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Neural correlates Underlying successful memory In the hippocampus

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ABSTRACT

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One of the most intriguing of the human brain's complex functions is the ability to store information provided by experience and to retrieve much of it at will. This capability of memory processing is critical to human's survival – that is, humans guide their actions based on a given stimulus (e.g., item) in an environment, and can do so even when the stimulus is no longer present owing to the memory of the stimulus.

A fundamental question of memory is why some experiences are remembered whereas others are forgotten. Since Scoville and Milner's characterization of patient H.M., who demonstrated severe recognition memory deficits following damage to the medial temporal lobe (MTL), the hippocampus has been extensively studied as one of the key neural substrates for memory. In line with this, several experiments have been conducted on exploring the roles of the

hippocampus in various ways. One is confirming the causality of the hippocampus in the memory process using direct electrical stimulation to the hippocampal region. The other is investigating the neural correlates of hippocampus using intracranial electroencephalography (iEEG) field potential and single neuron's action potential known as "spike" recorded directly from the hippocampus.

The present thesis is focused on providing direct electrophysiological evidence of human hippocampus in episodic memory that may help fill the gap that remained in the field for several years. Here, I will show how direct hippocampal stimulation affect human behavior and present characterized neural correlates of successful memory in the hippocampus.

In the first study, building on the previous findings on the hippocampus, I sought to address whether the hippocampus would show functional causality with memory tasks and elicit different neural characteristics depending on memory tasks applied. I found hippocampal stimulation modulated memory performance in a task-dependent manner, improving associative memory performance, while impairing item memory performance. These results of the task-specific memory modulation suggest that the associative task elicited stronger theta oscillations than the single-item task.

In the second study, I tested whether successful memory formation relies on the hippocampal neuronal activity that engaged preceding an event. I found that hippocampal pre-stimulus spiking activity (elicited by a cue presented just before a word) predicted subsequent memory. Stimulus activity during encoding (during-stimulus) also showed a trend of predicting subsequent memory but was simply a

continuation of pre-stimulus activity. These findings indicate that successful memory formation in human is predicted by a pre-stimulus activity and suggests that the preparatory mobilization of neural processes before encoding benefits episodic memory performance.

Throughout the study, the current finding suggests the possibility that the intervals of poor memory encoding can be identified even before the stimulus presented and may be rescued with targeted stimulation to the hippocampus even before the stimulus presented.

Keywords**:** hippocampus, episodic memory, brain stimulation, memory modulation, iEEG, single unit activity, pre-stimulus signal

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LIST OF ABBREVIATIONS

aHP, anterior hippocampus

AMY, amygdala

aTG, anterior temporal gyrus

BOLD, blood-oxygen level dependent (signal)

CA, Cornu ammonis

CAR, common average reference

CIP, caudal intraparietal area

CT, computerized tomography

DG, dentate gyrus

ECoG, electrocorticography

EEG, electroencephalography

ERC, entorhinal cortex

FCD, focal cortical dysplasia

FSIQ, full scale memory quotient

fMRI, functional magnetic resonance imaging

HF, hippocampal formation

HP, hippocampus

iEEG, intracranial electroencephalography

L, left

LFP, local field potential

LH, left hippocampus

ITG, inferior temporal gyrus

LTLE, left temporal lobe epilepsy

IQ, intelligence quotient

ISI, inter spike interval

LWM, limbic white matter

MTL, medial temporal lobe

mHP, mid-hippocampus

MR, magnetic resonance

MQ, memory quotient

OFC, orbitofrontal cortex

PDS, power spectral density

PHG, parahippocampal gyrus

PRC, perirhinal cortex

PSI, processing speed index

PRI, perceptual reasoning index

R, right

RA, right amygdala

RAVLT, Rey auditory verbal learning test

RH, right hippocampus

RT, reaction time

RTLE, right temporal lobe epilepsy

SD, standard deviation

SME, subsequent memory effects

SUA, single unit activity

STG, superior temporal gyrus

TLE, temporal lobe epilepsy

TP, temporal lobe

VCI, verbal comprehension index

VPA, verbal paired associates

WMS-IV, Wechsler memory scale fourth edition

WMI, working memory index

SECTION 1. INTRODUCTION

CHAPTER 1. Human Memory System

1.1 The hippocampus and memory

The medial temporal lobe (MTL) structures, encompassing the hippocampus and amygdala, as well as the neocortical regions of entorhinal cortex (ERC), perirhinal cortex (PRC), and parahippocampal cortex (PHC) are thought to play a crucial role in episodic memory and have been shown to play a role in different forms of learning and memory. Among these, the hippocampus, a structure within the temporal lobe, has been recognized as fundamental in the formation of episodic memory, in both semantic and episodic aspects.

In the study of human patient Henry Gustav Molaison, widely known as H.M. (1926-2008), showed that surgical resection of bilateral MTL to control his epileptic seizures caused memory deficits. The resection of MTL included hippocampal region of dentate gyrus, hippocampus, and subicular complex and induced moderate retrograde amnesia (a loss of memories prior to brain damage) and severe anterograde amnesia (a loss of the ability to form new memories) (Scoville and Milner, 1957). This Scoville and Milner's finding pointed to the hippocampus that is necessary for memory formation. Following this observation, several studies have been performed on other patients with hippocampal damage or in lesioned animal models, confirming the role of hippocampus in memory formation [see (Squire, 1992) for review].

1.2 The structure of the hippocampus

From an anatomical point of view, the hippocampus is folded and curved in a complex three-dimensional shape organized in three layers (archi-cortex). The entorhinal cortex, the major input and output structure of the hippocampal formation, possesses most of the afferent and efferent neurons. It transmits information to all hippocampal areas, and, in turn, receives signals back from them in a loop-circuit—the so-called trisynaptic organization of the hippocampus. This circuit begins with granule cells in the dentate gyrus that receive information from the entorhinal cortex via axons of the perforant pathway. These cells, then, make axonal projections via the mossy fiber pathway and synapse on pyramidal cells in the CA3 region. CA3 pyramidal cells then project via the Schaffer collateral pathway to synapse on pyramidal cells in the CA1 region, and these cells then project to the subiculum. All these fields are also interconnected with one another and to the entorhinal cortex, which makes projections to both the prefrontal cortex and hypothalamus (**Figure 1**).

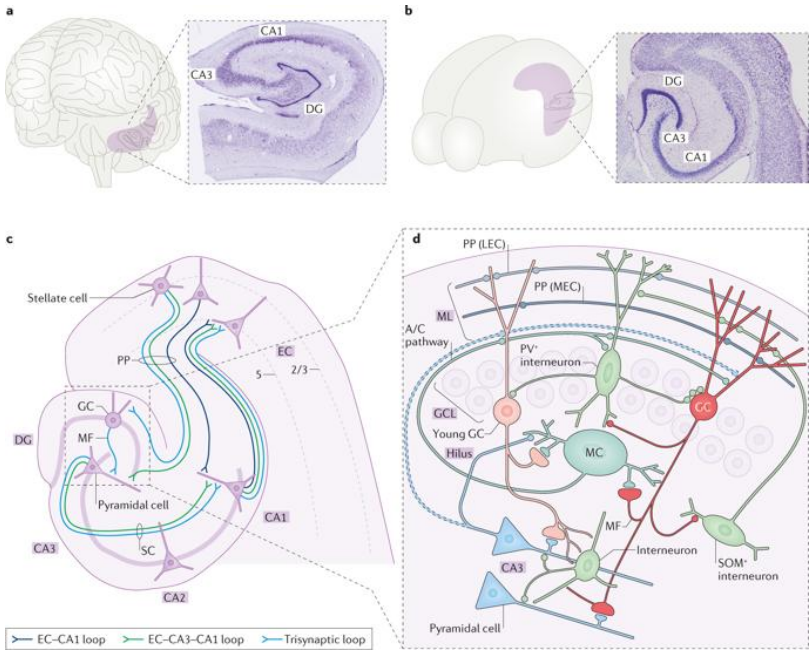

Figure 1. Anatomical organization of the hippocampus. A. Human brain and Nissl-stained section through the hippocampus. B. same as part for a mouse brain. C. Schematic of mouse hippocampal formation and its main synaptic connections. D. same as part for a human hippocampal formation. [Figure adapted from (Hainmueller and Bartos, 2020)]

CHAPTER 2. Human electrophysiological studies: *how to see a memory*

For decades, the role in episodic memory has been studied extensively in fMRI experiments [see (Eichenbaum et al., 2007) for review], but to date no mechanism has been identified to explain how neuronal ensembles enable encoding or retrieval of memory episodes. Consequently, one of the greatest challenges of modern neuroscience is to develop technologies that will be able to record electrical activity of population of neurons and individual neurons, both local and widespread activity of distributed but connected neuronal networks. These activities span a wide range of spatial and temporal scales. Beginning from action potentials of single neurons fired within a timeframe of 1 ms and ending on slow-wave oscillations generated by large-scale neuronal populations, there are several orders of magnitude separating the different levels of brain architecture and dynamics. These powerful technological innovations in human neuroscience in the past decades are enabling researchers to uncover fundamental rules about how individual memories form, organize and interact with each other.

2.1 Clinical rationale for invasive recordings with intracranial electrodes

Most human iEEG studies are conducted in patients with epilepsy. About 1/3 of epilepsy patients suffer from medication-resistant epilepsy (Kwan et al., 2011),

therefore they can gain seizure freedom if the source of their seizures can be identified and surgically removed (Engel, 1996). Prior to this, iEEG monitoring is often needed to identify the precise origin of seizures. Before the patient is implanted for invasive monitoring, clinicians use non-invasive diagnostic tools (e.g., Scalp-EEG, structural brain MRI, and brain FDG-PET) to measure about the approximate origin of patient's seizures. After preoperative evidence is strong enough to suggest laterality and lobar origin of seizure, either grids or depth electrode are placed over the suspected lobe or targeted deeper areas (e.g., hippocampus). To determine the source of the seizure, the patient is stayed to bed for several days (about 5-7 days) and during these days of monitoring and in this clinical setting, neuroscience experiments are conducted.

One of the main difficulty of the iEEG method is that it is only doable in clinical settings at few hospitals and by specially trained teams of clinicians and investigators. This introduces a significant limitation of accessibility. Furthermore, the experimental subjects suffer from a pathological condition. Clinical and hospital constraints do not permit experimental setups for sophisticated psychophysics measurements, and the experiments often suffer from low number of trials and simplicity of design. Moreover, the location of electrodes is decided clinically and once implanted in the operating room, cannot be changed - unlike animal recordings in which the investigator penetrate the cortex many times until the responsive neurons are found.

2.2 Human intracranial EEG

The first recordings of human electrical brain activity with electrodes attached to the scalp in patients with skull bone removed was performed by Hans Berger (1873-1941), inspired by the reports of electrical signals recorded directly from the brains of rabbits, cats, dogs, and monkeys (Mascari, 1988). Intracranial electroencephalography (EEG) is referred when recordings are obtained with intracranial electrodes either in the form of electrocorticography (ECoG) using strips or grids of electrodes implanted in the subdural space, or stereotaxic-EEG (sEEG) using wires of electrodes penetrating the brain and targeting deeper sites (e.g., hippocampus and amygdala) without open craniotomy.

Electrodes used in sEEG method are cylinder shaped with contact length of ~2mm, diameter of ~1mm, and total surface area of ~10mm^2 penetrating the cortical layers. By comparison, grid or strip electrodes used in ECoG are circular plates with a diameter of ~2mm and surface area of ~4mm^2 placed over the bare cortex. Thus, iEEG electrodes capture signals from a relatively large and diverse population of cells. Given the diameter of human iEEG electrodes and the known estimates of the number of neurons per cortical area, one may assume that there are ~500,000 cells underneath or surrounding these electrodes.

iEEG signals recorded from such a large and diverse population of cells is understandably complex and carry information in different bands of oscillatory activity (e.g., delta (1–3Hz), theta (4–7Hz), alpha (8–12Hz), beta (13–20Hz), and gamma (21 to ~40 or 50Hz)) as well as higher frequency activity (HFA) known as high-gamma activity (above 50Hz).

2.3 Single unit activity recording and spike sorting in human

In the 1950s, the first single-units recordings from the human cortex were performed during surgery for epilepsy (Ward and Thomas, 1955). In the early 1970s, this recording was performed by inserting micro-wires through the depth electrodes (Babb et al., 1973). The cornerstone of these recordings is the placement of electrodes in brain tissue to register the extracellular activity of neurons. In these recordings, the electrode is placed in the space between neurons, as opposed to other techniques such as patch clamp or intracellular recordings, in which the electrode is attached to one cell. The electrical potential changes measured at the electrode tip reflect current flows in the extracellular medium. The electrodes, platinum-iridium micro-wires (with a 40 μm width) inside the lumen of the probe containing the depth iEEG electrodes, which protrude 3-5 mm from the tip of the probe. The micro-wires have high impedance (300-3000 kΩ) and are suitable for recording action potentials and LFPs. The data I present in this study, both for illustrating single cell responses and for studying the stability of these responses, were collected using commercially available (Ad-Tech) depth electrodes, where each bundle had eight active micro-wires and one low-impedance micro-wire that was used as local reference.

Action potentials (spikes) detected from implanted micro-wires might come from several neurons close enough to the electrode tip (Quian Quiroga and Panzeri, 2009). The first step to process these recordings is to detect the spikes in the background noise (mainly generated by the activity of neurons further away), and to determine which spike corresponds to which neuron based on the spike shapes, a

process called spike sorting (Quiroga, 2012). For the data (Part II experimental study) presented here, spike detection and sorting was performed using *Wave_Clus* (Quiroga et al., 2004). Briefly, the steps typically involved in spike detection and sorting are: (i) band-pass filtering the data, for example between 300 and 3000 Hz, using a zero-phase filter to avoid distortion in the spike waveforms (Quiroga, 2009); (ii) computation of a detection threshold based on a robust estimate of the noise statistics; (iii) feature extraction of the spike shapes, in the case of *Wave_Clus* using the wavelet transform; and (iv) clustering of the waveforms to identify the firing of the different units.

2.4 Direct brain stimulation

Direct electrical stimulation (DBS) shows potential as a treatment for a variety of neurological conditions and as a tool for studying neuropsychiatric disorders and cognition. For years, direct electrical stimulation has been used to effectively treat motor disorders, such as Parkinson's disease, essential tremor, dystonia, and epileptic seizures (Benabid et al., 1987; Koller et al., 1997; Coubes et al., 2000; Fisher and Velasco, 2014), and have been extended to modulate brain circuits of neuropsychiatric and cognitive disorders such as Alzheimer's disease (Kuhn et al., 2015; Lozano et al., 2016). Additional experimental approaches have focused on patients with epilepsy undergoing intracranial monitoring, evaluating the effects of stimulation via depth electrodes on memory formation and recall (Ezzyat et al., 2017).

For the mechanism of DBS, DBS electrodes are surrounded by thousands of different axons, which can project to or from the area of implantation or simply pass by on their way to a completely different brain region. The electric field generated by DBS polarizes all the axons that are sufficiently close to the stimulating electrodes, resulting in action potentials traveling orthodromically and antidromically along the axon (McNeal, 1976). The axonal response to extracellular electrical stimulation may be dictated by 3 fundamental biophysical principles: (i) the variable axonal excitability with fiber diameter, (ii) the current-distance relationship, and (iii) the strength-duration relationship.

A better understanding of DBS mechanisms from physiological research is being translated into novel technical solutions. The variable effects may result from

different mechanisms, such as increased activity across specific regions. Further work is still needed to an effective stimulation strategy for developing more effective therapy.

CHATPER 3. Human Memory Research

3.1. Functional role of human intracranial oscillatory activity in successful memory mechanism

Fluctuations in postsynaptic potentials produce local oscillations. As neurons oscillate, the effectively open and close their window to both send and receive information. Neural oscillations have been linked to various cognitive phenomena in humans. There is not an exact mapping of oscillatory rhythms to specific cognitive processes. Instead, neural oscillations in a specific frequency range in one brain region may function in one cognitive process and in another cognitive process in another brain region. In addition, different neural oscillations can each function in a specific cognitive phenomenon. Several studies have reported that memory processing is modulated by specific brain oscillations such as theta and gamma activities (Klimesch, 1996).

3.1.1 Theta Oscillations

Theta oscillations were first discovered in the rabbit hippocampus in 1938 (Jung and Kornmüller, 1938), where they were found to occur both spontaneously and as a reaction to painful stimuli. A first link to memory was established when researchers followed up this discovery by showing that the duration of cortical theta oscillations recorded in rats after an aversive foot shock correlates with later memory for that foot shock (Landfield et al., 1972). Whereas early studies failed to identify a clear homologue of rodent theta in the human brain.

In particular, theta rhythm, traditionally defined in the 4-10 Hz range in rodent studies (Buzsaki and Moser, 2013), seems to correlate consistently with episodic memory (Klimesch, 1999), described as the ability to remember past experiences and autobiographical events (Tulving, 2002). Theta rhythm may be considered as the local field potential (LFP) that represents an electrophysiological marker of hippocampal activity. The link between theta oscillations and memory encoding in rodents has been unequivocally established through experiments demonstrating that pharmacological or surgical interruption of theta generation leads to deficits in memory formation (Fedor et al., 2010).

In humans, however, evidence supporting the importance of theta oscillations for memory encoding and retrieval have been somewhat mixed. Prior studies have shown variable evidence for theta oscillations during human episodic memory; some studies report memory-related theta increases while others report decreases. In many cases memory-related theta increases, and decreases were found in the same study, depending on when and where in the brain oscillatory power was examined. A key outstanding ambiguity regarding hippocampal theta oscillations in humans is whether there are functional differences between oscillations in the 2–4 Hz and the 4–10 Hz frequency ranges. Memory–related effects in the 2–4 Hz range have previously been reported during episodic memory encoding (Lega et al., 2012), spatial memory encoding and retrieval (Ekstrom et al., 2005), and recognition memory tasks (Rutishauser et al., 2010).

However, phase locking of single unit activity in the 4–10 Hz range has been reported to differ according to rated confidence in a recognition memory paradigm

(Rutishauser et al., 2015) and oscillatory differences in this band (such as phase reset and cross frequency coupling) may support working memory (Mormann et al., 2005). I separately analyzed activity in these two frequency bands seeking to elucidate whether these oscillations represent distinct phenomena by testing whether they exhibit different properties during associative memory formation and retrieval.

3.1.2 Gamma oscillations

High-frequency activity (HFA), a brain response with iEEG signals for episodic memory formation, provides spatiotemporal properties of memory encoding, with millisecond temporal resolutions. HFA refers to fast fluctuations in neuro-electrophysiological recordings that manifest as an increase in spectral power at frequencies above 60 Hz. The neural origin that gives rise to such fast activity is a topic of on-going research; HFA has been linked to asynchronous signals related to increased multi-unit activity (Manning et al., 2009; Milstein et al., 2009; Ray and Maunsell, 2011). An increasing number of studies have leveraged HFA as a marker of underlying neural activation (Lachaux et al., 2012), and thus HFA is regarded to represent categorized regional activation during memory encoding (Burke et al., 2014). Although HFA has been shown to be a potential biomarker for mapping, targeting, and modulating neuronal assemblies at a high temporal resolution during memory (Lachaux et al., 2012; Burke et al., 2015; Johnson and Knight, 2015), a complete description of the neural correlates of memory formation is yet to be provided.

3.2 Brain stimulation for memory enhancement

Researchers and clinicians have found that stimulation produces a wide range of memory-related behavioral effects. Cortical stimulation was first linked to memory in Wilder Penfield's pioneering studies where stimulating an awake patient's temporal lobe caused them to spontaneously recall old memories (Penfield and Perot, 1963). Penfield's subsequent work showed that the particular location that was stimulated greatly affected the way in which patients re-experienced old memories.

Following this discovery, many studies applied direct electrical stimulation to the temporal lobe using a variety of stimulation parameters. These studies were wide-ranging, emphasizing the complexity of precisely modulating human neuronal activity with stimulation (Selimbeyoglu and Parvizi, 2010). Some studies showed that stimulation impaired recall of complex scenes (Halgren et al., 1985), subsequent item recognition (Coleshill et al., 2004), spatial, and verbal memory recall (Lacruz et al., 2010; Jacobs et al., 2016). However, several studies have also shown improvements to verbal, visual, and spatial memory (Suthana et al., 2012; Fell et al., 2013; Miller et al., 2015; Ezzyat et al., 2017) including our own recent study (Jun et al., 2019).

In studies using brain stimulation to treat other neurological diseases also found inconsistent cognitive effects (Lang and Lozano, 1998a, b; Mayberg et al., 2005; Gutman et al., 2009). Hence, even though direct electrical stimulation holds potential to treat patients with neurological disorders who cannot be treated pharmacologically, understanding more fully how stimulation differentially affect neural activity is important for optimizing such therapies.

3.3 Single unit activity for predicting successful memory

In the late 1970s, the first studies on single-cell activity in the human MTL during memory tasks took place (Halgren et al., 1978). In the following years, several experiments to study different aspects of human cognition – including language, memory, perception, emotion – were developed (Fried et al., 1997) and in 2000, the presence of category-specific neurons (some responding only to faces, others to objects, others to animals, etc.) in the MTL was reported (Kreiman et al., 2000). Following these findings, neurons in the MTL with highly selective and invariant responses were found (Quiroga et al., 2005), and it was argued that these so-called 'concept cells' or 'Jennifer Aniston neurons' represent the meaning of the stimulus for declarative memory functions (Quiroga et al., 2008).

Recent findings using single unit activity recordings in the medial temporal lobe inform current neural network models of memory [see (Rutishauser, 2019) for review], and may lead to a more comprehensive understanding of the neural basis of memory-related processes. These recordings have shown that cells in the hippocampus appear to support declarative learning by distinguishing novel and familiar stimuli via changes in firing patterns. Some cells with highly selective and invariant responses have also been described, and these responses seem to represent abstract concepts such as identity, rather than superficial perceptual features of items. Importantly, however, both selective and globally responsive cells are capable of changing their preferred stimulus depending on the conscious demands of the task. Firing patterns of human medial temporal lobe neurons indicate that cells can be both plastic and stable in terms of the information that they code; although some cells show

highly selective and reproducible excitatory responses when presented with a familiar object, other cells change their receptive fields in line with changes in experience and the cognitive environment.

The idea that understanding comprehensive neural mechanisms supporting memory encoding is an essential precursor to any investigation of how the mechanism interact and will give an important implication for the interpretation of indirect brain investigation studies. However, there is limited research on the impact of how neuronal level activity reflect memory formation.

CHAPTER 4. Purpose of the Present Study

In this thesis, I took advantage of the relatively rare opportunity to record intracranially from humans to provide direct electrophysiological evidence of successful memory. Here, I explore hippocampal single unit activity (SUA) and iEEG field potential recorded from micro- and macro-electrode signals together with direct hippocampal stimulation to investigate the neural correlates in determining successful memory. As of this study, I expect, recordings from the neural population as well as individual neurons in patients have opened the possibility to narrow down the gap between neurophysiological studies in animals and non-invasive (e.g., fMRI) investigations in human memory system.

In chapter 1, I tested the functional causality between the hippocampus and episodic memory by directly stimulating the hippocampus. I simultaneously conducted iEEG recordings from the hippocampus while the patients were actively engaged the memory task to elucidate the neural correlates of successful memory. I hypothesized that the hippocampus would exhibit different oscillatory patterns from different memory tasks that may demonstrate differential behavioral effects with direct hippocampal stimulation. The research presented in chapter 1 has been published in Brain Stimulation (Jun et al., 2020).

In chapter 2, I provide further insight into characteristics of neuronal activity and distributed neuronal population in successful memory formation using hippocampal single unit activity (SUA) and iEEG field potentials, while patients were engaged in an episodic memory. Based on the previous finding that neural activity

during the time preceding stimulus presentation is sensitive to episodic memory performance, I hypothesized that successful memory formation is supported by neuronal preparation that precedes an event.

SECTION 2. EXPERIMENTAL STUDY

Chapter 5. The importance of the hippocampal oscillatory activity for successful memory:

Direct brain stimulation study

5.1 Abstract

Despite its potential to revolutionize the treatment of memory dysfunction, the efficacy of direct electrical hippocampal stimulation for memory performance has not yet been well characterized. One of the main challenges to cross-study comparison in this area of research is the diversity of the cognitive tasks used to measure memory performance. I hypothesized that the tasks that differentially engage the hippocampus may be differentially influenced by hippocampal stimulation and the behavioral effects would be related to the hippocampal oscillatory activity. To investigate this issue, I recorded intracranial EEG from and directly applied stimulation to the hippocampus of 10 epilepsy patients while they performed two different verbal memory tasks of a word pair associative memory task and a single item memory task.

Hippocampal stimulation modulated memory performance in a task-dependent manner, improving associative memory performance, while impairing item memory performance. In addition, subjects with poorer baseline cognitive function improved much more with stimulation. iEEG recordings from the hippocampus during non-stimulation encoding blocks revealed that the associative memory task elicited stronger theta oscillations than did item memory and that stronger theta power was related to memory performance. I show here for the first time that stimulation-induced associative memory enhancement was linked to increased theta power during retrieval. These results suggest that hippocampal stimulation enhances associative memory but not item memory because it engages

more hippocampal theta activity and that, in general, increasing hippocampal theta may provide a neural mechanism for successful memory enhancement.

5.2 Introduction

The hippocampus is a pivotal structure in episodic memory (Tulving and Markowitsch, 1998) and has been one of the main target structures of electrical brain stimulation aimed at manipulating the neural circuits underlying memory formation and, ultimately, at improving memory performance (Eichenbaum et al., 2007). Surprisingly, however, previous studies using direct hippocampal stimulation have broadly converged on the finding that stimulation has adverse effects (Lacruz et al., 2010; Jacobs et al., 2016; Goyal et al., 2018) or no effect (Suthana et al., 2012) in enhancing memory, with only a few having reported favorable effects (Berger et al., 2011; Hampson et al., 2012; Fell et al., 2013) including in our own recent study (Jun et al., 2019). These human and animal studies have underscored the causal role of the hippocampus in memory but raised many questions in the field regarding the factors that determine the efficacy of its stimulation.

One prominent theory of hippocampal function postulates that the hippocampus has a special role in relating or binding different attributes together to form memory for prior episodes into an integrated memory trace (Eichenbaum and Cohen, 2001; Battaglia et al., 2011; Marilina Mastrogiuseppe, 2019). As such, the hippocampus is often characterized as a hub of information, engaged to a greater extent for associations among memory elements than it is for individual elements. (Eichenbaum et al., 1994; Squire and Zola, 1998; Eichenbaum et al., 2007). Amnesic patients with selective hippocampal lesions exhibit greater impairment in item-item

associative memory than in memory for the items themselves (Kroll et al., 1996; Giovanello et al., 2003; Turriziani et al., 2004), and similar findings have also been reported in studies of primates (Zola-Morgan et al., 1986; Baxter and Murray, 2001) even within the same individual (Pascalis and Bachevalier, 1999). Underlying the differential behavioral effects could be a difference in hippocampal neuronal responsiveness in the two types of memory tasks employed (Kreiman et al., 2000). Indeed, neuroimaging studies demonstrated greater hippocampal activation during encoding of associative than item information (Brown and Aggleton, 2001; Giovanello et al., 2004) and claimed that the hippocampus preferentially contributes to associative memory (Giovanello et al., 2003; Mayes et al., 2004).

If the effect of applying direct electrical current to the hippocampus is dependent on its latent activity (Ezzyat et al., 2017), then it is possible that tasks that differentially engage the hippocampus (e.g., item vs. associative memory) may be differentially influenced by hippocampal stimulation. To explore this issue, the present study added patients with different memory task to compare the effect of direct hippocampal stimulation on a word pair associative memory task for which we previously reported positive stimulation effects (Jun et al., 2019) and a single word item memory task that was slightly modified from previous studies that reported negative effects of stimulation (Jacobs et al., 2016; Goyal et al., 2018).

More importantly, unlike our recent study, the present study aims to investigate the hippocampal oscillatory activity elicited by the task at hand. The relevance of the hippocampal theta rhythm on cognition is well-documented

(Rutishauser et al., 2010; Lega et al., 2012; Buzsaki and Moser, 2013), and past findings on human iEEG indicate that hippocampal theta rhythm enhances context-dependent retrieval of sequences (Hasselmo, 2005a, 2007). Thus, I hypothesized that associative memory and item memory would be differentially affected by hippocampal stimulation, and that stimulation-induced memory enhancement would be related to an increase in hippocampal theta power.

5.3 Materials and Methods

5.3.1 Patients

Ten intractable temporal lobe epilepsy patients [4 males and 6 females; average age, 30.5 ± 10.5 years; Memory Quotient (MQ) > 60]. Data were collected in a numerical order (**Table 1**) using the same methods at Seoul National University Hospital (Seoul, South Korea) had hippocampal depth electrodes implanted (**Figure 2A**) and then performed two different verbal memory tasks (i.e., word pair associative memory and single word item memory) with or without stimulation to the hippocampus. The local IRB approved the study protocol (H-1407-115-596) and all subjects provided written informed consent to participate in the present study.

5.3.2 Electrode localization

All electrodes were implanted for clinical purpose only. Electrodes (AdTech Medical Instrument Corporation, Racine, WI, USA) targeting medial temporal structures were depth electrodes (platinum, surface area of 0.059 cm^2, placed 6mm apart). Depending on clinical need, subdural grid electrodes were placed on the cortical surface (diameter of 4 mm, placed 10mmapart) with stainless steel contacts. Prior to electrode implantation, each patient underwent a magnetic resonance (MR) imaging in a Magnetom Trio, Magnetim Verio 3-tesla (Siemens, München, Germany) or Signa 1.5-tesla scanner (GE, Boston, MA, USA). Computed tomography (CT) images were recorded using a Somatom sensation device (64 eco; Siemens München, Germany). Additional MRI and CT scans were performed following electrode implantation.

Table 1. Demographics and clinical characteristics of patients

Subject	Demographics		Clinical characteristics				Stimulation parameter	
	Age	Sex	Seizure type	Pathology	Resection	Seizure onset	Anode	Cathode
Sub1	31	F	TLE	HP neuronal loss	aTG, aHP	STG	L.mHP	LWM
Sub2	55	F	TLE	DG dispersion	HP	PHG	L.mHP	LWM
Sub3	27	F	TLE	Temporal lobe FCD	ITG	TP, STG	L.mHP	LWM
Sub4	27	M	TLE	PHG reactive gliosis	PHG	aTG,	R.mHP	LWM
Sub5	28	F	TLE	FCD heteropia	PHG, AMY	AMY	R.mHP	LWM
Sub6	21	M	TLE	HP neuronal loss	AMY, PHG	OFC	R.mHP	LWM
Sub7	24	F	TLE	Temporal lobe FCD,	aTG, AMY	AMY	R.mHP	R. mHP
Sub8	25	F	TLE	left occipital lobe FCD	Occipital gyrus, HP	occipital lobe	L.mHP	LWM
Sub9	46	M	TLE	Temporal lobe FCD	aTG, AMY, aHP	temporal lobe	L.mHP	LWM
Sub10	20	M	TLE	AMY neuronal loss	aTG, AMY, HP	AMY	L.mHP	LWM

Abbreviations: R. = Right; L. = Left; HP = hippocampus; mHP = mid-hippocampus; aHP = anterior-hippocampus; AMY = amygdala; LWM = limbic white matter; PHG = parahippocampal gyrus; DG = dentate gyrus; aTG = anterior temporal gyrus; STG = superior temporal gyrus; ITG = inferior temporal gyrus; TP = temporal pole; TLE = temporal lobe epilepsy; FCD = Focal cortical dysplasia; OFC = orbitofrontal cortex

Subject demographic data are presented together with clinical observations from clinically identified seizure onset zones, and pathology in subjects who underwent corresponding surgery. Anode and cathode indicate brain regions of stimulation in each subject. In all subjects, the stimulation location was either the left or the right mid-hippocampus, the mean current was 2 mA, and the mean charge density was 360 $\mu C/cm^2$/phase.

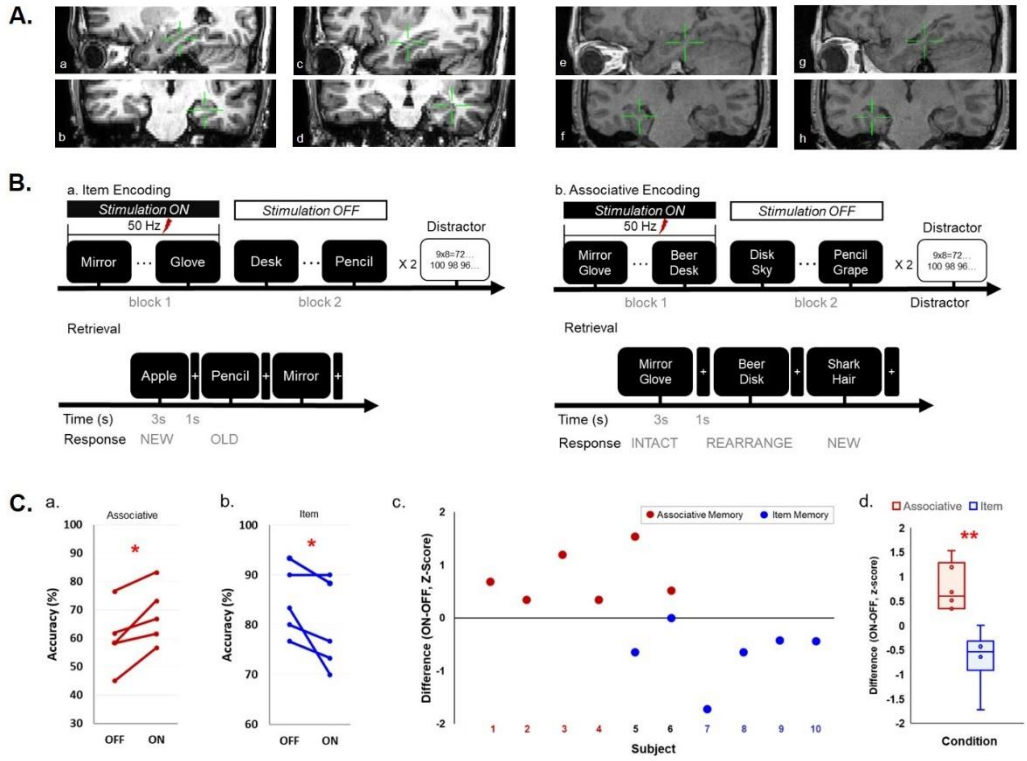

Figure 2. Location of contacts, memory paradigm, and behavioral results. A. Location of stimulation contacts in the medial temporal lobe. Preoperative high-resolution MR imaging co-registered with a postoperative CT scan (not pictured) showing the location of depth electrodes. The green crosshair denotes the location of the stimulation electrode in the right middle hippocampus in sagittal anode (a), sagittal cathode (b), coronal anode (c), and coronal cathode (d) sections in Subject 4. (e–h) The left middle hippocampus electrode in Subject 3. B. Paradigms of verbal memory tasks with stimulation. Example of the timeline of (a) word item memory and (b) a word pair associative paradigm. The 50 Hz stimulation was delivered in 5 s trains only at the encoding phase and was randomly assigned to two of four blocks. Lightning bolts denote periods when stimulation may be applied. C. Effects of stimulation on verbal memory performance. (a–b) The proportions of correctly

recognized words under the stimulation-off and -on conditions. Accuracy differences between the two conditions were significant across subjects (Wilcoxon signed-rank test, *p < .05). Colors denote associative task (red) and item task (blue). (c) The Z-score difference accuracy in between stimulation-on and -off in each individual subject. Note that Subjects 5 and 6 performed both tasks. (d) Mean difference accuracy across conditions for each task (Mann-Whitney *U* test, ** p < .01). Error bar indicates standard error of mean (SEM).

Target hippocampal electrodes for stimulation were inserted into the mid-body of the hippocampus gray matter using a temporo-lateral approach. A neuroradiologist identified each electrode contact using a thin section postimplant CT scan. The brain model and implanted electrodes were reconstructed from individual preoperative MR images and postoperative CT images using CURRY 7.0 (Compumedics Neuroscan, Charlotte, NC, USA). A neuroradiologist and neurosurgeon then confirmed the hippocampal electrodes within the medial temporal lobe (MTL). Each patient had at least one hippocampal electrode in the region of interest. The stimulation was applied between two adjacent contacts on the same depth electrode. Given that the electrodes within the MTL were 6 mm apart, the adjacent two stimulation target electrodes were identified as the hippocampal gray matter and in the temporal white matter and that the anode/cathode was assigned, accordingly.

5.3.3 Memory task

To assess the effect of stimulation on task-dependent memory, I asked each

subject to perform the two different verbal memory tasks, both of which are known to recruit the medial temporal lobe including the hippocampus during memory encoding (Axmacher et al., 2008), using STIM2 software (Compumedics Neuroscan, Victoria, Australia). Each memory task comprised three successive stages: encoding, distractor, and retrieval (**Figure 2B**). For word pair associative memory, six subjects completed sequentially 120-word pairs modified based on previous studies (Atri et al., 2004); the pairs consisted of two Korean concrete nouns with a mean frequency of 105.11 (SD = 3.35, IQR = 122.5). For word item memory, six subjects performed 120-word items of one concrete noun, randomly shown one at a time.

During encoding, a word pair was visible for 4 s, followed by a white fixation with a black screen of 1 s. To ensure a deep encoding, I used previously reported encoding feedback (de Vanssay-Maigne et al., 2011) in which participants were encouraged to respond by pressing with their index finger if they judged the appeared word on the screen as “pleasant” or “unpleasant”. Following the final word of the encoding block, subjects took a 10 min break and then performed a 30 s math distractor task consisting of a series of arithmetic problems for “A-B =?”, where A and B were randomly chosen integers ranging from 1 to 100.

In retrieval, a word pair associative test that was visible for 3 s, the subjects were asked to press one of three keyboard buttons as accurately and quickly as possible, depending on whether the word pairs had been presented before in the same pair (“intact”; button #1), whether the two words had been presented before but as parts of different pairs during encoding (“rearranged”; button #2) or whether both

words were all new (button #3). No words pairs appeared twice. In retrieval on the item task, subjects were to respond whether the word had been presented before ("old"; button #1) or was new ("new"; button #2) or the subject was not sure ("familiar"; button #3). For the main experimental session, no patients were exposed to the same experimental task more than once.

5.3.4 Brain stimulation

Stimulation was given only in the encoding phase by passing an electrical current between two adjacent electrodes using biphasic symmetric squared wave pulse of 300 ms per phase, at a frequency of 50 Hz, which was reported to have a positive effect on memory performance in past studies (Suthana et al., 2012). A Grass S12X stimulator (Natus, RI, USA) delivered a cycle of 5 s trains using 2.0 mA current equally in all subjects. Total energy was between 30 and 57 ($mC/cm^2/ph$) of charge per phase and square centimeter that was demonstrated to be safe and well tolerated in patients with epilepsy.

The encoding phase consisted of two sessions, with two blocks in each session. Stimulation was given during one of the two blocks in each session. During the stimulation-on block, the stimulator was active during the learning of a word (or a word pair), turned on or off for each trial. The stimulation was activated at the presentation of the word and lasted continuously for 5 s, extending until the following fixation: the stimulator was then inactive for the following word and fixation. The stimulation was randomized to occur during one of the two blocks in each session.

Note that for the present study, I compared stimulation effects between the stimulation-on and stimulation-off blocks.

5.3.5 Neuropsychological memory test

Neuropsychological assessments were conducted on all subjects before surgery (within one month) as part of routine clinical practice (Shin et al., 2009). I measured the Memory Quotient (MQ) using the verbal immediate and delayed recall subtests from the Korean version of the Rey auditory verbal learning test (RAVLT). The RAVLT requires immediate recall a list of 15 words presented audibly at intervals of 1 s, and this procedure is repeated five times (verbal immediate recall) after 20 min' recall for the list of words (verbal delayed recall). I measured WMS word associative memory using the verbal paired associates subtest from the Wechsler memory scale fourth Edition (WMS-IV). The WMS requires that patients learn seven pairs of unrelated words presented audibly and then listen to the first word of each pair and recall immediately the other word in the pair (verbal paired associates immediate, VPA1). After 30 min, the first word of the pair was presented and then the patient was required to recall the other word in the pair (verbal paired associates delayed, VPA2). For the purpose of revealing the relationship between individual memory capacity and the memory performance with hippocampal stimulation, I used the subjects' FSIQ, MQ, and WMS word associative memory in this study. Details of neuropsychological test scores are presented in **Table 2.**

Table 2. Results of neuropsychological memory test of patients

Subjects	Neuropsychological memory test		
	Full Scale IQ	MQ	WMS word associative memory
Sub1	91	90	11
Sub2	77	94	30
Sub3	78	81	12
Sub4	97	112	18
Sub5	85	111	9
Sub6	110	60	22
Sub7	57	66	5
Sub8	65	74	10
Sub9	97	89	17
Sub10	101	79	24
Average	85.8(15.8)	86.2(20.2)	15.8(7.4)

Data presented as mean (SD).

Subject pre-operative neuropsychological results. A clinical psychologist employed the Wechsler Adult Intelligence Scale-Korean version (K-WAIS-IV) for Full Scale Intelligence Quotient (IQ). The Rey-Kim Memory test was used to assess Memory Quotient (MQ) and the Wechsler Memory Scale (WMS) IV was used for word associative memory.

5.3.6 Analysis of memory performance and electrophysiological data

Behavioral data were analyzed with SPSS 23 (IBM, Armonk, NY, USA). I quantified the stimulation-on memory performance in this task by computing the proportion of learned words that were successfully recognized during stimulation-on versus those words learned during stimulation-off. To test whether items learned during stimulation were remembered more accurately than items learned without stimulation, I compared the accuracy of memory scores between stimulation-on and stimulation-off trials within blocks; I used the Wilcoxon signed-rank test and assessed the statistical significance of changes, and then compared memory differences with neuropsychological memory scores using the Spearman's rank correlation analysis with bootstrap confidence intervals calculated using 1000 resamples with a significance level of 95 %.

Intracranial EEG data including depth and ECoG were recorded using a 128-channel digital video monitoring system (Telefactor Beehive Horizon with an AURA LTM 64- & 128- channel amplifier system) digitized at a sampling rate of 1600 Hz. The impedance of the electrodes was between 0.3 and 1 kΩ when implanted. Analyses of intracranial EEG focused on oscillations in iEEG of field potentials recorded from the hippocampus in two patients who performed both the word pair associative and word item memory tasks. Main interests were whether oscillatory activity in the iEEG of field potentials would differ between the two task conditions and further testing the characteristics in successfully recognized words.

To this end, iEEG data were recorded during experimental testing from the

same targeted hippocampal electrodes for stimulation. Given the electrical stimulation produced substantial electrical artifacts in the recording channels, and volume conduction effects in nearby channels, iEEG data were not recorded when stimulation was given. I investigated the neural mechanism underlying the effects of hippocampal stimulation-on by analyzing iEEG data from memory retrieval as well as stimulation-off phase during encoding in the two subjects who performed both the tasks.

Analyses of iEEG data were conducted with MATLAB (Mathworks, Natick, MA, USA). Prior to data processing, all channels clinically identified within the ictogenic zone, or those electrodes observed as corrupt during recordings, were excluded from all data analysis. Electrodes were also excluded from subsequent analyses if there were any motion artifacts. All data preprocessing was performed at a single electrode level. For each subject, all non-excluded electrodes were first digitally filtered with a low-pass filter of 100 Hz. To attenuate 60 Hz line noise, a notch filter at 60 Hz was applied. The recorded data were then re-referenced to the common average reference (CAR).

To quantify specific changes in different frequency ranges in the hippocampal gray matter with a continuous time complex value representation of the signal, I conducted a time-frequency analysis. I performed spectral decomposition (1 frequency from 1 to 10 Hz, 2 frequencies from 10 to 20 Hz, and 4 frequencies from 30 to 100 Hz, logarithmically spaced; Morlet wavelets; wave number= 2.48) for the 0-4 s epoch relative to word onset in the encoding phase and 0-3 s epoch relative to word onset in the retrieval phase. Mirrored buffers (length = 2 s) were included before

and after the interval of interest and then discarded to avoid convolution edge effects. Transformed single trial data were squared for calculating power and then normalized by the mean and standard deviation of the baseline power (-1 to 0 s of word presentation onset) of each frequency.

To test the significance between stimulation-on versus stimulation- off during memory retrieval phase, I extracted t-values using the means and SDs with the independent two sample *t*-test. For visualization of time frequency map, epoched single trials were averaged across all trials. Then, to investigate the theta power changes in the hippocampus, I analyzed the signal across three different frequency ranges that have been implicated in episodic memory and plotted time series data with averaged t-value in each of the three frequency bands. To obtain hippocampal power during the encoding phase, I performed the same procedure and same spectral decomposition method described above.

For statistical analysis, trials were split into 2 groups based on whether the stimulus was associative memory or item memory. The averaged t-value across the presentation for stimuli (0-3 s) in all trials of each three frequency bands was compared between associative memory and item memory. This result was then used to perform group-level comparison in each of 6 subjects (**Figure 3C**). For the analysis of encoding phase, I performed the same procedure and same spectral decomposition methods described above. Note that the independent two sample t-test for encoding phase was conducted with all trials between two different memory tasks within subject (**Figure 4C and D** right panel). For power spectral density (PSD) analysis,

analyses for each patient, and state were calculated separately in MATLAB. PSD analysis used the Welch method (*pwelch* function in MATLAB with a 512 ms window, 256 ms of overlap, see Fig. 3A for an example of PSD in Subject 5). I determined significance using nonparametric statistics that controlled for multiple comparisons (Maris and Oostenveld, 2007).

5.4 Results

5.4.1 Hippocampal stimulation improves associative memory but impairs item memory

I firstly examined how subjects performed two different memory tasks while they were given the stimulation in their hippocampus. In this study, 10 subjects including two within-subjects with implanted electrodes performed two different verbal memory tasks while hippocampal stimulation was applied during some encoding trials (**Figure 2A**). I designed these tasks specifically to assess the differential effects of electrical stimulation in the hippocampus on memory encoding (**Figure 2B**).

I assessed the effect of stimulation on memory by examining behavior in the subsequent recognition phase of each task. In the item task, I defined successful memory as correctly identifying old items as "old". The mean percentage of correct responses across all trials was 83.6 ± 7.3 %. I defined successful memory in the associative task as the combined accuracy in the "intact" and "rearranged" trials because in order to correctly identify a pair as "rearranged", the subject must not only recognize that the words are all familiar but, they also recognize that the words are not in the correct pairing arrangement. The mean percentage of correct responses on the associative task was 63.5 ± 9.8 %. Note that the behavioral result of the associative task is the same as in our recent study (Jun et al., 2019).

I then assessed individual memory performance for the two memory tasks on

stimulation-on blocks compared with stimulation-off blocks and conducted nonparametric statistical within-subject's comparison of mean accuracy between stimulation-on and stimulation-off trials to measure significance (**Figure 2C**). For associative memory, the average accuracy in the six subjects improved significantly with hippocampal stimulation (off = 59.3 ± 10.1 %; on = 67.3 ± 9.7 %; Wilcoxon non-parametric paired t test, df = 5, p = 0.027). In contrast, for item memory, the average accuracy in the six subjects was significantly lower with hippocampal stimulation (off = 86.1 ± 6.5 %; on = 81.1 ± 8.0 %; Wilcoxon non-parametric paired t test, df = 5, p = 0.042).

Crucially, I conducted a comparison analysis of the effect of stimulation (i.e., the difference scores for stimulation-off and -on transformed to Z-scores) across the two conditions and found + 0.77 ± 0.45 for associative memory, but - 0.65 ± 0.53 for item memory (Mann-Whitney U test, Z = -2.9, p = 0.004); this statistically significant difference confirmed a task-dependent effect of stimulation.

5.4.2 Stimulation-induced memory enhancement is reflected in increased theta power during retrieval

I next sought to identify neural correlates of the observed memory enhancement in the hippocampus during memory retrieval. In this analysis, I used neural oscillations during the 3 s of stimulus presentation during retrieval trials to investigate how prior hippocampal stimulation during the encoding phase affected

subsequent hippocampal oscillatory activity during retrieval. I first looked at two subjects who performed both tasks for within-subject comparisons. **Figure 3A** shows the baseline normalized spectral power in the hippocampus for both subjects, calculated by the oscillatory activity difference between correctly remembered words in the stimulation-on and stimulation-off conditions in both the associative and the item memory tasks.

The overall results exhibited that oscillatory power in the theta range (2-10 Hz) increased significantly in the associative memory task but not in the item memory task. Successful recognition in associative memory but not in item memory exhibited increased power in the theta frequency range for remembered words previously followed by stimulation compared with remembered words without stimulation (two-sample t-test, $p < .01$).

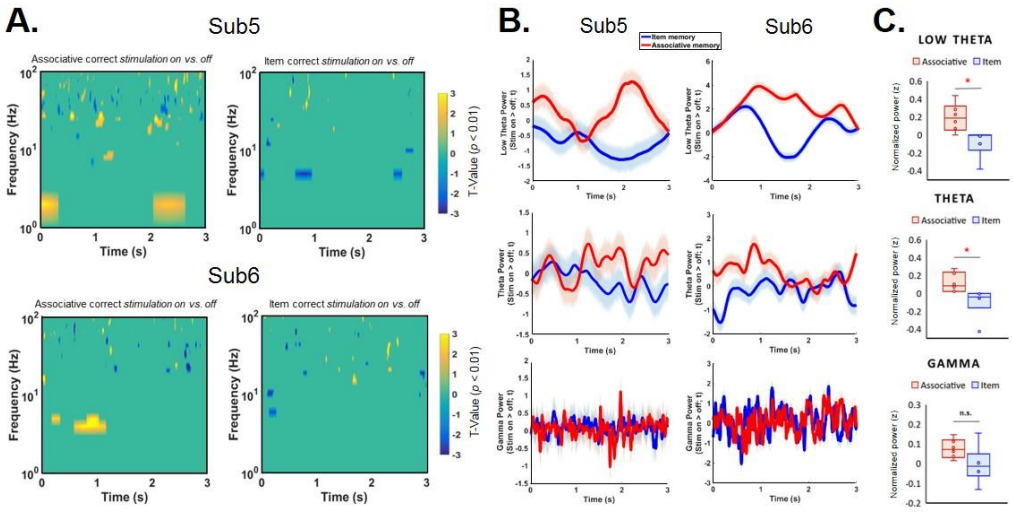

Figure 3. Memory effect in the hippocampus during retrieval. A. Individual within-subject time-frequency maps for Subjects 5 and Subject 6; a mean difference is shown in normalized power in correctly recognized trials between stimulation-on and -off during encoding. Baseline normalized power changes at each of 10 log-spaced frequencies between 1 and 100 Hz during the associative memory (left) and item memory (right) tasks in each subject. Colored regions indicate significance at $p < .01$ resulting from two-sample t-test comparing t-valued power. B. hippocampal time courses of 2–4 Hz (low theta), 4–10 Hz (theta), and 30–100 Hz (gamma) power during word presentation. C. Group-level comparison of baseline normalized theta power changes across six patients in each task. The results showed significant differences in the low theta and theta ranges but not in the gamma range (Mann-Whitney U test, $p < .05$ adjusted p value with post-hoc, $n = 6$).

I next analyzed the signal across three frequency ranges that have been previously implicated in episodic memory (Hasselmo, 2005b; Nyhus and Curran, 2010; Buzsaki and Moser, 2013; Watrous et al., 2013; Jacobs, 2014; Goyal et al., 2018; Lee et al., 2018), 2-4 Hz ("low theta" or "delta"), 4-10 ("theta"), and 30-100 Hz ("gamma"). I averaged the oscillatory power in each of the frequency bands and plotted during the retrieval period (0-3s) in which the word stimulus was visible on the screen. Associative and item memory showed a significant power difference in the low theta and theta ranges but not in the gamma range (two-sample t-test, $p < .01$, **Figure 3B**).

In addition, I also found that the memory effect in each of the other subjects who performed the associative memory task showed significant theta power increase but that did not occur with the item memory task (**Figure 4**). Group-level theta power

comparison between the associative and item memory tasks, after correction for multiple comparisons, confirmed significance difference (Mann-Whitney *U* test, 2 - 4 Hz: Z = -2.69, p = 0.004; 4 - 10 Hz: Z = -2.88, p = 0.002; 30 - 100 Hz: Z = -1.92, p = 0.065, adjusted p value with post-hoc, n = 6, **Figure 3C**). These results indicate that hippocampal stimulation during associative encoding may promote subsequent memory retrieval by influencing theta activity in the hippocampus, which is important for memory association.

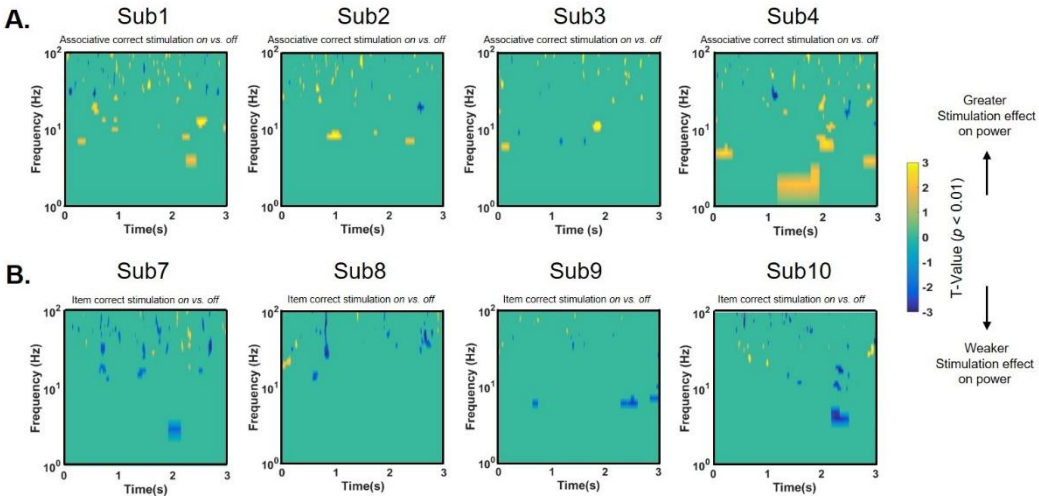

Figure 4. Memory enhancement during retrieval. Illustration of memory effect in hippocampus during retrieval. Time-frequency spectrograms show mean differences in normalized power of correctly recognized trials between stimulation-on and -off conditions during encoding. Changes in power are shown as log-spaced frequencies between 1 and 100 Hz from 0 s to 3 s. The color indicates significant cluster of changes in power resulting from t-statistics (two-sample t-test, $p < .01$). A. Individual subjects who performed the associative memory task. B. Individual subjects who performed the item memory task.

5.4.3 Associative memory elicits higher theta power than item memory during encoding

As I started with the assumption that in our study based on past studies, the associative memory would elicit higher theta activity than would item memory. Although the site of stimulation and patient characteristics (such as sex and cognitive ability) were largely matched across the two conditions, it is possible that the difference across the two task conditions could be due to a factor other than task. Because two of our subjects (Subject 5 and 6) participated in both tasks, I was able to make a closer, within subject comparison between neural activities during the two tasks for these subjects.

To address the hypothesis that neural signals during each task may correlate with the variations in the behavioral outcomes of hippocampal stimulation, I analyzed the brain activity in the stimulation-off trials during encoding. First, to determine whether there were reliable differences in hippocampal activity between the two tasks, I calculated changes in power during encoding relative to the pre-trial baseline, in which the patients were gazing at the white fixation cross. In general, the trend across the subjects' data showed that the theta range (2-10 Hz) power were significantly elevated for the associative task, but not for the item task (**Figure 5A and 5B**).

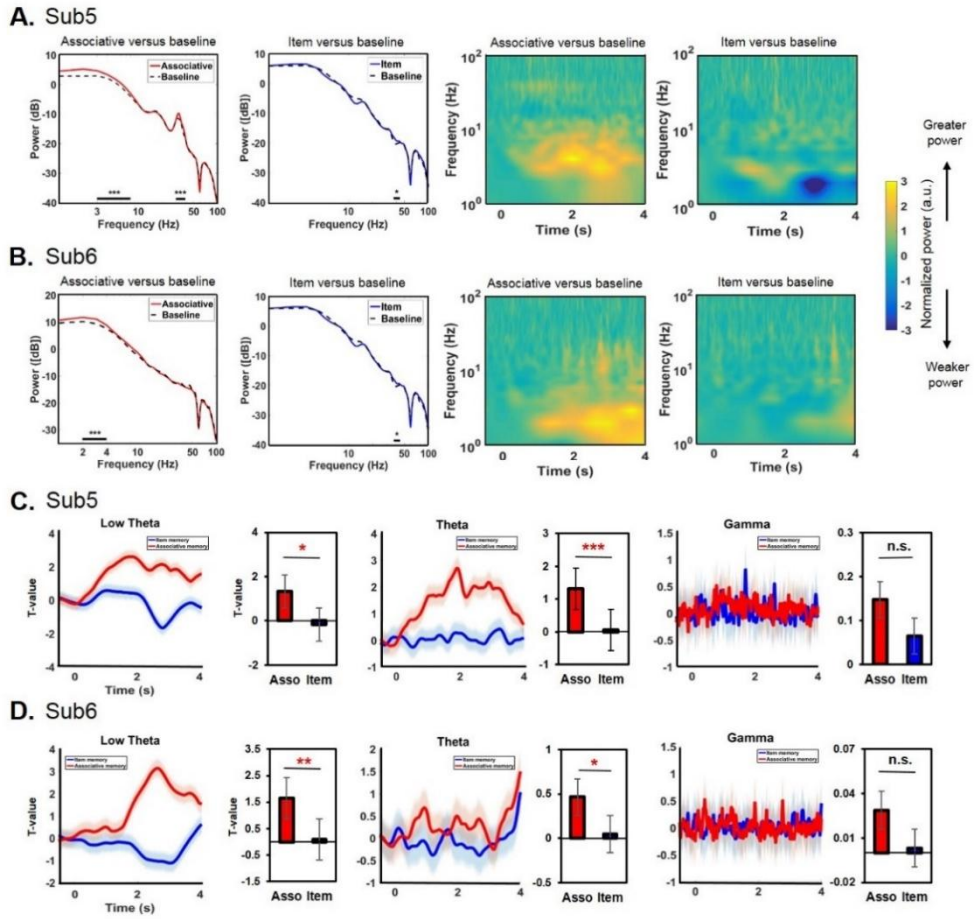

Figure 5. Neural evidence of verbal memory encoding. A-B. The left two panels show frequency spectrograms for the associative memory (red) and item memory (blue) tasks, respectively, for Subjects 5 and 6, showing mean difference in power between task and baseline (*p < .05, ***p < .001). The right two panels indicate baselined power changes shown at each of 10 log-spaced frequencies between 1 and 100 Hz for the associative memory and item memory tasks. C-D. Memory-related oscillatory activity of verbal memory encoding. Time courses of 2–4 Hz, 4–10 Hz, and 30–100 Hz power in the hippocampus during word presentation for the two subjects who performed both tasks (Subject 5 and Subject 6, respectively). All power values are baseline normalized to the pre-stimulus baseline. Shaded error regions are

± 1 within-subject SEM. The bar represents averaged power during item presentation, shown with 95% confidence intervals (*p < .05, **p < .01, ***p < .001). n.s. indicates not significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

I also calculated the power in each of three bands and averaged across the 0-4 s of the encoding period during which the word stimulus was visible on screen. There was a significant increase in power following the onset of stimuli in the low theta and theta but not the gamma range on the associative task, but not on the item task (two-sample t-test, Subject 5, 2 - 4 Hz: $t(98) = 1.67$, $p = 0.04$; 4 - 10 Hz: $t(98) = 3.51$, $p = 0.001$; 30-100 Hz: $t(98) = 1.07$, $p = 0.29$, Figure 3C; Subject 6, 2 - 4 Hz: $t(73) = 3.17$, $p = 0.002$; 4 - 10 Hz: $t(73) = 2.03$, $p = 0.04$; 30-100 Hz: $t(73) = 0.51$, $p = 0.61$, Fig. 3D). Taken together, these results demonstrate a fundamental difference in hippocampal oscillatory activity between the two tasks.

5.4.4 Successful memory encoding elicits higher theta power in both memory task

Past studies of verbal item memory (Sederberg et al., 2003; Lega et al., 2012) reported that hippocampal theta power during memory encoding was higher for subsequently remembered trials than for forgotten trials (a positive subsequent memory effect). **Figure 6** shows the subsequent memory effect in each of our subjects, labeled according to whether the learned stimuli were correct or incorrect. Replicating past studies, I found that correct items in both the associative and item memory tasks

showed higher theta power during encoding (both two-sample *t*-tests, $p < .05$). These results add to the existing literature that successful memory encoding in the hippocampus is positively correlated with the strength of theta oscillations.

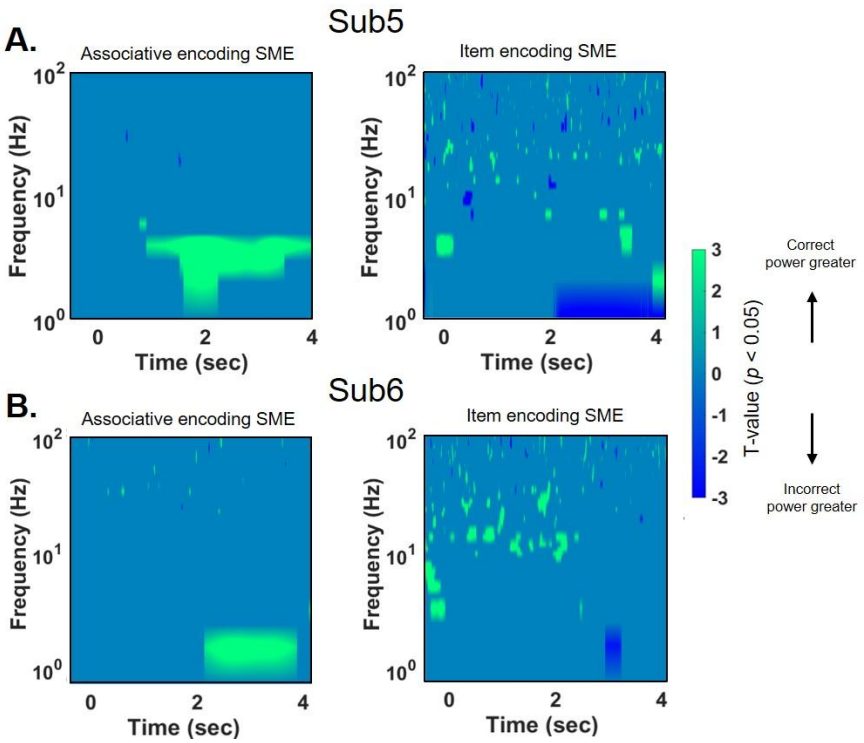

Figure 6. Illustration of subsequent memory effects (SME) in the hippocampus during the associative and item memory tasks. To identify the neural signatures of successful memory encoding, the time-frequency map shows mean differences in normalized power between correct and incorrect stimuli in each memory task (A: Subject 5, associative and item memory task, respectively, B: Subject 6). Changes in the right panel of time-frequency map represent the critical t for significance (two-sample t-test, $p < .05$).

5.4.5 Stimulation-mediated memory effect is greater in subject with poorer baseline cognitive function

I further considered the possibility that stimulation effects could be related to baseline memory function, and thus analyzed the correlations between the hippocampal-mediated memory effect and baseline cognitive capacity including memory in all patients (**Figure 7**). Overall, the patients with poorer baseline cognitive performance tended to improve much more with stimulation on the associative memory during retrieval. Conversely, the patient with higher baseline cognitive performance tended to show the greater stimulation-mediated impairment in item memory.

Across all subjects, the magnitude of the associative memory enhancement showed negative correlations with three different baseline neuropsychological performance, showing that only the correlation with WMS associative memory task presented as significance. (Spearman's rho, Full-Scale IQ, associative; $r(6) = -.145$ $p = .784$; MQ, associative: $r(6) = -.203$, $p = .7$; WMS word associative memory, associative: $r(6) = -.841$, $p = .036$). On the contrary, although the correlation with MQ did not present as significant (item: $r(6) = -.116$, $p = .827$), the memory impairment for item memory showed significantly positive correlations with baseline performance measures (Full-Scale IQ, item: $r(6) = .928$, $p = .008$; WMS word associative memory, item: $r(6) = .812$, $p = .05$).

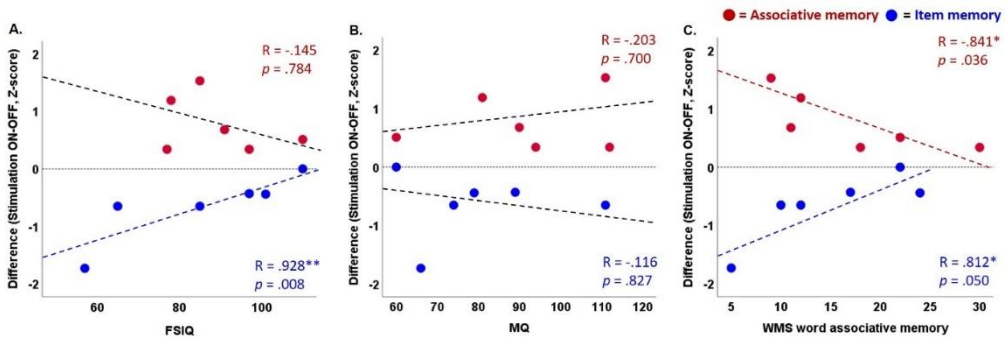

Figure 7. Correlation coefficient between the hippocampal stimulation mediated memory effect and baseline cognitive capacity. A. Full-Scale Intelligence Quotient (IQ), B. Memory Quotient (MQ), and C. WMS word associative memory were selected because they relate to the verbal memory task used in the study. The patients with worst baseline cognitive performance tended to show the greatest stimulation-mediated improvements in associative recognition and the greatest stimulation-mediated impairment in item recognition (Spearman's *rho*, *p < .05, **p < .01).

5.5 Discussion

5.5.1 Summary

With the present study, I demonstrated that direct 50 Hz electrical stimulation of the human hippocampus improved a word pair associative memory but impaired single-item memory. The task-specific memory modulation may be related to the fact that the associative task elicited stronger theta oscillations than the single-item task. During retrieval, memory enhancement was accompanied by neural oscillations that reflected increased theta activity in the hippocampus. Altogether, these findings indicate that cognitive effects of brain stimulation are dependent on the tasks employed and suggest that theta oscillations may provide a mechanism by which hippocampal stimulation enhances memory performance.

The present study provides for the first-time direct evidence of task specificity on the efficacy of direct hippocampal stimulation on memory in humans and demonstrates that theta activity is linked to this stimulation-induced memory enhancement. This finding extends prior non-invasive stimulation studies that implied the specific role of the hippocampus on associative memory (Wang et al., 2014; Matzen et al., 2015; Wang and Voss, 2015), and the selective stimulation influence on associative memory success in contrast with item memory (Tambini et al., 2018). Notably, our data reveal that hippocampal stimulation specifically influenced theta-dependent task in the hippocampus, and that this task-dependent neural activity associated with memory enhancement was observed even within the same subjects.

5.5.2 Task-dependent effects of hippocampal stimulation on memory

The behavioral finding of impaired item memory by stimulation corresponds with prior findings of observed item memory impairment by hippocampal stimulation (Jacobs et al., 2016; Goyal et al., 2018). Interestingly, however, I also found enhancement in associative memory (Jun et al., 2019). There are several differences, not necessarily mutually exclusive, that may underlie these behavioral discrepancies. One potential explanation is that the brain's encoding state could have an impact on the behavioral outcome of the stimulation. That is, the memory tasks that recruit different neuronal processes may be differently affected by stimulation through the activation of different neuronal pathways (Silvanto and Cattaneo, 2017).

A robust body of previous evidence indicates that the hippocampus supports encoding associative or relational information whereas item memory can be supported by extra hippocampal structures (Davachi, 2006; Diana et al., 2007; Mayes et al., 2007; Battaglia et al., 2011; Olsen et al., 2012). Indeed, the preferential engagement of the hippocampus for associative memory rather than item memory (i.e., non-associative memory) has been found in humans at the level of single hippocampal neurons in a recent study (Staresina et al., 2019), which reported elevated hippocampal firing selectively during successful associative memory retrieval.

In fact, I showed in the behavioral results a selective influence on a word pair associative memory task compared with single-item memory. I further confirmed that the neural activation levels differed depending on the tasks applied even within the

same individuals during memory encoding (**Figure 3**), indicating that the stimulation generated differing behavioral effects depending on its targeted neuronal activities.

This reliance of stimulation effect on different brain activity is in line with the MTL single unit activity in primates: A small difference in the specific neuronal population of the MTL could produce opposite behavioral effects through stimulating certain stimulus (i.e., task) selective neurons (Tamura et al., 2017). In similar reasoning, prior human iEEG study explored brain's encoding state-dependent modulation, showing that the effect of the stimulation on brain function depend on the state of neural activity at the time the stimulation is applied (Ezzyat et al., 2017).

This study estimated, unlike in the present study for which I focused on local hippocampal activity, global brain encoding states derived from whole-brain patterns of neural activity and showed that decoding the latent brain states can improve the chances of influencing memory outcomes through stimulation methods. On a related note, a non-invasive transcranial magnetic stimulation (TMS) study exhibited that the effect of stimulation is strongly reliant on the state of the stimulated region (Silvanto et al., 2008), suggesting that the difference in excitability of neurons could have a critical role in determining the behavioral outcomes of stimulation (Silvanto and Cattaneo, 2017).

Besides the differences in hippocampal oscillatory activity, the effects of stimulation could be sensitive to several stimulation parameters. In the present study, stimulation characteristics of phase, frequency and pulse width were similar to those in previous studies (Suthana et al., 2012; Jacobs et al., 2016); however, there were

still minor differences in factors such as stimulation sites, amplitudes, and duration. The stimulation sites in our study (i.e., cathodes) were located in temporal white matter together with the hippocampus gray matter; hence I hypothesize that the net effect of stimulation was to increase the activation of neurons projecting from the site of stimulation that preferentially mediated axons rather than the cell bodies (Perlmutter and Mink, 2006). Accordingly, this may have driven hippocampal activity by eliciting excitatory responses upon electrical stimulation (Ranck, 1975).

In addition to the stimulation site, our protocol was of slightly higher stimulation amplitude and of longer stimulation duration, which could have increased the total energy delivered to the tissues (Moro et al., 2002). Previous studies on animal and human deep brain stimulation exhibited that brain structures respond differently to stimulation parameters (Hamani and Temel, 2012; Hescham et al., 2013). Thus, setting precise parameters is an important factor for consistent effects of brain stimulation (Hamani et al., 2010).

5.5.3 Theta activity as a neural signature for memory enhancement

In iEEG data, theta activity increased only during associative memory and during successful memory encoding of item memory. The findings are consistent with those from several prior neuroimaging studies, suggesting more activity in the hippocampus for associative than for item memory (Davachi and Wagner, 2002; Giovanello et al., 2003; Jackson and Schacter, 2004; Kirwan and Stark, 2004) and increased theta activity during encoding for successfully remembered memory (Molle

et al., 2002; Sederberg et al., 2003).

Could the increase in theta power for associative retrieval be a result of excitatory activity by stimulation, resulting in subsequent memory improvement? As aforementioned, stimulation's effect on physiology depends on the excitability of the targeted neuron (Pollen, 1977); therefore, cognitive effects of stimulation could be modulated by the ongoing neural activity at the time (Ezzyat et al., 2017). Particularly, hippocampal stimulation could alternately incur either long-term potentiation or depression depending on whether the theta phase is at the peak or at trough at the time of stimulation delivery (Pavlides et al., 1988; Hyman et al., 2003). As such, stimulating the hippocampus may respect intrinsic brain states (i.e., theta activity) and dynamics, accordingly, suggesting that the hippocampal theta oscillations may play a role in stimulation-induced memory enhancement.

In the context of our study, I might speculate that the higher theta power in associative memory, which is generally associated with better memory performance, reflects a high level of hippocampal engagement in memory encoding; consequently, electrical stimulation when the brain is active in this manner might have induced positive stimulation effects on memory performance.

5.5.4 Clinical implications

Patients with epilepsy often exhibit cognitive deficits as a consequence of chronic seizures, antiepileptic medications, and associated neural dysfunction (Jacobs et al., 2016; Ezzyat et al., 2017). Thus, an important question was whether the benefit

of hippocampal stimulation was attenuated in patients with poorer baseline cognitive function. I found that the patients with poorer baseline cognitive performance tended to improve in memory much more with stimulation, while the enhancement effect was more limited in patients with higher baseline cognitive scores. This implies that those who have poor cognitive function and therefore need help might benefit the most from brain stimulation.

The neuropsychological test in which the patterns of test scores illustrate profiles of cognitive strength and weakness (Lezak, 1995) was designed to examine a variety of cognitive abilities. Because the tests I referred to cover verbal item memory, verbal associative memory, or general Intelligence Quotient (IQ; see Materials and Methods, Neuropsychological test), the correlations between the neuropsychological test and the effects of stimulation on memory performance may reflect the task specificity of the two different verbal memory tasks. For instance, the result was that the effect of stimulation on item memory was positively correlated with the Full-Scale Intelligence Quotient (FSIQ) score. In contrast, the stimulation effect on associative memory was negatively correlated with the Korean Wechsler Memory Scale (K-WMS) associative memory scores. Given the differential task characteristics, it seems rather obvious that these differences in correlation are apparent, and in fact, the FSIQ score reflect the attributes of verbal item memory function rather than associative memory function.

Addressing these issues can potentially translate into clinical practice, as the finding that electrical stimulation in the hippocampus might provide a hint regarding

why some patients with bilateral hippocampal lesion showed worse performance on the associative memory task than with item memory (Gold et al., 2006) and why some patients who undergo surgical removal of this region have associative verbal memory deficits.

5.5.5 Limitations

As in any study examining the effects of direct electrical stimulation in patients undergoing intracranial electrode monitoring, this study necessarily included patients with intractable epilepsy. Therefore, because of clinical constraints across patients including idiosyncratic variables (e.g., seizure locus, etiology, specific location of stimulation electrode), the present study raises several technical considerations. First, since the electrodes were implanted only for clinical purpose as part of pre-surgical evaluation for drug-resistant seizure, our study did not directly test a control region for stimulation. However, the selective modulation effect of hippocampal stimulation has been demonstrated previously in non-invasive studies (Wang et al., 2014; Wang and Voss, 2015). For example, while non-invasive primary motor cortical stimulation did not exhibit any reliable changes in cortical hippocampal connectivity or associative memory performance (Wang et al., 2014), targeted hippocampal stimulation demonstrated a selective influence on associative memory success (Tambini et al., 2018).

Second, I investigated a small number of subjects, and thus, I cannot claim with any certainty that the statistical power was sufficient. However, despite these

limitations, I observed a consistent memory effect of stimulation in all six subjects following each memory task. In addition, although not all subjects performed both memory tasks, I accounted for the differential effect of stimulation on memory tasks across patients by using a within subject design and comparing two patients' performance and electrophysiological responses during the memory process.

I note, however, that although the subjects' cognitive level was largely matched, it is possible that variability in other factors such as memory strategy or memory load may have interacted with hippocampal stimulation-induced memory modulation. To further investigate such issues, future work will aim to independently manipulate such factors from the stimulation itself. Given that invasive stimulation is highly localized and given the heterogeneous nature of neural responses at small scales, further study using smaller electrodes or micro-stimulation is needed to understand more precisely the relationship between stimulation parameters and the response elicited from small pieces of neuronal tissue (Titiz et al., 2017). Furthermore, considering the importance of mechanically characterizing the causal effects of stimulation on brain activity during memory encoding, it is crucial for the entire field that new and improved methods to minimize stimulation artifacts be developed.

5.5.6 Conclusion

By depicting that hippocampal stimulation is most likely to improve memory when the underlying hippocampal activity is specifically related to theta activity, our data offer valuable insights into the inconsistencies reported in behavioral effects of

hippocampal stimulation so far and provides the foundation for future work that maximizes the effectiveness of brain stimulation for treating memory disorders.

Chapter 6. Hippocampal pre-stimulus activity Predicts later memory success

6.1. Abstract

Neural activity during the time preceding stimulus presentation is sensitive to episodic memory performance. I hypothesized that successful memory formation is supported by neuronal preparation that precedes an event. To address this issue, I assessed the activity of single neurons recorded together with the iEEG field potentials from the hippocampus in humans, while they were engaged in a word item memory task. Human hippocampal single unit activity elicited by a cue presented just before a word increased the firing rates whether the word item would be recollected in a later memory test. Furthermore, the neuronal firing rate was correlated with the coordination of spike timing with the local high gamma oscillation. This finding proposes that successful memory formation in human is predicted by a pre-stimulus activity and suggests that the preparatory mobilization of neural processes before encoding benefits episodic memory performance.

6.2. Introduction

The neural activity elicited by an event when it is initially encountered is an important determination of whether the event will be remembered later (Sanquist et al., 1980; Rugg and Coles, 1995; Paller and Wagner, 2002). In prior work, the focus of much research in this activity has been centered on memory-predictive brain activation during encoding periods using 'subsequent memory effect (SME)' approach. The SME is typically measured as the difference in during-stimulus neural activity between subsequently forgotten and subsequently remembered events at encoding (Paller et al., 1987; Paller and Wagner, 2002). That is, subjects encode a series of items while neural activity is recorded and later take a memory test for those items. This neural activity, recorded during the encoding period, is sorted according to whether items are remembered or forgotten in the subsequent memory test. As follows, brain activities differentiated between subsequently remembered and forgotten items – encoding related activity, transient neural activity elicited by individual items – are taken as a neural marker in successful memory formation. This approach has yielded important insights into the neural mechanisms underlying successful memory encoding.

However, its application has been restricted to the investigation of encoding-related activity set in train by stimulus event. Learning from novel experiences is considered to depend on a variety of brain processes and mechanisms. The rationale that for real brains in real world, experiences and memoranda do not exist in isolation,

but rather proceed on a temporal continuum: time present is at least partially a consequence of time past and is expected to provide cues concerning time future. It is only natural, therefore, to expect that at any given point in time, brain activity resulting from experiences or expectations will affect the memory of the information encountered.

The finding from fMRI, neural activity which tonically maintained across a succession of stimulus event involved in successful episodic memory encoding (Otten et al., 2002), suggests the possibility that the brain activity which occurred immediately before encoding is also predictive of subsequent memory performance. In a follow-up study, a similar effect was also observed in the hippocampus (Park and Rugg, 2010). This idea is important for a comprehensive understanding of the different neural mechanisms supporting memory encoding and is an essential precursor to any investigation of how the mechanisms interact. However, there is limited research on the impact of how neuronal level activity prior to stimulus presentation reflect subsequent memory performance, per se, successful memory formation.

To address this issue, I hereby investigated whether a further aspect of encoding-related neural activity, namely, the activity that precedes a stimulus event (pre-stimulus activity) predicts later memory success. I addressed this question by using the 'pre-stimulus subsequent memory effect (pre-SME)' procedure in human single unit activity during pre-stimulus interval, furthermore, SUA's relationship with iEEG field potentials. The present study provides a characterization of neuronal bases

of pre-stimulus activity which will be an important implication for the interpretation of indirect brain investigation studies (i.e., fMRI, EEG) of successful memory formation (Wagner et al., 1998b; Paller and Wagner, 2002; Otten et al., 2006).

6.3. Material and methods

6.3.1. Patients

A total of 3 epileptic patients (3 male) aged 25 to 64 y (M = 39. 7, SE = 17.3) were implanted with hybrid depth electrodes for chronic seizure monitoring. Their average stay on the monitoring ward was 6 to 7 d. The patients volunteered for the study and gave informed consent. The experiment was approved by the institutional review boards of the Seoul National University Hospital (H-1407-115-596). I evaluated all patients using standard neuropsychological test (**Table 3**).

Table 3: List of patient demographics, pathology, and neuropsychological evaluation.

Subject	Age	Sex	Epilepsy diagnosis	WAIS-IV					WMS-R
				VCI	PRI	WMI	PSI	FSIQ	MQ
Sub 1	30	M	RTLE	116	86	98	84	95	66
Sub 2	25	M	LTLE	100	107	98	55	90	73
Sub 3	64	M	RTLE	114	94	112	92	103	77
Average	**39.7(17.3)**	**-**	**-**	**110(7.1)**	**96(8.7)**	**103(6.6)**	**77(15.9)**	**96(5.4)**	**72(4.6)**

Intelligence was measures with the Korean Wechsler Memory Scale (K-WMS) and memory with the Wechsler Memory Scale (WMS). Abbreviations: right temporal lobe epilepsy (RTLE), left temporal lobe epilepsy (LTLE), verbal comprehension index (VCI), perceptual reasoning index (PRI), working memory index (WMI), processing speed index (PSI), full scale IQ (FSIQ), and memory Quotient (MQ).

6.3.2. Electrodes

Nine micro-wires (8 high-impedance recording electrodes, 1 low-impedance reference; Ad-Tech, Racine, WI) protruding from the shaft of the depth electrodes were used to record signals from MTL neurons. Electrode location were chosen according to clinical criteria alone. Target locations were verified using a human hippocampal atlas using postoperative CT image that were reconstructed from individual preoperative MR images using CURRY 7.0 (Compumedics Neuroscan, Charlotte, NC, USA). Magnetic resonance images were acquired on Signa 1.5-tesla scanner (GE, Boston, MA, USA). Only electrodes that could be localized to the hippocampus or the amygdala were included in this experiment. All patients had good recognition memory as well as clearly distinguishable spiking activity on at least one electrode.

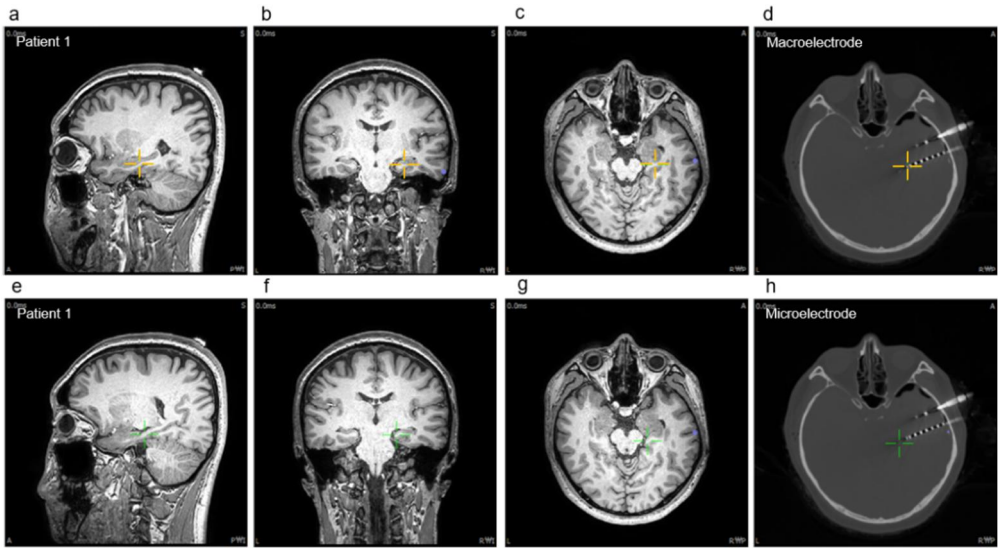

Figure 8: Electrode localization with structural MRIs. Shown are examples from one patient (see table 1 for demographic information). In the axis plane, the patient's left brain is on the left side. All images are T1 images, acquired with a Signa 1.5-tesla scanner (GE, Boston, MA, USA). Orange and green arrows always indicate an electrode in the macro electrode in the hippocampus, green in the microelectrdoe in the hippocampus. A-D. Example of macroelectrdoe localization A. Saggital image of the left side (T1). B. Coronal T1 image. C. Axial T1 image. D. Axial CT image. E-H. Example of microelectrode localization. E. Saggital image of the left side. F. Coronal image G. Axial image. H. Axial CT image of hippocampal microelectrode.

6.3.3. Task and stimuli

To assess the effect of stimulation on task-dependent memory, I asked each patient to perform the episodic memory of word item memory task, using STIM2 software (Compumedics Neuroscan, Victoria, Australia). Memory task comprised three successive stages: encoding, distractor, and retrieval (**Figure 8**). For word item memory, subject completed sequentially 60-word items of one concrete noun, randomly shown one at a time.

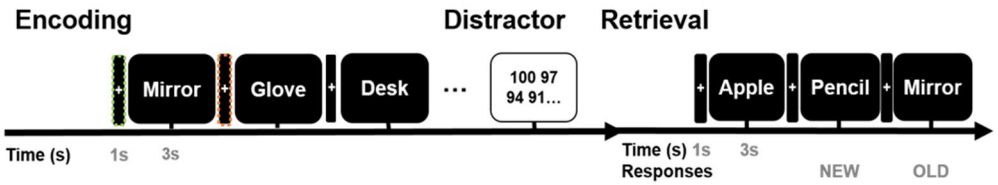

Figure 9: Word item memory task. Example of the timeline of a visual item memory task. Memory task comprised three successive stages of encoding, distractor,

and retrieval. Each patient completed 60 words of item during encoding one at a time, followed by a white fixation cue with a black screen of 1 s. The green and orange dashed line boxes indicate pre-stimulus periods for successfully remembered item and forgotten item, respectively.

During encoding, a word was visible for 3 s, followed by a white fixation cue with a black screen of 1 s. To ensure a deep encoding, I used previously reported encoding feedback in which patients were encouraged to respond by pressing with their index finger if they judged the appeared word on the screen as "pleasant" or "unpleasant". Following the final word of the encoding block, patients took a 10 min break and then performed a 30 s math distractor task consisting of a series of arithmetic problems for "A-B =?", where A and B were randomly chosen integers ranging from 1 to 100. In retrieval on the item task, patients were to respond whether the word had been presented before ("old"; button #1) or was new ("new"; button #2) or the subject was not sure ("familiar"; button #3). For the main experimental session, no patients were exposed to the same experimental task more than once.

6.3.4. Electrophysiological recordings and spike sorting

I recorded extracellular neural activity using 40-um micro-wires inserted in a clinical depth electrode, which was implanted in the hippocampus and amygdala. Spiking and iEEG field potentials were amplified and recorded using a 32-channel Neuralynx ATLAS system (Bozeman, MT). Spike data from the same recording

electrode with macro-electrode were obtained by band-pass filtering the raw electrode signal from 600 - 9000 Hz. For the spikes, the sampling rate was 32 kHz, and signals were referenced against one of the low-impedance reference electrodes and spikes were detected by the application of a threshold (30 uV) from the raw trace. Spikes sorting was performed using *wave_clus* (Quiroga et al., 2004). After sorting, each cluster was graded as being noise, multiunit activity (MUA) or single-unit activity (SUA) based on criteria such as the waveform shape, size of the waveform relative to noise, evidence of a refractory interval, and lack of power line interference, using the criteria described previously (Valdez et al., 2013). For visualization of the spikes aligned with the memory task, a raster plot was built by aligning spike timestamps with reference to the timestamp for the visual stimuli presentation (bin size = 200 ms, time window = 1 s before and 3 s after stimuli).

6.3.5. Analysis of iEEG field potentials

I study the spiking activity together with iEEG field potentials and their correlation with the neuron's firing. To quantify and compare the single unit activity (SUA) and macro-field oscillations, I computed the instantaneous power from hippocampal macro channel simultaneously recorded with hippocampal SUA using the squared magnitude of the Hilbert transform after band-pass filtering. Analyses of iEEG field potentials were conducted with MATLAB (Mathworks, Natick, MA, USA). Prior to data processing, all channels clinically identified within the ictogenic zone, or those electrodes observed as corrupt during recordings, were excluded from

all data analysis. Electrodes were also excluded from subsequent analyses if there were any motion artifacts. All data preprocessing was performed at a single electrode level. For each patient, all non-excluded electrodes were first digitally filtered with a low-pass filter of 200 Hz. To attenuate 60 Hz line noise, a notch filter at 60 Hz was applied. The recorded data were then re-referenced to the common average reference (CAR).

To quantify specific changes in different frequency ranges in the hippocampus with a continuous time complex value representation of the signal, I conducted a time-frequency analysis and performed spectral decomposition (1 frequency from 1 to 10 Hz, 2 frequencies from 10 to 20 Hz, and 4 frequencies from 30 to 200 Hz, logarithmically spaced; Morlet wavelets; wave number = 2.48) for the -1-3 s epoch relative to word onset in the encoding phase. Mirrored buffers (length = 2 s) were included before and after the interval of interest and then discarded to avoid convolution edge effects. To test the significance between successfully remembered item and forgotten item during pre-stimulus baseline and encoding phase, I extracted t-values using the means and standard deviations (SDs) with the independent two sample *t*-test. For visualization of time frequency map, epoched single trials were averaged across all trials.

6.4. Results

6.4.1. Behavioral results

I recorded single neuronal activity and iEEG field potentials in 3 patients with pharmacologically intractable epilepsy, who were implanted with intracranial electrodes for clinical reasons. I had patients encode visually presented 60 words which were pseudorandomized, and each trial started with a white fixation cross of 1 s as a cue for upcoming stimuli. Memory was tested by intermixing previously presented words with new words, and participants responded with an old/new judgement. Overall patient's behavioral result showed that patients remembered well: the mean percentage of correct responses across all trials was 80.0 $\pm$ 3.95 % (mean + s.e.m.) and the average sensitivity, *d'*, was 1. 7 $\pm$ 0.27 (mean $\pm$ s.e.m., $p < 0.05$), indicating that they had a good sense of the quality of their memories. The overall reaction time (RT) for recognition was 1220.23 $\pm$ 239.73 ms for correctly remembered words and 1717.18 $\pm$ 885.66 ms for forgotten words (mean $\pm$ s.e.m.).

6.4.2. Spiking properties of hippocampal neurons

I recorded spiking activities of singe units in the hippocampus and amygdala while patients performed the word item memory task. I isolated 32 single neurons from hippocampus and amygdala (30 from the hippocampus and 2 from amygdala: 29 single units and 3 multi units). Only single units that met isolation criteria including the inter-spike intervals (ISIs) were smaller than 3 ms and the cluster number of neurons lie below 0.5% (n = 29) were used in final analyses. 93% of most

units (n = 27 out of 29) were regular-spiking cells, and others as unclassified cells (2/29) based on autocorrelograms (Bartho et al., 2004). Unit recordings were made from the hippocampal CA3 for patient 1 (**Figure 10**), and patient 3. Neurons fired, on average, 1.63 spikes per second. The mean firing rate of the hippocampal neurons is 1.71 which is higher than that of 1.47 in amygdala.

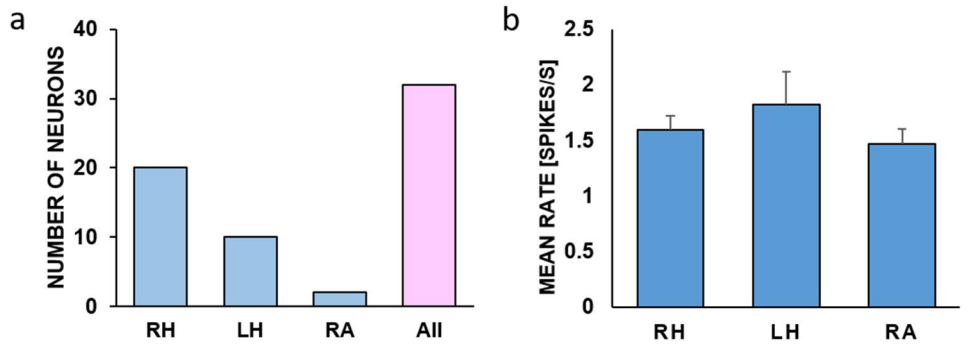

Figure 10. Mean firing rates and numbers of neurons from different brain areas. A. The number of units for each area was as follows, from left to right: 20, 10, and 2. B. Mean firing rates of neurons for each area. All neurons are included, regardless of firing rates. The mean firing rates of the hippocampal and amygdala is 1.71 and 1.47, respectively. Error bars are ± s.e.m. Abbreviation; RH: right hippocampus, LH: left hippocampus, RA: right amygdala.

6.4.3. Hippocampal pre-stimulus activity correlates with successful memory

I firstly examine how encoding activity around the presentation of words (i.e., task periods) differ to resting states before engage the encoding. I analyzed mean firing rates in the hippocampus both resting states and memory encoding for a given session. The result showed that the spiking activity for memory encoding was significantly higher than the activity for resting states, indicating that neurons exhibited a task specificity (**Figure 11B**, for a representative electrode site, Rank sum test, $p < 0.001$). In agreement with prior studies, the hippocampal spiking activity for subsequently remembered words was relatively greater to subsequently forgotten words for both before and after word stimuli. However, the raw spiking data showed that for the subsequently remembered words and forgotten words, the difference between pre-stimulus and during-stimulus spiking activity in each of condition was negligible, indicating that the during-stimulus activity was a continuation of the pre-stimulus activity (**Figure11C**, for a representative electrode site, Rank sum test, $p = 0.0524$).

To quantifying the pre-stimulus subsequent memory effect (pre-SME), mean firing rates were measured across the 1 s interval of the fixation cue period between remembered trials and forgotten trials. A unit was considered as pre-stimulus activity if the firing rates is responsive to a fixation cross cue during pre-stimulus interval and showed a consistent elevated pattern of firing in all trials of that fixation cross. Overall, the 12 units (38%, 10 hippocampus and 2 amygdala) showed a significant pre-

stimulus activity, i.e., consistently increased firing rate in all trials in a least 200-ms segment of time. The firing rates differed reliably before word onset according to later memory performance. The mean firing rates of single units elicited by fixation cues preceding encoding words showed significantly increased patterns with the firing rates of subsequently remembered words compared to forgotten words. Hippocampal spiking activity exhibited trends of higher during-stimulus spiking level for subsequently remembered vs. forgotten words, but the trend was not significant (**Figure 11D**, for a representative electrode site, Rank sum test, $p < 0.05$).

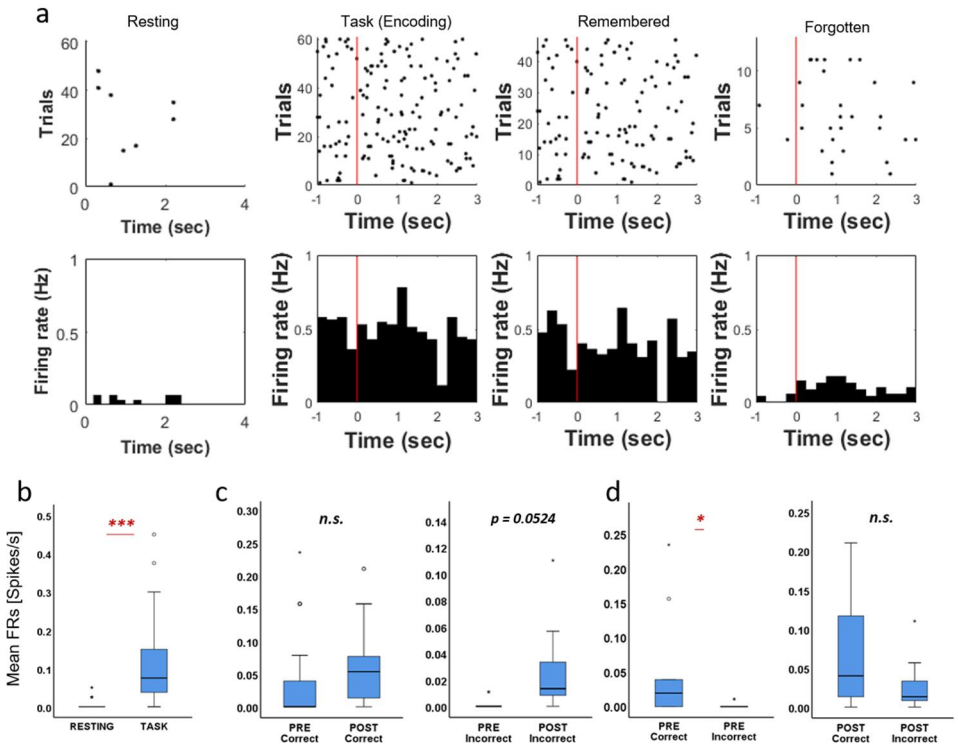

Figure 11: Hippocampal neuronal responses to subsequently remembered and forgotten word items. (A) Pre-stimulus and during-stimulus raster and histogram of

firing rates during resting and encoding sessions. Red line marks stimulus onset at time 0, offset is at 3 secs (x axis). Raster rows represent single trials, and each dot an action potential. (B) Mean firing rates between resting states and encoding. Mean firing rates for encoding duration is significantly larger than resting state ($p < 0.001$, Rank sum test). (C) Hippocampal spiking activity indicated the difference between pre-stimulus and during-stimulus onset activity was negligible ($p = 0.0524$). (D) The hippocampal pre-stimulus SUA is significantly larger in subsequently remembered items compared to subsequently forgotten items ($p < 0.05$). This neuron was selective during pre-stimulus interval with similar increases in activity during the during-stimulus interval.

These results were then used to perform group-level comparison in each of 12 units (**Figure 12**). Mean firing rates across neurons (n=12) during resting states vs. memory encoding showed a significant increase in firing rates for memory encoding compared to resting states (**Figure 12A** Rank sum test, $p < 0.001$). In addition, the group level comparison exhibited trends of higher hippocampal activity for subsequently remembered vs. forgotten words both in the pre-stimulus and during-stimulus, indicating that the during-stimulus activity was a continuation of the pre-stimulus activity (**Figure 12B**). In addition, in line with the individual result, the firing rates preceding words that were later remembered were significantly higher than those preceding words that were later forgotten (**Figure 12C** left, Rank sum test, $n = 12$, $p < 0.01$).

This result could be found in almost half of the hippocampal neurons that had significant responses during the pre-stimulus interval in later subsequently remembered items. Thus, the present data, as has been reported previously that words

that were subsequently remembered elicited more positive-going ERPs than words that were subsequently forgotten (Otten et al., 2006), also showed that pre-SME in the SUA were elicited by the encoding words.

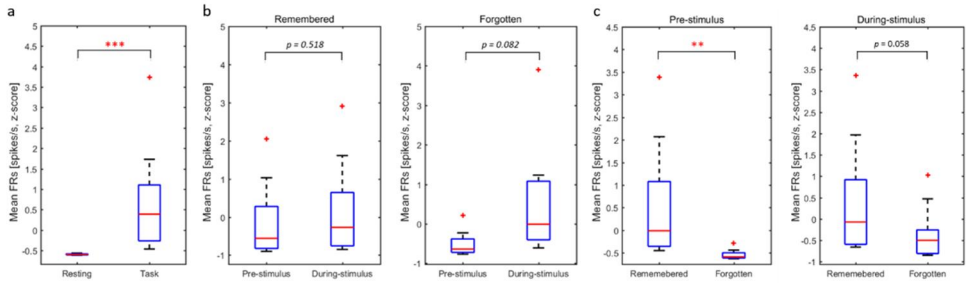

Figure 12: **Group-level comparison of spiking activity in the hippocampus.** (A) Mean firing rates across neurons (n=12) during resting states vs. memory encoding showed a significant increase in firing rates for memory encoding compared to resting states (Rank sum test, ***$p < 0.001$). (B) Pre-stimulus and during-stimulus mean firing rates as a function of subsequently remembered (left) vs. forgotten words (right). Both remembered and forgotten words, the difference between pre-stimulus and during-stimulus was not significant (Rank sum test, $p > 0.05$, respectively). (C) Group-level comparison of pre-stimulus and during-stimulus hippocampal mean firing rates. Hippocampal activity was higher for subsequently remembered words compared to subsequently forgotten words during pre-stimulus (left, Rank sum test, **$p < 0.01$). However, the trend of during-stimulus hippocampal activity was not significant (right) (Rank sum test, $p = 0.058$).

6.4.4. Hippocampal pre-stimulus spiking activity correlates with high gamma field potentials

For the channels with significant iEEG field potentials and unit responses, I firstly computed the instantaneous power using the squared magnitude of the wavelet transformed power. Then the pre-SME between remembered and forgotten items was investigated across patients. **Figure 13** shows the normalized average responses of time-frequency map, where I observed that the mean high gamma power bands (80 to 150 Hz) activation increases at the same time around - 550 and - 500 ms of spiking activity before the word presentation (two sample *t*-test, $p < 0.01$). To ensure that this result was not related to other brain regions, I confirmed that increase gamma power in area of temporal white matter. The cluster showed significance within the gamma for subsequently remembered compared forgotten items during pre-stimulus interval, only from in the hippocampus not from the temporal white matter (**Figure 13A-B** for hippocampus and **C-D** for temporal white matter).

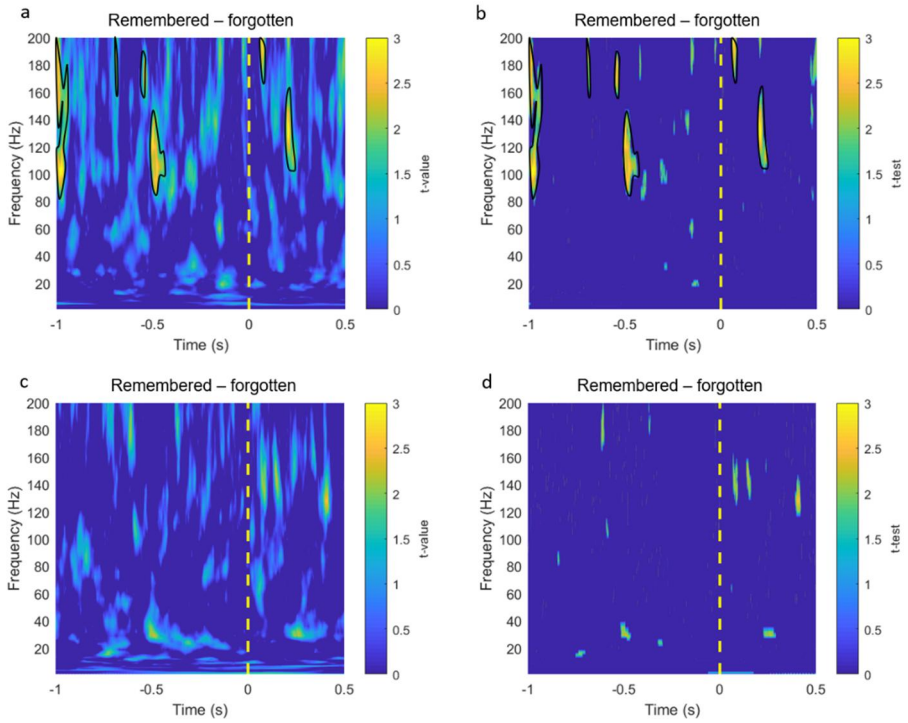

Figure 13. The time frequency for subsequently remembered versus forgotten difference. A. Time courses of the difference power value in the hippocampus between remembered versus forgotten items. The latency, relative to stimulus onset, of the most distinct difference was from -550 to -500 ms. B. Significance of the difference expressed as *t*-test. C-D. Same as in the non-hippocampal area (i.e., temporal white matter)

So far, I have examined each of hippocampal SUA and hippocampal field potentials during pre-stimulus intervals. To further explore the relationship these two signals, I computed the correlation between spikes and iEEG oscillatory activity, the spike field correlation, timing for each of unit responses during pre-stimulus intervals. The grand average of oscillatory activity showed a strong correlation with the spikes

to the gamma frequency range (84 to 120 Hz) during the pre-stimulus intervals (**Figure 14A** for examples from individual response of all trials, two sample *t*-test, $p < 0.05$).

The comparison of correctly remembered items versus forgotten items, the spike field correlation in the gamma ranges showed a concomitant significance only for remembered items but not for forgotten items (**Figure 14B,** 72 to 104 Hz for remembered items, two sample *t*-test, $p < 0.05$). I further quantified these observations with a phase-locking analysis between spikes and iEEG oscillatory activity. As shown in **Figure 14D-F**, however, there was no significant distribution of phase-locking values in the broad band (1 to 200 Hz), no frequency band showed significantly strong correlation during pre-stimulus intervals.

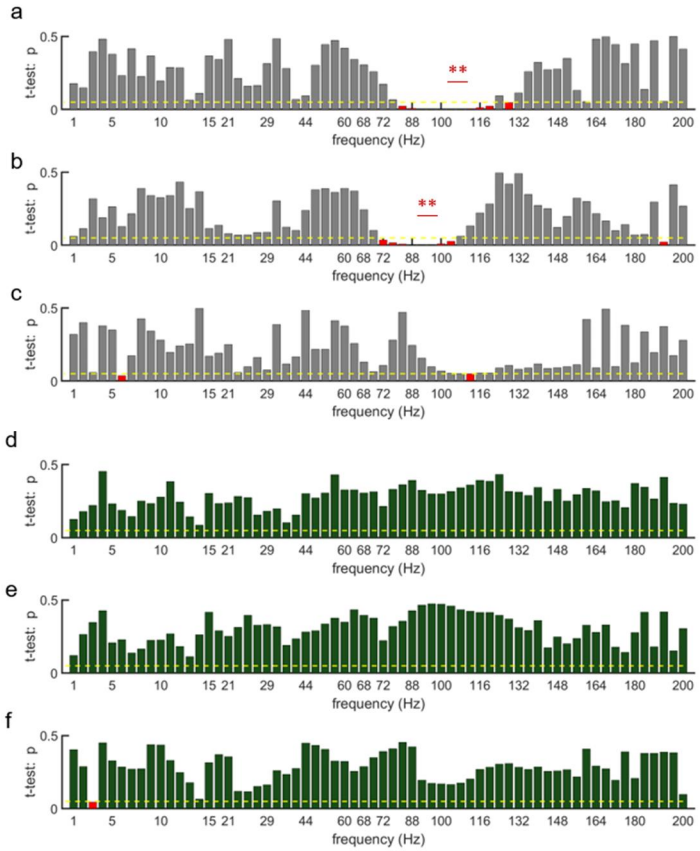

Figure 14. Spike field correlation and phase locking values in remembered versus forgotten items during pre-stimulus interval. A. p values for spike-field correlation between hippocampal SUA and iEEG oscillatory activity indicates that pre-stimulus activity exhibited significantly strong correlation with the spikes to the gamma frequency range (84 to 120 Hz, $p < 0.05$). B-C. The spike field correlation in the gamma ranges showed a concomitant significance only for remembered items but not for forgotten items (72 to 104 Hz, $p < 0.05$). D-F. p values for phase-locking values indicate there were no significant distribution of phase-locking values in the broad bands (1 – 200 Hz, $p > 0.05$). Yellow dashed line indicates p value under 0.05 and red bar showed the frequency bands under p values of 0.05.

6.5. Discussion

6.5.1. Summary

In this study, I evaluated whether spiking activity prior to stimulus predicted later memory success in a word item episodic memory task. I showed that hippocampal SUA, elicited by a fixation cue presented just before a word, increased the firing rates whether the word item would be remembered in a later memory test. During-stimulus activity also showed the trend of predicting later memory success, but the activity level was merely a continuation of pre-stimulus activity level. Moreover, the hippocampal neuronal firing rate was correlated with the coordination of spike timing along with the local high gamma oscillations (> 60 Hz). These findings provide that the hippocampal pre-stimulus activity modulates successful memory formation and predicts later memory success and the high gamma oscillations during the pre-stimulus interval may reflect attentional selection, providing an excitatory advantage to the hippocampal cells involved in coding the cue.

6.5.2. Comparison with previous findings

In previous literatures, studies showing pre-stimulus activity have largely been investigated in ERPs, allowing the identification of those features of the response that covary with successful encoding. For example, neural activity, in the preparatory time or pre-stimulus interval, has been shown to differ between items that are subsequently remembered versus forgotten. Other EEG studies support this idea

that pre-stimulus oscillatory activity is sensitive to episodic memory performance (Klimesch et al., 1999; Fell et al., 2011; Gruber et al., 2013; Merkow et al., 2014; Salari and Rose, 2016). Similar finding has been reported in studies of fMRI (Otten et al., 2002; Park and Rugg, 2010). This pre-SMEs also have been shown to be influenced by the task characteristics during encoding, such as stimulus modality (Wagner et al., 1998a; Golby et al., 2001; Otten et al., 2006; Park and Rugg, 2010). In this sense, pre-SMEs are thought to reflect, at least in part, preparatory mobilization of material-/task-specific and domain general processes that contribute to memory performance.

Concerning pre-SMEs using direct invasive electrophysiological studies, relatively little has been published using SUA and iEEG. Given the variability in the timing and manifestation of pre-SMEs, it is regarded that neuronal activity or oscillatory activity from iEEG field potentials would be more sensitive than that from non-invasive measures for detecting pre-SMEs that may be missed with ERPs or blood oxygen level dependent (BOLD) signals. The present study, for the first time, showed neuronal activity of pre-SMEs with markedly higher temporal resolution, providing an important implication for previous non-invasive studies.

6.5.3. Possible mechanisms underlying pre-stimulus activity

What might be responsible for modulating the amount of pre-stimulus hippocampal activity? One of the possibilities is that activity reflects attentional or preparatory state which the pre-stimulus cue recruits in anticipation of upcoming stimulus. Notably, the study task required subjects to shift unpredictably between

preparing for an upcoming visual study item. In this case, preparation required a shift of attentional focus from the task cue to the upcoming study item. Therefore, pre-stimulus hippocampal activity was enhanced on those items where processes set in train by the presentation of the cue culminated in a relatively optimal preparatory state.

A possible neurochemical mechanism underlying the above attentional shifts is suggested by the finding of (Adcock et al., 2006), which reported that hippocampal pre-SMEs were accompanied by effects in the ventral tegmental area - the origin of the mesolimbic dopaminergic system. In this study, subjects received a cue informing them whether accurate memory for the upcoming study item would be associated with high or low monetary reward, and result showed that on 'high-reward' items' activity during the cue-interval was enhanced for later recognized compared to missed items in bilateral anterior hippocampus. Although this study presented activation of the system by the manipulation of the 'reward value' of the pre-stimulus cue, however, pre-SMEs also have been reported in this system for cues signaling of the novelty of an upcoming study item (Wittmann et al., 2007). I speculate that the present finding may reflect a fixation cue driven by activation of the mesolimbic dopaminergic system which may play a role in the modulation of pre-stimulus hippocampal activity in circumstances other than reward anticipation.

In **Figure 11**, the raw spiking data exhibited that during-stimulus spiking level was a continuation of the pre-stimulus spiking level for both the subsequently remembered and the subsequently forgotten words. If pre-stimulus and during-

stimulus activity during encoding may be related to each other, how might pre-stimulus hippocampal activity determine encoding efficiency? In prior work, increasing excitability in neurons results in those neurons being biased to represent a new memory (Cai et al., 2016). Hence, more excitable neurons preferentially fire in response to the presentation of a stimulus, and synaptic changes in these neurons constitute the memory trace (Rogerson et al., 2014). Conceivably, in the present study, the more excitable neurons were already active at the time of stimulus presentation and created stronger memory traces (Han et al., 2007).

In the present study, the hippocampal neuronal firing rate was correlated with the coordination of spike timing along with the local high gamma oscillations (> 60 Hz). Gamma oscillations, because of their high frequency, are ideally suited for operations that require neuronal coordination on a time scale that is beyond the range of conscious perception. This type of fast coordination is thought to be needed during many fundamental operations of the hippocampus, including rapidly selecting inputs, grouping neurons into functional ensembles, retrieving memories needed to correctly perform a previously learned task, and determining which aspects of an experience will later be remembered [see (Colgin and Moser, 2010) for review].

Gamma synchronization of neurons may also mediate attentional selection processes in the hippocampus. Selecting the environmental cues that are important for performing hippocampal-dependent tasks and ignoring extraneous cues that are task-irrelevant requires attention (Muzzio et al., 2009b; Fenton et al., 2010). Gamma-facilitated transfer of inputs related to the task-relevant cues would provide an excitatory advantage to the hippocampal cells involved in coding these cues, enabling

them to be activated more reliably. Considering that attention largely determines which experiences will be remembered [see (Muzzio et al., 2009a) for a review], cell selection by gamma oscillations likely also plays a major role in regulating the information that will be retained in long-term storage. In this sense, the gamma power increase may play a role in selection of inputs during memory formation in the hippocampus.

6.5.4. Conclusion

In summary, the present study adds substantially to prior observations that the likelihood of a stimulus event will be successfully encoded is associated not only with the pattern of neural activity elicited by the event itself, but also with the activity that immediately precedes the event. When the brain, especially the hippocampus, is ready to encode into long-term episodic memory. Therefore, hippocampal-mediated episodic memory success may be potentiated by high levels of pre-stimulus activity.

SECTION 3. GENERAL CONCLUSION

Chapter 7. General Conclusion and Perspective

The present studies aim to improve our understanding of both the hippocampal oscillatory network mechanism and single unit activity during successful memory. In the first study, I highlight the different mechanisms responsible for the effect observed during electrical stimulation. I found the memory enhancement is modulated in a task-dependent manner. This finding will allow for a better understanding of why the stimulation effects are task-dependent.

In the second study, I found successful memory formation is predicted by a pre-stimulus activity. This result extends the knowledge of the spiking activity of memory formation and provide a more detailed brain states and hippocampal excitability. In this respect, it will be interesting to determine how pre-stimulus activity affects encoding in individuals who have trouble with episodic memory processes, such as Alzheimer's patients and the elderly (Hedden and Gabrieli, 2004).

Throughout these studies, it may be possible to improve memory by optimizing the probability that encoding-related activity before an event is engaged. This may be accomplished with rewards, or by teaching individuals to prepare themselves in such a way that benefits encoding. This association may allow to develop a new informed close-loop brain stimulation paradigm.

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Abstract in Korean（국문 초록）

성공 기억에 대한 해마의 역할과 기전

전소연

인간의 복잡한 뇌 기능 중 흥미로운 하나는 경험에 의거하여 정보를 저장하고 의지에 따라 저장된 정보를 재인하는 ‘기억 능력’ 이다. 인간은 주어진 자극에 기반하여 행동을 정하며 심지어 자극이 없는 상황에서도 자극에 대한 기억을 바탕으로 행동을 결정하기 때문에 기억 능력은 생존에 있어 매우 결정적이며, 이러한 기억과 관련된 가장 기본적인 질문은 기억의 저장 메커니즘, 즉, ‘어떤 기억은 저장이 되고 어떤 기억은 잊혀지는 가’일 것이다. 스코빌과 밀너가 처음 보고한 기억상실증 환자 H.M.은 측두영역의 손상을 입은 후 심각한 인지 기억 능력의 장애를 보였고, 이후 사람 뇌의 해마 영역은 기억을 관장하는 뇌의 중요한 영역 중 하나로 널리 연구되었다.

해마가 기억에 미치는 영향과 역할에 대해서는 다양한 방법으로 실험이 진행되어 왔다. 그 중의 하나는 뇌에 직접적인 전기자극을 가해 기억 과정 중 해마의 역할을 확인하는 방법인데, 이는 뇌전증 환자의 모델을 통해 사람의 뇌에 접근이 가능해지면서 이루어져 왔다. 두 번째 방법은 전기생리학적 방법을 통하는 것인데 세포 외 활동 전위인 “스파이크”를 통해 성공기억에서의 뉴런의 활동성을 밝히는 것이다.

이 논문은 이 분야에서 오랫동안 논란이 되었고 부족했던 성공 기억에 관련된 해마의 역할과 기전을 물리적 자극 및 신경세포의 신호를

측정해서 전기생리학적 특성을 제시하는데 초점을 맞추고 있다. 논문에서 본 저자는 사람의 성공기억형성과 재인에 대해 뇌 자극과 단위세포활동을 보고할 것이다. 해마와 기억의 인과관계 및 기억 과정 중의 해마의 뇌 기전과 관련된 기존의 실험적, 행동적 발견들에 근거하여 본 저자는 (ㄱ) 해마에 직접적인 전기 자극을 주고 기억 수행능력의 차이 및 기억 과제에 따른 해마의 신경 기전을 밝히고, (ㄴ) 성공 기억이 형성되는 과정에서 나타나는 신경세포의 발화 패턴의 특성을 살펴보았다. 본 연구를 통해 저자는 향후 기억의 형성 과정에서, 자극이 제시되는 구간뿐 만 아니라 자극이 주어지기 전 단계에서도 해마를 타깃 하여 전기 자극을 줌으로써 기억 실패로 이어질 수 있는 자극을 성공 기억으로 저장할 수 있도록 유도할 수 있을 것이라 기대한다.