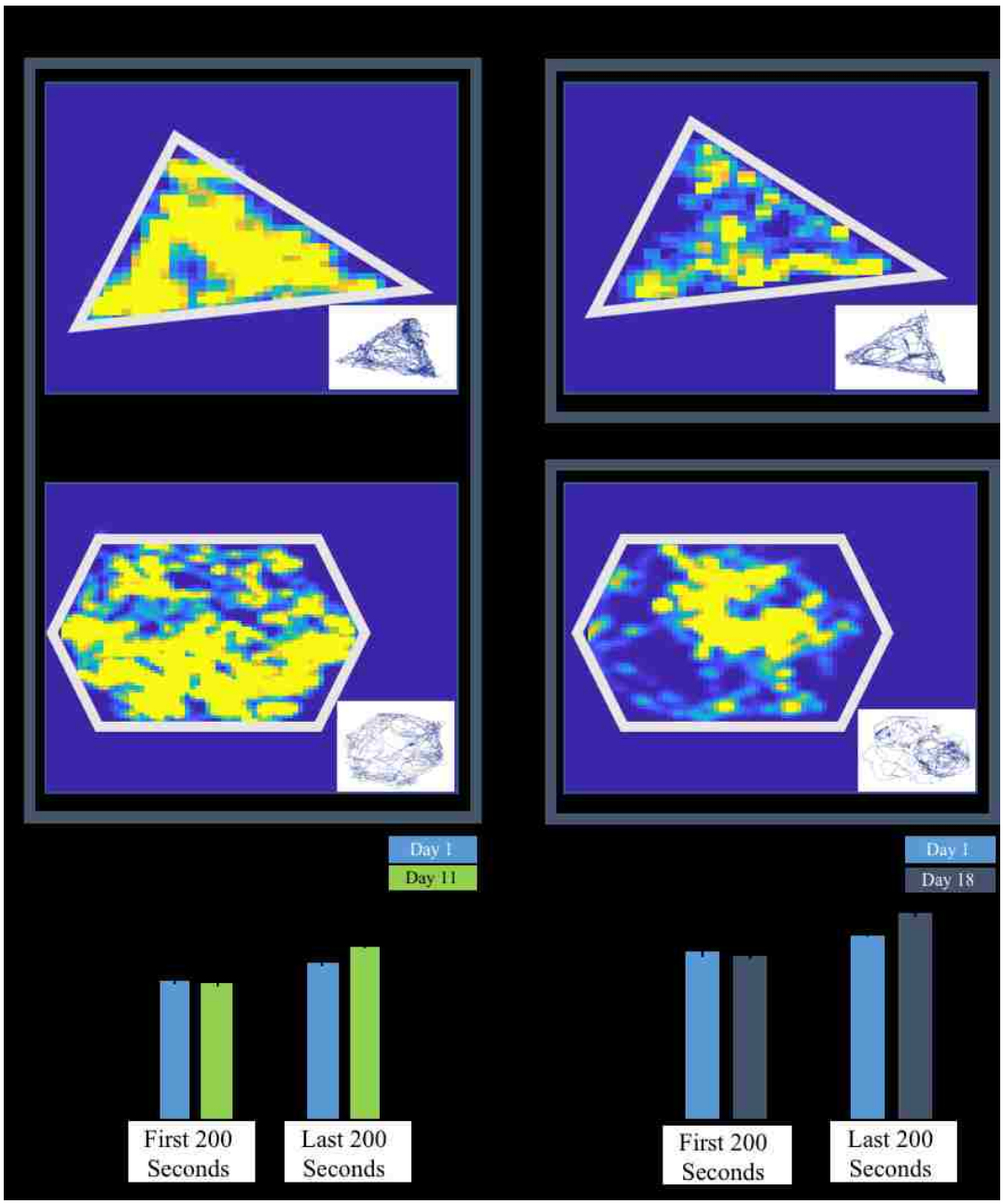


Figure 4. Time spent plots show exploration decreases during recall conditions. (A) The last 200 seconds of movement data from environment A (triangle) and B (hexagon) on day 1, bottom right subplot is corresponding path. (B) The last 200 seconds of movement data from environment on day 11, bottom right subplot is corresponding path. (C) The last 200 seconds of movement data from environment B on day 18, bottom right subplot is corresponding path. (D) Mean of the area under the curve values for environment A. Note the similarities during the first 200 seconds of each day and the difference between values during the last 200 seconds. (E) Mean of the area under the curve values for environment B. Note the similarities during the first 200 seconds of each day and the difference between values during the last 200 seconds. The space covered figures were taken from a representative subject and data from other animals were similar.

exploration of environment A on day 1 and day 11 to test recent recall. For remote recall, we analyzed the first and last 200 seconds of exploration in environment B on day 1 and day 18. In order to account for the discrepancies in shape and size of each environment, we separated each shape into a unique number of squares. Environment A was separated into 220 individual squares and environment B was separated into 300 individual squares, this allowed us to look at the amount of time the animal spent in each quadrant. We concluded that when animals spend an equal amount of time in each quadrant it is indicative of exploration, but when they spent the majority of time in a limited number of squares, minimal exploration was occurring.

To test how behavior changed, we calculated the amount of time spent in each square for eight fifty second time bins (first and last 200 seconds) and then calculated percent time spent value (shown above). Next, we generated the cumulative sum of the percent time spent in each quadrant. Then, we calculated the area under the curve for the first twenty values of the cumulative sum for environment A. A paired sample t-test revealed no significant difference between behavior during the first 200 seconds of exposure of environment A ($t(19) = 1.77$, $p = 0.09$; mean of day 1 = 0.5; s.e.m. = 0.08 and day 11 = 0.56; s.e.m = 0.13). However, the area under the curve for the last 200 seconds of environment A revealed a significant difference between days, indicating that there was a decrease in exploratory behavior during the last part of the recent recall day suggesting subjects habituated more rapidly ($t(19) = 2.321$, $p = 0.03$; mean day 1 = 0.49; s.e.m = 0.010, day 11 = 0.62; s.e.m = 0.013) (see Figure 4D). Because environment B was larger, we calculated the area under the curve for the first twenty-four values. A paired sample t-test revealed similar findings for the first 200 seconds of exposure for environment B revealed no significant difference between behavior ($t(23) = 1.17$, $p = 0.25$; mean of day 1 = 0.60; s.e.m.= 0.11 and day 18 = 0.66; s.e.m. = 0.09). However, the same analysis on the last 200 seconds revealed that subjects exhibited

significant decreases in exploratory behavior between days ($t(23) = 3.19$, $p = 0.004$; mean of day 1 = 0.60; s.e.m. = 0.02, day 18 = 0.74; s.e.m = 0.018) (see Figure 4E).

In addition to analyzing behavioral changes, we also examined if there were any increases or decreases theta band frequency power (see Figure 5A & B), note the highly powered signals at 8 Hz in both ACC and HC spectrograms. We separately analyzed ACC and HC LFPs recorded during the first 200 seconds of environment exposure. Because our analysis was strictly theta based, we extracted power from the theta range

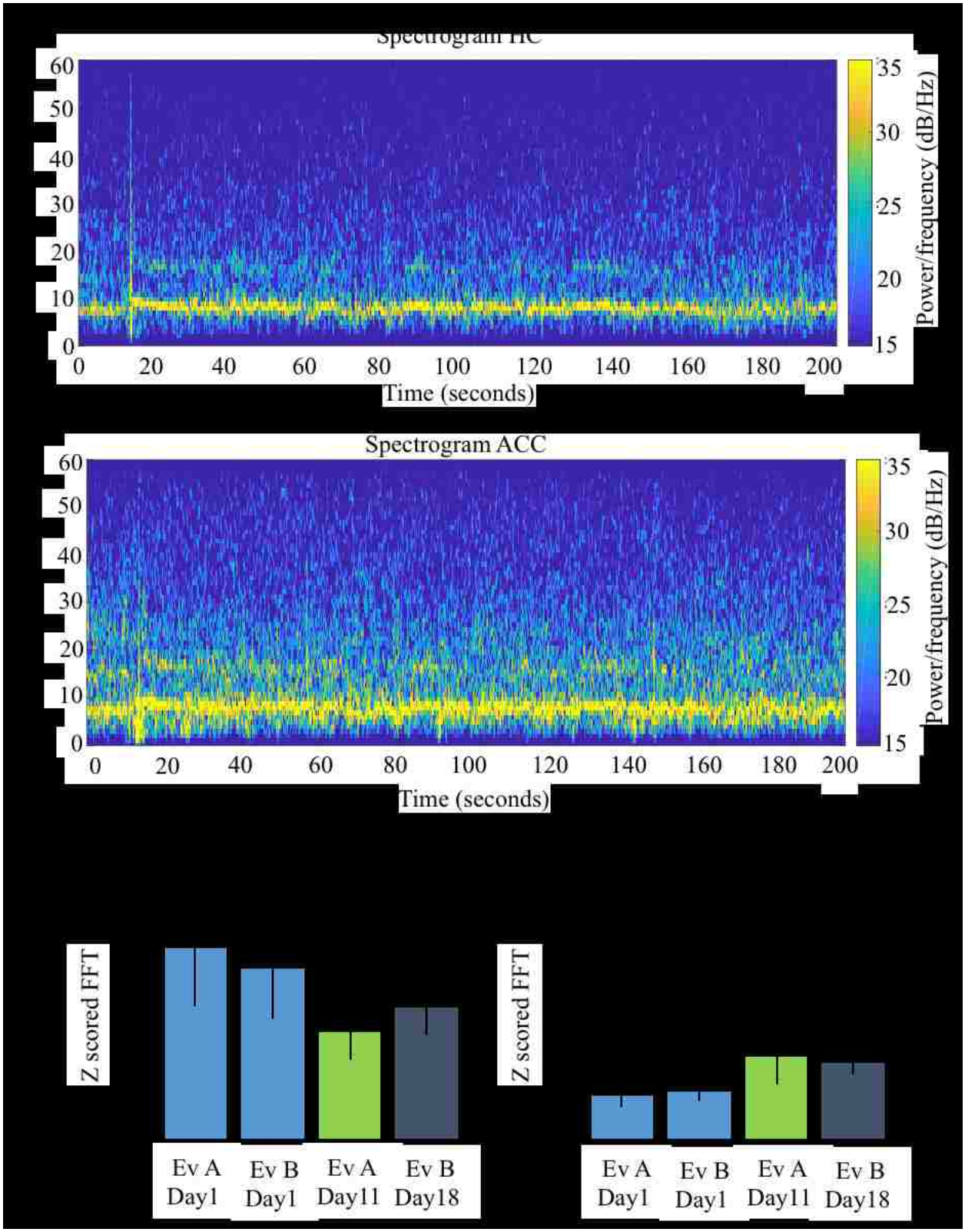

Spectrogram HC
Power/frequency (dB/Hz)
Time (seconds)
Spectrogram ACC
Power/frequency (dB/Hz)
Time (seconds)
Z scored FFT
Ev A Day1
Ev B Day1
Ev A Day11
Ev B Day18
Z scored FFT
Ev A Day1
Ev B Day1
Ev A Day11
Ev B Day18

Figure 5. Spectrograms and z-scored FFT means. (A) Spectrogram of HC activity during the remote recall condition (day 18), note the high-powered theta band activity. (B) Spectrogram of ACC LFPs during the remote recall condition. (C) The z–scored means of the FFT of HC wire average for the first 200 seconds each environment exposure during day 1, day 11, and day 18; there were no significant differences ($p > 0.05$). (D) The z–scored values of the FFT of ACC wires for the first 200 seconds in each environment exposure during day 1, day 11, and day 18; there were no significant differences ($p > 0.05$).

(5 – 12 Hz) before transforming the values into z scores. For the HC, we computed a one-way ANOVA on z scored FFTs. The means for environment A (mean = 3.11; s.e.m.= 0.98) and B (mean = 1.74; s.e.m.= 0.46) during day 1, environment A (mean = 2.77, s.e.m. = 0.83) during day 11, and environment B (mean = 2.14, s.e.m.= 0.46) on day 18 were not significantly different ($F(3,188) = 0.746$, $p = 0.53$). Similarly, a one-way ANOVA for ACC values during the same time window in environment A (mean = 0.79, s.e.m.= 0.21) and B (mean = 1.36, s.e.m. = 0.44) on day 1, environment A (mean = 0.83, s.e.m.= 0.18) on day 11, and environment B (mean = 1.26, s.e.m.= 0.17) on day 18, also revealed no significant differences in theta power ($F(3,188) = 1.863$, $p = 0.13$; see Figure 5C). These results indicate that any changes in future analysis were not merely the result of increases or decreases frequency power within each brain area.

Collectively, this data illustrates that animals had no significant differences in behavior or frequency power during the first 200 seconds of environment exposure. It also indicates that subjects habituated more quickly to the environments on subsequent more time exploring each environment. During both recent and remote recall days, the animals habituated significantly faster as seen by our percent time spent calculation. To control for behavioral differences in our data, future analysis is only during the first 200 seconds of environment exposures, a time when behavior was not significantly different.

Coherence

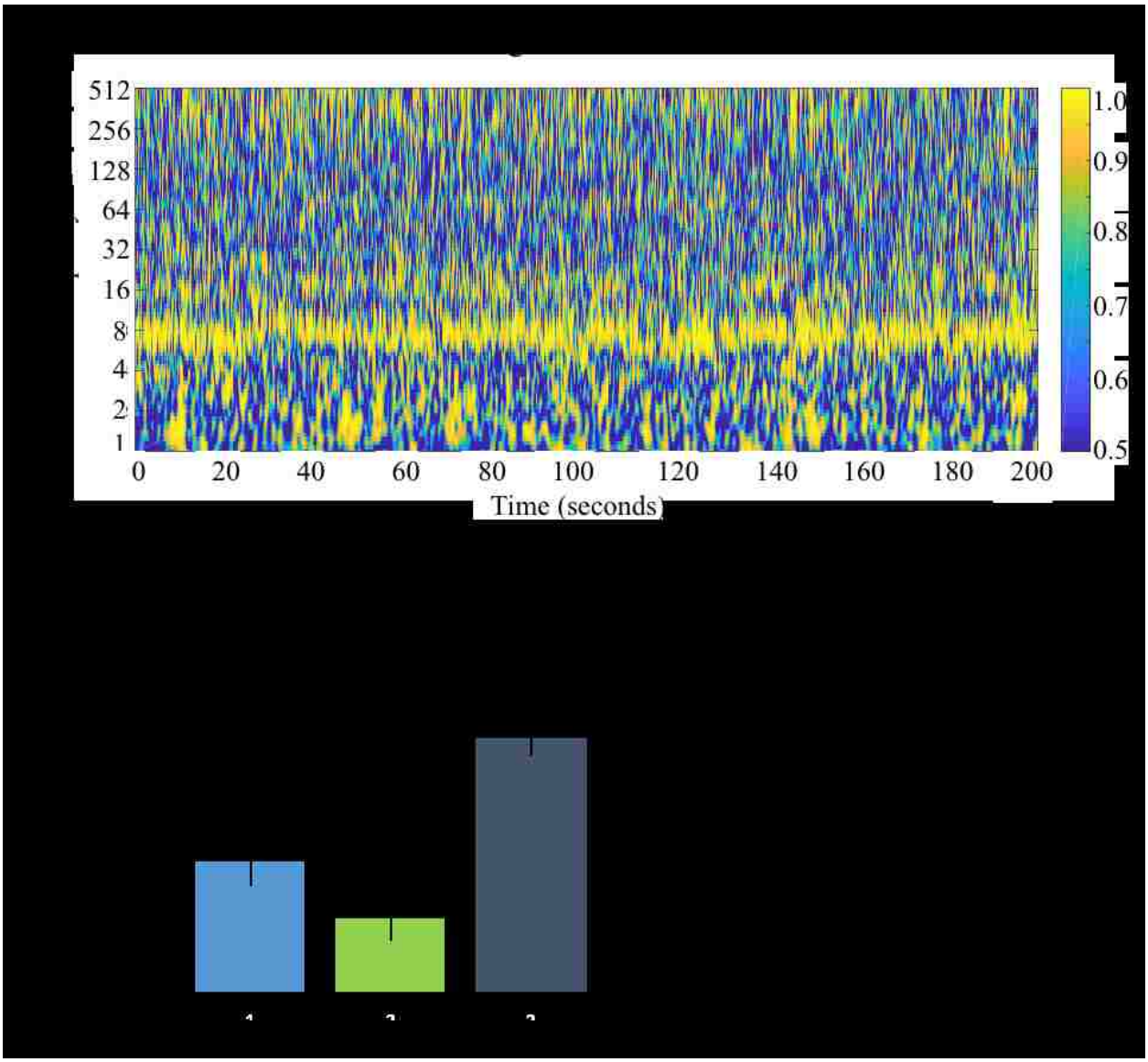
After confirming memory recall in behavior, and confirming that no significant changes in FFT scores occurred during first 200 seconds, all future analysis only used these times. We analyzed coherence between the ACC and HC and found that both areas were highly coherent in the theta range during the remote recall condition. This can be seen clearly with the light-colored areas in the coherogram around 8 Hz (see Figure 6A). To identify

how interactions between the ACC and HC change as a result time, we filtered LFP data between 5 and 12 Hz. It was predicted that the more remote the spatial memory was, the greater the reliance on oscillatory interactions between the ACC and HC would be. After computing theta band coherence values, we compared the values between days. A one-way ANOVA revealed a significant main effect of delay period for theta band coherence

between ipsilateral ACC and HC ($F(2, 197) = 20.82$, $p = 6.26e^{-9}$). We next used the Tukey – Kramer test to identify what day the theta coherence changed. We found the coherence value for initial exposure (mean = 0.21; s.e.m.= 0.04) and recent recall (mean =0.12; s.e.m. = 0.04) were significantly lower than the remote recall condition (mean = 0.41, s.e.m.= 0.03) $p < 0.0001$ (see Figure 6B). These data suggest that recall of older, or more remote, memories relies on the functional interactions between the ACC and HC. During recall of more recent memories we did not find any significant differences in ACC – hippocampal theta band coherence, which suggests that some type of neural network reorganization occurs between the recent and remote recall conditions.

Lead-Lag Correlation

After identifying significant changes in coherence between the ACC and HC


512
256
128
64
32
16
8
4
2
1
0
20
40
60
80
100
120
140
160
180
200
Time (seconds)
1.0
0.9
0.8
0.7
0.6
0.5

during the remote recall condition, we were interested in identifying which brain area initiated these interactions. Coherence between the ACC and HC was imitated by one of three possible outcomes; first, the HC initiated coherence with the ACC, second, coherence was generated by some other brain area, and last, ACC theta activity caused coherence with the HC. Previous reports have shown hippocampal activity to lead prefrontal activity during spatial working memory tasks (Siapas et al., 2005), and during contextually guided object sampling, ACC activity leads hippocampal activity (Place et al., 2016). Bidirectional interactions between these two areas have led researchers to the hypothesis that that the HC sends spatial information via theta band oscillations to the mPFC, and the ACC guides memory retrieval (Navawongse & Eichenbaum, 2013; Jin & Maren, 2015). If this is the case, then we should see ACC activity leading HC activity. We hypothesized that after memories became dependent on the ACC, theta oscillations effectively allow the two areas to communicate during memory recall, ACC theta band oscillations should lead hippocampal activity. To test this, we analyzed lag differences between LFPs in the HC and ACC during day 1, day 11, and day 18, (see Figure 7A), note on day one, the hippocampal signal is leading the ACC signal, on day 11 the results were highly variable with no consistent pattern emerging, and on day 18 ACC activity led the HC. To quantify these changes, we calculated the cross correlations the ACC and HC LFPs at specified increments. ACC signals were shifted forward and backward in time at 1 ms increments up to 100 ms in each direction, at each time point a cross-correlation was calculated between the ACC and HC LFPs. This created a distribution of cross-correlation coefficients over each time shift, allowing us to see the temporal offset of signals and infer whether one signal leads the other. Results from the lead-lag

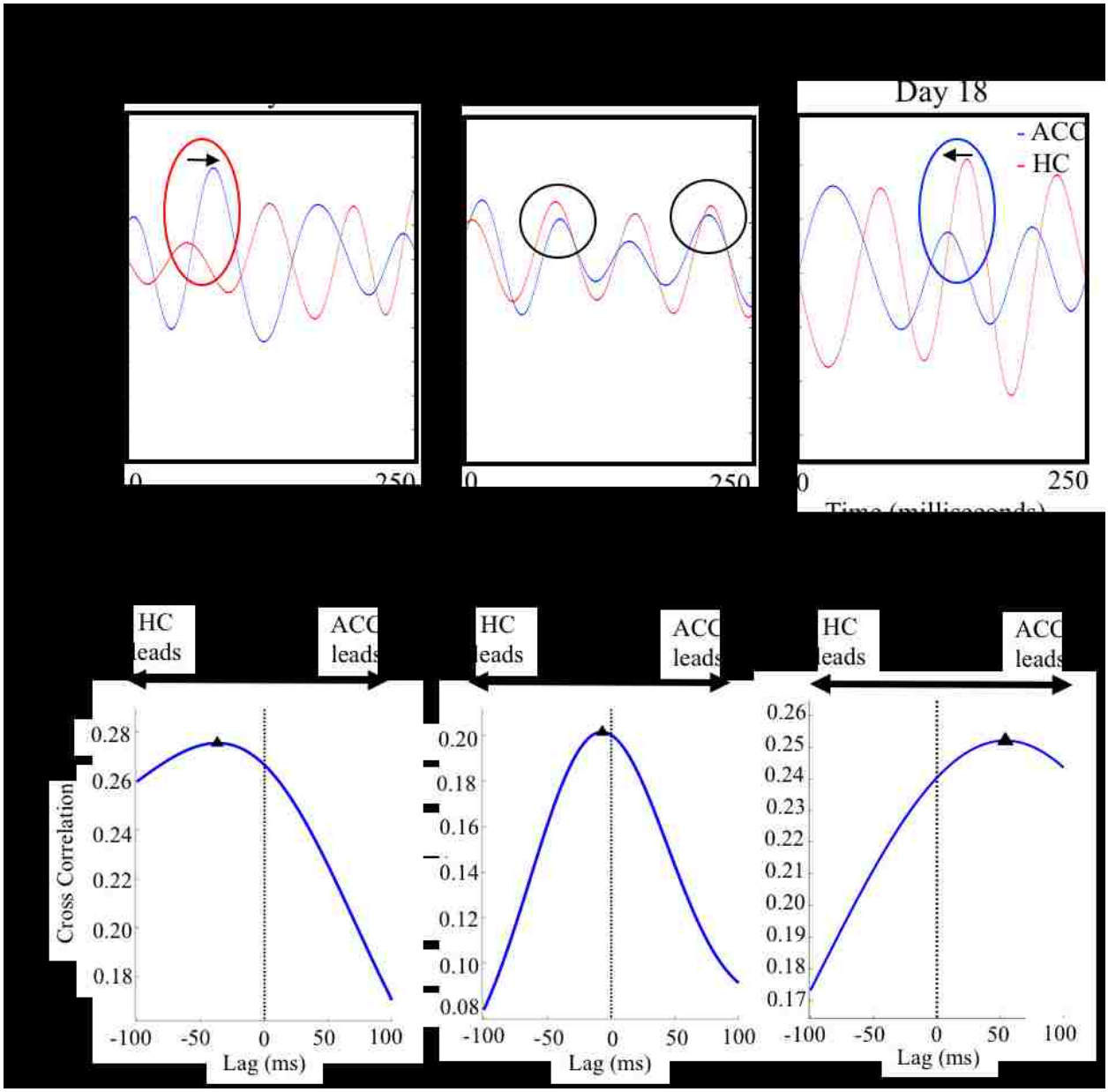


Figure 7. Filtered LFP trace and Lead lag correlation for day 1, day 11, and day 18. (A) Representative figures of HC and ACC signals during day 1, day 11, and day 18 conditions. Note the HC (red) reaching its peak before the ACC (blue) on day 1 (left). On day 11 (middle), notice that either signal consistently leads the other. On day 18 (right) the ACC signal peaks prior to the HC signal, which is an indication that ACC activity leads the HC during the remote recall condition. (B) distribution of lag time for HC and ACC signal for day 1 (left), day 11 (middle), and day 18 (right). Note on day 1 the highest correlation between the two signals occurs when the HC signal is offset backwards in time. This changes during the remote recall condition such that the highest correlation between the two signals occurs when the HC signal is moved forward in time. These figures were taken from a representative subject and data from other animals were similar.

correlations for day 1 (mean lag time = -17.0 ms, median = -11) and day 11 (mean lag time = -16.4 ms, median = -8) had the highest cross-correlations when ACC signals were shifted backwards in time, thus indicating that the hippocampal signal led the ACC signal during these days. However, on day 18 the highest cross-correlation occurred when the ACC was shifted forward in time (mean = 30.8 ms, median = 16.5) (see Figure 7B) indicating that the ACC was leading the HC during remote memory recall. We then used the Wilcoxon rank sum test to identify if the changes in lag time were significantly greater than zero. The difference between max lags for day 1 and day 11 was not significantly different from zero ($p > 0.05$, Wilcoxon's rank sum test). The difference between max lags for day 11 and day 18 was not significantly different from zero ($p > 0.05$, Wilcoxon's rank sum test). However, the difference between the max lag day 1 and day 18 was significantly different ($p < 0.05$, Wilcoxon's rank sum test). These results provide evidence in support of bidirectional interactions between the ACC and HC. On day 1 and day 11. The findings are consistent with previous reports of that show the hippocampal theta directing prefrontal unit activity (Benchenane et al., 2010) and theta oscillations (Hyman et al, 2010) during spatial learning. These findings support models of PFC guided memory recall (Eichenbaum, 2017; Frankland & Bontempi, 2005). The change in directionality of theta activity during the remote recall condition could be an indication that the ACC initiates recall of spatial memories, therefore providing an explanation for why ACC ablation causes temporally graded memory impairments (Bontempi et al., 1999; Liu et al., 2009; Weible et al., 2012).

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CHAPTER 5

DISCUSSION

This study aimed to identify electrophysiological evidence that could explain how the ACC-hippocampal network interact during remote memory recall. We recorded LFPs from the ACC and HC, and performed coherence and lead-lag cross-correlation analysis on these signals. The results revealed that re-exposure to environments during a more remote delay period (18 days) produced significant increases in coherence, which suggests increased communication between theses brain areas (Fries, 2005). Moreover, lead-lag analysis provided evidence that support the hypotheses of an ACC mediated recall. These findings are consistent with previous reports that have identified the importance of the ACC in remote memory recall (Bontempi et al, 1999; Restivo et al., 2009; Weible et al., 2012), however, to our knowledge, this is the first electrophysiological evidence that explains how the ACC initiates memory recall through communication with the HC.

We first set out to identify if subjects exhibited memory recall by comparing exploratory behavior on initial exposure to subsequent exposures. It appeared that subjects did recall environmental information, as analysis of different time windows revealed that when subjects revisited environments, the amount of exploratory behavior significantly decreased. This finding indicates that that the animals had recalled enough information about the environment such that it was no longer novel. The memory task used in this experiment takes advantage of the rodent's natural curiosity. When exposed to a novel environment rats will vigorously explore their new surroundings, but on subsequent exposures, rats will decrease the amount of exploratory behavior (Terry, 1979; Leussis & Bolivar, 2006).

After confirming the animals successfully recalled environmental information, we aimed to identify changes in ACC and HC LFP activity. Coherence, which measures the extent at which

two independent signals change frequency and amplitude together, can be indicative of the degree to which two separate brain areas are communicating (Fries, 2005; Bastos et al., 2015; Fries, 2015). Previous reports have shown that the HC and ACC exhibit elevated theta band coherence during a number of spatial working memory tasks (Siapas et al., 2005; Jones & Wilson, 2005; Hyman et al., 2010), and while it is true that more research is needed to better understand the importance of those interactions, mounting evidence suggests that these interactions may cause the reorganization of neural spike patterns which could contribute to learning (Benchenane et al., 2010; Benchenane et al., 2011).

Here, we analyzed theta band coherence between the HC and ACC while subjects learned new contextual information, and also during recall conditions during both recent and more remote delay periods. Despite not finding any significant differences in behavior or frequency power during the first 200 seconds of environment exposure, analysis of coherence revealed significant increases in ipsilateral ACC-hippocampal interactions. Interestingly the change did not occur until the remote memory recall condition, suggesting that as time passes, and the consolidation process begins, successful recall of these memories may become reliant on interactions between the ACC and HC. Considering the importance of the ACC for remote memory recall (Bontempi et al., 1999; Teixeira et al., 2006; Ding et al., 2008), the electrophysiological marker, which supports previous findings, is of great significance, and an important step in identifying how both the HC and ACC have continued involvement in these memory processes.

We next set out to identify the mechanism that could described the role of the ACC in remote memory recall. We performed the lead-lag correlation analysis technique which describes the directionality, or initiation, signal generation. During the learning phase of this experiment, our findings were consistent with previous reports such that the HC led ACC activity, and during

the recent recall day there did not seem to be a directional relationship between the two areas. However, during the remote recall phase, the data indicated a change in directionality and the ACC actually led the HC, suggesting that the ACC was initiating the coherent interactions during remote memory recall. It seems that the ACC initiates memory recall with the HC which might explain why there are changes in ACC and HC coherence after the passage of time. These findings offer support for a proposed model of memory recall initiation (see figure 8), which shows the change in recall control after the delay period, note that during learning the HC is more important than the ACC for recall, but over time, the ACC has a more active role in the recall process. Additionally, these data support multiple trace theory as it appears that there is continued involvement of the HC during recall of these types of memories.

Implications

In the present study, we identified a novel electrophysiological marker that may illustrate why the ACC is so important for remote memory recall. The increases in ACC-hippocampal coherence suggest that both areas become more aligned during remote memory recall. In addition, the directional changes of theta activity indicate that the ACC initiates memory recall. Together, the findings from this project indicate that the ACC has a more active role in memory recall, but are unable to specify exactly what neural

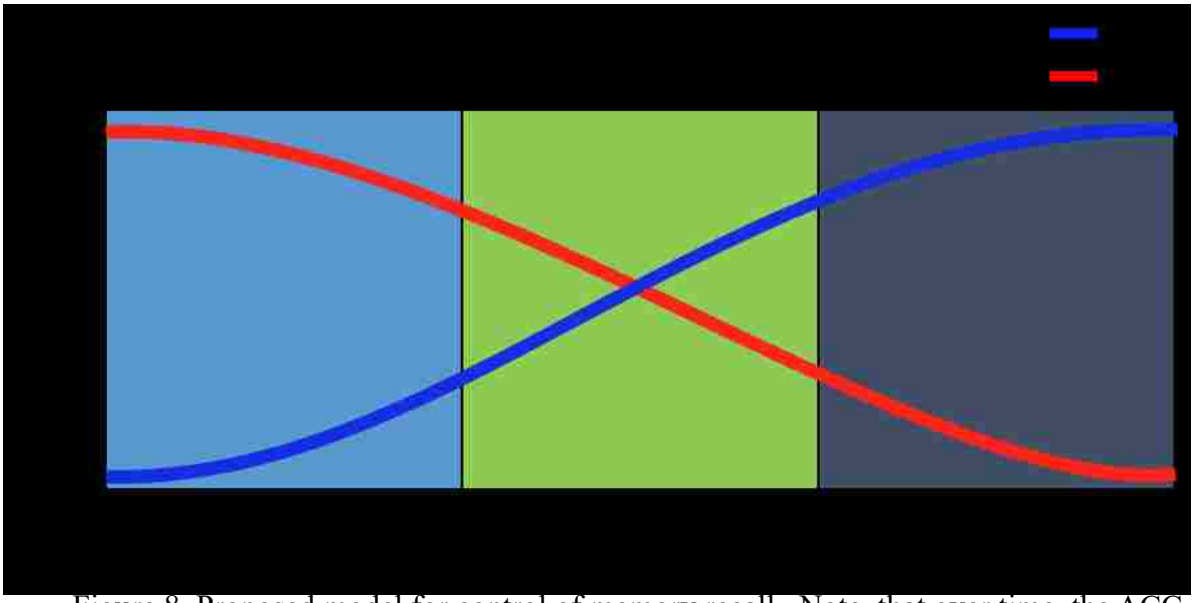

Figure 8. Proposed model for control of memory recall . Note, that over time, the ACC has a more active role in initiating recall, and the HC has less.

changes occur during the delay period, or what role the ACC has in the memory consolidation process. However, there are a number of supported conceptualizations of how the ACC contributes to memory recall.

Memory processes are heavily reliant on numerous structures within the limbic system (Rajmohan, & Mohandas, 2007). Specifically, the HC for its role in forming new memories and mediating the consolidation process (Eichenbaum, 2007), but it is clear that some aspects of spatial memories eventually become dependent on extrahippocampal brain areas. However, according to MTT, retrieval of contextual episodic memories is continuously dependent on an intact HC (Nadel & Moscovitch, 1997) because some aspect of the memory trace is still within the HC. Our data offer support for MTT because we show the relationship between the HC and ACC becomes more robust as time passes. But what is the role of the ACC? Results from the lead-lag correlation suggest that the ACC is sending some type of information to the HC.

In neural networks learning is thought to result in permanent changes in neural structure. These changes occur throughout the brain including the ACC. Maviel and colleagues (2004) were interested in how these changes relate to recall of remote spatial memories. After training animals on a reward location in a five–arm maze, the examination of the growth – associated protein 43, or GAP–43, revealed that animals exhibit increases of GAP-43 in prefrontal areas after a significant delay period. Similar findings have been reported in separate tasks (fear conditioning) by Restivo and colleagues (2009). Additionally, Einarsson & Nader (2012) were able to block learning effects with injections of anisomycin directly into the ACC. Crucially, these findings were supported by electrophysiological evidence and a functional mechanism by Takehara & McNaughton (2008). After training subjects in contextual fear conditioning chamber Takehara re-exposed animals at differing delay periods (up to 6 weeks) and analyzed changes in neural spiking activity. They report reorganization of mPFC neuronal firing patterns after the delay period indicating functional changes to ACC activity, suggesting, that over time, as the fear memory became consolidated, something related to that memory was stored in ACC circuits. The changes to ACC neural structure and spike timing might relate to a change in dependency of remote memories.

Numerous experimental reports and computational models have aimed to identify and explain the memory trace. The Tonegawa group has been central to this endeavor with their numerous discoveries, especially the ability to manipulate memory traces in the rodent hippocampal formation (Lui et al., 2012; Ramirez et al., 2013), but the continued role the HC has in memory recall is still a topic of debate (Morris, 2006), and engrams of hippocampally independent memories have been quite elusive. After consolidation, memories are spread out across a complex network throughout the brain (Squire & Alvarez, 1995; McClelland,

McNaughton, & O'Reilly, 1995). In recent years a few studies have been able to initiate contextual fear memory recall through optogenetic stimulation of medial prefrontal circuits (Rajasethupathy et al., 2015), suggesting that as memory traces in the HC naturally fade, engrams within the ACC become increasingly important (Kitamura et al., 2017). These studies have been monumental, but there is still uncertainty regarding what type of information occurs in ACC neural networks.

Limitations

It is true that we used a subtle gauge of memory recall, However, despite our recall measure being strictly movement based, it is consistent with other experimental findings that employed more stringent memory tasks such as taste aversion (Ding et al., 2008) and contextual fear conditioning (Kitamura et al., 2017). And, while it is possible that the observed changes could have arisen due to increases in or decreases in frequency power, we controlled for behavior and only analyzed coherence and lead-lag correlations during the time windows that did not have significant differences in movement or FFT. Moreover, other factors such as the slight changes in recording areas due to movement of tetrodes influenced our findings. Lastly, it is possible that another brain area is influencing ACC activity and ACC acts as a conduit between the HC and that other brain area. Future work should attempt to account for these possible limitations

Future directions

Future work should aim to identify what changes occur in the ACC that allow this area to entrain hippocampal theta activity, and what role the HC has during recall of remote spatial memories. Additionally, these investigations should attempt to identify if the observed changes in this network are a result of memory traces forming in the ACC, or if there is some unknown mechanism related to working memory that drives the electrophysiological changes. Analysis of other frequency bands and spike patterns within these brain areas will aid in the discovery of novel

mechanisms for remote memory recall.

In order to understand memory processes future research should test whether the changes in network interactions between the ACC and HC are caused by increased task difficulty, consolidation, or some other cognitive or neural process. Future work could utilize a memory task in which environmental similarities are an additional variable. If increased coherence is a result of task difficulty, then when animals are exposed to two similar environments, there should be at least some discernable changes in interactions based of more difficult or less difficulty recall situations. While the experiment described above might reveal some insight into how difficulty impacts the interactions between the ACC and HC, it is unlikely that the findings would reveal directional changes based solely on task difficulty. Siapas et al. (2005) and Hyman et al. (2005) utilized a number of spatial tasks that ranged in difficulty and did not report directional changes in the hippocampal-prefrontal network, indicating that task difficulty is probably irrelevant. More likely, it is possible that the observed changes in coherence and directionality were a result of time passing and reorganization of memory traces than task difficulty.

Identifying the cause of this change in neural activity will help to formulate a better understanding of memory recall but exploring other mechanisms that contribute to recall is equally important. Our experiment focused on theta band frequencies, but further investigation is required to better understand the neural correlates related to other rhythmic changes occurring during remote memory recall. However, our data also found high power in beta (15 – 30 Hz) and gamma (30 – 80 Hz) band frequencies (Wang, 2010). To this end, additional work is needed to investigate how the neurons change firing patterns as a result of learning, and how this affects unit phase locking to external LFPs.

Beta oscillations are a harmonic of theta oscillations and such might be an important

contributor to HC cellular function and hippocampal communication with cortical brain areas (Igarashi et al., 2014). During visual cue onset beta band power decreased in the PFC but PFC-HC synchrony increased (Brincat & Miller, 2016) which might suggest the importance of this network for internal memory processing or at least visual working memory states. The extent of beta band oscillations in hippocampal function is still largely unknown. This oscillation was thought to exert control over motor systems (Bouyer et al., 1987) but more recent studies have identified that it has an integral role in integrating sensory systems (Witham et al., 2007). Considering the possibility that the ACC acts as an area that mediates memory recall by integrating cortical network information, understanding how beta band communication between the ACC and HC might yield some interesting findings.

In addition to examining the role of beta oscillations in memory recall, understanding the effect of gamma band is also important. The gamma cycle is found throughout the brain, particularly in hippocampal (Montgomery et al., 2008), parietal, and frontal areas (Bouyer et al., 1981). It is closely related to a number of physical properties of neurons such that the time window of this oscillation is closely aligned with the outcome of GABA and AMPA signaling (Johnston & Wu, 1994). Traditionally, gamma band oscillations are associated with neuron to neuron communication during perceptual processing (Gray & Di Prisco, 1997) and attentive states (Fries et al., 2001) but using genetic manipulations, researchers have been able to increase attention by stimulating prefrontal networks at the gamma band frequency (Kim et al., 2016). Additionally, through cross frequency coupling, hippocampal theta coupled with gamma oscillations has been shown to entrain neocortical neurons which are an important mechanism for neural function (Sirota et al., 2008).

Investigating how other frequency bands such as beta and gamma oscillations change will

provide additional insight into how brain areas change communication patterns change as a result of time passing analyzing spike patterns in neurons would provide insight into the types of processing that is taking place during this type of learning and memory recall. In recent years a number of studies have reported changes in neural firing patterns, or spike timing reorganization, that are directly related to learning (Benchenane et al, 2010; Benchenane et al, 2011) and memory consolidation (Takehara & McNaughton, 2008) in the mPFC. These studies indicate that prefrontal areas are undergoing some type of change related to learning processes and systems consolidation. In our study, we focused primarily on LFPs, but future work should attempt to identify if there are changes in either ACC or hippocampal activity relating to the passage of time. This work would provide additional insight into how both areas functionally reorganize as a result of new memory

Future research should also investigate the effect of outer area neural oscillations have on HC neurons. Place et al. (2016) previously reported prefrontal oscillations leading hippocampal activity during object exploration, our data also show this effect during remote memory recall. However, to my knowledge, and despite the abundance of reports showing how HC oscillations affect other brain areas, (Siapas et al., 2005; Rutishauser, Ross, Mamelak, & Schuman, 2010; Jones & Wilson, 2005; Belluscio, Mizuseki, Schmidt, Emptor, & Buzsáki, G. (2012) none have shown the ACC or mPFC to influence hippocampal unit activity. While identifying how outer area phase locking influence cellular activity in the HC could help to explain the role of the HC in memory recall, drawing conclusions about this relationship continue to be difficult. Because of this, future research should investigate how ACC theta phase locking influences hippocampal cellular activity.

Conclusion

This project only begins to examine the vast functions of the ACC – hippocampal network and additional work is needed to further assess how reciprocal information transfer between these

brain areas contributes to all aspects of memory. Examination of broadband frequency changes in coherence could offer to provide fundamental information relating to how the two areas communicate. Moreover, investigations of neural population changes could provide information on the specific role each area has during memory recall. The data provided here only offer minimal insight into the complex workings of memory systems but as more research emerges, we will eventually begin to disentangle these complex neural processes.

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Curriculum Vitae
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Education

Expected 2021	**University of Nevada Las Vegas** Ph.D. in Psychology with an emphasis in neuroscience
2015	**California State University Northridge** Bachelor of Arts in Psychology completed concurrently with a minor in Biology

Interests

Systems neuroscience
Learning & memory
Decision making

Current Position

2017 - present	**University of Nevada Las Vegas** Graduate Teaching Assistant ~ Introduction to Psychology

Past Positions

2015 - 2017	**University of Nevada Las Vegas** Graduate Assistant for Dr. James M. Hyman ~ Neurobiology of Learning and Memory
2013 - 2015	**California State University Northridge** Lab Manager with Dr. Jose P. Abara
2014 - 2015	**California State University Northridge** Teaching Assistant ~ physiological psychology with Dr. Jose P. Abara
2013 - 2014	**California State University Northridge** Teaching Assistant ~ Advanced Research Methods with Dr. Sheila Grant

Publications

2017 Wirt, R. A., & Hyman, J. M., (2017). Integrating spatial working memory and remote memory: Interactions between the medial prefrontal cortex and hippocampus. *Brain Sciences,* 7,43.

Abstracts and Professional Presentations

2017 **It's About Time: electrophysiological evidence for temporally mediated consolidation of spatial memories.**
Wirt, R.A., Crew, L.A., Zha, K.R., Kaplan, N.L., Francis, R.M., Hyman, J.M. Presented at the Society for Neuroscience, Washington DC.

Bio-plausible models of ACC function in a novel probabilistic reversal learning task.
Francis, R.M., Wirt, R.A., Hyman, J.M. Presented at the Society for Neuroscience, Washington DC.

2016 **Increases in Cross-Hemispheric Medial Prefrontal Cortex LFP Coherence for Remote Memories**
Wirt, R. A., Ortiz, A. A., Hyman, J.M. Presented at the Society for Neuroscience, San Diego, CA.

Using the Rescorla-Wagner Equation to Model Choice Behavior
Francis, R. M., Ortiz, A. A., Ender, L. A., Green, K. E., Wirt, R. A., & Hyman, J. M. Presented at the Society for Neuroscience, San Diego, CA

Construction and Assembly of a Hyper-Drive Recording Implant
Ortiz, A. A., Wirt, R. A., Hyman, J.M. Presented at the AANAPISI research symposium.

Increases in Theta Coherence between ACC and HC for Delayed Spatial Recall.
Zayas, S., Wirt, R. A., Hyman, J.M. Presented at the AANAPISI research symposium.

2015 **Non-Monotonic Relationship Between Social Anhedonia and Attention as Indexed by The Contingent Negative Variation**
Trieu, T., Morales, J., Neswald, J., Wirt, R. A., Alamdari, A., Abara, J., Sergi, M. Presented at the Association for Psychological Science annual meeting, New York, NY.

Anticipatory Response is Decreased in Schizotypy with Anhedonia
Wirt, R. A., Neswald, J., & Abara, J.P. Presented at the Western Psychological Association, Las Vegas, NV

An ERP Study of Motor Preparation in Schizotypy and Anhedonia
Neswald, J., Wirt, R. A., Pterosaur, S., Sarkissians, S., Morales, J., Abara, J.P., & Sergi, M. Presented at the Western Psychological Association, Las Vegas, NV.

The Benefits of Neurofeedback Procedure in Aiding the Recovery for Veterans with Symptoms of PTSD
Wirt, R. A., Kelson, C., Connors, A., Wilson, A., Roque-Flores, F., Abara, J.P. Presented at the Society for Behavioral Medicine, San Antonio Texas.

2014 **Anticipatory Response Indexed by Contingent Negative Variation in Persons with Schizotypy and Anhedonia**
Pterosaur, S., Neswald, J., Sarkissians, S., Morales, J., Wirt, R. A., Abara, J.P., & Sergi, M. Presented at the Society for Neuroscience, Washington DC.

An ERP Study of Expectation and Motor Preparation following Neurofeedback Procedure
Pongpipat, E. E., Magana, V. M., Sarkissians, S., Neswald, J., Camacho, V., Wirt, R. A., & Abara, J. P. Presented at the Western Psychological Association, Portland, OR.

Awards & Prizes

2018 **1st place UNLV GPSA research forum**
Patricia Sastaunak Scholarship
UNLV graduate college summer session scholarship

2017 **Graduate and Professional Student Association travel award**

2015 **CSUN undergraduate outstanding research award**
CSUN undergraduate research travel award

2014 **CSUN college of undergraduate research travel award**

Service

2016 - Present

Brain Awareness Week Campaign
Educating elementary school children about basic concepts in neuroscience while also illustrating the importance of helmet safety.

2016 – 2017

Las Vegas Brain Bee Along with other members of the Nevada Brain Bee Association, I helped recruit students to the neuroscience competition. The bee is designed to increase neuroscience awareness and to encourage high school students to pursue careers in neuroscience.

Professional Societies

2013 - present **Society for Neuroscience**
2017 - present **Society for the Teaching of Psychology**
2017 - present **American Association for the Advancement of Science**