STRESSING SLEEP: THE IMPACT OF PSYCHOSOCIAL STRESS ON
SLEEP AND EMOTIONAL MEMORY CONSOLIDATION

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Abstract

by

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Emotional experiences create durable memory traces in the brain and tend to be incredibly well remembered. Importantly, moderate stress responses have been linked to increased performance on emotional memory tests, but suppressed ability to remember neutral information. Evidence suggests sleep also enhances memory consolidation, but the way stress affects sleep remains unclear. In the present study, participants encoded scenes of varying degrees of emotional arousal. After, participants completed a psychosocial stress task or a control task prior to sleep. Participants then completed a recognition task in which the objects and backgrounds were presented separately and one at a time. Stress subjects demonstrated an increase in memory for negative objects but poorer memory for their matched neutral backgrounds, resulting in a greater emotional memory trade-off compared to controls. Thus, HPA axis activation after encoding may “tag” the emotional object as important to remember, enabling sleep to selectively increase memory consolidation while concurrently suppressing neutral information.
This is for my parents, my first and biggest fans.
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CHAPTER 1:
INTRODUCTION

1.1 Overview

Emotional experiences tend to have privileged status in long-term memory such that they are typically better-remembered than their neutral counterparts, even after long delays. This bias to remember emotionally salient information is likely adaptive, as remembering an emotional experience is often necessary for survival and for understanding the complexities of day-to-day social interaction. Laboratory studies have reported that participants show a boost in vivid memory for emotional events and stimuli (Heuer & Reisberg, 1990; Kensinger & Corkin, 2003; see Buchanan & Adolphs, 2002 and Hamann, 2001 for review), especially when the stimuli are perceived as negative and elicit an arousal response (Abercrombie, Chambers, Greischar, & Monticelli, 2008; Bradley, Greenwald, Petry, & Lang, 1992; Hamann, Cahill, and Squire, 1997; Cunningham, Crowell, Alger, Kensinger, Villano, Mattingly, & Payne, 2014a; Ochsner, 2000). Emotionally salient stimuli consistently elicit greater physiological responses than neutral stimuli (Abercrombie et al., 2008; Lang et al, 1993; Lang, 1995). The degree of change that the presentation of an emotional stimulus (i.e., the material to be learned with emotional content) has on the emotional arousal (i.e., a state the
participant/rat is in during/after encoding) of the subject is governed by the intensity of arousal that the viewer associates with it (Lang et al., 1993). Research on the neurobiology of this phenomenon suggests that a stimulus perceived as negatively arousing can elicit changes in autonomic nervous system (ANS) output (Hauschildt et al., 2011; Lang et al., 1993) and increased activity in brain regions important for emotional processing (Garavan et al., 2001; Hamann et al., 2002). For example, simple presentation of an emotionally arousing image can trigger changes in heart rate (HR), skin conductance response (SCR), facial movements (electromyogram; EMG; Lang et al., 1993; Pace-Schott et al., 2011), event-related potentials (ERPs; Diedrich, et al., 1997; Schupp et al., 2006), and amygdala activation (Garavan et al., 2001), as well as increase subjective ratings of arousal (Lang et al., 1993; Lang, 1995).

Differences in the encoding of negative and neutral information that may lead to the distinct durability of memory traces between these types of stimuli can be seen at the neural level. While neutral declarative memories depend primarily on activation in the hippocampus and other regions of the medial temporal lobe (MTL) regions (Moscovitch, Rosenbaum, Gilboa, Addis, Westmacott, & Grady, C, 2005; Scoville & Milner, 1957), memory consolidation of emotional events and stimuli involves a complex interchange between these MTL regions and the amygdala, a subcortical structure essential for processing emotions (Hamann, Ely, Grafton, & Kilts, 1999; Hamann, Ely, Hoffman, & Kilts, 2002; Kensinger & Schacter, 2006; McGaugh, 2004; see Alger, Chambers, Cunningham, and Payne, In Press for review). Neuromodulators released during emotional arousal, such as epinephrine and cortisol, boost the
amygdala’s influence on MTL structures such as the hippocampus and caudate nucleus, which in turn enhances memory for the events that initiated the arousal (McGaugh, 2000; 2004). For instance, Ritchey and colleagues (2008) determined that activity in the amygdala at the time of encoding predicted emotional, but not neutral, memory. They also found that connectivity between the amygdala and the MTL can predict memory for emotional stimuli, especially after a longer delay (Ritchey, et al, 2008).

While negative valence coupled with arousal has been shown to selectively benefit memory for emotional stimuli over neutral stimuli, evidence suggests that not all aspects of the emotional stimuli are equally remembered. For instance, the encoding of complex emotional scenes with negative objects placed on plausible, neutral backgrounds typically leads to a trade-off effect in which memory for the central emotional object is enhanced at the cost of memory for the peripheral, non-emotional elements of the scene. When scenes feature a neutral central object (rather than a negative and arousing one), memory for the object and the peripheral elements of the scene are equivalent. (Kensinger, Garoff-Eaton, & Schacter, 2007b; Payne, Stickgold, Swanberg, and Kensinger, 2008)

Because human memory is limited, this ability to selectively preserve memories that are high in emotional salience and therefore most relevant and most likely to assist in our survival is incredibly important. Our ability to retain this information is so vital that several mechanisms have developed to enhance our ability to successfully encode, store, and retrieve emotional memories. Specifically, discrete lines of research have indicated that both sleep (Hu, Stylos-Allan, & Walker, 2006; Payne et al., 2008; Wagner,
Gais, and Born, 2001) and stress (Bennion, Mickley Steinmetz, Kensinger, and Payne, 2013; Cahill, Gorski, and Le, 2003; Payne, Jackson, Hoscheidt, Ryan, Jacobs, & Nadel, 2007; Payne, Jackson, Ryan, Hoscheidt, Jacobs, & Nadel, 2006) enhance the ability to recall emotionally salient information. The goal of this study was to combine these two separate lines of research and explore how sleep and stress impact one another and work collectively to affect emotional memory consolidation. To begin, however, it is necessary to understand how each of these mechanisms individually affects emotional memory and each other.

1.1.1 The influence of stress on emotional memory

The term “stress” is a notoriously ambiguous term that can describe both the event a person experiences and the physiological reaction a person undergoes in response to that event. For the purpose of this review, stress will refer to the latter, specifically to the stress-response or measures that the body takes to restore homeostasis (Sapolsky, 1994). Stress response in humans and non-human animals involves stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, the link between the central nervous system (CNS) and the hormonal system of the body. When faced with a stressor, the hypothalamus is activated, leading to the production of corticotropin-releasing hormone (CRH). CRH travels to the nearby pituitary gland, triggering the release of adrenocorticotropic hormone (ACTH). This hormone enters the bloodstream and makes its way to the adrenal cortex located adjacent to the kidneys. Activation of the adrenal cortex elicits the release of stress hormones, such as
glucocorticoids (GCs; e.g., cortisol in humans and most primates and corticosterone in most laboratory animals). The stress hormones make their way back to the brain, turning off additional production of CRH and ending the cascade of effects through a negative feedback loop (see Figure 1.1; Kudielka and Kirschbaum, 2005; see Tsigos & Chrousos, 2002 for review). The production of GCs creates a host of negative and positive effects on cognition and behavior in humans and laboratory animals, and can create drastic alterations in other physiological processes, such as sleep (Born, Späth-Schwalbe, Schwakenhofer, Kern, & Fehm, 1989; Cheeta, Ruigt, van Proosdij, and Willner, 1997) and memory (Cahill et al., 2003; Payne et al., 2006).

Critically, evidence suggests that when exposed to a moderate increase in GCs, the HPA axis works in concert with the adrenergic system to increase the durability of memory for emotional information (McGaugh, 2004). This boost in emotional memory is often accompanied by a decrease in memory for neutral information (Payne et al., 2006, 2007; see Wolf, 2008 for review). When humans encode and consolidate a memory while under certain levels of stress, the simultaneous release of GCs and norepinephrine (NE) acts on the amygdala, augmenting the interactions between the amygdala, hippocampus, and other brain regions important for memory consolidation, above and beyond what is experienced through emotional arousal at encoding alone (McGaugh, 2004; Roozendaal, McEwen & Chattarji, 2009). As detailed above, this

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1 It is important to note that while there is a large literature suggesting that stress enhances emotional memory consolidation and hinders neutral memory, separate bodies of work suggest that stress globally impairs memory (e.g., Schwabe and Wolf, 2010), or even impairs emotional memory and enhances neutral memory (e.g., Rimmele et al., 2003).
Figure 1.1: The Hypothalamic-Pituitary-Adrenal (HPA) axis is activated in response to a stressor. It leads to the release of glucocorticoids (GCs) into the system (cortisol in humans). The axis is shut off through a negative feedback loop once the GCs reach the brain and signal the hypothalamus and pituitary gland to stop producing CRH and ACTH, respectively. (Rosenbach, 2008)
crosstalk within the limbic system is what drives the preferential consolidation of emotionally salient information over neutral information (McGaugh, 2000; Ritchey et al., 2008), and it is becoming increasingly clear that stress potentiates this effect (see Hamann, 2009; Payne & Nadel, 2004 for review). Due to the sluggish time-release of some of these neuromodulators (e.g., cortisol), however, evidence suggests that they may have their greatest impact during the consolidation stage of memory formation, which takes place during the variable period of time between encoding and retrieval. (McGaugh, 2000, 2004)

Behavioral studies in humans manipulating either exposure to a stressful event leading to a moderate endogenous increase in cortisol or administration of exogenous cortisol support the theory that stress benefits the consolidation of emotionally salient information. Cahill and colleagues (2003) utilized a cold-pressor task to manipulate stress after encoding (during the consolidation) of scenes with varying levels of emotional arousal. Those that experienced the stress task demonstrated an increase in memory for the emotionally arousing slides compared to controls after a 1 week delay, but there was no difference in neutral memory between groups. In another study, the Trier Social Stress Test (TSST; Kirschbaum, Perke, and Hellhammer, 1993) was utilized as a psychosocial stressor to endogenously increase cortisol levels. The investigators found that stress exposure at encoding impaired long-term neutral memory, but enhanced long term emotional episodic memory (Payne, et al, 2007). Buchanan and Lovallo (2001) found that even the simple administration of 20mg of cortisol prior to encoding scenes of varying emotional arousal led to an increase in cued recall performance after a week
delay for pictures they rated as emotionally arousing, but not the images that they did not consider arousing. A similar study using various doses of cortisol (i.e., 20 mg, 40 mg, and placebo) found a benefit for the memory consolidation of both emotional and neutral stimuli with cortisol administration after just a two day delay (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003). Importantly, they found evidence for an inverted U-shaped dose response curve across the stimuli; with memory facilitation observed most predominantly when administered 20-mg of cortisol, and less or no benefit to memory in the 40-mg group. This provides evidence that the concentration of GCs may be an important factor in this effect, with moderate amounts of stress benefiting emotional memory consolidation while an overabundance can be damaging.

Importantly, several reports from both human and non-human animal studies suggest that while the flood of GCs after exposure to stress benefits emotional memory, it does not globally promote memory. If not aroused at encoding or during the early stages of consolidation, then stress may have no, or even detrimental effects on memory consolidation (Abercrombie, Speck, & Monticelli, 2006; de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Roozendaal, Okuda, de Quervain, & McGaugh, 2006; Yang, Han, Cao, Li, & Xu, 2006). Additionally, evidence suggests that stress manipulation must occur at encoding or during consolidation to see any beneficial effects of cortisol release on memory performance. Studies investigating elevated GC concentration at retrieval of any kind of memory have primarily indicated a negative effect on performance (de
In addition to emotional arousal at encoding, there are several other important caveats to consider when exploring how stress modulates memory. First, the impact of HPA activation on memory has been found to differ between men and women, with cortisol increase having a stronger effect in men than their female counterparts (Wolf, Schomer, Hellhammer, McEwen, and Kirschbaum, 2001). Second, the effects of cortisol on memory may also depend on the dose administered or endogenous response to a stressor. For example, one study administered 25mg of cortisol or a placebo prior to testing on verbal and nonverbal declarative memory. Low responders (< 68.25 nmol/L) demonstrated an increase in verbal memory performance compared to controls, while high responders (> 68.25 nmol/L) performed worse (Domes, Rothfischer, Reichwald, & Hautzinger, 2005). This finding supports the inverted-U theory first established by Yerkes and Dodson (1908), which suggests that minimal and maximal concentrations of cortisol may hinder memory, but moderate amounts of cortisol may allow for optimal performance. A large body of both human (Abercrombie et al., 2003; Domes et al., 2005; see de Kloet, Joels, and Holsboer, 2005 and Joels, 2006 for review) and non-human (Ghosh, Laxmi & Chattarji, 2013; Vyas, Mitra, Rao, & Chattarji, 2002) empirical evidence supports this theory. Research in rodents has shown that while amygdalar plasticity and function is enhanced by the presence of GCs in the brain, hippocampal and prefrontal cortex (PFC) function is impaired (Ghosh, Laxmi & Chattarji, 2013; Vyas, Mitra, Rao, & Chattarji, 2002). Human imaging studies have demonstrated that stress
prior to encoding led to a significant deactivation of the hippocampus (Pruessner, Dedovic, Khalili-Mahani, Engert, Pruessner, Buss, & Lupien, 2008) but an increase in amygdala activation leading to better memory for emotional information (van Stegeren, Wolf, Everaerd, Scheltens, Barkhof, & Rombouts, 2007). Together these studies have provided the framework to begin to unravel the neural bases of these effects.

A third important consideration is that emotional memories are not necessarily preserved in their entirety as precise, veridical replicas of the original experience. The next step in understanding how stress affects emotional memory is to explore what components of emotional memory are influenced by stress. A naturalistic example of this can be seen in the weapon-focus effect in which a victim may have vivid memory for the weapon they were exposed to, but relatively poor memory for other details of the event, such as the face of their attacker (Stanny & Johnson, 2000). As noted above, this effect has been studied experimentally through the use of complex scenes in which a negative or neutral central object is placed on a neutral, peripheral background (e.g., Bennion, et al., 2013; Kensinger, et al., 2007b; Payne et al., 2008, Payne and Kensinger, 2010; Waring, Payne, Schacter & Kensinger, 2010; Cunningham et al., 2014a; Cunningham, Chambers, and Payne, 2014b). For example, participants could see either a parked car (neutral object) or a car accident (negative object) placed on a background of a street (plausible, neutral background; see Figure 1.2) at encoding, followed by a recognition session in which the objects and backgrounds are presented separately and one at a time. Kensinger and colleagues (2007) found that even after a short delay the emotionally salient objects were remembered better than the neutral objects, but the
backgrounds paired with the negative objects were more poorly remembered than those matched with the neutral objects. Individual differences such as anxiety, working memory capacity, and executive function have been shown to moderate this effect (Waring, et al., 2010). Memory for objects in this “emotional memory trade-off” has also been found to correlate with resting levels of cortisol prior to a 12 hour delay of nocturnal sleep (Bennion, et al., 2013), but the effect of manipulating cortisol during consolidation of this memory paradigm remains unexplored.

1.1.2 The influence of sleep on emotional memory

A typical night of sleep can be divided into two phases: rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM). NREM sleep can be further subdivided into Stage 1, Stage 2, and deeper slow wave sleep (SWS). A histogram of a healthy night of sleep can be seen in Figure 1.3 (Payne, 2011). Stage 1 sleep is a transition state as a person initially drifts off to sleep. As a person continues to fall into deeper sleep, they will then enter into Stage 2 sleep characterized by brain activity in the 5-8 Hz range, sleep spindles (high frequency, 12-15 Hz bursts in the sigma band) and K-complexes (momentary high amplitude, negative, high-voltage peaks, approximately 100 µV followed by a slower positive complex) (Stickgold et al., 2001; Smith, 1995; Smith, Aubrey, & Peters, 2004). This stage is subsequently followed by SWS, which can be identified by high-amplitude, low frequency oscillations known as delta waves (0.5-4 Hz) (Steriade, Nuñez, & Amzica, 1993). About 90 minutes after the onset of sleep the first epoch of REM will occur, marked by a reduction in muscle tone and low-amplitude,
Figure 1.2: Emotional memory trade-off paradigm. Central objects with varying degrees of valence and arousal are placed on neutral backgrounds. At encoding the subjects are presented with the complex, plausible scenes. At recognition the objects and backgrounds are presented separately and one at a time (Payne, 2008)
high frequency EEG patterns, as well as periodic eye movements and muscle twitches 
(Carskadon & Dement, 1989). The first half of the night is archetypally heavy in SWS, 
while the latter half of the night is generally more REM-heavy. (Payne, 2011) 
Similar to stress, a period of sleep during the consolidation phase has been shown to 
benefit memory processing as well. Unlike stress, however, processes during sleep have 
been shown to benefit both negative (Hu, et al., 2006; Payne et al., 2008; Payne and 
Kensinger, 2010) and neutral information (Backhaus, Hoeckesfeld, Born, Hohagen, & 
Junghanns, 2007; Peigneux, Laureys, Fuchs, Collette, Perrin, Reggers, Phillips, Degueldre, 
Del Foire, Aerts, Luxen, & Maquet, 2004; Plihal and Born, 1997; Scullin and McDaniel, 
2010), and some theories suggest that certain stages of sleep may be important for 
certain types of learning and memory consolidation (see Diekelmann and Born, 2010; 
found that declarative memory for word pairs was enhanced in children that slept after 
encoding compared to a group that remained awake for a 12 hour period. Plihal and 
Born (1997) took advantage of the natural, cyclic rhythms of sleep by employing a split-
night paradigm, in which participants learned a task and then were allowed either 3 
hours of early, SWS-heavy sleep or 3 hours of late, REM-rich sleep. They found that 
recall of word pairs improved more significantly after the earlier period rich in SWS 
compared to late sleep or a matched period of wakefulness. Performance on a 
procedural mirror tracing task, however, benefited more from the late REM-heavy than
Figure 1.3: This profile represents the sleep architecture of a healthy night of sleep. Slow wave sleep dominates the first half of the night, but during the latter half, REM sleep is the majority. (Payne, 2011)
the early sleep or period of wakefulness suggesting distinct roles in memory processing for these stages of sleep.

Hu and colleagues (2006) examined the effects of sleep on emotional episodic memories by having participants encode a set of negative and neutral pictures prior to a 12 hour delay of nocturnal sleep or daytime wakefulness. At the next session, participants completed a recognition test and were asked to discriminate between the original pictures and a novel set by responding “remember” (recalled specific details), “know” (the picture seemed familiar but lacks specific visual detail), or entirely new. A night of sleep improved “know” judgment memory accuracy for emotionally arousing pictures and memory bias became more conservative for “remember” judgments to emotional scenes compared to a delay of wakefulness. These findings suggest that memory for emotionally salient information may preferentially develop during sleep. A more recent study examined the neural correlates of emotional memory and found that both three days and 6 months after encoding, recollection of emotional images was associated with greater activity in the amygdala and ventromedial prefrontal cortex (vmPFC) for participants that slept after encoding compared to sleep-deprived subjects (Sterpenich, Al bouy, Darsaud, Schmidt, Vandewalle, Dang-Vu, Maquet, 2009). Wagner and colleagues (2001) utilized a split night design similar to Plihal and Born (1997) and found that 3 hours of late night REM-rich sleep benefited long term memory for arousing narratives containing negative content compared to equivalent delays of early SWS and wakefulness. This finding implicates REM sleep, a period in which the amygdala and hippocampus are among the most active brain regions (Maquet, Péters, Aerts,
Delfiore, Degueldre, Luxen, & Franck, 1996), as a potentially critical stage for emotional memory processing.

Sleep has also been shown to be an important mechanism in the processing and consolidation of different aspects of emotional memories over time. In a seminal paper examining the “emotional memory trade-off” detailed above (see The Influence of Stress on Emotional Memory), Payne and colleagues (2008) found that a delay including sleep potentiated the magnitude of the emotional memory trade-off effect by preserving memory for the negative objects but not their backgrounds. This finding suggests that the two components of the scene are consolidated separately and undergo different processing during sleep. A recent extension of this paradigm investigated how the staging of sleep is involved and found a positive correlation between REM sleep and the selective consolidation of the central, negative objects contained within complex scenes (Payne, Chambers, and Kensinger, 2012). These results strongly suggest that post-encoding processes can affect and play a critical role in the durability of emotional memory traces.

1.1.3 The influence of stress on sleep

The flood of glucocorticoids saturating our central nervous system during and after experiencing a stressor does more than affect our memory. HPA axis activation has been shown to have substantial effects on metabolism, immune function, reproduction, perception of pain, and most notably for the purpose of this review, sleep structure (Sapolsky, 1994; Born et al., 1989; Meerlo, Easton, Bergmann, and Turek, 2001).
Evidence suggests that REM sleep is particularly susceptible to the influence of stress (Cheeta et al., 1997; Kant, Pastel, Bauman, Meininer, Maughan, Robinson, Wright, and Covington, 1995; Meerlo et al., 2001; Vandekerckhove, Weiss, Schotte, Exadaktylos, Haex, Verbraecken, and Cluydts; 2011; Vazquez-Palacios et al.; 2004). Interestingly, however, there is strong disagreement amongst reports in which some studies find a decrease in REM sleep following the increase in hormones associated with HPA axis activation (Born, 1989; Kant et al., 1995; Vanderkerckhove et al., 2011), while others indicate a stark increase (Cheeta et al., 1997; Meerlo, 2001; Vazquez-Palacios et al., 2004).

Kant et al. (1995) placed rats in a chronic severe stress condition involving a random periodic foot shock for a duration of two weeks. The rats were either given the ability to stop the shocks by pulling a chain or were yoked to a rat in the former condition and had no control. Both groups showed a similar decrease in REM during the first 24 hours. REM sleep in the uncontrolled stress group rebounded to normal levels after the first day, while REM sleep in the controllable stress group remained diminished through day three. After seven days of chronic stress, REM in both groups returned to baseline (Kant et al., 1995). The authors suggested that the controllable stress group may have been more affected by the stressor because the stressor not only created distress, but also required a physical action (pulling the chain) which may have required a greater degree of arousal from sleep. Additionally the graphs reported by the authors show that the two groups seem to recover at similar rates, but the controllable stress
group had higher baseline levels of REM sleep requiring more recovery to match pre-
stress levels (see Figure 1.4).

Another study exogenously manipulated HPA axis activity in humans by
intravenously delivering 6 mg of cortisol as constant rate infusions continuously
throughout the course of a night of sleep from 10pm-7am (Born, et al., 1989). They
found a significant reduction in the amount of REM sleep, as well as an enhanced
amount of SWS compared to a night in which a placebo was administered in the same
manner. In this same study, exogenous ACTH delivery (0.55 U/h) to the same subjects
created an endogenous increase of cortisol to levels even higher than that of the cortisol
administration condition, which also led to a decrease in REM, but had no effect on SWS
activity. The disparity observed after exogenous versus endogenous increases in cortisol
indicates that the simple administration of cortisol has differential effects on sleep
architecture compared to when other components of the HPA axis are activated to
produce cortisol. While ACTH administration, which leads to the activation of the
adrenal glands to increase cortisol production, was also found to affect REM, the natural
cascade of responses appeared to have less of an effect on other stages of sleep
compared to the blunt force of direct cortisol administration. This indicates a very
important methodological distinction for future research. Additionally, the type of
stressor (physical vs. psychological), timing of stressor, and manner in which the
hormones are manipulated could potentially be important factors as well.
Figure 1.4: Average recovery of rats in controllable stress and rats in uncontrollable stress group from Kant et al., 2005. Authors report that the controllable stress group takes more time for REM to rebound when in a continual stress condition. The above graph shows, however, that the groups recovered at similar rates, but baseline levels of the controllable stress group was also higher than those in the uncontrollable stress group. Controllable stress group = white bars; Uncontrollable stress group = dark bars (Kant et al., 2005)
In a study that manipulated HPA axis activation through a psychological stressor prior to sleep, Vanderkerckhove et al. (2011) had participants go through a stressful negative mood induction task just before bed. The task involved failure feedback on their performance to different cognitive tasks that they were told would measure their intelligence. While no direct measures of HPA activity were obtained, the failure task did induce a significant increase in negative affect as measured by the PANAS compared to a neutral, control night. On nights that the subjects performed the negative mood induction task they displayed significant sleep fragmentation as expressed by decreased total sleep time (TST), sleep efficiency [i.e., TST/Time in Bed (TIB)] and percentage of REM, as well as increased wake after sleep onset (WASO) latency, total time awake, latency to SWS, number of awakenings, and number of awakenings from REM compared to a second night in the lab in which they did not undergo the stressful task (Vanderkerckhove et al., 2011). Importantly, while the authors did allow an adaptation night followed by a baseline night of sleep in the lab to rule out any acclimation effects, they do note a potential confound in having the neutral condition sometimes occur after the failure condition. The experimenters counterbalanced the conditions to avoid natural order effects, but this meant that there were times in which the participant may have had a stress response in anticipation of a negative performance based on the previous experience failure condition. This anticipatory stress could also have altered the dependent variables in the study, making it more difficult to discern any specific effects of the stressor on sleep. The authors attempted to control for this by comparing the sleep physiology of the neutral night to the baseline night (after the initial
adaptation night), which did not yield significant results. Based on their results, the authors concluded that an emotionally distressing event not only led to a decrease in REM, but it also caused pronounced effects across several measures of sleep quality. This study suggests further support that the type of stressor (psychosocial) and timing of cortisol manipulation (prior to sleep) may be important factors in understanding the effect of stress on sleep. Importantly, this naturalistic stressor had a much broader impact on multiple parameters of sleep than administration of hormones during sleep. When comparing the effects of these two methods on REM sleep alone, however, the exogenous cortisol had a greater effect size ($\eta^2 = 0.7$) compared to the emotionally distressing task ($\eta^2 = 0.32$). This suggests that the administration of cortisol had a much more powerful effect on REM sleep than the endogenous release. Numerous potential explanations, however, still need further exploration before we can determine the cause of this substantial difference (e.g., the continual administration of cortisol throughout the night compared to a brief stressor before bed, the exogenous administration of GCs to levels higher than can be achieved naturally, etc.).

While the previous studies suggest that stress and high levels of cortisol concentration lead to a reduction in REM sleep, other studies report an increase in REM sleep after stress (Cheeta et al, 1997; Marinesco, Bonnet & Cespuglio, 1999; Meerlo et al., 2001; Rampin, Cespuglio, Chastrette, and Jouvet; 1991; Vazquez-Palacios et al., 2004). A group of male Lister hooded rats subjected to a battery of chronic, mild stress situation (e.g., soiled cage, continuous lighting, cage tilt, short-term water deprivation, etc.) showed a decrease in wake and deep sleep and an increase in REM duration and
transitions into REM sleep, as well as a reduced latency into the first epoch of REM compared to a control condition (Cheeta et al., 1997). A second rodent study by Meerlo et al. (2001) examined the difference that a restraint stressor would have on two different strains of laboratory bred mice; C57BL/6J (C57BL; a common inbred strain of laboratory mouse) and BALB/cJ (BALB; an albino, laboratory-bred strain known for exhibiting high levels of anxiety). They found that both strains had similar increases in corticosterone after the restraint stressor and a slight initial suppression in REM sleep (2-3hrs after stress), but only the C57BL mice had a disproportionately large increase in REM during the next dark phase. The authors note that this increase was above and beyond the initial loss of REM sleep resulting in an overall increase in REM sleep for the C57BL mice after the restraint stressor compared to baseline and gentle handling. The BALB mice only showed a slight increase in REM sleep during the first half of the next dark phase, which could be explained as a rebound effect after the initial dip in REM and was not significantly different from baseline or gentle handling. The authors left the question of why restraint stress affected these two strains differently open for future research. They do note, however, that based on their results individual differences and the type of stressor are important in understanding how subsequent sleep is affected, making it imprudent to speak in general terms about the effects of stress on sleep (Meerlo, et al., 2001). Support for this position can be found in studies in which the stress of social defeat had no effect on subsequent REM in rats and mice (Meerlo, Pragt, and Daan, 1997; Meerlo and Turek; 2001). Importantly, however, other studies have replicated this increased REM after immobilization in rats (Marinesco et al., 1999;
Rampin et al., 1991; Vazquez-Palacios et al., 2004). Vazquez-Palacios et al. (2004) not only found an increase in REM sleep in rats after a restraint stressor, but additionally they found that administering the drug naltrexone (NTX; a selective blocker of the opioidergic system) after the stressor prevented the change in sleep. Delivery of NTX, however, did not prevent the endogenous rise of corticosterone. Together these studies indicate that an interaction of hormones or activation of multiple brain structures or networks may be necessary to affect subsequent sleep rather than just the presence of stress hormones alone.

In summary, the literature is currently mixed on how stress and HPA axis activation affects subsequent sleep, and very little is known about these interactions in humans. Most notably, some studies indicate that increases in stress hormones lead to a decrease in REM duration, while others find an increase in measures of REM. Despite this contrast, these results are not necessarily mutually exclusive. Factors such as the type of stressor (e.g. chronic vs. specific, severe vs. mild, physical vs. psychological), the method of hormonal delivery (exogenous vs. endogenous), the timing of HPA axis activation before onset of sleep, and the host experiencing the stressor (laboratory animal vs. human) could all be important factors in determining exactly how sleep following an increase in HPA axis activation will be affected.

1.1.4 The influence of sleep and stress on memory

While a there has been a great deal of investigation on the influences of sleep and stress on emotional memory separately, there is a dearth of exploration on how the
two may interact to influence emotional memory, yet the two homeostatic systems are invariably intertwined. Basal cortisol concentrations in humans follow a diurnal rhythm that reaches a minimum during the early, SWS-rich sleep and rises during late night REM sleep (Plihal & Born, 1999), until it reaches its peak immediately after morning awakening. The latter half of the night is marked by a steep increase in cortisol concentration with large bursts occurring in the periods between REM epochs (Born, Kern, Bieber, Fehm-Wolfsdorf, Schiebe, & Fehm, 1986). Only a few studies have attempted to understand if and how these fluctuations in neurochemistry interact with processing during sleep staging to effect memory consolidation. One such study found that the infusion of low dose cortisol during early, SWS-rich sleep impaired the consolidation of neutral word pairs by blocking hippocampal-dependent processing (Born & Wagner, 2004). This study is important for understanding the mechanisms involved in the consolidation of neutral declarative memories, but does not provide any insights on the consolidation of emotional memories. Wagner and colleagues (2005) attempted to understand the effects that elevated cortisol during late night REM sleep has on emotional memory consolidating by administering either a placebo or metyrapone, a cortisol synthesis inhibitor, after encoding emotional and neutral texts and prior to bed. Metyrapone administration successfully blocked the late night rise in cortisol and reduced SWS resulting in reduced memory for neutral texts. Metyrapone did not affect the percentage of sleep spent in REM and emotional memory performance was enhanced compared to the placebo group. The authors suggest that this may indicate that the increase in cortisol naturally seen during the REM heavy
stages of sleep may help to protect from excessive emotional memory formation (Wagner, Degirmenci, Drosopoulous, Perras, & Born, 2005).

While the previous two studies manipulated neurochemistry during sleep, Bennion and colleagues (2013) examined how pre-encoding baseline cortisol, prior to delays of sleep and wake, would affect performance on the emotional trade-off paradigm. They found that elevated pre-encoding cortisol concentration correlated with better memory for negative objects, but only if sleep occurred during the delay between encoding and retrieval. While Wagner et al. (2005) manipulated cortisol concentrations during sleep and Bennion et al. (2013) examined how baseline cortisol concentration at encoding affects emotional memory consolidation over delays of sleep and wake, no study to-date has manipulated cortisol concentration during the early stages of consolidation prior to sleep and examined how this influences subsequent sleep and emotional memory.

1.1.5 The role of depression

A clinical diagnosis of depression offers a unique opportunity to examine the interaction of sleep, stress, and emotional memory when the systems are not functioning properly. For instance, while depression alone has been shown to have a deleterious effect on declarative memory for neutral information (Kizilbash et al., 2000), studies have found that depressed patients have a bias in memory for negative information (Watkins, 2002; Pyszczynski et al., 1989). A recent study by Liu et al. (2012) found that when presented with positive, negative and neutral words, depressed
patients showed a reduction in pleasure and arousal to positive words, but an increase in experienced arousal to negative words. Depressed subjects also had overall lower memory performance in a recall measure, but still maintained a bias in memory for the negative information.

In general, depression has a disruptive effect on sleep quality. However, certain measures of REM sleep are particularly affected. A comprehensive study on sleep and depression in 2001 found that sleep disturbances are highly common in depressed patients (see Figure 1.5; Riemann et al., 2001). The most prevalent changes in sleep architecture in depressed individuals include a reduction of SWS and a general disinhibition of REM sleep which includes a shortening of REM latency, an increase in the first epoch of REM sleep, and an increase in REM density. Zarcone & Benson (1982) found an increase in REM eye movement density in subjects with even subclinical depression. Interestingly most effective antidepressants on the market today dramatically suppress REM sleep (Riemann et al., 2001).

In addition to disrupting sleep, strong evidence suggests that depression leads to a hyperactive cortisol response and sluggish HPA negative feedback loop (Young, Lopez, Murphy Weinberg, Watson, and Akil, 2000; see Abercrombie, 2009 for review). For instance, Mitchell and Smythe (1990) found a positive correlation between Hamilton Rating Scale for Depression (HAMD) scores and morning baseline plasma cortisol levels (collected between 8:30-9:30am) suggesting a hyperactive HPA system. Two studies explored depressed individuals response to a psychosocial stress compared to matched
Figure 1.5: Comparison of sleep profiles between a depressed female not taking any medications and a matched healthy female control. The depressed patient shows many atypical features in her sleep architecture such as increased awakenings, disinhibition of REM, and a severe reduction in SWS. MT, Movement time; BM, body movements; EM, rapid eye movements. (Image from Riemann, 2001)
controls. One study found that depressed individuals had a normal cortisol response to
the social stress task despite an increased baseline cortisol concentration (Young et al.,
2000), again indicating a trait characteristic of HPA activity rather than specific HPA
reactivity issues. The second study found a significantly greater ACTH response (and a
similar but not significant cortisol response) to the stressor in a depressed group
compared to their matched controls, however this increase was entirely driven by a
subset of depressed individuals with comorbid anxiety disorders (Young, Abelson,
Cameron, 2004). Stetler and Miller (2011) completed an extensive meta-analysis on four
decades of research on depression and HPA activity. They also found that depressed
patients tended to display increased cortisol and ACTH levels (not CRH concentrations).
Importantly, however, when the analyses were limited to studies that met their minimal
methodological standards, the magnitude of the cortisol effect was reduced nearly by
half, suggesting that this finding may not be as clear as previously thought. They also
found that studies using older subjects had greater differences and cortisol differences
emerged for atypical, endogenous, melancholic, and psychotic forms of depression. In
general there is a great deal of support suggesting that depression leads to a
hyperactive HPA axis, but factors such as the type of depression, the measure of
depression, comorbidity with other disorders, the measure of HPA axis
activity/reactivity, and other characteristics of the individuals may be important to
consider in this line of research.

In summary, while not all of the details are fully understood at this point, it is
clear that the symptoms of depression are intricately woven with the mechanisms
involved in emotional memory, stress response, and sleep. The concurrent exploration of the impact of depression on each of these systems along with the examination of healthy functioning may not only help to shed light on how the optimal performance of these critical processes, but could have important clinical implications as well.

1.2 Present study

The primary goal of the present study was to investigate how a psychosocial stress manipulation would affect subsequent sleep and emotional memory performance. As detailed above, reports on the effect of HPA activity on both sleep and memory consolidation remain equivocal, with factors such as timing, the organism (laboratory animal vs. human), sex differences, the type of stress (endogenous vs. exogenous), and the response or dose of glucocorticoid all acting as potential confounds. To this effect we used psychosocial stress exposure as a means of targeting the effects of endogenous cortisol increase on the consolidation process in humans. While a small body of work has previously examined the effects of manipulating cortisol concentration before (Abercrombie et al., 2006) or during sleep (Born et al., 1989) and investigated the relationship of baseline cortisol at encoding on memory performance after sleep and wake (Bennion et al., 2013), to our knowledge this was the first to endogenously manipulate cortisol concentrations during the early stages of consolidation, allow for a recovery period prior to sleep, and then examine the effects of the stressor on subsequent sleep and emotional and neutral memory performance compared to a control condition. To do this, participants encoded a set mixed set of
complex scenes varying in degree of emotional arousal. Each scene had a central negative or neutral object placed on a peripheral neutral background. After encoding, participants were then asked to complete a psychosocial stress test or a matched control condition. This was followed by a consolidation delay that included a night of polysomnograph-recorded sleep. Upon awakening participants completed a recognition test in which the objects and backgrounds were presented separately and one at a time. The use of this “emotional memory trade-off” paradigm (Kensinger et al., 2007b; Payne et al., 2008) allowed us to determine if a stress manipulation prior to sleep differently influenced the consolidation of distinct aspects of negative arousing scenes.

Consistent with prior literature on stress and memory retention (e.g. Buchanan & Lovallo, 2001; Domes et al., 2005; Payne et al., 2007; Wolf, 2009 for review), we predicted that stress administration would lead to an increased preference for the consolidation of the emotional components of scenes at the expense of their neutral backgrounds, increasing the magnitude of the emotional memory trade-off effect. We further predicted that this memory effect would correlate with the magnitude of the HPA axis response to the stressor. While human studies have reported a stark decrease in REM following the exogenous manipulation of glucocorticoid administration (Born et al., 1989), only one has used a stressful task to endogenously manipulate stress. Vanderkerckhove et al. (2011) involved a negative mood induction task immediately prior to sleep which led to a substantial breakdown in total sleep architecture, not just
REM sleep\(^2\). Several non-human animal studies, however, have indicated an increase in REM after an endogenous stress manipulation (Cheeta et al, 1997; Marinesco et al., 1999; Meerlo et al., 2001; Rampin, et al, 1991; Vazquez-Palacios et al., 2004) and evidence suggests that a recovery from the stressor may be needed before the increase in REM occurs (Meerlo, et al., 2001). Due to the lack of human evidence and given that the present design allowed for a recovery period prior to the onset of sleep, we hypothesized that the stress manipulation would lead to an increase in the percentage of sleep time spent in REM. Also in line with the potential role for REM sleep in emotional memory consolidation (Payne et al., 2012; Wagner et al, 2001; Wagner et al., 2005), we hypothesized that the amount of time spent in REM would predict memory for the emotional components of scenes.

Finally, we were also interested in how symptoms of depression affect the neuromodulation of sleep and emotional memory by cortisol (see 1.1.5 The Role of Depression). In a preliminary exploration of this question, we piloted a handful of subjects who scored in the mild-moderate range on a validated depression questionnaire at the time of experiment participation and examined their behavioral patterns. The small sample size of this pilot group prevented us from exploring any stress reactivity or sleep related theories at this time.

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\(^2\) Cortisol concentrations were not collected in this study either to verify an increase in HPA axis activity.
2.1 Participants

Forty-eight participants (female = 26) from the University of Notre Dame (mean age 19.8 ± 1.5) completed the study for class credit or cash compensation. All participants were fluent English speakers with normal or corrected-to-normal vision. All participants were asked hydrate well before the study and not to smoke, eat or perform any strenuous exercise for one hour prior to each session. They were also asked to refrain from alcohol, tobacco and caffeine for the duration of the study. Forty-four of the participants were healthy, medication-free students and were randomly assigned to participate in the stress (n = 21) and control (n = 23) conditions [scored ≤10 on the Beck Depression Inventory (BDI)]. Data from one participant in the stress group was lost due to a campus-wide power outage (final stress group n = 20). Four additional participants were recruited as depressed pilots (stress n = 3; control n = 1). Depressed participants had elevated scores on a modified Beck Depression Inventory (no suicidality question due to confidentiality concerns) sent out with the Notre Dame Psychology Department Pre-screen Survey at the beginning of the semester. In order to be included, subjects also had to score 11-28 (cutoff scores for mild-moderate depression; Beck et al., 1988),
at the time of participation on the full Beck Depression Inventory (BDI) to account for symptom remission (i.e., to ensure that their depression symptoms had not significantly decreased from their initial scores on the pre-screen survey taken sometimes weeks earlier). Depressed participants were also medication-free at the time of participation to ensure no medication or antidepressant effects on sleep. Aside from the pre-screening and BDI scores, the protocols for the depressed and non-depressed subjects were identical.

2.2 Materials

The scenes depicted negatively salient or emotionally neutral objects placed on plausible neutral backgrounds (Kensinger et al., 2007; Payne et al., 2008). For each of 64 scenes (e.g., a car on a street), we created eight different versions by placing each of two similar neutral objects (e.g., two images of a car) and each of two related negative objects (e.g., two images of a car crash) on each of two neutral backgrounds (e.g., two images of a street). Creating 8 different but related versions of each scene allowed for the development of 8 different but related lists. The use of each list was counterbalanced across participants to account for possible list effects. An additional 32 scenes were deconstructed and served as lures on the subsequent recognition memory test. Participants in a prior experiment had rated the objects and backgrounds on dimensions of valence and arousal using 7-point Likert scales (Kensinger, Garoff-Eaton, & Schacter, 2006). All negative objects had an average arousal rating of 5 to 7 (with higher scores representing an arousing image) and average valence ratings lower than 3.
(with lower scores representing a negative image). All neutral items (both objects and backgrounds) had been rated as neutral in valence (valence ratings between 3 and 5) and nonarousing (arousal values lower than 4).

Self-assessments administered during the study included the Stanford Sleepiness Scale (SSS; Hoddes, et al., 1973), the State-Trait Anxiety Inventory (STAI; Spielberger, 2010), the Positive and Negative Affect Schedule (PANAS; Watson, et al., 1988), the Beck Depression Inventory (BDI; Beck, et al., 1988), the Beck Anxiety Inventory (BAI; Beck, et al., 1988) and the Mood and Anxiety Symptoms Questionnaire (MASQ; Watson & Clark, 1991).

2.3 Equipment

Images were presented at encoding and recognition the using E-Prime (Psychology Software Tools) on a 22” Lenovo Intel® Core ™ i5CPU Desktop running Windows 7®. The screen was approximately 19” away from the participant. Night time sleep was recorded and staged using a Grass Comet polysomnography system on a separate HP Compaq Elite SFF® PC utilizing Windows 7 Professional®. Cortisol radioimmunoassays were completed using Coat-A-Count assay kits from Siemens Healthcare Diagnostics (Duluth, GA) and were completed in the wet lab of the Emotion & Stress Physiology Lab at Notre Dame.
2.4 Procedure

All participants were instructed to arrive in the lab in the mid-afternoon (15:30-16:30) in order to control for the natural diurnal rhythm of cortisol and this first session typically lasted approximately two hours. Upon arrival, participants were randomly assigned to the stress or control group and completed informed consent and a standard set of lab questionnaires (i.e., demographics, PANAS, STAI, SSS) for at least 15 minutes. After this acclimation period, participants rinsed their mouths out with water and provided a baseline salivary cortisol sample. All salivary cortisol samples were collected through passive-drool techniques for the duration of the study. After sample completion, subjects were escorted to a computer station and instructed to rate a series of scenes, varying in degree of emotional arousal. In order to maximize encoding the participants were asked to rate each scene on a 7-point scale as to whether they would approach (positive) or back away (negative/arousing) if they encountered the scene in real life. Each participant viewed a total of 64 scenes, 32 neutral and 32 negative. Each scene was displayed for 5 seconds to allow time for the participant to fully view the picture, and then the subject was asked to give their rating prior to moving on to the next scene.

Following encoding, subjects in the stress condition completed the Trier Social Stress Task (TSST), a validated psychosocial stressor shown to increase endogenous levels of cortisol (Kirschbaum, Pirke, & Hellhammer, 1993). Participants were given 10 minutes to write a speech as to why they would be the best candidate for their dream job. They could write about any position, the only requirement was that they had to use
factual information. They were then instructed to present the speech without notes for 5 minutes to a panel of two confederate judges dressed in lab coats, followed by a 5 minute out-loud arithmetic task. Judges were trained to maintain a flat affect and offer no positive feedback for the entire duration of the task. Those in the control condition performed a control version of the task in which they were asked to prepare the same speech, but were allowed to read the speech from their notes in an empty room. Subjective measures of anxiety were also administered before and after completion of the TSST. Immediately after completion of either task, four additional saliva samples were collected in 15 minute intervals to determine HPA axis reactivity to the task. Cortisol secretion through HPA activity varies from person to person and changes in concentration can take time to reach saliva (up to an hour), so this collection method enables the measurement of the full delayed arc of cortisol change by collecting several samples over various time points during that delay. During the sampling participants were completed a second battery of self-report assessments. Self-assessments included measures of affect (PANAS, Watson, et al., 1988) and state anxiety (STAI, Spielberger, 2010) which were collected at baseline (20min after arriving in the lab), immediately after completion of the stress/control task, and again the next day prior to recognition testing.

After the sampling period (between 17:30-18:30) they were dismissed for dinner and a recovery period prior to returning at 22:00 to the lab for a night of polysomnograph (PSG) recorded sleep. Electrodes were attached to allow for digital PSG
recording while participants watched a non-arousing video\textsuperscript{3} for approximately 1 hour. The PSG montage included 7 electroencephalogram (EEG) leads (O1, O2, C1, C2, Cz, F1, F2), two electromyogram (EMG) and two electrooculogram (EOG) leads, with each electrode referenced to the contralateral mastoid. Immediately prior to sleep participants provided another saliva sample and lights out commenced between 23:00-00:00. After approximately 8.5 hours of sleep opportunity the participants were awoken and asked to immediately provide a saliva sample followed by 3 additional saliva samples at 15 minute intervals to test separate hypotheses.

Once the morning sampling was complete, participants performed an unexpected, self-paced recognition task in which the previously encoded objects and backgrounds were presented separately and one at a time, in random order. Some of these objects and backgrounds were identical to the scene components that had been encoded (e.g., the same car accident), others were the alternate version of the object of background and thus shared the same verbal label but differed in specific visual details (e.g., a similar car accident), and others were objects or backgrounds that had not been seen at encoding (new). Similar pictures were included for analysis of ‘gist’ memory for scenes (see Data Analysis) and participants never saw both the same and the similar version of an item at test. For each item, participants indicated whether it was an exact match to a previously viewed component ("same"), similar but not an exact match ("similar"), or not seen before ("new"). The recognition task included 32 same objects

\textsuperscript{3} Participants were given the option to watch a movie from the original Shrek trilogy or a documentary piece from the series Planet Earth
(16 negative, 16 neutral), 32 similar objects (16 negative, 16 neutral), 32 new objects (16 negative, 16 neutral), 32 same backgrounds (16 previously presented with a negative object, 16 previously presented with a neutral object), 32 similar backgrounds (16 previously presented with a negative object, 16 previously presented with a neutral object), and 32 new backgrounds. Upon completion of the recognition task all participants were debriefed on the purpose of the study, with a particular emphasis on ensuring that participants in the stress paradigm understood that they were not actually being judged during the stress task, but rather that the task was designed to elicit a stress response (for a complete diagram of the study conditions, see Appendix A).

2.5 Data analysis

2.5.1 Memory Analysis

To investigate varying degrees of memory detail following the stress manipulation, we calculated both specific and general recognition memory scores. Consistent with prior studies, a less conservative general recognition score was computed by summing the number of “same” and “similar” responses to same items, as this score reflects memory for at least some aspects of the studied item (Kensinger et al., 2007a; Payne et al., 2008). That is, for same items given either a “same” or “similar” response, participants had to remember at least that a particular type of object or background had been studied (e.g., that they had seen a car accident or a street), because otherwise they would have instead indicated that the item was “new”. Thus, general recognition memory is a measure of a participant’s ability to remember at least
the gist of the items (with or without specific detail). The more conservative specific recognition score (i.e., summing only “same” responses to same items), was computed to capture veridical memory for the precise visual details of a studied object or background. Specific and general recognition scores were computed for each type of scene components (negative and neutral central objects and peripheral neutral backgrounds that had been studied with either a negative or neutral object).

Analysis revealed that the false alarm rate (“same” or “similar” responses to “new” items) across subjects was low (less than 5% for “same” responses to “new” items and less than 18% for “similar” responses to “new” items) and did not differ between groups (p = 0.65 and 0.14, respectively). As in prior research, due to the low rates of false alarm we only report uncorrected recognition scores (Payne, et al., 2008). For our purposes, ‘memory trade-off’ was defined as the difference between object and background memory. To calculate the memory trade-off score we created difference scores by subtracting the corresponding background memory score (proportion of backgrounds correctly remembered) from the object memory (proportion of objects correctly remembered) score for each valence (e.g., negative objects – negative backgrounds) within each group (stress and control). This was done for both specific and general memory scores. For example, if a person’s negative object score was 0.70 and their negative background score was 0.50, their memory trade-off score would be 0.20.
2.5.2 Cortisol Assessment

Participants used the passive drool method using a straw to expectorate (i.e., no gum, cotton, or other saliva flow stimulants were used) and were instructed to fill the test tube to the 5mL line. They were allowed to drink sips of water throughout the experiment session, but only immediately after a sample. After each data collection session, vials were capped and frozen until later processing, which was done in-house and began with three freeze-thaw cycles and centrifugation. Cortisol levels were determined by solid-phase $^{125}$I radioimmunoassays (Coat-A-Count, Siemens Healthcare Diagnostics, Duluth, GA), using the protocol described by Wirth & Schultheiss (2006). Four assays were necessary to assay all samples. Samples with an initial coefficient of variation (CV) greater than 60 were then reassayed. The average lower limit of detection (LLD; $B_0 - 3 \times SD$ method) was 0.26 ng/ml. After removing samples under the LLD, mean intra-assay CV was 15.8%. Inter-assay CVs for Morning and Evening combined pools of saliva averaged 4.6% and 13.5% respectively.
CHAPTER 3:

RESULTS

3.1 Results

All results reported include only the non-depressed, healthy subjects except for the Preliminary Analysis: Depression section.

3.1.1 Stressor efficacy and cortisol response

To assess the efficacy of our stressor and control task and to determine their impact on affect, both objective cortisol measures and subjective reports of state anxiety (STAI) and affect (PANAS) were administered pre- and post-task. First, to ensure the subjective validity of the stressor we conducted mixed ANOVAs, with time of assessment as the repeated measure, on ratings of state anxiety and negative affect in the stress and control groups. We found that in addition to main effects of group on reported anxiety \(F_{1, 38} = 13.1, \ p = 0.001, \ \eta^2_p = 0.19\) and negative affect \(F_{1, 37} = 7.8, \ p = 0.008, \ \eta^2_p = 0.17\), there were also group x time interactions of assessment on reported anxiety \(F_{1, 38} = 8.6, \ p = 0.006\) and negative affect \(F_{1, 37} = 24.4, \ p < 0.001, \ \eta^2_p = 0.4\). As can be seen in Figure 3.1, participants in the stress condition showed a significant change and reported both more anxiety and more negative affect after the speech task than did participants in the control group.
Figure 3.1: Changes in self-report measures of anxiety and negative affect (NA) pre-and post-TSST and Control tasks. *indicates p < 0.05
Healthy participants in the stress condition also showed elevated levels of cortisol following stress exposure (see Figure 3.2). To assess the impact of the TSST vs. control manipulation on cortisol responsivity, a 2 (group) x 5 (time: t0, t1, t2, t3, t4) mixed ANOVA, with time as a repeated factor was performed. There were no main effects of group $[F_{1, 41} = 1.6, p = 0.2]$ or time $[F_{4, 38} = 2.22, p = 0.09]$, nor a time x group interaction $[F_{4, 38} = 0.84, p = 0.5]$. Due to high variance of cortisol response to the TSST the following independent-samples comparisons between groups were not significant $[t1: t (41) = 1.5, p = 0.15; t2: t (41) = 1.8, p = 0.08; t3: t (41) = 1.2, p = 0.25; t4: t (41) = 0.7, p = 0.5]$. Area under the curve with respect to increase (AUCi) was also calculated as another measure of the delayed arc of cortisol response over time (Pruessner et al., 2003). AUCi was calculated across t0-t4 and between group comparisons failed to reach significance ($p > 0.28$).

Due to the high level of variability in response to the TSST, another possibility supported by previous research was to divide the healthy stress group into high- vs. low-responders based on changes in salivary cortisol level from baseline to post-stressor (Domes et al., 2002; Elzinga & Roelofs, 2005; Nater, Moor, Okere, Stallkamp, Martin, Ehlert, & Kliegel, 2007; Takahashi, Ikeda, Ishikawa, Tsukasaki, Nakama, Tanida, & Kameda, 2004; Wolf et al., 2001). We performed a post-hoc median-split within the stress group (Nater et al., 2007) based on AUCi from t0-t4 $[t (18) = 4.3, p < 0.001]$.

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4 TSST Time points: t0 = Baseline, t1 = Immediately after TSST, t2 = 15 min after TSST, t3 = 30 min after TSST, t4 = 45 min after TSST
Figure 3.2: Average HPA reactivity to stress and control tasks as measured by salivary cortisol concentration over time. Time is in relation to the end of the TSST task.
median split at $AUC_i = 11.1$; see Figure 3.3a] resulting in a group of high responders ($n = 11$) and a group of low responders ($n = 9$). High responders had significantly greater $AUC_i$ $t(18) = 4.3, p < 0.001$ compared to the low responder group. High responders and low responders also differed in the magnitude of change in salivary cortisol concentration from baseline to the sample taken immediately following the TSST $t(18) = 4.8, p < 0.001$, 15 min post-TSST $t(18) = 3.4, p = 0.003$ and 30 min post-TSST $t(18) = 2.2, p = 0.04$. High responders significantly differed from the control group in $AUC_i$ $t(32) = 2.9, p = 0.006$ and in the magnitude of change in salivary cortisol concentration from baseline to immediately following the TSST $t(32) = 3.0, p = 0.005$ and 15 min post-TSST $t(32) = 2.9, p = 0.007$, despite no difference in baseline cortisol concentration $t(32) = -0.2, p = 0.8$; see Figure 3.3b]. There were no differences in any measure of cortisol reactivity between the control group and low responders (all $p$’s > 0.1). While the separating the stress condition into these discrete groups resulted in small sample sizes in each cell reducing the power of the effects (i.e., increased risk of Type I error), this design created the opportunity to explore two important questions. First, we were able to examine how sleep and memory behavior was affected for all participants that experienced a psychosocial stressor. Second, the separation of the responder groups enabled us to preliminarily explore if mounting a significant physiological stress response during consolidation is necessary for any changes in these sleep or memory consolidation systems caused by stress.
Figure 3.3: A) Average HPA reactivity to stress and control tasks as measured by salivary cortisol concentration. Stress group was further subdivided into Responders vs. Low responders. B) Salivary cortisol concentration comparison of just High responders and the Control group.
3.2 Behavioral measures

3.2.1 The impact of stress on specific memory

Given the growing literature on stress and memory consolidation, our primary objective was to determine how exposure to stress would differently impact the consolidation of specific negative and neutral information compared to a control condition. Thus, we first conducted a 2 (condition: stress, control) x 2 (scene component: object, background) x 2 (valence: negative, neutral) mixed analysis of variance (ANOVA), with scene component and valence as repeated measures, on specific recognition memory. This analysis revealed a main effect of scene component \(F_{1,41} = 70.9, p < 0.001, \eta^2 = 0.63\) and a 2-way interaction between scene component and valence \(F_{1,41} = 35.8, p < 0.001, \eta^2 = 0.47\), which confirmed the existence of the trade-off effect (Kensinger et al., 2007). Negative objects were better recognized than neutral objects \(t (42) = 3.3, p = 0.002\), but the backgrounds on which the objects were placed were more poorly recognized when they were associated with negative objects than when they were associated with neutral objects \(t (42) = -4.8, p < 0.001\). Critically, scene component and valence also interacted with the between-subject factor of condition resulting in a 3-way interaction of condition x scene component x valence \(F_{1,41} = 4.6, p = 0.037, \eta^2 = 0.102;\) see Figure 3.4), indicating that the pattern of memory results was affected by the addition of a stressor during the consolidation period. Although specific object memory was significantly better than background memory within negative scenes for both the stress and control conditions \(t (19) = 8.1, p < 0.001\)
and $t(22) = 7.3, p < 0.001$ respectively], negative objects were remembered better than neutral objects only when the delay included the stressor [$t(19) = 4.2, p = 0.001$] but not after the control task [$t(22) = 1.1, p = .30$]. This suggests that stress bolsters memory for negative information while suppressing memory for neutral information.

To further unpack how the magnitude of memory trade-off was affected by the stress manipulation, memory trade-off scores were calculated for each valence (negative and neutral) in both conditions (stress and control; see Data Analysis). As expected, the magnitude of the negative memory trade-off was greater than the difference in object and background memory within the neutral scenes for both the stress [$t(19) = 5.7, p < 0.001$] and control [$t(22) = 2.8, p = 0.01$] conditions. Critically, and consistent with our predictions, between-group comparisons revealed a marginally significant increase in negative memory trade-off for those that experienced the TSST during the consolidation period compared to those in the control condition [$t(41) = 2.0, p = 0.05$; see red braces in the first and third columns of Figure 3.4] while neutral memory difference scores remained equivalent between groups [$t(41) = -0.61, p = 0.55$].

3.2.2 The impact of stress on gist memory

As noted previously, in addition to understanding the effect of stress on specific detailed memory, we were also interested in how it might affect more general, ‘gist’ memory (memory with or without specific detail). Thus, general recognition memory scores were also calculated (see Data Analysis). We once again began by conducting a 2
Figure 3.4: Specific memory, All Stress vs. Control. Behavioral results found a 3-way interaction in which stress creates a larger disparity in specific memory between negative objects and the backgrounds on which they were placed compared to the control group.
(condition: stress, control) x 2 (scene component: object, background) x 2 (valence: negative, neutral) mixed analysis of variance (ANOVA), with scene component and valence as repeated measures, only this time we utilized general recognition memory scores. This analysis also revealed a main effect of scene component \( F_{1, 41} = 100.9, p < 0.001, \eta_p^2 = 0.71 \) and a 2-way interaction between scene component and valence \( F_{1, 41} = 80.6, p < 0.001, \eta_p^2 = 0.66 \), replicating the trade-off effect within the recollection of gist information. Similar to specific memory, negative objects were better recognized than neutral objects \( t (42) = 6.2, p < 0.001 \), and backgrounds associated with negative objects were remembered more poorly than those associated with neutral objects \( t (42) = -4.7, p < 0.001 \). In contrast with specific memory, scene component and valence did not interact with condition \( F_{1, 41} = 2.7, p = 0.1, \eta_p^2 = 0.06 \); see Figure 3.5), suggesting that stress does not significantly alter gist memory. Not only was gist object memory significantly better than gist background memory within negative scenes for both the stress \( t (19) = 9.1, p < 0.001 \) and control conditions \( t (22) = 8.1, p < 0.001 \), but negative objects were also better remembered than neutral objects in both groups as well \( \text{stress: } t (19) = 6, p < 0.001; \text{control: } t (22) = 3.4, p = 0.003 \). Additionally memory trade-off score comparisons resulted in equivalent difference magnitudes between groups for both negative \( t (41) = 0.96, p = 0.34 \); see green braces in figure 3.5) and neutral \( t (41) = -1.0, p = 0.32 \) information. The lack of a stress effect on gist memory suggests that stress has its greatest effects on specific detailed memory while the easier gist memory is less affected.
Figure 3.5: Gist memory, All Stress vs Control. Both the stress and control group showed increases in negative object gist memory, but there were no differences between groups.
3.2.3 High responder vs. low responder vs. control ANOVA results

As indicated previously, while all stress participants displayed an overall average increase in anxiety and negative affect, not all participants exposed to the TSST experienced an increase in HPA activity as measured by salivary cortisol concentration. This distinction led to the division of the stress group into responders and low responders (see Data Analysis). To begin to explore the relationship between these distinct groups, a 3 (condition: High responder, stress low responder, control) x 2 (scene component: object, background) x 2 (valence: negative, neutral) mixed analysis of variance (MANOVA), with scene component and valence as repeated measures was performed on specific memory. This resulted in a main effect of scene component \(F_{1, 40} = 65.3, p < 0.001, \eta_p^2 = 0.62\) and a 2-way interaction between scene component and valence \(F_{1, 32} = 37.7, p < 0.001, \eta_p^2 = 0.49\). Interestingly, this ANOVA also produced a marginally significant 3-way interaction \(F_{1, 40} = 3.3, p = 0.05, \eta_p^2 = 0.14\); see Figure 3.6], indicating differences between each of the three conditions. Running this same analysis on gist memory scores, resulted in a similar main effect of scene component \(F_{1, 40} = 83.3, p < 0.001, \eta_p^2 = 0.68\) and 2-way interaction between scene component and valence \(F_{1, 32} = 78.5, p < 0.001, \eta_p^2 = 0.66\). The three-way interaction, however, failed to reach significance for gist memory \(F_{1, 40} = 2.9, p = 0.07, \eta_p^2 = 0.12\). To further unpack how performance in each of these subgroups differed, memory analyses comparing stress high responder and low responder behavior to the control group were ran separately.
Figure 3.6: Image depicts the significant three way interaction of group x scene component x valence. Resp. = High responder, Nonresp = Stress Low responder, Neg = Negative, Neu = Neutral.
High responder memory was examined to specifically target how increased HPA axis activity affects emotional memory consolidation. A 2 (condition: High responder, control) x 2 (scene component: object, background) x 2 (valence: negative, neutral) mixed analysis of variance (ANOVA), with scene component and valence as repeated measures, on specific recognition memory again resulted in a main effect of scene component \( F_{1,32} = 44.1, p < 0.001, \eta^2_p = 0.58 \) and a 2-way interaction between scene component and valence \( F_{1,32} = 35.8, p < 0.001, \eta^2_p = 0.52 \). The 3-way interaction that was found previously across all stress participants was replicated, only omitting low responders led to an increase in effect size despite the reduction in power \( F_{1,32} = 6.9, p = 0.01, \eta^2_p = 0.18 \; \text{see Figure 3.7} \). The rest of the responder memory results mirror the results when considering the entire stress group. Negative objects were better recognized than neutral objects \( t (33) = 3.0, p = 0.005 \) and the backgrounds on which the objects were placed were more poorly recognized when they were associated with negative objects than when they were associated with neutral objects \( t (33) = -4.1, p < 0.001 \). Specific object memory in the high responder group was significantly better than background memory within negative scenes \( t (11) = 6.4, p < 0.001 \) and negative objects were still remembered better than neutral objects \( (11) = 8.0, p < 0.001 \).

Memory trade-off analysis within the responder group also revealed that the negative memory trade-off was greater than the difference in neutral object and background memory \( t (10) = 6, p < 0.001 \). The between-group comparison of the
Figure 3.7: Specific Memory, High responders vs Control. Limiting the stress group just to responders replicated the between group interaction with an even stronger effect size than when the entire stress group was included.
magnitude of the negative trade-off between just the High responders and control
group failed to reach significance \[t (32) = 1.83, p = 0.077; \text{neutral memory trade-off}
differences not significant; see braces in the first and third columns of Figure 3.7\]. In
summation, the finding that a majority of specific memory results were replicated
within just the high responder subset of the stress group, and in several cases resulted
in even stronger effects despite the reduction in power, provides preliminary support
that an increase in HPA axis reactivity drives the enhanced emotional memory
processing seen during consolidation after stress.

3.2.4 Stress low responder and specific memory results

To determine how behavioral results differed when subjects underwent the
stress task but did not mount an endogenous cortisol response, a 2 (condition: stress
low responder, control) x 2 (scene component: object, background) x 2 (valence:
negative, neutral) mixed analysis of variance (ANOVA), with scene component and
valence as repeated measures, was done utilizing only low responders from the stress
group. While the main effect of scene component \[F_{1, 30} = 55.6, p < 0.001, \eta^2_p = 0.65\] and
the 2-way interaction between scene component and valence persisted \[F_{1, 30} = 13.5, p =
0.001, \eta^2_p = 0.31\], there was no longer a three way interaction between valence, scene
component and condition \[F_{1, 32} = 0.65, p = 0.43, \eta^2_p = 0.02; \text{see Figure 3.8}\]. Additionally,
while negative objects were remembered significantly better than their matched neutral
backgrounds in the stress low responder group \[t (8) = 4.8, p = 0.001\], specific memory
Figure 3.8: Specific Memory, Stress Low responders vs Control. Preliminary results indicate that low responders did not differ from control group in any measure of specific memory.
for negative and neutral objects was equivalent [t (8) = 1.3, p = 0.2]. Finally there was no
difference in the magnitude of the memory trade-off scores between stress low
responders and the control group [t (30) = 1.4, p = 0.15]. Together these preliminary
results suggest that the enhanced emotional memory consolidation after experiencing a
stressor is dramatically reduced if it is not matched with a physiological increase in HPA
axis activity.

3.2.5 Stress low responder and specific memory results

Gist memory results within the High responder subgroup offer additional
support for the enhancement in emotional memory consolidation after stress. A 2
(condition: High responder, control) x 2 (scene component: object, background) x 2
(valence: negative, neutral) mixed analysis of variance (ANOVA), with scene component
and valence as repeated measures, on gist recognition memory resulted in the same
main effect of scene component \(F_{1,32} = 68.1, p < 0.001, \eta_p^2 = 0.68\] and 2-way interaction
between scene component and valence \(F_{1,32} = 92.9, p < 0.001, \eta_p^2 = 0.74\]. Critically,
after the stress low responders were removed from the stress group the 3-way
interaction of condition x scene component x valence became significant \(F_{1, 32} = 6.9, p =
0.01, \eta_p^2 = 0.18; \) see Figure 3.9) suggesting that gist memory may be affected by a stress
manipulation, but only when the stressor leads to a significant physiological stress
response. Although negative object memory is equivalent between High responders and
the control condition \(t (32) = -0.8, p = 0.4; \) both over 85% gist recognition rates,
backgrounds associated with negative objects and neutral object recognition
Figure 3.9: Gist memory, High responders vs Control. When the stress group is limited to responders, gist memory does appear to be affected by stress. Most notably there is suppression in memory for neutral objects and neutral backgrounds paired with negative objects.
was marginally worse in the High responders compared to the control condition

\[ \text{negative backgrounds: } t(32) = -2.0, p = 0.05; \text{ neutral objects: } t(32) = -2.0, p = 0.05 \]

indicating that consolidation of gist memory is affected by the increase in HPA activity such that negative information is preserved but the storage of neutral information is hindered. Analysis of the magnitude of the trade-off effects revealed no significant differences between groups \( t (32) = 1.5, p = 0.15 \).

3.2.6 Stress low responder and gist memory results

Analyses of gist memory were run again substituting the High responder group with the low responder group to determine if a lack of HPA axis response created a different effect on general memory performance. A 2 (condition: stress low responder, control) x 2 (scene component: object, background) x 2 (valence: negative, neutral) mixed analysis of variance (ANOVA), with scene component and valence as repeated measures, on gist recognition memory resulted in the main effect of scene component \( F_{1,30} = 50.8, p < 0.001, \eta_p^2 = 0.63 \) and 2-way interaction between scene component and valence \( F_{1,30} = 33.0, p < 0.001, \eta_p^2 = 0.52 \), but unlike the High responder group, the three way interaction was not significant when considering the low responders \( F_{1,30} = 0.06, p = 0.8, \eta_p^2 = 0.002 \). As can be seen in Figure 3.10, the stress low responder group did not differ from the control group in any measure of gist memory, suggesting that an elevation in cortisol concentration is necessary for preferential processing of general emotional memory as well.
Figure 3.10: Gist memory, Stress Low responders vs Control. Stress low responders did not differ from the control group in any measures of gist memory.
3.2.7 Preliminary analysis: Depression

As noted in the introduction, one goal of this thesis was to begin a preliminary investigation of how the interaction of sleep, stress, and memory is affected by elevated depression symptomology. As noted above, subjects were recruited through their responses to a modified BDI measure (no suicidality question due to confidentiality concerns) on the University of Notre Dame Psychology Department Pre-screen Survey at the beginning of the semester. Only 11 students scored within the required range on the modified BDI and despite repeated contact, participants within this subset were reluctant to sign up and often cancelled sessions. As a result, data has been fully collected for only three depressed subjects in the stress group and one depressed subject in the control group. While detailed analysis of their behavioral results is not yet meaningful, the pattern of these early results is nonetheless interesting and worthy of brief comment (specific memory, see Figure 3.11a; gist memory, see Figure 3.11b). Most notably, the negative memory trade-off seems to be quite small for specific and gist memory in the stress and control condition, possibly indicating a generalizing effect in which all veridical details of a negative event are better remembered in depression (see Discussion). Correlational analyses to determine if BDI scores in the healthy controls related to emotional memory results also failed to reach significance (all p’s ≥ 0.15).
Figure 3.11: Pilot Depression Data- a) Specific and b) Gist memory scores for those scoring mild to moderate depression on the BDI in the stress (n=3) and control (n=1) condition.
3.3 Sleep measures

3.3.1 The effect of stress on sleep

All participants spent the night between the encoding and recognition task in the Sleep, Stress and Memory Lab (SAMLab) at the University of Notre Dame to allow for PSG-recorded sleep and analysis. Sleep data was not used for two control participants [one participant had a sleep efficiency (SE; TST/total time in bed) 3 standard deviations below the mean, and data from the other was lost due to equipment failure] leaving 20 subjects in the stress group and 21 subjects in the control group for full sleep analysis.

Sleep data can be found in Table 3.1. Interestingly the stress group had a marginal reduction in Stage 1 measures [Stage 1 minutes: $t(39) = -2.0, p = 0.05$; Stage 1 percent: $t(39) = -2.0, p = 0.05$] and a strong trend for an increase in Stage 2 percent [$t(39) = 1.8, p = 0.079$], but all other measures of sleep quality as well as measures of REM and SWS were equivalent between the stress and control groups, contrary to what was predicted. This held was also true when low responders were removed from the stress group and only those that mounted a significant HPA response to the stressor were compared to the control group (all $p$'s $> 0.2$).

3.3.2 Correlations of sleep with emotional memory

Based on our a priori hypothesis that stress prior to sleep would lead to changes in emotional memory processing, we focused our analysis on how measures of sleep predicted specific and gist memory performance for the emotional scene components and the magnitude of the negative trade-off. Across all participants, minutes spent in
SWS and SWS percent both negatively correlated with specific memory for negative objects of the scenes \[r (41) = -0.32, p = 0.04\] and \[r (41) = -0.32, p = 0.04\] respectively; see Figure 3.12a and 3.12b]. Neither of these SWS measures correlated with gist memory across groups (all p’s > 0.15) and when the correlation was run separately within each group, the negative correlation between SWS and specific negative object memory was no longer significant. Again, contrary to expectation, no measure of REM sleep or any other sleep measure correlated with specific or gist emotional scene component score or trade-off memory score.

3.4 Stress measures

3.4.1 Correlations of stress with emotional memory

To determine whether the enhancement of the emotional memory trade-off effect in the entire stress group was driven by individual differences in reported and experienced anxiety in response to the stressor, we correlated emotional memory trade-off scores with subjective state anxiety and cortisol reactivity measures. Since it was determined that the stress group had elevated cortisol concentration and on average reported significantly higher anxiety after the stress task (see 3.1.1 Stressor Efficacy and Cortisol Response), the stress and control groups were combined to increase the variability and the power of the correlations. Across all participants, cortisol concentration immediately after the stress manipulation task (t1) significantly correlated with the magnitude of the trade-off for specific memory between negative objects and their paired backgrounds \[r (43) = 0.31, p = 0.045\]; see Figure 17]. Additionally, post-stress subjective anxiety as measured by the STAI was also
### TABLE 3.1

SLEEP PARAMETERS FOR OVERNIGHT IN LAB

<table>
<thead>
<tr>
<th>Sleep Parameter</th>
<th>Stress Group Mean ± SD</th>
<th>Control Group Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sleep Time</td>
<td>466.9 ± 25.8</td>
<td>463.7 ± 27.2</td>
</tr>
<tr>
<td>Wake After Sleep Onset</td>
<td>35.6 ± 25.6</td>
<td>35.7 ± 22.3</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>90.8 ± 5.3</td>
<td>91.0 ± 5.3</td>
</tr>
<tr>
<td>Sleep Latency</td>
<td>9.1 ± 7.3</td>
<td>9.5 ± 7.0</td>
</tr>
<tr>
<td>Stage 1 min*</td>
<td>39.4 ± 11.7</td>
<td>49.6 ± 19.6</td>
</tr>
<tr>
<td>Stage 1 %*</td>
<td>8.5 ± 2.6</td>
<td>10.1 ± 4.9</td>
</tr>
<tr>
<td>Stage2 min</td>
<td>248.6 ± 36.4</td>
<td>232.2 ± 29.3</td>
</tr>
<tr>
<td>Stage 2 %*</td>
<td>53.1 ± 6.2</td>
<td>50.0 ± 5.0</td>
</tr>
<tr>
<td>SWS min</td>
<td>91.0 ± 19.4</td>
<td>94.9 ± 25.0</td>
</tr>
<tr>
<td>SWS %</td>
<td>19.6 ± 4.4</td>
<td>20.4 ± 5.1</td>
</tr>
<tr>
<td>REM min</td>
<td>88.0 ± 23.8</td>
<td>87.0 ± 21.3</td>
</tr>
<tr>
<td>REM %</td>
<td>18.8 ± 4.7</td>
<td>18.7 ± 4.1</td>
</tr>
</tbody>
</table>

Table Notes: Sleep parameters of stress and control group as recorded by PSG in the Notre Dame sleep lab. All measures in minutes unless reported as a percentage. Sleep Efficiency = TST/total time in bed, SWS = slow wave sleep, REM = rapid eye movement sleep, Sleep Latency = latency to sleep onset. *indicates p = 0.05, † indicates p < 0.08
Figure 3.12: Across groups, SWS in a) minutes and b) percent negatively correlated with specific memory scores for negative objects. This correlation disappears when the subjects are separated by group.
significantly correlated with the size of the specific negative memory trade-off \( r (39) = 0.38, p = 0.028 \); see Figure 3.14\(^5\). Together these results provide preliminary evidence that physiological HPA activation and subjective anxiety levels during the early stages of consolidation of complex emotional scenes may be associated with subsequent long-term memory performance.

\(^5\) Post-task negative affect as measured by the PANAS did not correlate with any measure of memory (all \( p \)'s > 0.33)
Figure 3.13: Across all participants, salivary cortisol concentration immediately after the stress or control condition positively correlated with the magnitude of the emotional memory trade-off score.
Figure 3.14: Across all participants, subjective anxiety as measured on the STAI immediately after the stress or control condition positively correlated with the magnitude of the emotional memory trade-off score.
CHAPTER 4:

DISCUSSION

4.1 Discussion

The primary goal of the current study was to assess the impact of psychosocial stress reactivity on subsequent sleep and the consolidation of individual scene components from both negative and neutral scenes. Previous studies have found that even after a short delay memory for the emotional, central object is preferentially remembered over their paired neutral backgrounds, while memory for neutral objects and their backgrounds remains equivalent (Kensinger et al., 2007b). This emotional memory trade-off effect can be potentiated when certain consolidation enhancing processes, such as sleep (Payne et al., 2008; Payne and Kensinger, 2010, Cunningham et al., 2014a), occur during the delay. Like sleep, moderate stress during consolidation leads to superior memory for emotionally salient information following long term delays (Cahill et al., 2003; Payne et al., 2007). Previous studies have also found that manipulating cortisol levels and natural cortisol concentrations during sleep (Wagner et al., 2005; see Born & Wagner, 2004 for review), as well as baseline cortisol levels at encoding prior to sleep (Bennion et al., 2013) affect neutral and emotional memory consolidation. The present study directly manipulated psychosocial stress after encoding
and prior to sleep during the early stages of memory consolidation. This allowed us to examine the impact of increased cortisol concentration on subsequent sleep architecture and explore how these distinct consolidation processes interact to affect the selective preservation of different aspects of emotional memory.

Our results show that the experience of a psychosocial stressor during the consolidation period of neutral and emotional scenes significantly increased the magnitude of the negative memory trade-off compared the control condition, but did not alter memory consolidation within neutral scenes. Additionally, within the stress group, specific memory for negative objects was significantly greater than memory for neutral objects after stress. Specific emotional and neutral object memory performance was equivalent within the control group. These findings suggest that stress after encoding enhances the consolidation of emotional material while simultaneously suppressing neutral memory. This increase in the magnitude of the trade-off in emotional memory reflects an increase in the selective processing of the individual components of the emotionally salient scenes following stress exposure. Correlations with stress measures reaffirm this finding. Both subjective reports of anxiety and cortisol concentrations immediately after the stress test correlated with the size of the trade-off for specific memory between negative objects and their paired backgrounds. Interestingly, stress did not appear to have the same effect on the consolidation of gist memories when all stress participants were included. Original analysis of gist memory performance revealed no differences between the stress and control conditions, despite the average increase in subjective anxiety reported by those that underwent the
stressor. This is in line with previous research, in which negatively arousing content at encoding has also been shown to directly enhance memory for specific, detailed memory (Kensinger et al., 2006). Importantly, however, while subjects in the stress group demonstrated an average increase in subjective reports of anxiety and negative affect to the TSST, not all subjects mounted a significant physiological response to the stressor as measured by acute increases in salivary cortisol concentrations. This led to the division of the stress group into high responders and low responders, and separate, preliminary behavioral analyses of these groups resulted in markedly different memory patterns. High responders showed an enhancement in negative memory consolidation, particularly for specific memory in which negative objects were remembered better than neutral objects. When comparing just high responders to the control group, not only did limiting the stress group result in a pattern of specific memory recognition that mirrored the results of the entire stress group (including a numerically larger emotional memory tradeoff), but the size of the effect increased despite the reduction in power. Interestingly, when low responders were omitted, gist memory results also revealed an effect of stress. Most notably there was a suppression of consolidation for neutral objects and neutral backgrounds paired with negative objects. Additionally, we did not find any preliminary group differences in specific or gist memory consolidation between low responders and the control group. All measures of performance, including memory for any particular scene component and the magnitude of memory trade-offs, were equivalent between these two groups. It is important to note, however, that the low responder group was quite small and additional subjects are needed to strengthen
these results. Still, these findings provide preliminary evidence that the post-encoding cascade of neuromodulators released by an HPA axis response to a psychosocial stressor may be an important part of the selective processing of emotional components of scenes and an overall suppressive effect on neutral memory.

Contrary to our predictions, completing the stress test during early stages of consolidation prior to sleep had no effect on subsequent sleep architecture, even when those that did not mount a significant HPA activation were removed. Likewise, no sleep stage correlated with performance on any measure of emotional memory for either group. This result was surprising in light of the large body of work suggesting that stress affects sleep detailed in the Introduction and the behavioral data from this study showing clear differences in emotional memory processing between the stress and control conditions. While speculative, we offer two possible explanations for these results. First, it is possible that the TSST might not be a traumatic enough experience to create lasting effects on sleep structure. Given the population of high achieving college students, a public speaking/out loud math task might be a relatively minor stressor or a stressor that they have developed effective coping strategies for from past experience. Additionally, in order to control for the natural diurnal rhythm of cortisol, all participants completed the stress task in the midafternoon, approximately 4-6 hours before sleep. With this extended delay prior to sleep, it is possible that any enhancements that stress has on emotional memory processing may have been nearing completion by the time sleep occurred, leading to relatively minor effects on sleep.
A second possibility is that while stress prior to sleep may not significantly alter the structure of the subsequent sleep period, perhaps the level or density of processing going on during these stages is intensified. Previous research has found that the measures of prefrontal theta power during REM sleep predicts emotional memory consolidation (Nishida, Pearsall, Buckner, & Walker, 2009), while Stage 2 spindle density and NREM spectral power predicts performance on procedural and neutral declarative memory tasks, as well as attention and executive function capacities (Anderson & Horne, 2003; Mander, Rao, Lu, Saletin, Ancoli-Israel, Jagust, & Walker, 2013; see Fogel & Smith, 2011 for review). It is interesting to note that the stress group showed a reduction in the lightest stage of Stage 1 sleep, suggesting that perhaps instead of an increase in the amount of deeper stages of sleep, stress may increase the intensity of sleep stages important for memory processing during the night. Despite finding only minor effects on the structure of sleep after stress, it is tantalizing to consider how the degree of processing during sleep may be affected. In this regard, spectral analysis and REM theta density analysis can be applied to this and future data in order to explore this possibility.

The experience of emotional arousal at encoding has been shown to play a critical role in the enhancement of memory by cortisol in both human (Abercrombie et al., 2006) and laboratory animal models (Okuda, Roozendaal, and McGaugh, 2004; Roozendaal et al., 2006). This emotional arousal then leads to a release of norepinephrine and increased activity in the amygdala, which modulates activity in areas important for declarative memory formation, such as the hippocampus.
Stress that temporally coincides with emotional arousal (either simultaneously or concurrently) leads to the production of both cortisol and norepinephrine which coalesce to potentiate amygdalar activity and functional connectivity with areas of the brain important for memory consolidation, such as the hippocampus and certain areas of the prefrontal cortex (McGaugh, 2004; Abercrombie et al., 2006; Roozendaal, et al., 2009; Sterpenich et al., 2009). It is important, therefore, for laboratory studies such as these to use experiences and stimuli that generate an arousal during encoding. The negative images used in this study have not only had their subjective arousal ratings previously verified (Kensinger et al., 2007a), but have also been shown to elicit greater physiological responses as measured by heart rate and skin conductance compared to the neutral images (Cunningham et al., 2014).

Our results suggest that experiencing a psychosocial stressor early in the consolidation phase leads to the greatest impact on the specific details the scenes, which relies heavily on hippocampal function. Gist memories, however, are thought to rely more on neocortical structures (Payne, Schacter, Propper, Huang, Wamsley, Tucker, Walker, & Stickgold, 2009; see Nadel & Moscovitch, 1997 for review). While the enhancement of emotional gist memory appeared less affected by the increase in cortisol, there did seem to be a general inhibition of gist memory for neutral content after stress, especially when limited to subjects that mounted a significant HPA activation after the stressor.

Because sleep and stress have both shown to provide neurobiological milieus that are beneficial for emotional memory consolidation (e.g., Diekelmann & Born, 2010;
Payne et al., 2008; Payne et al., 2007; Buchanan & Lovallo, 2001), we suggest that the arousal associated with emotionally salient pictures at encoding interacts with elevated cortisol after stress to provide a “tag” on the emotional component of the scene. This “tag” then leads to optimal processing during subsequent consolidation. Critically, the cortisol-induced boost in activity led to an enhancement of this effect, even greater than after a night of sleep alone, which has previously been shown to already boost this emotional trade-off effect (Payne et al., 2008). This was made evident by comparison of the stress group, who experienced a stressor and then were allowed to sleep, to individuals in the control condition, who were also allowed to sleep but did not experience additional stress during consolidation. The concurrent reduction in consolidation of the associated untagged, neutral information (i.e., the neutral backgrounds) could possibly result from a lack of attention during the encoding as the emotional item is being tagged, or a lack of consolidation processing into long-term memory as the memory system recognizes it as less important (i.e., untagged). Further research will be necessary to further elucidate the mechanisms behind this effect. Real world examples of this stress-enhanced tagging of emotional information for preferential processing are abundant and often necessary for survival. For instance, it is adaptive to remember the poisonous snake that tried to attack you, regardless of where it may next appear.

The current study successfully isolated the influence of HPA axis activity on the consolidation phase of memory formation. Despite the profound effects that stress prior to sleep had on memory performance, however, there were no measurable effects of
stress directly on sleep, adding no further clarity to the relationship between these two processes. Interestingly, the increased magnitude of the emotional memory trade-off described above is very similar to what has been shown previously after a sleep-filled consolidation period compared to an equivalent period of wakefulness. As noted previously, Bennion et al. (2013) found that higher baseline cortisol concentrations at encoding correlated with memory for negatively arousing objects in the trade-off paradigm, but only after a delay including sleep. They also theorized that this elevation in cortisol enables better tagging of the emotional information, leading to its preferential consolidation. This raises the question of whether arousal and cortisol concentrations might have been an unknown factor in previous studies that showed a benefit of sleep for emotional memory (e.g., Hu et al., 2006; Payne et al., 2008). It is important to note, however, that many of these studies compare performance of a daytime wakefulness group to a nocturnal sleep group, and cortisol concentrations are necessarily higher at encoding for the wake group due to its natural diurnal rhythm. Clearly then, cortisol concentration at encoding and early phases of consolidation is not enough to drive this effect alone, and some sort of partnership must exist with processes going on during sleep.

An additional consideration also must be made for the correlations of HPA activity and subjective anxiety with the pattern of emotional memory consolidation. For both measures, only post-task measures correlated with the magnitude of the emotional memory trade-off. There were no memory correlations with measures of the change in HPA reactivity or anxiety (e.g., AUCi), which would suggest a direct association
of stress responsivity with memory changes and eliminate the potential confound of trait features within the participants. Further exploration needs to be done to dissociate between these state and trait factors and how each may influence sleep and memory.

As noted in the introduction, a secondary goal of our study was to begin to pilot the same experimental design with individuals reporting higher levels of depression symptoms (mild-moderate), as depression offers a unique condition in which sleep, stress, and emotional memory consolidation are all dramatically altered. Thus far recruitment of this population has been slow with only 4 participants meeting criteria fully completing the study, but an interesting and unique pattern of results has already begun to develop. Previous research would suggest that depressed individuals tend to focus more on negatively arousing information and thus may show an increase in negative memory bias for the emotional components of the scenes leading to an exacerbation of the emotional memory trade-off. So far, however, the opposite pattern has emerged. Contrary to our predictions, the magnitude of the emotional memory trade-off seems to be reduced for both specific and gist memory, regardless of the conditions. While speculative, this pattern of results may allude to a generalizing effect in depression. Previous evidence has indicated that depressed individuals have increased veridical memory for negative information (Watkins, 2002; Pyszczynski et al., 1989; Liu et al., 2012). The memory paradigm used in this study is unique, however, in that it examines how different aspects of emotional memory are consolidated and retained over time. Depressed individuals may generalize the negative salience to the entire scene, potentiating memory for the backgrounds associated with negative scenes
along with the negative objects. In this same way other depressive symptoms, such as an overgeneralization of negative life events, may manifest in a similar fashion. Given that the variance is relatively small in the stress group despite the small number of subjects, further exploration and scrutiny into this subgroup merited.

4.2 Conclusion

This is the first to demonstrate that the manipulation of endogenous cortisol following the encoding of negatively arousing stimuli enhances their consolidation at the cost of memory for neutral, peripheral details. This stress effect leads to an increase in the disparity of memory for the central negative object compared to its peripheral, neutral background. Additionally subjective measures of anxiety and objective measures of HPA activity immediately after the stressor predict the magnitude of this emotional memory trade-off on a subsequent recognition task after sleep. The interaction of emotional arousal with HPA activity influenced the magnitude of the emotional memory trade-off effect such that the preferential consolidation of negative information was increased compared to the control condition, despite everyone receiving a night of sleep. Interestingly no measures of emotional memory or stress reactivity were related to sleep architecture during the consolidation period, however further analyses are necessary to determine if other measures of sleep, such as REM density or sleep spindles, may be affected by stress and play a role in the enhancement of emotional memory consolidation. This intersection of sleep, stress and emotional memory has important clinical implications as a great deal of psychopathology, such as Depression
and PTSD, are characterized by dysfunction in all three domains. For instance, previous research has reported significantly higher baseline cortisol concentrations in melancholic depressives compared to healthy controls, supporting the theory of a hyperactive HPA axis in depressed patients (Scott et al., 1998; Young, et al., 2000; 2004; see Abercrombie, 2009 for review). This increase in basal cortisol is paired with a decrease in REM latency and an increase in REM density (Riemann et al., 2001; Zarcone & Benson, 1982), as well as a bias in veridical memory for negative information (Liu et al., 2012; Pyszczynski et al., 1989; Watkins, 2002). Pilot results with participants reporting higher depressive symptoms are already showing a unique pattern of results in which negativity may be generalized to the entire emotional, complex scene.

Dysregulated HPA activity could set the stage for the over-consolidation of negatively arousing events during sleep, leading to increased recall for negative information. In a naturalistic setting, this over-consolidation could lead to rumination and skew their memory for recent life events to be more negative than they truly were. The long-term negative effects of dysregulation in this system may have substantial deleterious effects on an individual’s quality of life, especially in light of research indicating that measures of sleep disturbance can outperform depression and hopelessness scores as predictors of suicidal ideation and behavior (Ribeiro, J, Pease, J, Gutierrez, P., Silva, C., Bernert, R., Rudd, M, and Joiner, T, 2012). Thus, our finding that stress during consolidation affects emotional memory outcomes provides an important first step in understanding the influence of stress on sleep and the evolution of emotional memories. Further research
will be necessary to fully grasp these complex relationships and how they may be related to cognitive function and mental health.
Figure A.1 Diagram of the study procedure. Saliva samples 2-6 and 8-11 were taken in 15 minute intervals.
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