Theta Activity during REM and NREM sleep and Predicting Fear Learning Outcomes

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Submitted to the Graduate Faculty of the

Dietrich School of Arts & Sciences in partial fulfillment

of the requirements for the degree of

Bachelor of Philosophy

University of Pittsburgh

2015

UNIVERSITY OF PITTSBURGH

DIETRICH SCHOOL OF ARTS & SCIENCES

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Fear conditioning, extinction, extinction-recall, and renewal are all components of a well-established paradigm used for studying fear learning and response in the laboratory setting, and are used to model anxiety disorders in animals and humans. Studies have recently begun to investigate the role of the brain electrical activity during sleep in emotional learning and memory. Emotionally relevant learning and memory has been associated with a particular activity band, theta rhythm, which can be measured during sleep. Previous studies have suggested theta activity during sleep may be an indicator of maladaptive stress responses. Despite this, there has not been a thorough investigation of theta activity during a full night of sleep and its relationship to subsequent fear responses.

The goal of the present study was to assess the relationship between theta activity and fear learning and memory, as well as, theta activity and extinction learning and memory using well-validataed paradigms to study fear responses in humans. Participants (16 females; 15 males; mean age = 23.6; SD = 3.80) underwent two consecutive nights of polysomnographic (PSG) recording in the Sleep and Chronobiology Laboratory at Western Psychiatric Institute and Clinic, before and after fear learning and extinction. Quantitative electroencephalographic spectral analysis was used to measure theta activity during sleep on both nights. Our objective was to evaluate the extent to which theta power during sleep predicts outcomes of fear learning and extinction memory the

following day. Results showed higher theta power during the second night of the experiment predicted greater retention of the extinction memory during the fear renewal task completed the following morning.

Understanding the relationship between theta power during sleep and fear and extinction learning and memory may identify markers of risk or resilience to anxiety disorders in trauma exposed individuals.

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PREFACE

Many people have aided me throughout the entirety of the Bachelor of Philosophy process, and I am truly appreciative of their guidance. My sincerest thanks to:

Dr. Anne Germain, my faculty advisor, for providing me with an excellent foundation of research knowledge and values, answering my countless questions, and believing and trusting in me during my experience under her supervision.

Dr. Terri Hastings, for giving me an incredible and unparalleled experience during the Honors College Health Sciences Research Fellowship - her passion for mentoring students during the summer made the program truly special

The University Honors College, for giving me the opportunity to focus exclusively on my research project during the Honors College Health Sciences Research Fellowship

The faculty and staff of the Military Sleep Tactics and Resilience Research Team, for all of their assistance and support

Dr. Julio Fernandez-Mendoza and Dr. Sangeeta Chakravorty, for their integral role in the Bachelor of Philosophy process

My family and friends, for providing me with their endless encouragement

1.0 INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Describing Sleep in Humans

Sleep is defined as a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment. Despite it being inactive behaviorally, sleep is an active, dynamic biological state. This first chapter summarizes the methods used to study sleep, and succinctly describes the literature and data on the relationship between sleep, learning and memory. This is followed by an explanation of the paradigms that have been implicated in the pathogenesis of anxiety disorders, fear conditioning and fear extinction, and describes the literature on the relationship between sleep and these paradigms.

Sleep in humans consists of two distinct, alternating sleep states: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. NREM sleep, additionally, is divided into three stages: N1, N2, and N3 (formerly Stage 3 and Stage 4) or slow wave sleep. In adults, REM sleep constitutes approximately 20-25% of total sleep time (TST), while roughly 50% of TST is spent in N2 of NREM sleep. The remaining stages make up approximately 30% of TST. These stages of sleep progress in a cycle beginning with N1, advancing to N2 and N3, descending back into N2, and ending with REM sleep before the cycle starts over again at N1. The beginning of an

archetypal night of human sleep contains a much larger percentage of NREM sleep, and as the night progress, REM sleep episodes increase in duration (Carskadon & Dement, 2011).

Physiologically, NREM sleep is characterized by decreased brain metabolism (Nofzinger et al., 2002), blood pressure, respiration, body temperature, and a slower heart rate. Additionally, distinct neuroendocrine responses are found with NREM sleep including the release of growth hormone and prolactin (Cardinali & Pandi-Perumal, 2005). The stages of sleep are defined by using electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG), as the tool for measurement of the brains electrical activity. Stage N1 of NREM sleep is identified by slow eye movements, theta (4–8 Hz) waves, and vertex sharp waves. Stage N2, on the other hand, is defined by the presence of two morphologically distinct waveforms: K-complexes and sleep spindles. A K-Complex is typically defined as high amplitude, distinct negative sharp wave with a positive component immediately following the negative sharp wave, lasting between 0.5-1 seconds. Sleep spindles are composed a group of waves between 12-16 Hz, which quickly increase in amplitude, followed by a gradual decline in amplitude. Finally, stage N3 is associated with the release of growth hormone and high amplitude, low frequency delta waves (0.5-4 Hz) (Espie & Morin, 2012; Silber et al., 2007).

REM sleep is characterized by the presence of low amplitude, fast saw tooth waves recorded by EEG, rapid eye movements, and muscle atonia. Further, breathing becomes more rapid and shallow, blood pressure rises, and heart rate increases. REM sleep subdivides into two distinct types: tonic REM sleep and phasic REM sleep. Phasic REM sleep is epitomized by ponto-geniculo-occiptal (PGO) waves seen in animals, accompanied by bursts of rapid eye movements. There is now some evidence that PGO wave activity exists in humans in patients with Parkinson's and Epilepsy (Datta, 2000; Fernández-Mendoza et al., 2009; Lim et al., 2007). Additional features

include muscle twitches and middle ear muscle activity (Carskadon & Dement, 2011; Espie & Morin, 2012). Hallmark to tonic REM sleep is lower voltage electrocortical theta rhythm, brain temperature elevation, and decreased electromyogram amplitude (Baust, Berlucchi, & Moruzzi, 1964; Rechtschaffen, 1978).

The first account of the use of scalp electrodes to record neuronal activity from the surface with electroencephalography (EEG) in humans was reported by Hans Berger, in 1929 (Berger, 1929). The electrodes record the current that is the result of the summation of electrical fields generated by neuronal activity. With sufficient excitatory input, a neuron fires an action potential that causes an electrical signal to travel down the axon of the cell. The signal results in the release of neurotransmitters across the synaptic cleft, which, in turn, produces a current that flows within the dendrites of the target cell. The current yields an electrical field, and when a large number of neurons exhibit a similar pattern of electrical activity, with enough electric fields oriented similarly, the current generated is large enough to be recorded via electrodes placed on the surface of the scalp. The frequency and amplitude of the waves recorded with EEG can be categorized by reevaluating their characteristics. The most familiar classification uses the wave frequency and contains five clinically relevant categories: delta (0.5 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz), sigma (12-15), beta (15 – 30 Hz), and gamma (30 – 80 Hz) waves.

Today, the measurement method that encompasses EEG and other physiological signals to detect sleep stages and phasic events is known as polysomnography (PSG). PSG includes recordings of muscle activity using electromyogram (EMG) and eye movements using an electrooculogram (EOG). EMG and EOG are combined with EEG to categorize sleep stages.

EEG oscillatory activity is comprised of several waveforms, with the predominating frequency determining the observed activity. That is, the observed activity found during stage N3

of sleep is dominated by the high amplitude, delta waves, with little activity coming from additional waveforms. Using physiological parameters in addition to the aforementioned waveforms, sleep stages can be determined. An “architecture” can be produced using the structure and pattern of sleep (Achermann, 2009). The observed waveforms can be analyzed further using spectral analysis techniques, the goal of which is to uncover the structural components of a signal at a given time point. The oscillations observed can be represented with relative percentages of each activity (e.g. power), which give information about cognitive state and, less commonly, disease pathology (Achermann, 2009; Pivik et al., 1993). For instance, slow wave activity (0.5-4 Hz) is most prominent in slow wave sleep in the first half of the sleep period, and is amplified in individuals suffering from sleep terrors (Broughton, 1968). Theta activity (4-8 Hz) during wakefulness is associated with drowsiness, but elevated during REM sleep (Merica & Blois, 1997; Wallace, 1970). Beta activity is thought to reflect cognitive activity and arousal during both wakefulness and sleep (Espie & Morin, 2012).

Frequency bands of interest can be observed in terms of relative or absolute power. Absolute power can be biased in certain populations due to varying degrees of skull conductivity, while relative power accounts for these individual differences (Carrier, Land, Buysse, Kupfer, & Monk, 2001). The present study focuses on theta activity because of its potential relationship to emotional learning and memory, especially as it relates to fear conditioning and extinction learning and memory.

1.1.2 The Association between Sleep, Learning, and Memory

While the functions of sleep are not fully understood, there is substantial evidence that sleep is involved in learning and memory. Despite wide usage of the terms “learning” and “memory”,

adequately explaining the processes involved is no easy task. Firstly, definitions of these terms must be adequately described to avoid confusion and misinterpretations commonly found in the literature. Henceforth, memory can be defined as a neural change caused by an experience, while learning is a process by which a memory is acquired and a new neural connection is made (Okano, Hirano, & Balaban, 2000). Memory has typically been separated into two categories or classifications: declarative and non-declarative memory, each containing distinct subcategories. Declarative memory is typically defined as consciously accessible memories of fact-based information. Its subcategories include semantic (general knowledge, not associated with an event) and episodic (memory of past events) memory. Non-declarative, or nonconscious memory, includes procedural memory, which can be learned implicitly. Procedural memory is memory for the performance of actions, such as habits, like brushing teeth, and other cognitive skills, like reading (Tulving, 1985; Walker & Stickgold, 2004). Other forms of non-declarative memory include, conditioning, non-associative, and priming memory.

The focus of the present study is on associative-conditioning learning and memory. Associative learning is a process by which a relationship between two stimuli is learned and remembered. One type of associative learning is classical conditioning, often referred to as Pavlovian conditioning. Classical conditioning can be obtained using a wide range of stimuli. The basic requirements consist of a biologically significant stimulus, such as food, electric shock, or a context (e.g. room), which evokes a reflexive response. The “biologically significant” stimulus is called the unconditioned stimulus (US); for example, an aversive, or conversely a pleasant stimulus. The response it elicits is known as the unconditioned response (UR) and can be responses such as freezing, changes in heart rate, breathing, or skin conductance. Additionally, a previously neutral stimulus, such as a tone or a light, is repeatedly presented just before the US, until the

neutral CS can evoke a response unaccompanied by the US called the conditioned response (CR; Gluck & Myers, 2001).

Analogous to the above concepts are the developmental stages of memory. The beginning stage is memory encoding, also referred to as acquisition, and can be followed by several post encoding stages, most notably consolidation. Encoding entails the formation of a representation within the brain, allowing for further processing (see Walker & Stickgold, 2004, for review). The recently formed memory can then be consolidated, a process by which the memory becomes increasingly resistant to interference from competing factors through the simple passage of time, without further practice or reprocessing (McGaugh, 2000). Other post encoding processes include the integration of a recently acquired representation with previously established memories. That is, the process by which the representation is associated with prior experiences and knowledge. Additionally, a memory representation can be translocated (anatomically reorganized within the central nervous system) or it can be subjected to erasure (see Walker & Stickgold, 2004, for review). If a memory is stabilized through the processes consolidation, it can be recalled. Recall is also a process that can destabilize the memory representation. The destabilized representation may reconsolidate, and if it does not reconsolidate, it can quickly degrade (Nader, 2003).

Memories associated with emotional experiences have been shown to persist for a considerably longer period, when compared to neutral memories (Kleinsmith & Kaplan, 1963; LaBar & Phelps, 1998; Walker & Tarte, 1963). Physiological responses accompanying an event influence the process by which it is encoded, with interactions between the amygdala and the hippocampus central to the idea (Richardson, Strange, & Dolan, 2004). Memory consolidation is modulated by the release of acetylcholine, norepinephrine, and other β-adrenergic onto basolateral amygdala (BLA), which goes on to project to several brain regions, including the central amygdala,

hippocampus, medial prefrontal cortex, bed nucleus of the stria terminalis, and nucleus accumbens. The BLA, in turn, influences the process of consolidation through the aforementioned projections (Mandyam, 2013; Mcintyre, Power, Roozendaal, & Mcgaugh, 2003). Memories acquired amid stress-related events and the effects these experiences have on the developmental stages of memories is particularly important to the study of Post-Traumatic Stress disorder (PTSD).

Sleep and memory have been functionally linked in the literature for quite some time. The effect of sleep on memory is largely beneficial. For instance, sleep increases the ability to retain information over time after initial learning (Benson & Feinberg, 1975; Graves, 1936; Richardson & Gough, 1963). This effect is likely due to the increased resistance to interference after sleep (Alger, Lau, & Fishbein, 2012; Ellenbogen, Hulbert, Jiang, & Stickgold, 2009; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006). In addition to retention, sleep has also been implicated in the memory consolidation. There is evidence that REM sleep may be significant to the consolidation of emotional information and procedural tasks (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Kuriyama, Stickgold, & Walker, 2004; Plihal & Born, 1999; Smith, 2004; see Payne, 2004 & Stickgold, 2005, for review). While, NREM, especially slow wave sleep, has been implicated in declarative and episodic memory (Plihal & Born, 1997; Schabus et al., 2004; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002; see Gais, 2004 & Stickgold, 2005, for reivew).

Further, neural activity produced during learning has been shown to be reinstated offline during REM sleep (Maquet, 2001; Stickgold, Hobson & Fosse, 2001). Studies have consistently found increases in REM sleep after learning in animals, yet the results in humans have been mixed. Nevertheless, there is still a rather substantial amount of evidence that increases in REM sleep persists after the learning of procedural memories (De Koninck, Christ, Hébert, & Rinfret, 1990;

Fishbein, Kastaniotis, & Chattman, 1974; Lucero, 1970; Mandai, Guerrien, Sockeel, Dujardin, & Leconte, 1989; C. Smith, Kitahama, Valatx, & Jouvet, 1974; C. Smith, 2001).

Sleep, also, has been hypothesized to aid in the strengthening of the factually based information, while reducing the associated emotional reactivity to the memory, referred to as "sleep to forget, sleep to remember". According this hypothesis, sleeping involves the decoupling of the hippocampal-associated activity (information) of an event and the amygdala activity (emotional reactivity) with the experience. Failure to achieve this decoupling can result in the persistence of the affective "charge" remaining within memory networks and could become a risk factor for anxiety disorders (Walker & van der Helm, 2009). Akin to this claim are the findings that emotional memories are strengthened through sleep, particularly REM sleep (Greenberg, Pearlman, Schwartz, & Grossman, 1983; Nishida, Pearsall, Buckner, & Walker, 2009; Wagner, Gais, & Born, 2001; Walker, 2009). The relationship between emotionally relevant experiences, more specifically fear learning paradigms, and sleep will be discussed in depth in section 1.1.5.

1.1.3 Theta Activity in Emotional Learning and Memory

Theta activity is most prominent in periods of synchronized activity between the 4-8 Hz frequency bands and is a hallmark of tonic REM sleep. Theta activity has been investigated in animals and, to some degree, in humans. Rodent experiments have shown that increases in hippocampal theta activity are associated with behavioral activities such as navigation and the encoding of working and episodic memory (Jacobs & Kahana, 2010; Jensen & Colgin, 2007). Another study reported increased coherence of theta between the hippocampus, medial prefrontal cortex, and amygdala during REM sleep promotes the consolidation of fearful memories (Popa, Duvarci, Popescu, Léna, & Paré, 2010). In addition, rats showed increased theta activity during REM sleep subsequent to

avoidance task training (Fogel, Smith, & Beninger, 2009) In humans, EEG theta activity recorded from the scalp was enhanced following a paired associative learning task (Fogel, Smith, & Cote, 2007). Theta power recorded via magnetoencephalography was the strongest oscillatory activity during a navigation task and was associated with an increased ability to recall dreams the morning following sleep (Araújo, Baffa, & Wakai, 2002).

One study associated REM theta power during an afternoon nap with enhanced emotional memory consolidation (Nishida et al., 2009). In Nishida's et al.'s study, individuals were shown emotionally negative and neutral picture stimuli during two "study" sessions and were randomized to a nap or no-nap group. The two study sessions were followed by a recognition test, in which participants were asked if they had been shown a picture in either one of the previous sessions. The first study session took place 4 hours before a recognition memory test, and a second session took place 15 minutes before the test. In between the study sessions, the nap group had the opportunity to sleep for up to 90 minutes, while the no-nap group remained awake. The nap group, overall, showed better performance in the recognition of negative emotional stimuli. Within the nap group, increased right prefrontal relative theta power during REM sleep was significantly correlated with improved recognition of negative emotional stimuli (Nishida et al., 2009). The authors proposed that REM sleep theta power during a nap session may favor the consolidation of negative emotional memory stimuli, and suggest that this may be involved in pathological conditioning such as PTSD. However, the recognition of negative pictures is not a validated paradigm to study trauma-like reactions and thus, it is unclear how their findings can translate to sleep-dependent, fear-specific learning and memory processes.

In another study, two groups of African American individuals underwent two consecutive nights of PSG recording. One group had a significant amount of trauma-exposure, but did not

develop PTSD (resilient group). The second group was composed of individuals who met DSM-IV criteria for current PTSD. Following spectral analysis of the second night of sleep, the resilient group had greater frontal relative theta power during the last REM sleep period when compared to the PTSD group. The authors suggested that increased theta power during REM sleep may be associated with increased resilience to traumatic experiences. This study provided evidence that REM sleep theta power may be associated with trauma-relevant emotional memory processing during sleep (Cowdin, Kobayashi, & Mellman, 2014). However, this study did not specifically assess sleep-dependent memory processes in the two groups and only observed theta power from trauma-exposed individuals. Additionally, there was no use of a paradigm modeling traumatic events (e.g. fear conditioning).

These two studies suggest a contradictory relationship between theta power and negative emotional memories. A nap-related increased frontal theta power during REM sleep was associated with increased negative emotional memory consolidation, but resilient, trauma-exposed individuals also showed increased frontal theta power during REM sleep. While both studies suggested that theta power during REM sleep may be significant in the context of PTSD and other fear-related anxiety disorders, neither used methods nor measures that would allow for specifically testing this hypothesis.

The present study sought to test the hypothesis that theta power during sleep may be a correlate of fear learning and memory, by using a validated paradigm of fear conditioning in early, acute post associative learning, the learning and recall of negative salient experiences.

1.1.4 Fear Learning Paradigms

Fear conditioning, extinction, extinction-recall, and renewal are all components of a well-established paradigm used for studying fear learning and response in the laboratory setting, and are used to model anxiety disorders in animals and humans. Fear conditioning is the process by which a neutral, conditioned stimulus (CS, e.g., a color) is repeatedly paired with an aversive stimulus, known as the unconditioned stimulus (UCS, e.g., mild electrical stimulation). In both animals and humans, the context in which this pairing occurs also becomes associated with the UCS. Eventually, the CS (and context), now associated with the US, evokes a conditioned fear response (CR) without the presentation of the US. This response is called the conditioned response (CR; Craske, Hermans, & Vansteenwegen, 2006). In a typical fear conditioning procedure, there is a neutral stimulus (CS-), which remains unassociated with any unconditioned stimulus, and at least one conditioned stimulus (CS+) paired with the US. Rat studies use freezing behavior to measure the magnitude of the conditioned response. Skin conductance response (SCR) is typically used, in humans.

The study of electrodermal activity dates back to 1880 (Neumann & Blanton, 1970). Typically, skin conductance is the unit by which electrodermal activity is measured. Experimentally, its measurement is conducted by applying a small electrical current between two electrodes placed on the palm and can be recorded from other locations, as well. This allows for the measurement of either resistance or conductance (by taking the inverse of resistance) between the two points. The tonic measure of SC is described as the skin conductance level, while the phasic measure is skin conductance response (SCR). SCR is commonly used as an index of sympathetic nervous system activity. In human studies, it is used as an index of conditioned fear responses in the study of fear learning and expression (Boucsein, 2012).

Fear extinction, is the gradual reduction in the behavioral or physiological response to the CS+, when the CS+ is repeatedly presented without the aversive US. The ability to reduce the fear response upon repeated presentations of the CS without the US is referred to as extinction learning. Originally assumed to be the erasure of the recently established fear-based response, fear extinction has been shown to be a separately learned memory that competes with the original conditioning memory to prevent the manifestation of a behavioral response (Milad & Quirk, 2002; Pavlov, 1927). This relationship is believed to be crucial to fear learning and memory, as these processes are proposed as putative mechanisms underlying anxiety disorders, including PTSD.

When using conditioning to model anxiety disorders, for instance PTSD, the traumatic experience is considered the US, and the repeated fear response with or without the presence of the US is seen as the CR (Grillon, Southwick, & Charney, 1996; Pitman, 1988). PTSD has been associated with the enhanced acquisition of fear responses. Further, a decreased ability to extinct fear responses has been observed, commonly resulting in the failure of extinction (Milad et al., 2007; Orr et al., 2000; Orr, Meyerhoff, Edwards, & Pitman, 1998; Wessa & Flor, 2007).

Fear extinction-recall is a procedure where the CS is presented without the US in the extinction context to produce the retrieval and expression of the extinction memory. Spontaneous recovery of the fear response is found during recall, after an arbitrary amount of time passes. Similarly, during fear renewal, the CS is presented without the US. However, the CS is shown in the conditioning context, generating an even larger recovery of the fear response than found in extinction-recall (Craske et al., 2006).

1.1.5 Sleep and Fear Learning

Studies have shown a bidirectional relationship between sleep and fear learning, leading to investigations of how manipulating sleep may affect fear learning and extinction. For example, animal studies have found that sleep deprivation after fear conditioning, impaired the consolidation of the fearful memory (Graves, Heller, Pack, & Abel, 2003). Rapid eye movement sleep deprivation following fear conditioning compromises extinction learning (Silvestri, 2005). On the other hand, fear conditioning disrupted REM sleep later in the evening (Sanford, Silvestri, Ross, & Morrison, 2001). In humans, one study showed that sleep promoted the generalization of extinction memory to a similar stimulus. Additionally, extinction memories were better recalled in the morning than in the evening, suggesting a circadian effect in fear learning and responses (Pace-Schott et al., 2009, 2013). However, the specific aspects of sleep that may explain the impact of sleep on fear learning and extinction memory remains unknown. The present study aims to evaluate whether theta power during REM sleep was associated with fear conditioning and extinction learning and memory in healthy young adults.

Because NREM sleep is also disrupted in anxiety disorders (see Wulff et al., 2010, for review), we also evaluated the relationship between theta power and fear conditioning and extinction learning and memory during NREM sleep.

1.2 STUDY AIMS AND HYPOTHESES

Sleep is involved in learning and memory. Similarly, there is an emerging body of work suggesting the existence of a role for theta activity in these processes. Fear paradigms such as conditioning,

extinction, extinction-recall, and renewal have a bidirectional relationship to sleep, as well. Despite this, there has not been a thorough investigation of theta activity during a full night of sleep and its relationship to subsequent fear responses. The present study aims to investigate this relationship in healthy young adults.

1.2.1 Study Questions

To evaluate the relationship between theta activity and fear-learning outcomes, the present study sought to answer the following questions:

1. Do REM and NREM sleep theta activity recorded the evening prior to fear conditioning procedures predict the index of conditioned fear responses measured as skin conductance response?
2. Do REM and NREM sleep theta activity the evening prior to fear conditioning procedures predict the magnitude of fear extinction?
3. Do REM and NREM sleep theta activity the evening prior to fear conditioning predict the retention of the extinguished memory during fear extinction-recall?
4. Do REM and NREM sleep theta activity the evening subsequent to fear conditioning, extinction, and extinction-recall predict the retention of the extinguished memory during fear renewal, the following morning?

1.2.2 Hypotheses

Because of the formerly discussed findings regarding theta activity and negative emotional memory consolidation, it was hypothesized that increased REM and NREM sleep theta activity

would favor the acquisition and consolidation of the fear conditioning memory. Given the greater quantity of theta activity found in REM sleep compared to non-rapid eye movement sleep and the previously considered findings, the results were predicted as such:

1. Increased theta activity in REM and NREM sleep during the night prior to the experiment will show smaller physiological responses during fear conditioning.
2. Increased theta activity in REM and NREM sleep during the night prior to the experiment will show greater fear extinction.
3. Increased theta activity in REM and NREM sleep during the night prior to the experiment will have less retention of the extinction memory during extinction-recall.
4. Increased theta activity in REM and NREM sleep during the night subsequent to fear conditioning, extinction, and extinction-recall will have less retention of the extinction memory during fear renewal.

2.0 METHODS

The larger study titled “Effects of Dose-Dependent Sleep Disruption on Fear and Reward” (DoD Funding: Log No. 11293006), from which a subsample of data obtained for the current study, was a cross-sectional study evaluating the relationship between doses of sleep disruption and the neural networks involved in fear responses and rewards processing. The study was approved by the Institutional Review Board (IRB) at the University of Pittsburgh and the Human Research Protect Office of Department of Defense. The present analysis obtained a subsample of polysomnographic and physiological measure of peripheral fear response data (e.g. skin conductance response) from 31 participants whom completed a full night of uninterrupted sleep during the experimental procedures.

2.1 PARTICIPANTS

2.1.1 Recruitment

Healthy young men and women between the ages of 18 and 30 years old were recruited from the Greater Pittsburgh Metropolitan Area to participate in a larger study investigating the relationship between sleep disruption and emotional processing. Recruitment was performed through a variety of public advertising media outlets including: online websites (e.g. veteranssleep.pitt.edu, craigslist.com), social media sites (e.g. Facebook, Twitter), newspapers, radio, television, and bus advertisements. Additional recruiting sources consist of former participant referrals, pull-tab

flyers, and Google advertisements. Either participants contacted the research staff directly via information on the recruitment resources, where staff conducted a brief telephone-screening interview, or participants filled out a prescreening questionnaire, which was found via a link on the veteranssleep.pitt.edu website. Eligible participants were then contacted by research staff to complete a brief telephone screening evaluation.

2.2 PROCEDURES

2.2.1 Screening Phase

2.2.1.1 Informed Consent

Informed consent for the larger study was obtained during an in-person discussion with the participant and a trained research staff member. Participants were encouraged to ask questions throughout the consent discussion. At the end of the meeting, all participants signed a consent form, providing informed consent to act as a participant in a research study. Screening consent for the larger study was acquired after performing certain screening procedures, but prior to performing any research interactions. Consent was, again, obtained prior to beginning of any experimental procedures.

2.2.1.2 Screening Phase

Interested individuals contacted the research staff directly, or filled out a prescreening questionnaire via a link on veteranssleep.pitt.edu. Research staff, then, either performed a telephone-screening interview with those individuals who contacted the research staff directly, or

contacted those whom were eligible after the completion of the prescreening interview. The telephone screening consisted of obtaining demographic and clinical data. Demographic information included age and sex. Clinical information included the existence of medical, psychiatric, and sleep disorders, current medications, current alcohol and drug use, caffeine and stimulant use, the existence of metal in the body, and claustrophobia. Succeeding the completion of the telephone-screening interview, eligible participants, who remained interested, were invited for a consent visit, and then completed the remaining screening phase procedures during an in-person, comprehensive diagnostic evaluation conducted at the University of Pittsburgh over the course of two to four visits.

At the first visit, a trained research staff member described the screening phase of the larger study in detail and obtained written informed consent from the participants for the screening procedures. Participants were additionally given an actigraph and sleep diary to estimate sleep-wake pattern over a minimum of seven consecutive evenings. The sleep diary consisted of two portions. One of which was to be completed at wake time, while the remaining portion was completed at bedtime. The wake time portion asked questions concerning the time the participant went to bed, amount of time required to fall asleep, details surrounding any nocturnal awakenings, such as the number, cause, and duration, final time the participant got out of bed, and total time spent asleep. The bedtime portion asked questions about the prior day's activities including: meal times, naps, and exercise completed. Furthermore, participants listed any caffeine, alcohol, tobacco, and medications used throughout the day.

The additional instrument used to evaluate sleep-wake patterns was an actigraph. An actigraph is a bracelet, similar in style to a watch, containing an accelerometer, which monitors the quantity of arm movements per 60-second epoch. The activity information is then saved in the

device's memory, so it can be analyzed by a computer program. The program uses the data to determine periods of restfulness and wakefulness throughout the data acquisition period. Participants wore the actigraph 24 hours per day for a minimum of seven days on their non-dominant hand. The Respironics Actiwatch 2® (Philips Respironics, Bend, OR) was the instrument used for actigraphy evaluation. Eligible participants had regular sleep patterns, with at least six hours of sleep, but no more than nine hours sleep.

The diagnostic evaluation entailed a rapid clinical screening evaluation using self-reported measures, physical health evaluation, diagnostic interviews completed by the study coordinator, and a medical evaluation administered by a licensed physician. A portable two-channel apnea-screening device called Apnealink (ResMed, San Diego, CA) was given to the participants for home use. The Apnealink was worn for one full evening of sleep to rule out the possibility of sleep apnea.

Participants were excluded from the study during initial screening procedures if they had current or past history of the following medical issues: medical illness (excluded if within the past year), seizures, neurological disorders, diabetes, sleep apnea, color blindness, phase delay sleep disorder, phase advance sleep disorder, or hearing impairments. Participants were additionally excluded if they were found to have a current or past history (< 1 year) of the following psychiatric disorders: mood disorders, anxiety disorders, sleep disorders (excluded if within the past year), alcohol or substance abuse (excluded if within the past three months), psychotic disorder, or bipolar disorder. Other exclusionary criteria included: the use of any medications known to affect sleep, blood flow, or neural functioning, regular night shift work, irregular sleep-wake patterns, greater than two caffeinated beverages per day, or the individual had an apnea-hypopnea index greater than 5 (five events per hour of sleep).

Participants were excluded for the ensuing criteria due to MR safety or data integrity concerns: left-handedness, severe obesity, implanted medical devices, shrapnel, or bullet fragments, or a fear of enclosed spaces. Women were excluded if they were pregnant or breastfeeding, used Mirena or any other intrauterine device, progesterone, triphasic or quadriphasic oral contraceptives, oral contraceptives that contain ethinyl estradiol at a dose greater than 50 micrograms per day, or the participant had a menstrual period of an abnormal length.

The screening self-report measures used to identify ineligible participants were as followed: Insomnia Severity Index (ISI), Pittsburgh Sleep Quality Index (PSQI), PSQI Addendum for PTSD (PSQI-A), Epworth Sleepiness Scale (ESS), Edinburgh Handedness Inventory (EHI), Medical History Questionnaire (MHQ), Current Medications and Substance Use List, PTSD Checklist – Civilian Version (PCL-C), Patient Health Questionnaire (PHQ), and the Trauma History Questionnaire (THQ). The diagnostic interview typically lasted 1.5 hours and was used to assess the presence and severity of trauma history, current and past psychiatric disorders, symptoms of sleep disorders, and current physical health. A consensus meeting was held weekly with the research investigators and study coordinator to review information obtained throughout the diagnostic interview and to determine the participant's eligibility. The diagnostic instruments used to evaluate the preceding items included: Clinician Administered PTSD Scale (CAPS), Structured Clinical Interview for DSM-IV (SCID), and Structured Interview for DSM-IV Sleep Disorders (SLD).

The ISI (Bastien, Vallières, & Morin, 2001; Morin, 1993) is a self-report questionnaire containing seven items used to subjectively assess severity of insomnia symptoms, overall satisfaction with sleep, the type and asperity of daytime cognitive impairments, and miscellaneous sleep-related concerns. Each of the seven items is scored on a scale of 0 to 4 point(s). A total score

from 0 to 7 represents clinically insignificant insomnia, while a score greater than or equal to eight and less than or equal 14 signifies subthreshold insomnia. A cut off score of 14 has been shown to distinguish between clinically significant insomnia and clinically insignificant insomnia. Participants remained eligible if they had an ISI score less than 7.

The PSQI (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) is self-report questionnaire consisting of 18 items assessing seven components of sleep quality including: subjective sleep quality, sleep latency, duration, efficiency, disturbances, the use of the sleep medication, and daytime dysfunction. Each of the components is rated on a scale from 0 to 3 and is determined by the frequency of each disturbance. Zero represents no disturbances in the past month, 1 equals less than once per week, 2 equals once or twice per week, and 3 equals three of more disturbances per week. The total score ranges from 0 to 21, where a cut off score of 5 discriminates between a good sleeper and a bad sleeper. Participants eligible for the study had a score of less than 5.

The PSQI-A (Germain, Hall, Krakow, Katherine Shear, & Buysse, 2005) is a self-report instrument assessing the frequency of disruptive nocturnal behaviors in seven items. Each of the items are rated on a scale of 0 to 3, with a rating system consistent with the PSQI (i.e. zero equals no disturbances in the past month, etc.). A global score with a range of 0 to 21 is obtained from the sum of all seven items. Three additional items regarding the co-occurrence of anxiety and anger are obtained for informative purposes. However, these items are not included in the total score. A cut off score greater than or equal to 4 is used to identify individuals with PTSD. Participants with a score less than 4 were included in the study.

The ESS (Johns, 1991) is self-report questionnaire containing eight items used to subjectively measure an individual's sleepiness. Each of the components rates one's propensity to become drowsy or doze off, in a given situation, on a scale from 0 to 3. The total score has a range

from 0 to 24, found by taking the sum of the eight components. A score of 10 or greater indicates significant daytime sleepiness and may require medical attention to investigate the presence of sleep-disordered breathing. Eligible participants had a score of less than 10.

The EHI (Oldfield, 1971) is a 10 question instrument used to determine hand dominance. Each question asks an individual's hand preference during a particular activity on a scale of 1 to 5. One represents "always left", while 5 represents "always right". The normalized total score ranges from -100 to 100, with a score greater than or equal to 40 denoting right-handedness. Individuals with a score of 40 or greater were included for the larger study.

The MHQ was a locally developed questionnaire consisting of a list of various chronic medical and psychiatric disorders. Participants were asked if they had the listed conditions and what medications associated with the medication were taken. Associated with the MHQ was a Current Medications and Substance Use List, which asked participants to list any medication, supplement, alcohol, tobacco, caffeine, or illicit drug use, and whether they are over-the-counter or prescribed. The questionnaire was used to identify if a participant was consuming any medications known to affect sleep or wake functions including, but not limited to: benzodiazepines, hypnotics, antidepressants, antipsychotics, anxiolytics, antihistamines, decongestants, beta-blockers, corticosteroids, and diuretics.

The PCL-C (Weathers, Litz, Hermans, Huska, & Keane, 1993) is a 17-item self-report measure of the DSM-IV symptoms of PTSD. The civilian version of the checklist asks about symptoms related to identifiable "stressful experiences". Each component is scored on a scale from 1 to 5, allowing for a range of 17-85. A cut off score of 50 is used to determine clinically significant PTSD symptoms. Eligible participants had a score of less than 50, and did not meet criteria for PTSD on the CAPS.

The PHQ (Spitzer, Kroenke, Williams, & Group, 1999) consists of questions regarding an individual's prior and current history of mood and anxiety symptoms, in addition to alcohol and substance dependence. It is a validated self-report version of the Primary Care Evaluation of Mental Health Disorders, which is a screening instrument used in a clinical setting.

As stated previously, the following instruments being described were used during the diagnostic interview, which lasted approximately 1.5 hours. The CAPS (Blake et al., 1995) is a structured interview used to make a categorical PTSD diagnosis, in addition to determine symptom severity. Diagnosis of past and current PTSD are based on the 1-2 rule, that is a frequency score of one out of a scale from 0 to 4 and a severity score of 2 on a scale from 0 to 4 are required for a symptom to meet criterion. The tool takes roughly 60 to 90 minutes to administer. Diagnosis of PTSD was determined using the DSM-IV diagnostic algorithm, which include one "B" criteria, three "C" criteria, and two "D", "A", "E", and "F" criteria. Participants who meet the diagnostic criteria for current or past PTSD based on the administration of the CAPs were excluded from the study and given resources for evaluation and treatment.

The SCID (First, Spitzer, Gibbon, & Williams, 2002) is a diagnostic exam used to determine the presence of current or past episodes of DSM-IV Axis I disorders. All modules for the non-patient addition were used to determine eligibility in the study, including mood episodes, psychotic screening, substance use disorders, anxiety disorders, somatoform disorders, and eating disorders. The assessment typically takes between 30 and 90 minutes to administer and was assessed to determine if participants required a rapid treatment referral. Individuals with no psychiatric disorders within the past year were eligible for the study, excluding bipolar and psychosis, in which any history would require exclusion.

The SLD is an in-house developed assessment tool used to determine both current and past episodes of the presence, frequency, and severity of DSM sleep disorder symptoms. Common sleep disorders not contained in the DSM-IV including obstructive sleep apnea and restless leg syndrome are assessed using the SLD. Participants with symptoms of insomnia, sleep apnea, narcolepsy, idiopathic hypersomnia, and restless leg syndrome were excluded from the study and referred to a sleep evaluation center for an in-depth assessment of symptoms.

2.2.2 Experimental Phase

Participants who remained eligible after completing the entirety of the screening procedures were invited to the experimental phase of the study. After obtaining informed consent, the participants scheduled three consecutive evenings of polysomnographic recording at the Sleep and Chronobiology Laboratory of the Western Psychiatric Institute and Clinic. For a week prior to the sleep lab stay, participants once again wore an actigraph and completed a sleep diary to confirm consistent sleep-wake patterns prior to their stay in the Sleep and Chronobiology Laboratory. Alcohol and caffeine use was also reevaluated during this time. Upon arrival at the Western Psychiatric Institute and Clinic, participants underwent a urinary drug screen, followed by beginning overnight polysomnographic recording. Participants were able to leave the morning subsequent to the first evening of recording for the duration of the daytime. Participants, then, returned to Western Psychiatric Institute and Clinic in the evening to prepare for the functional Magnetic Resonance Imaging (fMRI) scan series, in addition to the two remaining evenings of polysomnographic recording. The present study use PSG and qEEG data from the second and third night of recording.

The fear learning procedures were completed in the fMRI environment. A research staff member escorted the participant to the Magnetic Resonance Imaging Research Center (MRRC) at the University of Pittsburgh Medical Center Presbyterian for each of the four scans. Fear conditioning and extinction procedures were completed the morning following the second night of polysomnographic recording. Fear extinction-recall protocol took place in the evening, between 17:00 and 19:00 on Night Two of PSG recording. Fear renewal procedures were completed the morning following the third night of polysomnographic recording (Figure 1). Participants received $75 for the completion of the screening procedures, and another $270 for the entirety of the participant's stay in the Sleep and Chronobiology Laboratory.

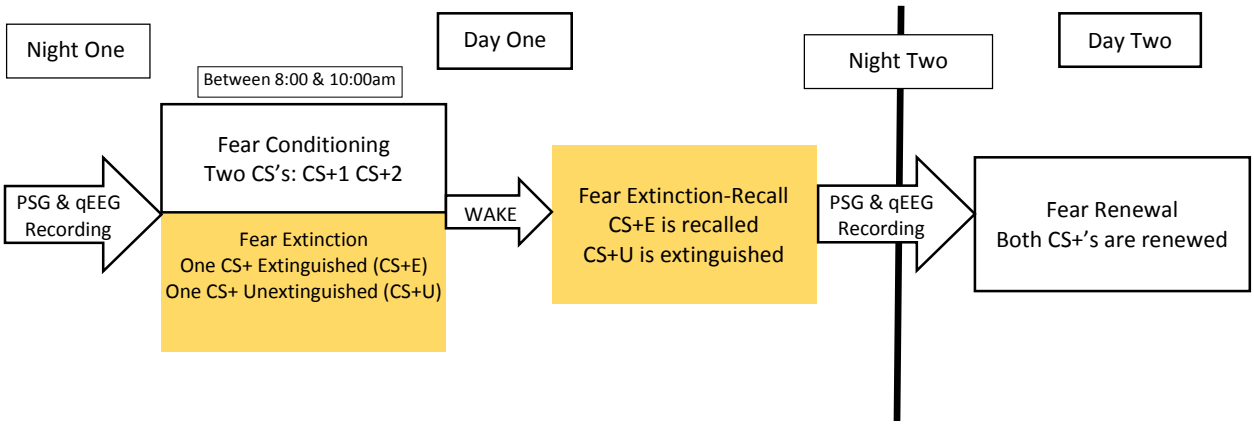

Figure 1. Experimental Design. Participants arrived at 5:00pm and underwent PSG and qEEG recording the first night of the experiment. Following the first night of recording, fear conditioning and fear extinction procedures took place with two conditioned stimuli (CS+1; CS+2). One conditioned stimulus was extinguished (CS+E) during fear extinction, and one conditioned stimulus was left unextinguished (CS+U). Approximately eight hours later fear extinction-recall took place. The morning following Night 2, fear renewal procedures were completed. During both fear extinction-recall and fear renewal, both conditioned stimuli were presented.

2.2.3 Polysomnography (PSG) and Quantitative EEG (qEEG)

Participants underwent three consecutive evenings of polysomnographic recordings in the Sleep and Chronobiology Laboratory at Western Psychiatric Institute and Clinic. PSG recording included a standard electroencephalogram montage with bilateral frontal, central, and occipital leads, two electroocculogram, and chin electromyogram leads. Grass Telefactor M15 bipolar Neurodata amplifiers and Stellate Harmonie collection software was used for recording for both waking and sleep EEG. All scorers had demonstrated a >90% concordance for scoring epochs with reference records. Spectral power for five frequency bands were attained: delta (0.5 Hz – 4.0 Hz), theta (4.0 Hz – 8.0 Hz), alpha (8.0 Hz – 12.0 Hz), beta (12.0 Hz – 40.0 Hz), and gamma (>40.0 Hz). The current analysis was restricted to the theta frequency band from the central leads; however, all frequency bands were obtained in order to calculate total power and obtain relative theta power. Relative power in the theta band was calculated by dividing the average power of the theta band by the entire spectrum and multiplying the results by 100.

2.2.4 Fear Conditioning, Extinction, Extinction-Recall, and Renewal

The fear learning protocol consisted of five phases: habituation, conditioning, extinction, extinction-recall, and renewal. Fear conditioning and extinction procedures were performed the morning following the second night of polysomnographic recording. The aversive, unconditioned stimulus was an electric shock, or mild electrical stimulation, administered using a Coulbourn Transcutaneous Aversive Finger Stimulator (Coulbourn Instruments, Allentown, PA) on the index

and major finger of the participant's dominant hand. Participants chose a shock level that was "highly annoying, but not painful" through the administration of increasing intensities of mild electrical stimulation from 0.2 mA to 4.0 mA. The level chosen was then used throughout the entirety of the fear conditioning procedures. Participants were told they may or may not be shocked for all subsequent fear learning procedures. However, mild electrical stimulation was only administered during fear conditioning protocol.

Figure 2. Conditioned stimulus photos with both contexts. The conditioned stimuli were digital photographs of three lamps each illuminated as a different color (red, blue, yellow) and displayed on a computer screen within two distinct photographic environments, or contexts, the "extinction context" and the "conditioning context".

The conditioned stimuli were digital photographs of three lamps each illuminated as a different color (red, blue, yellow) and displayed on a computer screen within two distinct photographic environments, or contexts (Figure 2). The two contexts consisted of an office with a computer and a conference room with a table. The office environment served as the "conditioning context" and was the only context where the conditioned stimuli were paired with mild electrical stimulation. The other context served as the "extinction context", in which the conditioned stimulus was shown to the participant without the administration of the aversive, unconditioned stimulus during extinction and extinction-recall procedures.

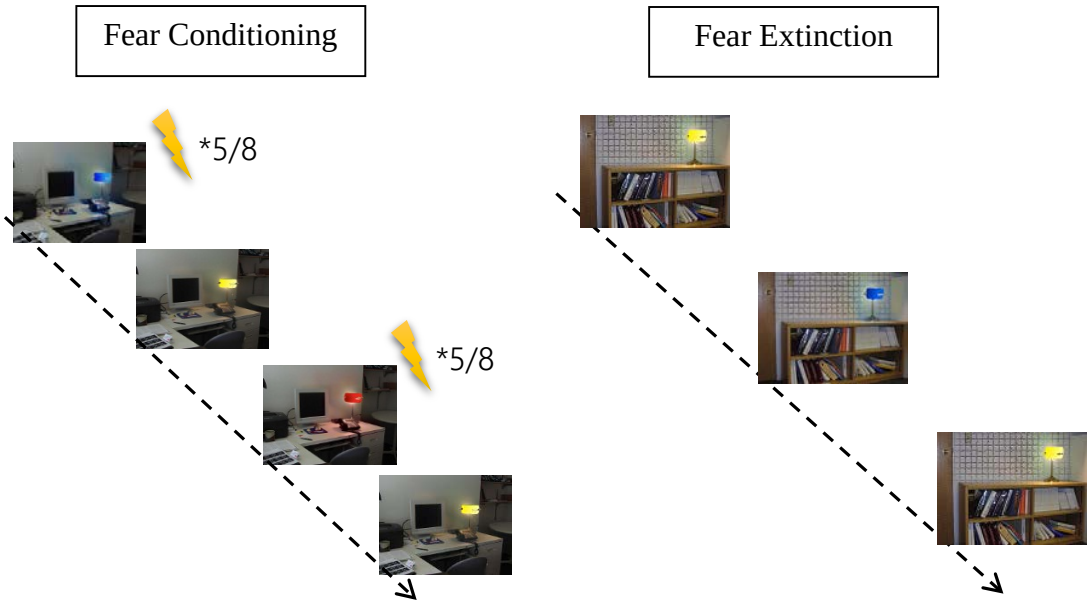

Figure 3. Fear conditioning and fear extinction temporal diagram. During fear conditioning (left), two conditioned stimuli (CS+1; CS+2; lamp colors) were repeated paired with the aversive, unconditioned stimulus (UCS; i.e. shock). A third conditioned stimulus (i.e. lamp color; CS-) was never accompanied by any mild electrical stimulation ("safe color"). During fear extinction (right), only one CS+ and the CS- was presented. The CS+ was presented sixteen times in the extinction context without the administration of the UCS, with sixteen randomly interspersed trials of the CS-.

Prior to fear conditioning protocol, a habituation phase was completed by the participants. The habituation phase showed all possible combinations of the lamp colors and the contexts to the participants. Subjects were told no shocks would be administered during this first phase.

For the fear conditioning phase (figure 3), two conditioned stimuli (CS+1; CS+2; lamp colors) were repeated paired with the aversive, unconditioned stimulus (UCS; i.e. shock). A third conditioned stimulus (i.e. lamp color; CS-) was never accompanied by any mild electrical stimulation ("safe color"). Another sixteen presentations of the conditioned stimulus unaccompanied by any shocks (i.e. CS-) were randomly interspersed throughout the CS+ trials. Consequently, the conditioning protocol consisted of 32 trials: eight trials of one CS+, eight trials of the additional CS+, and 16 trials of the CS-. Five of eight presentations of the two CS+'s were paired with a shock; for a total of 10 shocks. Each CS+ trial consisted of a three-second context-only presentation ("lamp light off"). This was followed by six seconds of one CS+ and the context were presented together. Finally, mild electrical stimulation was administered for 0.5 seconds immediately following the termination of the CS+ presentation (figure 4). The trials presenting the conditioned stimulus left unassociated with any shocks (i.e. CS-) consisted of a similar protocol,

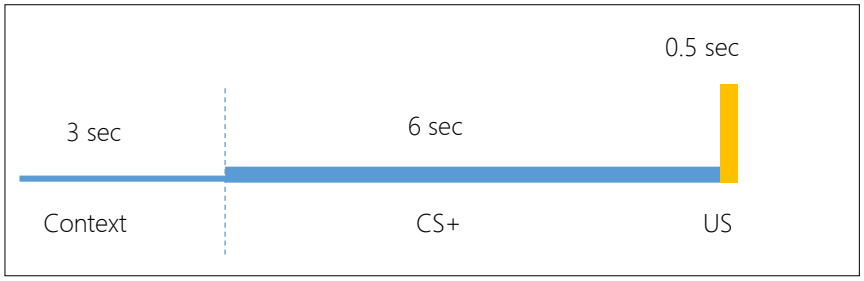

Figure 4. Diagram of a single CS+ trial during the conditioning phase. Each CS+ trial consisted of a three-second context-only presentation. After which, six seconds of both the CS+ and the context were presented together. Finally, mild electrical stimulation was administered for 0.5 seconds immediately following the offset of 10 of the 16 CS+ presentations.

two-second context-only presentation and six-second presentation of both the CS- and the context, excluding the administration of any mild electrical stimulation.

The fear extinction phase (figure 3) immediately followed fear conditioning. During the extinction phase, only one CS+ and the CS- was presented. The CS+ was presented sixteen times in the extinction context without the administration of the UCS, with sixteen randomly interspersed trials of the CS-. The CS+, therefore, was extinguished, becoming the extinguished conditioned stimuli (CS+E). The thirty-two total trials were the identical to the CS- trials during the conditioning phase. The second CS+ was not presented during the extinction phase, and remained unextinguished (CS+U). No shocks were administered throughout the extinction phase of the study. After the completion of fear extinction, participants were taken back to the Sleep and Chronobiology Laboratory by a research staff member. There, they remained monitored by a staff member and with PSG to verify that the participants remained awake during the day.

In the evening between 17:00 and 19:00, participants returned to the MRRC to complete the extinction-recall phase of the fear protocol. During this phase, all three of the lamp colors were presented in the extinction context, unaccompanied by any shocks. Eight trials of each CS+ were presented, in addition to 16 trials of the CS-, for a total of 32 trials. They, then, returned to the Sleep and Chronobiology Lab for the second night of undisturbed sleep with PSG recording.

The following morning between 8:00 and 10:00, they returned to the MRRC to complete the fear renewal phase. The fear renewal phase was synonymous with the recall phase; however, each type of conditioned stimulus (CS+E, CS+U, CS-) was presented in the conditioning context. Again, the participants were told they “may or may not be shocked” prior to this phase of the protocol.

2.2.5 Electrodermal Activity

The participants' skin conductance level (SCL) was recorded throughout the each phase of the fear protocol. SCL recordings were acquired from the hypothenar surface of the non-dominant hand using 9mm diameter Sensor Medics Ag/AgCl electrodes (BioPac Systems Inc., Goleta, CA) filled with a conductive solution and attached 14 mm apart from each other. A Coulbourn Lab Inc. Analog to Digital Converter (V19-16) digitized analog signals at 5 Hz and stored the values in a personal computer. Skin conductance response (SCR; Table 1) was computed by subtracting the mean SCL during the final two seconds of the context-only presentation from the maximum SCL during the six-second CS presentation. Indices of extinction-learning and extinction retention were calculated as outlined in the following section.

Table 1. Skin conductance response equation

Measure	Equation
Skin Conductance Response (SCR)	$Maximum\ SCL\ during\ six\ second\ CS\ presentation - mean\ SCL\ during\ the\ context\ only\ presenation$
*SCL – Skin Conductance Level	

2.2.6 Data Analysis

Analyses were conducted using SPSS 21/22/23 statistical software (IMB, Armonk, NY). Data was inspected for normality prior to running statistical evaluation. Additionally, paired t-tests of the average SCR across all eight trials of the CS- and its associated CS+ was completed to validate the fear conditioning procedure. Participants' SCRs were removed from the analyses as non-conditioners if the mean SCR to the CS- was greater than the mean SCR to both CS+'s recorded during the fear conditioning phase of the study. Linear regression analyses were, then, used to

investigate the relationship between theta activity the night prior to the fear learning procedures to determine fear learning outcomes. An alpha level of 0.05 was used as a threshold for all the analyses, and standardized beta coefficients were reported. Theta power in NREM and REM sleep was used in separate models. The fear renewal analysis used NREM and REM relative and average theta activity recorded the final night of the study as the independent variable.

For absolute and relative theta power and fear conditioning outcomes, the dependent variable was the square-root transformed SCR, recorded during the conditioning phase, averaged across all eight trials of each CS+.

Table 2. Equations used to compute outcomes of interest

Measure	Equation
Extinction Learning Index	$100 - 100 * \left[\frac{Average\ SCR\ of\ the\ final\ two\ trials\ of\ Fear\ Extinction}{Maximum\ SCR\ recorded\ during\ Fear\ Conditioning}\right]$
Extinction Retention Index - Fear Extinction Recall	$100 - 100 * \left[\frac{SCR\ recorded\ during\ the\ first\ trial\ of\ Fear\ Extinction\ Recall}{Maximum\ SCR\ recorded\ during\ Fear\ Conditioning}\right]$
Extinction Retention Index - Fear Renewal	$100 - 100 * \left[\frac{SCR\ recorded\ during\ the\ first\ trial\ of\ Fear\ Renewal}{Maximum\ SCR\ recorded\ during\ Fear\ Conditioning}\right]$

Key:
CS – Condition Stimulus
SCR – Skin Conductance Response

For fear extinction, the Extinction Learning Index (ELI; Milad et al., 2008) was computed and used as the dependent variable. To measure a participant's index of overall extinction learning, the average SCR of the final two CS+E trials of the extinction phase was divided by the maximum SCR found at any point of the conditioning phase and multiplied by 100 to produce remaining

conditioned response at the conclusion of extinction learning. This value was subtracted from 100 to yield an index of extinction learning, (ELI).

The extinction-recall and renewal analysis utilized the Extinction Retention Index (ERI; Milad et al., 2008) for the dependent variable in linear regression analysis. The ERI was calculated in two similar manners. For the extinction-recall phase, the SCR recorded during the first trial of extinction-recall of the extinguished conditioned stimulus (i.e. CS+E) was divided by the maximum SCR observed during the conditioning phase and multiplied by 100 to produce the percentage of conditioned response recovered. This, in turn, was subtracted from 100 to yield an index of extinction memory retained. The ERI used for the investigation of renewal, instead, divided the maximum SCR observed during the conditioning phase by the first trial of renewal for the extinguished conditioned stimulus and the unextinguished conditioned stimulus (i.e. CS+E; CS+U; Table 2).

3.0 RESULTS

Table 3. Demographic information, screening and sleep measures

Characteristic	N (REM group; n = 20)		N (NREM group; n = 31)
Race			
White	11 (55.0%)		19 (61.3%)
Black or African-American	3 (15.0%)		3 (9.68%)
Hispanic	1 (5.00%)		1 (3.23%)
Asian	4 (20.0%)		7 (22.6%)
Undisclosed	1 (5.00%)		1 (3.23%)
Gender			
Male	9 (45.0%)		15 (48.4%)
Female	11 (55.0%)		16 (51.6%)
Measure	N	Mean	Standard Deviation
Age	31	23.9	3.94
Insomnia Severity Index	31	1.32	1.40
Pittsburgh Sleep Quality Index	31	1.68	1.17
Pittsburgh Sleep Quality Index – Addendum	31	.161	.374
Epworth Sleepiness Scale	31	3.45	1.88
PTSD Checklist – Civilian Version	31	18.9	3.20
Sleep Latency N1 (mins)	31	12.5	13.1
Time Spent Asleep N1 (mins)	31	453	18.5
Sleep Efficiency N1 (%)	31	94.2	2.95
Percent NREM sleep N1 (%)	31	72.4	4.13
Percent REM sleep N1 (%)	31	27.6	4.13
Sleep Latency N2 (mins)	31	10.9	9.56
Time Spent Asleep N2 (mins)	31	448	30.3
Sleep Efficiency N2 (%)	31	93.3	6.10
Percent NREM sleep N2 (%)	31	72.1	3.98
Percent REM sleep N2 (%)	31	27.9	3.98

Participants were a subsample of 31 healthy, right-handed young men and women (16 females; 15 males) who completed laboratory PSG and functional magnetic resonance imaging recording (fMRI). For the purposes of this examination, participants who completed a full night of uninterrupted sleep were used for analysis. A total of 31 participants between the ages of 18 and 30 years-old (mean = 23.6; SD = 3.80; median = 23.1) were studied for the analysis of NREM theta activity and fear learning outcomes. Thirteen participants were excluded from REM sleep

analysis due to a large number of EEG artifacts found during sleep stage scoring. For the REM theta activity and fear learning outcomes analysis, 20 participants (11 females; 9 males), similarly, between the ages of 18 and 30 years-old (mean = 23.9; SD = 3.94; median = 23.1) were examined. Of the 31 participants, 61.3% were Caucasian, 22.6% were Asian, 12.9% were African-American, and one participant's race was undisclosed (Table 3). All participants were free of any psychiatric or sleep disorders and were medically cleared to participate in the study by a licensed physician.

3.1 DESCRIPTIVE STATISTICS AND PAIRED T-TEST

Descriptive statistics for the sample screening and sleep measures are summarized in Table 3. All participants were free of any psychiatric or sleep disorders and were medically cleared to participate in the study by the study physician.

To confirm that the fear conditioning procedures were successful, paired-samples t-tests were conducted to compare square-root transformed mean SCR during the CS- trials of conditioning coincided with the CS+1 (CS-1) and the CS+1 condition. That is, the CS-1 was the SCR recorded during the first eight CS- trials of fear conditioning. There was a significant difference in the square-root transformed SCR values for CS-1 (M = .12, SD = .214) and the CS+1 (M = .53, SD = .346) condition; $t(30) = -10.05$, $p < 0.001$. Additionally, a paired-samples was conducted to compare square-root transformed mean SCR during the CS- trials of conditioning coincided with the CS+2 (CS-2) and the CS+2 condition. That is, CS-2 was the SCR recorded during the final eight CS- trails of fear conditioning. There was a significant difference in the square-root transformed mean SCR values for the CS-2 (M = .026, SD = .182) and the CS+2 (M = .21, SD = .292) condition; $t(30) = -3.08$, $p = .004$. Together, these results confirmed that fear

conditioning was successful, as the mean SCR values during the CS- trials was significantly lower than the associated SCR values during the CS+ trials. Findings for the above analyses are summarized in Figure 5.

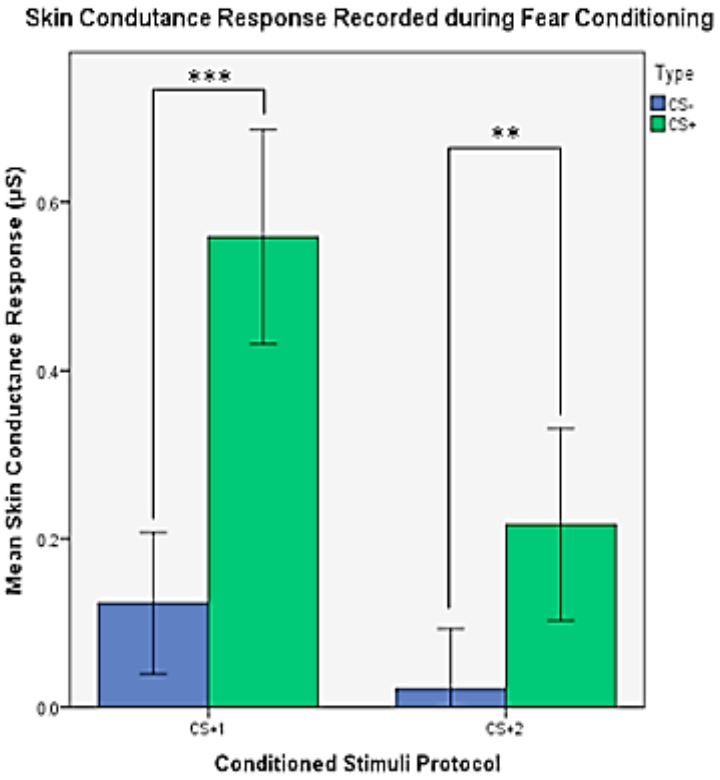

Figure 5. Graph of CS- and CS+. **p < .005; ***p < .001

3.2 REM THETA ACTIVITY AND FEAR LEARNING PARADIGMS

Descriptive statistics for the square-root transformed mean SCR recorded during fear conditioning, ELI, and ERI's are summarized in Table 4. Two participants were non-conditioners and removed from analysis. Nineteen of the 31 subjects had REM PSG data that was of high enough quality for data analysis.

3.2.1 REM Theta Power and Fear Conditioning

Descriptive statistics for relative and average (absolute) theta during REM sleep were obtained from the C3 and C4 electrodes for the night prior to the experiment are summarized in Table 5. Relative and average REM sleep theta power from the C3 and C4 electrodes the night prior to fear learning procedures did not significantly predict the square-root transformed mean SCR averaged across all trials of the CS+1 and CS+2 recorded during fear conditioning (Table 5). That is, the independent variable, REM sleep theta power from the C3 and C4 electrodes, did not significantly predict the index of conditioned fear responses measured as skin conductance response.

3.2.2 REM Theta Power and Fear Extinction

Further, relative and average theta power from the C3 and C4 sites during REM sleep, the night prior to fear learning procedures did not significantly predict the ELI measured during fear extinction procedures, or in other words, magnitude of fear extinction (Table 5).

Table 4. Mean and standard deviation for outcomes of interest

Measure	N	Mean	Standard Deviation
Shock Intensity (mA)	19	1.90	.622
SCR CS+1* (μS)	19	.460	.335
SCR CS+2* (μS)	19	.154	.257
CS- at CS+1	19	.148	.169
CS- at CS+2	19	.144	.366
ELI	17	93.7	14.7
ERI CS+E Recall	17	32.1	68.4
ERI CS+E Renewal	18	22.2	170
ERI CS+U Renewal	18	32.3	73.1
C3 REM Rel. Theta N1	19	.194	.057
C4 REM Rel. Theta N1	19	.195	.056
C3 REM Rel. Theta N2	19	.206	.056
C4 REM Rel. Theta N2	19	.202	.053
C3 REM Avg. Theta N1 ($\mu V^2/Hz$)	19	1.77	.940
C4 REM Avg. Theta N1 ($\mu V^2/Hz$)	18	1.70	.767
C3 REM Avg. Theta N2 ($\mu V^2/Hz$)	19	2.04	1.17
C4 REM Avg. Theta N2 ($\mu V^2/Hz$)	18	1.89	1.01

Note. Outliers were removed due to unreliable skin conductance data. Additional outliers removed from the regression analysis due to unreliable theta power measures but were included above.

*Square-root transformed values

Table 5. Simple linear regression analyses of relative and average REM theta power and outcomes of interest

	SCR CS+1*			SCR CS+2*		
Independent Variables	β	R^2	p	β	R^2	*p*
C3 REM Rel. Theta N1 (n = 19)	.003	.000	.989	.241	.058	.320
C4 REM Rel. Theta N1 (n = 19)	-.056	.003	.819	.123	.015	.616
C3 REM Avg. Theta N1 (n = 19)	-.319	.102	.183	-.239	.057	.325
C4 REM Avg. Theta N1 (n = 18)	-.286	.082	.249	-.264	.070	.290
	Extinction Learning Index (ELI)					
	β	R^2	*p*			
C3 REM Rel. Theta N1 (n = 17)	.106	.011	.686			
C4 REM Rel. Theta N1 (n = 17)	.096	.009	.715			
C3 REM Avg. Theta N1 (n = 17)	-.053	.003	.840			
C4 REM Avg. Theta N1 (n = 16)	-.229	.052	.394			
	Extinction Retention Index (ERI) CS+E Recall					
C3 REM Rel. Theta N1 (n = 17)	-.021	.000	.936			
C4 REM Rel. Theta N1 (n = 17)	-.069	.005	.793			
C3 REM Avg. Theta N1 (n = 17)	.326	.107	.201			
C4 REM Avg. Theta N1 (n = 16)	.113	.013	.677			
	ERI CS+E Renewal			ERI CS+U Renewal		
	β	R^2	*p*	β	R^2	*p*
C3 REM Rel. Theta N2 (n = 18)	.375	.141	.125	.320	.102	.196
C4 REM Rel. Theta N2 (n = 18)	.417	.174	.085**	.393	.155	.107
C3 REM Avg. Theta N2 (n = 18)	.338	.114	.171	.288	.083	.246
C4 REM Avg. Theta N2 (n = 17)	.265	.070	.289	.307	.094	.216

Note. *Skin conductance Response (SCR) was recorded during eight presentations of each condition stimulus (CS+1, CS+2; lamp colors) and averaged across all eight trials. Additional outliers were removed due to unreliable ELI and ERI measures.*

*Square-root transformed; ***p*<.1*

3.2.3 REM Theta Power and Fear Extinction-Recall

Similarly, relative and average REM sleep theta power from the C3 and C4 sites the night prior to the experiment did not a significantly predict the ERI observed during fear extinction-recall (Table 5). That is, the independent variable, REM sleep theta power, did not significantly predict retention of the extinguished fear memory, as observed during fear extinction-recall.

3.2.4 REM Theta Power and Fear Renewal

Relative theta power from the C4 site during REM sleep on the night subsequent to fear conditioning, extinction, and extinction-recall showed a trend to predict the ERI of the CS+E observed during fear renewal procedures. A simple linear regression was calculated to predict ERI based on REM sleep relative theta power from the C4 electrode ($F(1, 16) = 3.371$, $p = 0.085$). A trending relationship was found, with an R^2 of .417 and $p = 0.085$. Participants' predicted ERI was equal to 1302.06*relative theta power (μV/Hz) – 242.62 (Figure 6; Table 5). A positive relationship was found between the independent variable, REM sleep relative theta power from the C4 electrode, and the retention of the extinguished memory observed during fear renewal, the morning following a full night of sleep. One participant was excluded from analysis due to the residual lying outside three standard deviations of the average residual.

REM sleep relative and average (absolute) theta power recorded from the C3 and C4 electrode the night subsequent to fear conditioning, fear extinction, and fear extinction-recall did not significantly predict ERI of the CS+U observed during fear renewal (Table 5).

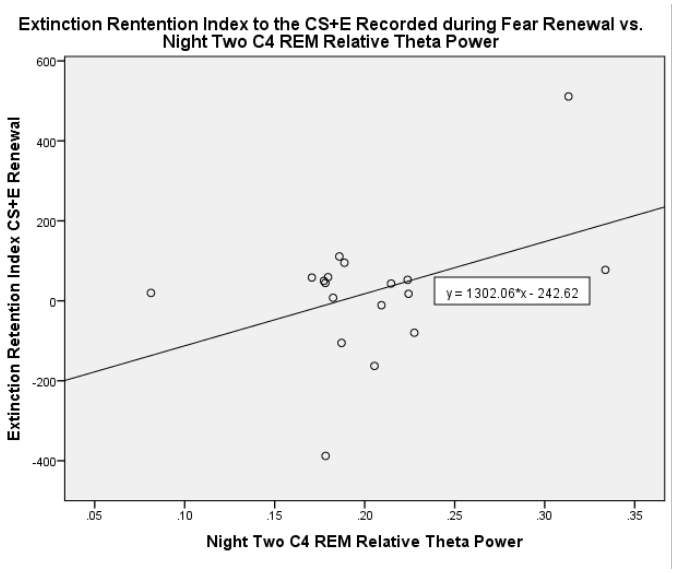

Figure 6. Scatterplot of ERI CS+E vs. Night Two C4 REM Relative Theta Power ($p = 0.085$; $\beta = 0.417$)

Additionally, REM average theta power recorded from the C3 and C4 electrode, and REM relative theta power recorded from the C3 electrode the night, subsequent to fear conditioning, fear extinction, and fear extinction-recall did not significantly predict the ERI of the CS+E observed during fear renewal (Table 5).

In summary, there was no significant relationship between theta power (relative or absolute measures) and fear learning outcomes. A trend-level relationship was observed between relative theta power from the C4 site during REM sleep on the night subsequent to fear conditioning, extinction, and extinction-recall and the ERI of the CS+E observed during fear renewal procedures.

3.3 NREM THETA ACTIVITY AND FEAR LEARNING PARADIGMS

Descriptive statistics for the square-root transformed mean SCR recorded during fear conditioning, ELI, and ERI's are summarized in Table 6. Two participants were non-conditioners and removed from analysis. Descriptive Statistics for NREM sleep relative and average theta obtained from the C3 and C4 sites for the evening prior to the experiment are summarized in Table 6.

3.3.1 NREM Theta Power and Fear Conditioning

Relative NREM sleep theta power from the C3 and C4 sites the night prior to fear learning procedures did not significantly predict the square-root transformed mean SCR averaged across all trials of the CS+1 and CS+2 recorded during fear conditioning (Table 7). That is, the independent variable, NREM sleep theta power from the C3 and C4 electrodes, did not significantly predict the index of conditioned fear responses measured as skin conductance response.

3.3.2 NREM Theta Power and Fear Extinction

Similarly, relative and average NREM sleep theta power from the C3 and C4 sites the night prior to fear learning procedures did not significantly predict the ELI measured during fear extinction procedures, or in other words, magnitude of fear extinction (Table 7).

3.3.3 NREM Theta Power and Fear Extinction Recall

Similarly, NREM sleep relative and average (absolute) theta power from the C3 and C4 sites the night prior to the experiment did not significantly predict the ERI observed during fear extinction-recall (Table 7). That is, the independent variable, REM sleep theta power, did not significantly predict retention of the extinguished fear memory, as observed during fear extinction-recall.

Table 6. Descriptive statistics for outcomes of interest

Measure	N	Mean	Standard Deviation
Shock Intensity (mA)	29	2.02	.776
SCR CS+1* (μS)	29	.494	.328
SCR CS+2* (μS)	29	.178	.251
CS- at CS+1	29	.139	.191
CS- at CS+2	29	.101	.301
ELI	26	94.5	12.7
ERI CS+E Recall	27	43.7	70.4
ERI CS+E Renewal	28	29.3	141
ERI CS+U Renewal	28	26.7	129
C3 NREM Rel. Theta N1	29	.081	.024
C4 NREM Rel. Theta N1	29	.081	.024
C3 NREM Rel. Theta N2	29	.086	.024
C4 NREM Rel. Theta N2	29	.083	.025
C3 NREM Avg. Theta N1 (μV^2/Hz)	28	3.27	1.26
C4 NREM Avg. Theta N1 (μV^2/Hz)	28	3.21	1.09
C3 NREM Avg. Theta N2 (μV^2/Hz)	27	3.65	1.42
C4 NREM Avg. Theta N2 (μV^2/Hz)	28	3.61	1.41

Note. Outliers were removed due to unreliable skin conductance data recorded during fear conditioning. Additional outliers removed due to unreliable theta power measures.

*Square-root transformed values

Table 7. Simple linear regression analyses of relative and average NREM theta power and outcomes of interest

	SCR CS+1*			SCR CS+2*		
Independent Variables	β	R^2	p	β	R^2	p
C3 Rel. NREM Theta N1 (n = 29)	-.115	.013	.554	.036	.001	.852
C4 Rel. NREM Theta N1 (n = 29)	-.152	.023	.430	-.015	.000	.937
C3 Avg. NREM Theta N1 (n = 28)	-.282	.080	.146	-.216	.047	.270
C4 Avg. NREM Theta N1 (n = 27)	-.263	.069	.186	-.241	.058	.226
	Extinction Learning Index (ELI)					
	β	R^2	p			
C3 Rel. NREM Theta N1 (n = 26)	.187	.035	.361			
C4 Rel. NREM Theta N1 (n = 26)	.259	.028	.201			
C3 Avg. NREM Theta N1 (n = 25)	-.167	.028	.426			
C4 Avg. NREM Theta N1 (n = 25)	-.202	.041	.332			
	Extinction Retention Index (ERI) CS+E Recall					
C3 NREM Rel. Theta N1 (n = 27)	.188	.035	.348			
C4 NREM Rel. Theta N1 (n = 27)	.010	.000	.960			
C3 NREM Avg. Theta N1 (n = 26)	.073	.005	.724			
C4 NREM Avg. Theta N1 (n = 26)	-.095	.009	.643			
	ERI CS+E Renewal			ERI CS+U Renewal		
	β	R^2	p	β	R^2	p
C3 NREM Rel. Theta N2 (n = 28)	.118	.014	.551	-.067	.004	.736
C4 NREM Rel. Theta N2 (n = 28)	.079	.006	.691	-.045	.002	.820
C3 NREM Avg. Theta N2 (n = 27)	.105	.011	.601	.121	.015	.546
C4 NREM Avg. Theta N2 (n = 27)	.134	.018	.505	-.142	.020	.479

Note. *Skin conductance Response (SCR) was recorded during eight presentations of each condition stimulus (CS+1, CS+2; lamp colors) and averaged across all eight trials. Additional outliers were removed due to unreliable ELI and ERI measures.*

*Square-root transformed

3.3.4 NREM Theta Power and Fear Renewal

NREM sleep relative and average theta power recorded from the C3 and C4 sites the night subsequent to fear conditioning, fear extinction, and fear extinction-recall did not significantly predict ERI of the CSU observed during fear renewal (Table 18). Additionally, NREM average theta power recorded from the C3 and C4 electrode and NREM relative theta power recorded from the C3 and C4 electrode the night subsequent to fear conditioning, fear extinction, and fear extinction-recall did not significantly predict the ERI of the CSE observed during fear renewal (Table 18).

4.0 DISCUSSION

Sleep is implicated in learning and memory processes, yet the specific indices of brain activity that support this association is not well established. There is an emerging body of work suggesting the existence of a role for theta activity in sleep-dependent learning and memory processes (Cowdin et al., 2014; Fogel et al., 2007; Nishida et al., 2009; Spoormaker, Gvozdanovic, Sämann, & Czisch, 2014). However, there has not been a thorough investigation of theta activity during a full night of sleep and its relationship to subsequent fear learning, extinction, and extinction–recall. The present study aimed to investigate the extent to which REM and NREM theta activity during a full session of nighttime sleep was related to physiological indices of fear learning and memory. As fear conditioning and fear extinction has been considered a source of pathology in anxiety disorders (Milad, Orr, Pitman, & Rauch, 2005; Milad & Quirk, 2012; Pace-Schott et al., 2009; Rauch et al., 2005), uncovering the degree of this relationship may allow for new clinical prevention, diagnosis, or intervention strategies that aid in the management of anxiety disorders.

Contrary to the hypotheses, there was no significant relationship between theta power during REM sleep or NREM sleep and fear conditioning, fear extinction, fear extinction–recall, or fear renewal outcomes. Therefore, the study findings do not support Nishida and colleagues' claim that theta power during REM sleep is associated with increased consolidation of negative emotional memories (Nishida et al., 2009). Further, the hypothesis that REM or NREM sleep theta power during night following the fear conditioning, extinction, and extinction-recall phases of the experiment would result in less retention of the extinction memory (or conversely, more retention of the conditioned fear response) during fear renewal was not supported. Instead, a trend-level relationship of the opposite was found. Specifically, higher theta power during REM sleep

predicted greater retention of the extinction memory during renewal at a trend level. This finding is consistent with the suggestion that theta power during REM sleep may be marker of resilience-related processes as proposed by Cowdin and colleagues (Cowdin et al., 2014), such as enhanced consolidation of fear extinction. However, the relationship between REM sleep theta power did not reach statistical significance, and the available sample of participants was relatively small. Thus, further investigation in a larger sample is warranted.

This study also found no evidence that NREM sleep theta power has a relationship to any of the fear learning and extinction components tested with our experimental paradigm. However, a role for other features of NREM sleep, such as delta power or beta power, in fear learning and memory cannot be ruled out based on the design and findings of the present study.

There are several possible explanations for the null findings in this study. First, theta power during either REM sleep or NREM sleep did not significantly predict fear conditioning and fear extinction responses on the following morning. Thus, theta power during sleep may not be an index of an individual's ability to develop fear responses following the pairing of an aversive stimulus with a neutral stimuli (i.e. fear conditioning), and it may not influence an individual's ability to extinguish fear responses and learn new and competing stimulus contingencies. While other features of sleep may still likely be involved in these processes, the present study suggests that it is improbable that theta power during REM or NREM sleep is a reliable index of this particular type of associative learning.

Second, theta power during either REM sleep or NREM sleep also did not significantly predict subsequent fear extinction-recall. However, it is important to note that the experimental design differed from the previously published methods (Pace-Schott et al., 2009) in that the fear extinction recall phase of the experiment was not preceded by a sleep period. In our experiment,

the fear extinction-recall phase occurred approximately six to eight hours after the fear conditioning and extinction phases, on the same day, and after participants remained awake for the entire interval. Thus, the present study cannot rule out the possibility that theta power during REM or NREM sleep may be predictive of fear extinction-recall when sleep is permitted between fear conditioning and extinction and subsequent testing of extinction-recall.

Although there was no significant relationship between theta power during REM sleep or NREM sleep and fear renewal, the trend-level finding that higher theta power during REM sleep predicted greater retention of the extinction memory during the renewal phase of the experiment may have been due to the full night of sleep that took place before fear renewal. The full night of sleep may have allowed for the adequate consolidation of the conditioning and extinction memories, but further analyses are required to confirm this hypothesis.

The contrast in the findings between Nishida and colleagues' and this study may be due to different testing paradigms: this study used a well-established fear learning and memory experiment used to model anxiety disorders in humans; whereas Nishida and colleagues compared neutral and negative affective images presented before and after an afternoon 90-minute nap opportunity (Nishida et al., 2009). Recognizing emotional pictures is markedly different from associative learning used in the present study. Specifically, the affective response associated with the images, whether positive or negative, is predetermined by prior life experience that occurs outside of the laboratory. The associations made during fear conditioning are learned during the task, making a previously neutral stimulus a negative one.

Testing the relationship between fear learning paradigms and theta power is significantly more relevant in the context of anxiety disorders. The images used in Walker and colleagues' work were validated images from the International Affective Picture System (IAPS; Lang, Bradley, &

Cuthbert, 2005), and it is still likely that REM sleep theta power is associated with increased negative emotional memory consolidation when compared to a set of neutral stimuli. However, it does not measure one's capacity to learn and recall conditioned and extinguished fear memories.

Based on the trend-level finding that higher theta power during REM sleep predicted greater retention of the extinction memory, individuals with higher REM sleep theta power may favor the consolidation of the extinction memory in comparison to the conditioning memory, and this mechanism may be malfunctioning in anxiety disorder pathology. As an alternative, the consolidation of the conditioning memory may be favored for these individuals.

The present study raises the possibility that, while the "sleep to remember, sleep to forget" hypothesis (Walker & van der Helm, 2009) may explain habitual sleep-dependent memory processing, it may not provide an adequate conceptual framework for sleep-related processing in fear-specific learning and memory. It may not be the case that sleep, specifically, decouples the negative affective memories from the declarative memories in the context of a traumatic event and putative resulting strengthened fear conditioning and/or failure of extinction. Instead, by considering the trend-level relationship between REM sleep theta power and fear renewal, the extinction memory may be strengthened after a night of sleep, while the conditioning memory may be degraded over the passage of time, a process likely independent of sleep. This could be due to the inhibition induced by the extinction memory and resulting lack of the expression of the conditioning memory. However, it must be noted that further analysis of alternative activity bands, in addition to theta, is required to evaluate adequately these assertions.

Evidence that sleep may favor the consolidation of the extinction memory may be supported in Cowdin's work, as REM sleep theta power has been found to be lower in individuals with current PTSD compared to those that are resilient (Cowdin et al., 2014). The resilient

individuals may be consolidating their "extinction" memories with significantly more efficacy than their traumatic, "conditioning" memory when re-exposed to a stimuli associated with this traumatic event in daily life. A prospective study of theta power during REM sleep early after trauma exposure and comparisons between individuals who do or do not develop PTSD is necessary to uncover the extent to which theta power during REM sleep is indeed associated with resilience or risk for PTSD. Studying changes in theta power during REM sleep following treatments of anxiety disorders could also clarify the relationship between theta power and remission from chronic maladaptive trauma responses.

These findings provide preliminary support for the hypothesis that REM sleep is a brain state particularly conducive to emotional memory consolidation (Paré, Collins, & Pelletier, 2002), whether that memory is positive or negative is unclear. The present study provides the first demonstration of theta power's role during a full night of sleep in the consolidation and recall of emotional memories in a widely used model of acute trauma, with REM sleep emerging as the primary sleep state responsible for this purposed relationship.

Previous studies have found that fragmented REM sleep subsequent to a traumatic experience were associated with the development of PTSD (Mellman, Pigeon, Nowell, & Nolan, 2007; Mellman, Bustamante, Fins, Pigeon, & Nolan, 2002). It can be proposed that those with less theta power during REM sleep may indeed be at a greater risk to develop PTSD following trauma, as theta power was associated with the consolidation and recall of extinction memory during renewal. Theta activity may be able to quantize an individual's ability to integrate and modulate the affective components of memories that serves to reduce reactivity to traumatic events. This does coincide with the "sleep to remember, sleep to forget" hypothesis (Walker & van der Helm, 2009) and that PTSD may reduce the therapeutic effects of REM sleep.

This study is not without its limitations. Firstly, larger samples are necessary to determine the robustness of this finding. Several of the correlations found, specifically during the analyses of REM sleep theta power and fear renewal outcomes (Table 5), may have reached significance, perhaps a larger sample was available. Additionally, there was not a sleep session before both fear extinction and after extinction, the outcomes found during fear renewal, could differ. Theta activity, also, is typically found during wakefulness when exploring novel contexts (Araújo et al., 2002). It is hypothesized that theta activity may serve as a marker of a memory representation for later offline reinstatement (Datta, 2000). This phenomenon may have influenced theta power during the second night of sleep, prior to which participants underwent conditioning, extinction, and extinction recall.

As previously stated, without a period of sleep between fear conditioning and fear extinction, it is difficult to conclude precisely theta activity's role in negative memory consolidation. Further, theta power was only investigated in the C3 and C4 electrodes. Investigating theta power at other recording sites could lead to additional findings. Additionally, an investigation of sleep theta power in comparison to theta power during wakefulness may also help in elucidating the relationship between theta activity and fear-related learning and memory from wakefulness into REM and NREM sleep.

Without a comparison to theta power during wakefulness, it cannot be concluded that fear memory consolidation may be exclusive to REM sleep from these findings. Theta power was only observed immediately before and after fear conditioning and extinction; and as such the present study did not evaluate whether similar relationship may be detectable later (Kookoolis, Pace-Schott, & McNamara, 2010; Nielsen, Kuiken, Alain, Stenstrom, & Powell, 2004). In addition, the

study only included healthy individuals; it is unknown whether similar relationships would be seen in subjects with current anxiety disorders.

The inclusion of variables such as childhood trauma and genetics may provide insight into how trauma effects theta activity after a significant amount of time has passed. Additionally, observing various activity bands during sleep in individuals with anxiety disorders, such as PTSD, in comparison to healthy individuals after a fear conditioning and extinction task may provide evidence involvement of theta activity in the consolidation of fear-related processes. Finally, investigating if there are any correlates between fMRI activity observed during fear conditioning and activity bands later in the evening could uncover possible daytime markers of consolidation that occurs later during sleep.

Considering that REM sleep theta power or other sleep-dependent features favor the consolidation and expression of extinction memories, several new approaches to treatment for anxiety disorders can be proposed. It may be possible to objectively track the progress of individuals currently undergoing treatment. Further, this concept can be used to measure the efficacy of current treatments, if in fact it is found that those who remit from certain anxiety disorders have detectable changes in other activity bands or other features during sleep. This could also be used in a personalized approach to monitor which treatments are working more effectively that others for a given individual.

Overall, a more thorough understanding on the relationship between sleep and fear learning and memory, including fear extinction-recall and fear renewal, may enhance available therapeutic approaches. For instance, the timing of an exposure-based therapy session relative to the main sleep episode (or naps) may improve treatment outcomes. Similarly, and if it is found that theta power during wakefulness has a role in the consolidation of extinction memories, neurofeedback

training could become a usable tool in treatment, as it has been shown to alter brain activity (Lubar, Swartwood, Swartwood, & O'Donnell, 1995; Reiner, Rozengurt, & Barnea, 2014; Saxby & Peniston, 1995; Zoefel, Huster, & Herrmann, 2011). Other methods such as transcranial magnetic stimulation (TMS) or transcranial direct-current stimulation may also be investigated as a form of adjunctive therapy, as TMS is already used in treatment-resistant major depressive disorder (Bersani et al., 2013).

In summary, the present study did not confirm the hypothesis that REM sleep or NREM sleep theta power predicts different components of fear learning or extinction. Nevertheless, the present study raises a number of important questions that can be empirically investigated, and that have the potential to enhance the treatment of anxiety disorders through leveraging the unique potential of sleep.

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