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Setting us straight! The Role of Sleep in Modulating Emotional Reactivity.

by

Els van der Helm

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Matthew Walker, Chair

Professor Dacher Keltner

Professor Ron Dahl

Dr. Matthew Brett

Fall 2013

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Els van der Helm

Abstract

Setting us straight! The Role of Sleep in Modulating Emotional Reactivity.

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Doctor of Philosophy in Psychology

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Folk wisdom has long implied a critical role for sleep in emotional processing, highlighted by sayings as 'Sleep on it and you'll feel better in the morning'. However, until very recently there was a lack of objective research investigating this claim. Now rapidly emerging evidence describes a powerful and causal relationship between sleep and affective brain regulation. These findings are mirrored by long-standing clinical observations demonstrating that nearly all mood and anxiety disorders co-occur with one or more abnormalities of sleep. This dissertation aims to (1) set forth a rapid-eye movement (REM) sleep hypothesis of affective brain homeostasis, optimally preparing the organism for next-day social and emotional functioning, (2) provide behavioral evidence of detrimental impairments in emotional functioning following sleep deprivation, and (3) outline affective brain and behavioral benefits of sleep when it is obtained. Finally, the discussion will describe how this model may explain the prevalent relationships observed between sleep and affective disorders, including relevant treatment mechanisms, with a particular focus on post-traumatic stress disorder (PTSD).

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Before I arrived at Berkeley there were previous mentors who helped me develop my early scientific career and the following three people have always supported me: Thank you Ysbrand van der Werf, Eus van Someren and Bob Stickgold.

During my time at UC Berkeley I realized I wanted to continue my career outside of academia and I would like to thank both Rich Ivry and Iris Mauss for their support, it was great to have people within academia who are just as open minded as I am about the career options for PhDs.

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Dedication

To my partner, family and friends for all of their love and support.

In particular my grandparents, Pake, Beppe, Opa, Oma & Mieke and parents Luit & Nynke for their encouragement and support of their (grand) children to pursue higher education.

Chapter 1. Introduction

1.1 Background

Folk wisdom has long implied a critical role for sleep in emotional processing with sayings as 'Sleep on it and you'll feel better in the morning'. However, until recently there was a lack of objective research investigating this claim. This thesis will not only put forth a theory on the function of dream sleep in emotional processing, it will also test this theory in a novel way by utilizing different study designs and combining several behavioral and neuroimaging techniques.

To put into perspective the studies and results of the current dissertation, I would like to start with discussing a number of critical factors that come into play when considering the relationship between sleep and emotion. This will hopefully highlight both the wide array of possibilities, as well as limitations in sleep and emotion studies. This discussion will then be followed by a description of the studies carried out in this dissertation.

In order to examine the relationship between sleep and emotion there are a number of key components to consider. Firstly, study design. A research study can be designed in one of several different ways. One method is to study the effects of the absence of sleep, by decreasing sleep or remove it altogether (sleep deprivation) and examine subsequent effects on emotional functioning. A second method to investigate the relationship between sleep and emotion, is to compare the effects of a night of sleep when it is obtained, for example by comparing it with the effects of a day of wake. A full night of sleep deprivation is often a powerful tool to observe effects on emotional or cognitive functioning, although it is a somewhat uncommon phenomenon in daily life. Therefore, the latter study design is a well-suited complement, mirroring a more natural sleep-wake cycle, which can shed light on what function sleep might have in a more naturalistic setting.

In addition to study design, another critical feature of sleep studies is the way sleep is measured and analyzed. For instance, sleep can be measured with sleep diaries (self-report), actiwatches that measure activity and light levels, or more invasive measures such as electroencephalography (EEG). Furthermore, depending on the type of measurement, sleep can subsequently be analyzed in a number of ways, e.g: timing of sleep, total duration, duration of specific sleep stages, number of sleep wave forms such as spindles or slow waves, or spectral analysis of waveforms occurring during specific sleep stages.

Finally, there are several methods to measure emotion. Emotion can be measured at a behavioral level using subjective measures such as questionnaires that depend on self-reported emotional state. Other paradigms focusing on behavioral measures are tasks that require participants to experience emotional stimuli such as pictures or video clips and/or respond to such stimuli. Emotional processing can additionally be measured with neuroimaging tools, such as EEG or functional magnetic resonance imaging (fMRI). These tools allow for insight into brain areas and mechanisms that underlie emotional behavior.

This is a limited list of critical factors, but it underscores the complexity and wide variety of sleep and emotion studies. As with any field of research, combining a wide array of study designs and methods allows for insight into mechanisms at a more detailed level. Unfortunately, many studies to date on sleep and emotion relied solely on self-reported changes in emotional

state(Dinges, et al., 1997; Zohar, Tzischinsky, Epstein, & Lavie, 2005). This is pertinent not only due to the limited scope of the study designs and methods, but also due to the known effects of sleep deprivation on (non-emotional) cognitive functioning(Durmer & Dinges, 2005; Ellenbogen, Hu, Payne, Titone, & Walker, 2007; Mander, Santhanam, Saletin, & Walker, 2011b; Stickgold & Walker, 2007; Yoo, Hu, Gujar, Jolesz, & Walker, 2007). Such effects on cognitive ability could influence the ability to self-monitor and interpret ones own emotional state.

Few studies to date have investigated sleep and emotion using a multi-modal approach of combining neuroimaging methods such as EEG and fMRI with behavioral tasks and subjective (self-report) measures.

1.2 Overview of Included Reviews and Studies

This dissertation has 3 specific aims.

> ***Aim 1***: The first aim is to set forth a rapid-eye movement (REM) sleep hypothesis of affective brain homeostasis, optimally preparing the organism for next-day social and emotional functioning. Two reviews by the author will form the basis for this discussion in Chapter 2.
>
> ***Aim 2***: The second aim is to provide behavioral evidence of the detrimental *impairments* in emotional functioning following sleep deprivation using an objective task (i.e. not dependent on any form of self-report). One behavioral study will form the basis for this discussion in Chapter 3.
>
> ***Aim 3***: The third aim is to outline affective brain and behavioral *benefits* of sleep when it is obtained from neuroimaging studies. This discussion is based on both an event-related fMRI study (Chapter 4) as well as a resting state fMRI study (Chapter 5).

Finally, in Chapter 6 the model and presented results will be tied together and I will describe how they may explain the prevalent relationships observed between sleep and affective disorders, including relevant treatment mechanisms, with a particular focus on post-traumatic stress disorder (PTSD).

Chapter 2. A model on the function of sleep

2.1 Overview

Cognitive neuroscience continues to build meaningful connections between affective behavior and human brain function. Within the biological sciences, a similar renaissance has taken place, focusing on the role of sleep in various neurocognitive processes, and most recently, the interaction between sleep and emotional regulation. In this chapter, I will survey an array of diverse findings across basic and clinical research domains, resulting in a convergent view of sleep-dependent emotional brain processing. Based on the unique neurobiology of sleep, I outline a REM-sleep model describing the overnight modulation of affective neural systems and the (re)processing of recent emotional experiences, both of which appear to redress the appropriate next-day reactivity of limbic and associated autonomic networks.

2.2 Background

The ability of the human brain to generate, regulate and be guided by emotions represents a fundamental process governing our personal lives, our mental health as well as our societal structure. Advances in cognitive neuroscience over the past two decades have provided important systems-level accounts of the mechanisms underlying affective brain processes (Critchley, 2005; Delgado, Olsson, & Phelps, 2006; Hartley & Phelps, 2010; Ochsner, et al., 2009), translationally bridging animal models of emotion regulation and relevant clinical disorders (Davidson, Pizzagalli, Nitschke, & Putnam, 2002; Delgado, et al., 2006; Drevets, Savitz, & Trimble, 2008; Etkin, 2010; Labar & Cabeza, 2006; Liberzon & Martis, 2006).

Independent of this research area, a recent resurgence has also taken place within the basic sciences, focusing on the functional impact of sleep on neurocognitive processes (Walker & Stickgold, 2006). However, surprisingly less research attention has been afforded to the interaction between sleep and emotional regulation or affective brain function. This is surprising considering the remarkable overlap between the known physiology of sleep, especially REM, and the associated neurochemistry and network anatomy that modulate emotions, as well as the prominent co-occurrence of abnormal sleep (including REM) in almost all affective psychiatric and mood disorders.

Here, I review evidence in humans suggesting an obligate symbiosis between sleep and affect; both maladaptive consequences caused by the absence of sleep and adaptive benefits following the presence of sleep, especially rapid eye movement (REM) sleep. From these findings, I set forth a neurobiological framework that may account for the observed interactions between sleep and affective brain function.

Although this review has a specific focus on human studies a number of animal studies similarly suggest an intimate link between fear conditioning, stress and REM-sleep in animals (DaSilva, et al., 2011; Liu, et al., 2011; Madan, et al., 2008; Sanford, Silvestri, Ross, & Morrison, 2001; Sanford, Tang, Ross, & Morrison, 2003; Sanford, Yang, & Tang, 2003; Sanford, Yang, Wellman, Liu, & Tang, 2010; Wellman, Yang, Tang, & Sanford, 2008; Yang, Wellman, Ambrozewicz, & Sanford, 2011). It should be noted that this focused review addresses the relationship between sleep and emotions. In this limited capacity, it does not consider the

nonetheless fascinating potential interaction between sleep and mood states, which I and others have considered different to emotions: emotions are short-lived events, often in response to external stimuli, while mood states are more sustained events, often internally generated (see for a review (Mendl, Burman, & Paul, 2010)). Although the relationship between REM-sleep and mood is an interesting topic as well, it is regarded to be outside of the scope of this review.

Neurobiology of the Sleeping Brain

Before considering the impact of sleep, and specifically REM sleep, on affective brain function, it is relevant to outline its neurobiological features that help explain a mechanistic link with emotional processing. Neuroimaging techniques have revealed significant elevations in activity in affect-related regions including the amygdala, hippocampus and 'extended limbic system' throughout the medial prefrontal cortex (mPFC) during REM sleep (Nofzinger, 2005). These dramatic changes in functional brain anatomy are paralleled by (and likely instigated by) equally profound alterations in neurochemistry (Kametani & Kawamura, 1990; Marrosu, et al., 1995).

Perhaps most remarkable is the dramatic reduction of noradrenergic activity during REM sleep, showing a marked drop during REM sleep (although not completely absent, as indicated by microdialysis studies (Ouyang, Hellman, Abel, & Thomas, 2004; Park, 2002; Shouse, Staba, Saquib, & Farber, 2000). As a consequence, REM sleep represents a brain state largely devoid of adrenergic neurochemistry, while cholinergic activity dominates (Kametani & Kawamura, 1990; Marrosu, et al., 1995). EEG waveforms during REM sleep are associated with oscillatory activity in the theta band range (4–7 Hz), together with higher frequency synchronous activity in the 30–80 Hz ("gamma") range (Cantero, et al., 2003; Llinas & Ribary, 1993; Steriade, Amzica, & Contreras, 1996). Despite power in the gamma range being the most dominant frequency band present during REM sleep, gamma power is significantly lower than it is during wakefulness (Scheffzuk, et al., 2011), likely due to the marked reduction in adrenergic input from the locus coeruleus during REM. These neurobiological features are relevant since many of the neuroanatomical and neurochemical systems altered during REM sleep overlap with the systems supporting waking brain mechanisms of emotion and memory (Dolcos, LaBar, & Cabeza, 2005). Additionally, they further overlap with systems that become disrupted following sleep loss.

Impact of sleep loss on emotional brain function

Emotional Reactivity

Together with impairments of attention, alertness and memory, sleep loss is commonly associated with subjective reports of irritability and affective volatility (Horne, 1985). For example, sleep restriction to only 5hr of sleep a night across a 1-week period leads to a progressive increase in mood disturbance (e.g. mental exhaustion and stress) in participants on the basis of questionnaire mood scales, together with diary documentation of increasing subjective emotional difficulties (Dinges, et al., 1997). Moreover, accumulated sleep loss in medical residents amplifies negative emotional consequences of disruptive daytime experiences while blunting the benefit associated with goal-enhancing activities (Zohar, et al., 2005).

Studies assessing physiological and neural measures have provided additional objective verification of emotional dysregulation following sleep deprivation, offering potential explanatory mechanisms of the aforementioned subjective disturbances. Using functional MRI (fMRI) it has been demonstrated that one night of sleep deprivation triggers a 60% amplification in reactivity of the amygdala to negative aversive picture images, relative to a normal night of sleep (**Figure 1a&b**) (Yoo, Gujar, Hu, Jolesz, & Walker, 2007a). Moreover, this increase in

amygdala activity following sleep loss was also associated with a significant reduction in functional connectivity of the amygdala with regions of the mPFC believed to exert top-down regulatory control of the amygdala (**Figure 1c&d**). In contrast, significantly greater amygdala connectivity was observed with a classical fight/flight adrenergic-activating brainstem center of the locus coeruleus under conditions of sleep loss. Congruent evidence has further demonstrated that sleep deprivation results in similar enhanced amygdala reactivity and reduction of connectivity with prefrontal regions during a working memory task that involves emotional distractors (Chuah, et al., 2010) and excessive pupil diameter responses (an index of autonomic reactivity) during the passive viewing of negative picture stimuli (Franzen & Buysse, 2008).

Importantly, sleep deprivation is not only associated with enhanced reactivity towards negative stimuli. Growing evidence suggests that sleep loss imposes a bi-directional nature of affective imbalance, also triggering amplified reactivity to positive, reward-relevant picture stimuli. For example, sleep deprivation significantly enhances responsivity throughout regions of the dopaminergic mesolimbic systems in response to pleasure-evoking emotional picture stimuli (Gujar, Yoo, Hu, & Walker, 2011). As with negative emotion reactivity, this enhanced mesolimbic sensitivity to rewarding emotional items was also associated with decreased functional connectivity in regions of the medial and orbital prefrontal cortex. Similar enhanced mesolimbic reactivity following sleep deprivation has also been reported using basic monetary reward incentive paradigms (Libedinsky, et al., 2011; McKenna, Dickinson, Orff, & Drummond, 2007b; Venkatraman, Chuah, Huettel, & Chee, 2007; Venkatraman, Huettel, Chuah, Payne, & Chee, 2011).

These findings collectively support a framework whereby sleep deprivation exaggerates subcortical limbic and striatal reactivity not only to negative but also to positive affective stimuli, both of which are associated with impoverished prefrontal cortex connectivity. The consequence appears to be a pendulum like, bi-directional reactivity of the brain to both ends of the emotional valence spectrum. Such a model is of clinical relevance for at least two areas of mental health. First, parallel findings of anatomical dysfunction, characterized by altered activity in limbic areas and limbic-prefrontal cortex connectivity, have been reported in a number of psychiatric mood and anxiety disorders that express co-occurring sleep abnormalities, including major depression, bipolar disorder and PTSD abnormalities (Davidson, 2002; Davidson, et al., 2002; Drevets, et al., 2008; Etkin, 2010; New, et al., 2007; Pezawas, et al., 2005; Rauch, et al., 2000; Rich, et al., 2006; Shin, Rauch, & Pitman, 2006; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007; Surguladze, et al., 2005). These commonalities directly raise the issue of whether sleep loss plays a causal role in the etiology of these conditions. Second, considering the known disruption of sleep in a number of addiction disorders (Arnedt, Conroy, & Brower, 2007; Brower & Perron, 2010; Ciraulo, Piechniczek-Buczek, & Iscan, 2003; Dimsdale, Norman, DeJardin, & Wallace, 2007; Pace-Schott, et al., 2005), this evidence may implicate sleep loss as a predisposing risk factor and therapeutic target in addiction vulnerability to reward-stimulating drugs. They may further intimate a potential role for sleep disruption in the maintenance of addiction habits, especially during attempted withdrawal.

Emotion recognition and expression

In contrast to the signature of amplified neural reactivity in response to emotional stimuli under conditions of sleep loss, a number of studies have reported what appears to be a paradoxical blunting, rather than over-estimation, in the subjective recognition and rating by sleep deprived participants in response to the expression of emotion by others. For example, sleep loss impairs

the degree of perceived emotion felt by deprived participants in response to emotional film clips (Minkel, Htaik, Banks, & Dinges, 2011). Intriguingly, sleep loss also decreased the degree of outward observable emotional expressiveness of the sleep deprived individual themselves. Similarly, a decrease in the vocal expression of positive emotion by deprived participants has been reported after a single night of sleep loss, suggesting multiple routes of emotional expression (facial muscles, vocalization) are compromised by sleep loss (McGlinchey, et al., 2011). Of concern, insufficient sleep appears to trigger as much, if not more, of an impact on emotional expression in young children. Recent evidence demonstrates that three-year-olds who do not obtain an afternoon nap show dysregulation of both positive and negative emotion expression in response to emotional stimuli as well as puzzle solving challenges, relative to those who have obtained a nap (Vandekerckhove, et al., 2011).

Such impairments in self-expression of emotions appear to be at odds with prior evidence describing amplified (rather than impaired) limbic reactivity following sleep deprivation. However, this disparity may be reconciled when considering the concomitant neural impairments in the prefrontal cortex. Not only are prefrontal regions implicated in top-down regulatory control of subcortical limbic networks, they critically *integrate* primary affective signals into second-order maps of the internal state of the organism (Craig, 2010, 2011; Critchley, 2005, 2009; Harrison, Gray, Gianaros, & Critchley, 2010; Medford & Critchley, 2010). It has been argued that only through such mapping and hence appreciation of the current body state, can the brain select appropriate behavioral actions for the organism (actions that include emotion expression) (Craig, 2010, 2011; Critchley, 2005, 2009; Harrison, et al., 2010). Set against this evidence, the above disparate findings may be resolved, such that the sleep-deprived brain suffers a mismatch between excessive subcortical reactivity yet impaired higher-order prefrontal function, the latter preventing optimal use and control of the former. As a consequence, there is failure of affectively guided judgments, decisions and, down-stream, emotive (re)actions.

Benefits of Sleep on Emotional Brain Function

Dissipation of emotional reactivity

In contrast to affective dysregulation caused by the absence of sleep, beneficial influences upon emotional perception and regulation have been described following the presence of sleep, and REM sleep in particular. For instance, a daytime nap has been shown to dissipate the intensity ratings of threat-relevant negative emotional face expressions (Fear, Anger), yet increase responsivity towards positive (happy) facial images (Gujar, McDonald, Nishida, & Walker, 2010). Interestingly, however, not all participants who slept demonstrated this resetting of emotional reactivity. Instead, only those participants in the nap group who obtained REM sleep during the nap displayed this resetting of affective reactivity. Also fitting with a sleep-dependent emotion depotentiation model, if participants are deprived of sleep the first night after being exposed to emotional picture stimuli (then given recovery sleep), upon subsequent re-exposure to these same emotional stimuli, no palliative dissipation of amygdala reactivity is observed . These studies support a model of sleep-dependent emotional depotentiation, demonstrating that the presence of sleep provides a neural dissipation of limbic reactivity to prior emotional memories, while sleep loss results in the persistence of such reactivity, even after several nights of recovery sleep.

Emotional Memory Consolidation

Beyond basic processes of affective reactivity and expression, sleep has additionally been

demonstrated to play an influential role in emotional memory modulation (for a review see (Payne & Kensinger, 2010; Walker, 2009; Walker & van der Helm, 2009b)). Most notable is a role for sleep in the "offline" consolidation of salient emotional experiences, including memory for individual words and pictures, selective consolidation of emotional elements within visual pictures, as well as the generalization of fear extinction (Kleinsmith & Kaplan, 1963; Levonian, 1972; Pace-Schott, et al., 2009; Payne, Stickgold, Swanberg, & Kensinger, 2008; Spoormaker, Andrade, et al., 2011; Wagner, Hallschmid, Rasch, & Born, 2006b; Walker & Tarte, 1963).

REM sleep, in particular, may be especially critical for emotional memory processing. In the case of basic aversive learning, pre-sleep fear conditioning responses, indexed by skin conductance and brainstem reactivity, proportionally decrease the probability of REM sleep occurring during a subsequent nap. This may indicate that excessive fear responses during wake can lead to subsequent disturbances of REM sleep, and with it, the functional affective benefits REM sleep provides (**Figure 2a&b**). Moreover, those who were able to obtain REM sleep after fear conditioning demonstrated a superior degree of ventromedial prefrontal cortex (vmPFC) activity during post-sleep fear extinction (**Figure 2c**), consistent with a reestablishment of prefrontal cortex emotional regulatory control permitted by REM, and without any expression of amplification of amygdala reactivity (Spoormaker, et al., 2010).

Beyond fear conditioning, emotional fact-based or "episodic" memory similarly demonstrates sensitivity to REM sleep. Early work reported that the overnight retention of emotional details relative to neutral details of a narrative story was superior following late-night sleep (a time period rich in REM sleep) (Wagner, Gais, & Born, 2001). It has subsequently been demonstrated that the speed of recognizing emotional face expressions presented prior to sleep is significantly improved the next day, the amount of which positively correlated with the amount of intervening REM sleep (Wagner, Kashyap, Diekelmann, & Born, 2007). Moreover, not only does the amount of time and speed of entry into REM sleep predict the degree of subsequent strengthening and hence offline consolidation of emotional (and not neutral) memory (**Figure 3a&b**), but it is specifically the amount of EEG theta activity (4 – 7 Hz) – a dominant electrical oscillation of REM sleep expressed over the prefrontal cortex – that predicts memory retention (**Figure 3c&d**; (Nishida, Pearsall, Buckner, & Walker, 2009). These findings have lead to the proposal that REM sleep represents a neurobiological brain-state particularly amenable to emotional memory processing (Hu, Stylos-Allen, & Walker, 2006; Pare, Collins, & Pelletier, 2002; Walker, 2009; Walker & van der Helm, 2009b), with theta oscillations proposed as a carrier frequency that potentially allows disparate brain regions that initially encode information to selectively interact offline. By doing so, REM sleep theta may afford the ability to strengthen distributed aspects of specific emotional memory representations across related but different anatomical networks, and/or promote their integration into pre-existing autobiographical memory networks (Cahill, 2000; Jones & Wilson, 2005). However, strengthening of the experience of an emotional event may only be one of two specific functions that REM sleep provides emotional memories, as we next describe.

REM Sleep Homeostasis of Affective Brain Function: A Hypothesis

Although there is a wealth of evidence to suggest that emotional experiences persist in our autobiographies over time (strengthening of the memory) (Dolcos, et al., 2005), an equally remarkable but less noted change is a reduction in the affective tone associated with their recall (depotentiation of emotion). The reason that affective experiences appear to be remembered more robustly than neutral memories is due to well characterized autonomic neurochemical

reactions elicited at the time of the experience (McGaugh, 2004). These neurochemical reactions are believed to adaptively prioritize the formation (and hence long-term retention) of salient information, creating what is commonly termed an "emotional-memory" (**Figure 4a**). However, the later recall of these memories tends not to be associated with anywhere near the same magnitude of autonomic (re)activation as that elicited at the moment of experience – suggesting that, overtime, the affective "blanket" (the emotion) that originally tagged the memory at the time of learning has been removed, whereas the information of the experience (the memory) persists (**Figure 4b**).

I offer the hypothesis that such decoupling of emotion from memory preferentially takes place overnight, during the unique neurobiological state of REM, such that we *sleep to forget* the emotional tone, yet *sleep to remember* the tagged memory of that experience. This model further posits that if such a process is not achieved, the magnitude of affective "charge" would persist, resulting in the potential condition of chronic anxiety within autobiographical memory networks.

I suggest that the state of REM sleep provides an optimal biological milieu within which this form of "overnight therapy" can be achieved, based on three associated features: neuroanatomical, neurophysiological and neurochemical (**Figure 4a**). First, the prominent increase in activity within limbic and paralimbic structures during REM sleep (Nofzinger, 2005) supports the ability for reactivation and hence (re)processing of previously acquired affective memories. Second, the neurophysiological signature of REM sleep involving dominant theta oscillations within subcortical as well as cortical nodes offers large-scale network cooperation during REM for the strengthen of distributed aspects of the emotional memory representation (e.g. perceptual, contextual), across such related but different anatomical networks, resulting in enhanced consolidation and integration of that memory. Third, these interactions during REM sleep (and perhaps through the conscious process of dreaming) critically and perhaps most importantly take place within a brain that is low in aminergic neurochemical concentration (Pace-Schott & Hobson, 2002), particularly noradrenergic input from the locus coeruleus (associated with stress and anxiety responses) and dominated by cholinergic neurochemistry (Itoi & Sugimoto, 2010; Ramos & Arnsten, 2007; Sullivan, Coplan, Kent, & Gorman, 1999; Valentino & Van Bockstaele, 2008). Therefore, REM sleep is proposed to offer a unique biological condition in which to achieve, on one hand, a strengthening and consolidation of the informational core of emotional experiences (the memory), yet additionally depotentiate and ultimately ameliorate the autonomic arousing charge originally acquired at the time of learning (the emotion). Through the process of developing stronger cortico-cortical connections (connections between cortical areas), integration and assimilation of the affective event(s) in the context of past knowledge is supported. As a result, emotional experiences are preferentially retained long-term, but importantly the emotion, which was initially critical to signify salience and priority at the time of learning, has been dissipated. The brain therefore preserves a memory of an emotional event, but which itself is no longer emotional.

This model complements pioneering psychological theories of dreaming by Greenberg and colleagues (Greenberg, Pearlman, & Gampel, 1972; Greenberg, Pillard, & Pearlman, 1972) as well as Cartwright and associates (Cartwright, Agargun, Kirkby, & Friedman, 2006; Cartwright, Luten, Young, Mercer, & Bears, 1998; Cartwright, Kravitz, Eastman, & Wood, 1991), which suggest that the process of REM sleep mental activity aids in the resolution of previous emotional conflict, resulting in reduced next-day negative affect. In fact, the strong emotional tone of mental activity that occurs during sleep (often referred to as *dream mentation*; (Hobson, Pace-Schott, & Stickgold, 2000)) has long encouraged speculation of sleep-dependent

affective processing (for reviews, see (Levin & Nielsen, 2009; Nielsen & Levin, 2007; Stickgold, 2002)).

Specific predictions emerge from this model. As partially demonstrated, one prediction would be that the degree to which the information of those emotional experiences are retained, long-term, would be proportional to the amount of post-encoding REM sleep obtained, how quickly it is achieved (REM latency), as well as the power of theta oscillations during REM. Evidence for all these predictions exists (Nishida, et al., 2009; Pare, et al., 2002). Also, the inverse REM-relationship would hold for the magnitude of emotional depotentiation after sleep. This prediction is explicitly tested in the study discussed in Chapter 4. The implications of this model for psychiatric conditions will be described further in Chapter 6.

Figures

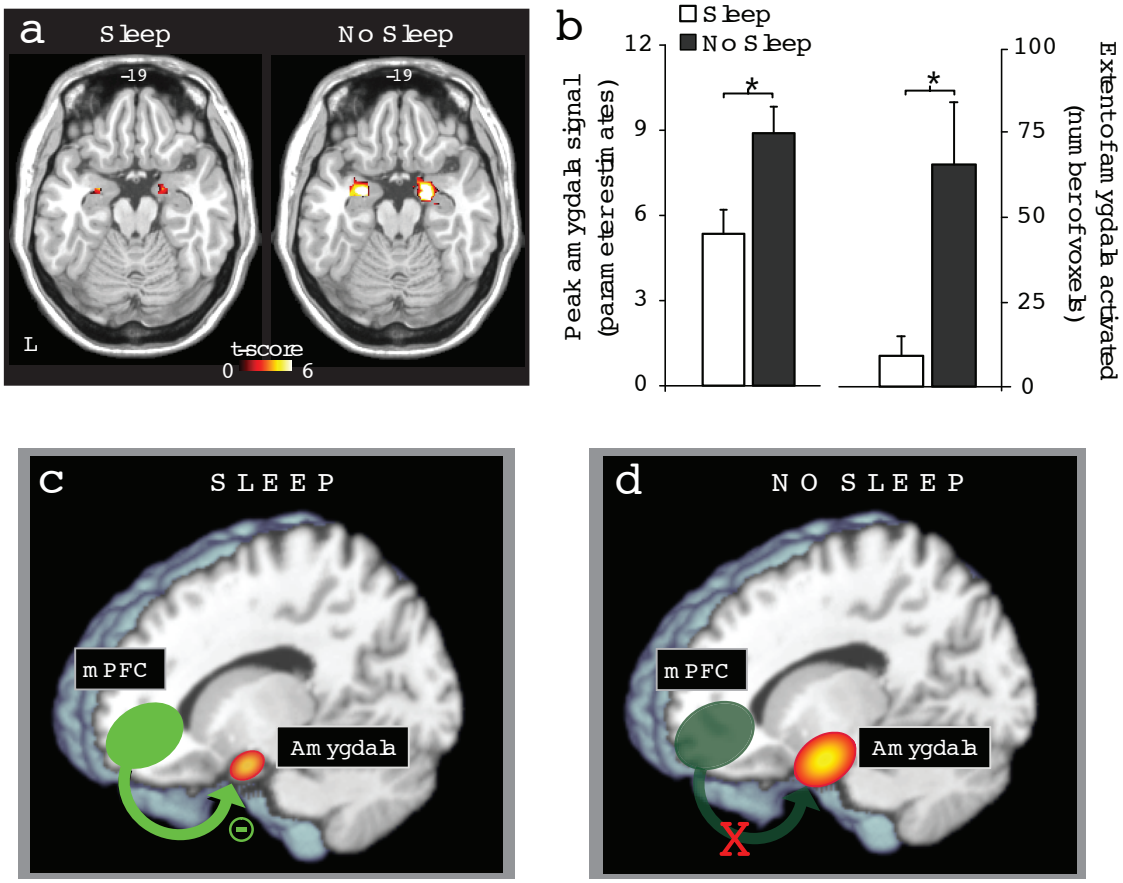

Figure 1. The impact of sleep deprivation on emotional brain reactivity and functional connectivity. a) Amygdala response to increasingly negative emotional stimuli in the Sleep deprivation and Sleep control groups, and b) Corresponding differences in intensity and volumetric extent of amygdala activation between the two groups (average ± s.e.m. of left and right amygdala), c) Depiction of associated changes in functional connectivity between the medial prefrontal cortex (mPFC) and the amygdala. With sleep, the prefrontal lobe was strongly connected to the amygdala, regulating and exerting and inhibitory top-down control, d) Without sleep, however, amygdala-mPFC connectivity was decreased, potentially negating top-down control and resulting in an overactive amygdala. *$p < .01$; error bars indicate S.E.M. (Modified from (Yoo, Gujar, Hu, Jolesz, & Walker, 2007b).

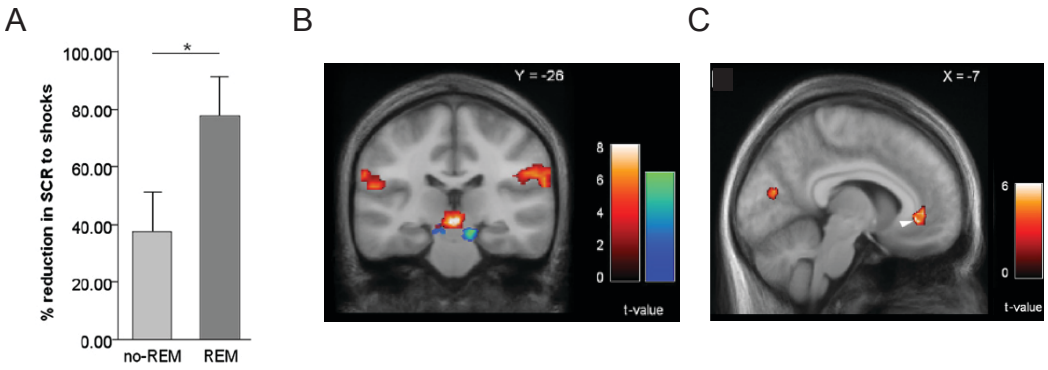

Figure 2. Fear conditioning and extinction related to REM-sleep. (a) Significantly greater decreases in skin conductance response across the fear conditioning trials prior to an afternoon nap in those participants obtaining REM-sleep relative to the participants who did not enter REM-sleep. Error bars represent S.E.M. (b) fMRI response to mild electrical shocks applied during the conditioning session. Hot colors depict areas of significant activation in response to shock stimuli versus safety, including regions of brainstem, thalamus and bilateral insula, while cool colors depict regions that declined more (linearly) across the conditioning trails in the REM group than in the no-REM group, also including the brainstem. (c) Post-sleep changes in brain fMRI activation during extinction, demonstrating greater ventromedial prefrontal cortex (vmPFC) activity in those who obtained prior REM sleep relative to those who did not, p cluster <0.05. Modified from (Spoormaker, et al., 2010).

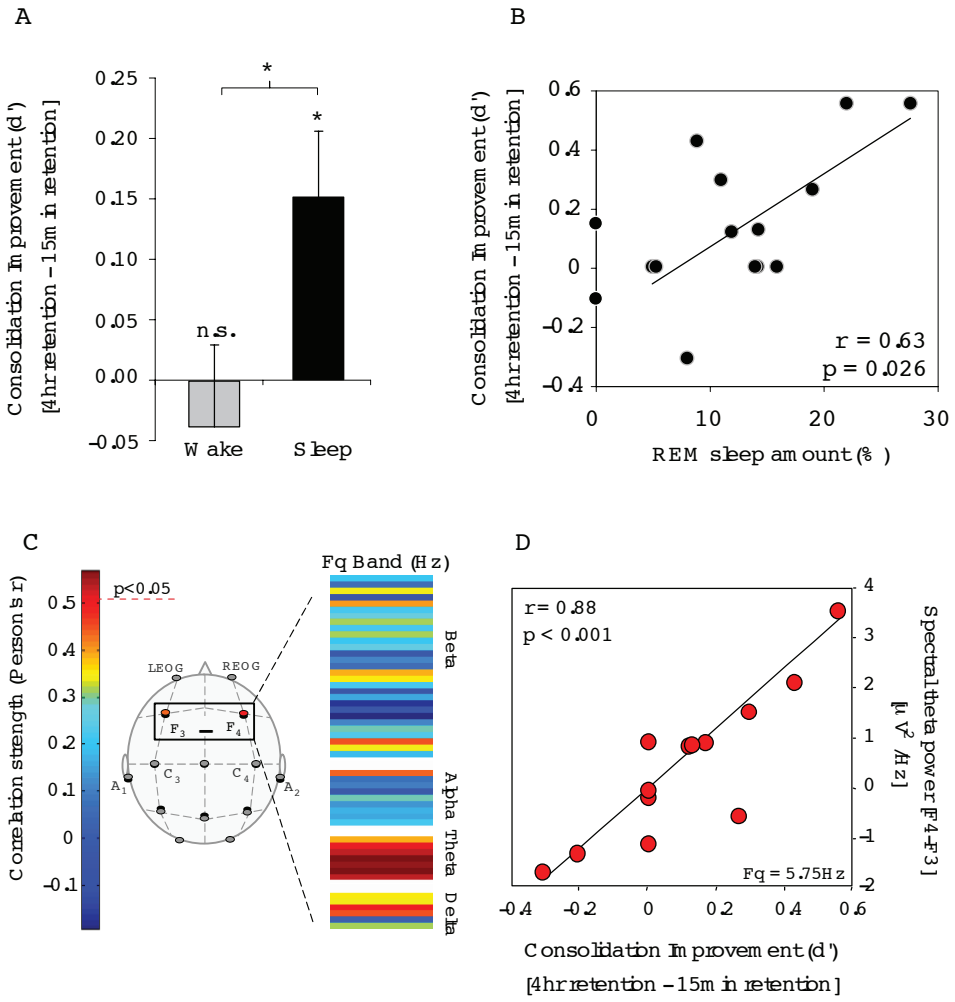

Figure 3. REM-sleep enhancement of negative emotional memories. A) Offline benefit (change in memory recall for 4 hr versus 15 min old memories) across the day (wake, grey bar) or following a 90 min nap (sleep, filled bar); B) Correlation between the amount of offline emotional memory improvement in the nap group (i.e. the offline benefit expressed in filled bar of figure a), and the amount of REM-sleep obtained within the nap; C) Correlation strength (Pearson's *r*-value) between offline benefit for emotional memory in the sleep group (the benefit expressed in filled bar of figure A) and the relative right versus left prefrontal spectral-band power ([electrode F4 – electrode F3]) within the delta, alpha, theta and beta spectral bands, expressed in average 0.5 Hz bin increments. Correlation strength is represented by the color range, demonstrating significant correlations within the theta frequency band (hot colors), and D) exhibiting a maximum significance at the 5.75 Hz bin. *$p < .05$; error bars indicate s.e.m. Modified from (Nishida, et al., 2009).

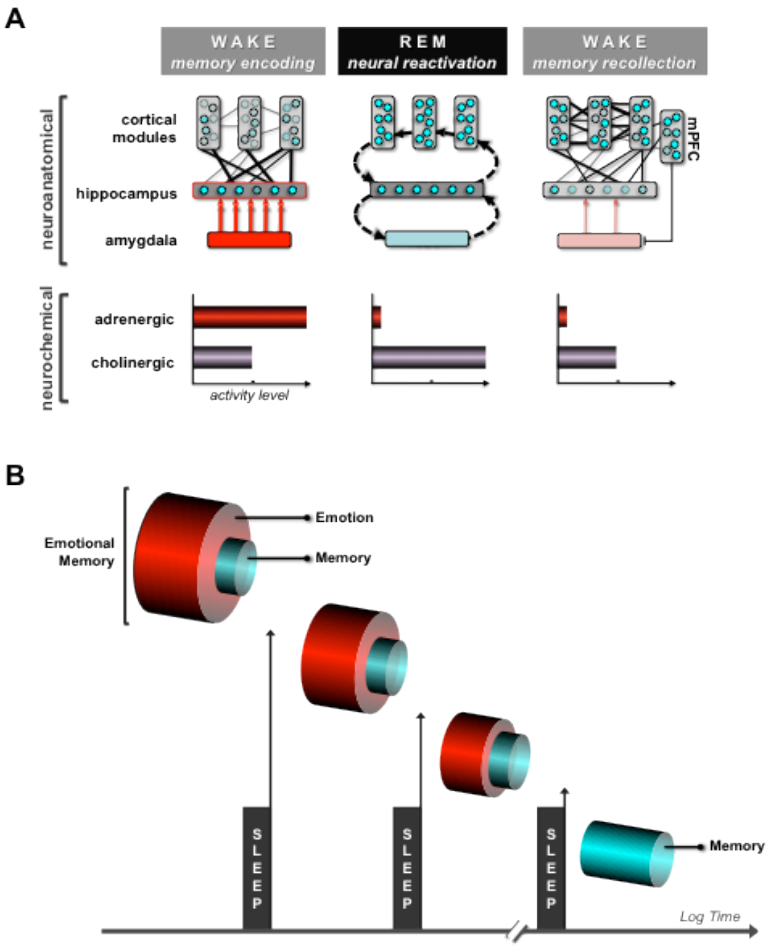

Figure 4. The sleep to *forget* and sleep to *remember* (SFSR) model of emotional memory processing: A) Neural dynamics. The formation of an episodic emotional memory during wake involves the coordinated encoding of hippocampal-bound information within cortical modules, facilitated by the amygdala, and modulated by high concentrations of aminergic neurochemistry. During subsequent REM-sleep, these same neural structures are reactivated, the coordination of which is made possible by synchronous theta oscillations throughout these networks, supporting the ability to reactivate previously encoded emotional experiences. However, this reactivation occurs in a neurochemical milieu devoid of aminergic modulation, and dominated by cholinergic neurochemistry. As a consequence, emotional memory reactivation can achieve, on the one hand, a depotentiation of the affective tone initially associated with the event(s) at encoding, while on the other, a simultaneous and progressive neocortical consolidation of the information. The latter process of developing stronger cortico-cortical connections additionally supports integration into previously acquired autobiographical memories, further aiding the assimilation of the affective event(s) in the context of past knowledge, the conscious expression of which may contribute to the experience of dreaming. The cross-connectivity between structures is represented by number and thickness of lines. The circles within cortical and hippocampal structures represent information nodes; shade reflects extent of connectivity: strong (filled), moderate (grey) and weak (clear). Color fill of amygdala and arrow thickness represents magnitude of co-activation with and influence on the hippocampus. B) Conceptual outcome.

Through multiple repetitions of this REM-sleep mechanism across the night, and/or across multiple nights, the long-term consequence of such sleep-dependent reprocessing would allow for the strengthening and retention of salient information previously tagged as emotional at the time of learning. However, recall no longer activates an affective, aminergic charge, allowing for post-sleep recollection with minimal autonomic reactivity (unlike encoding), thereby preventing a state of chronic anxiety.

Chapter 3. Sleep deprivation impairs the accurate recognition of human emotional expressions

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3.1 Overview

Although a growing literature on the effects of sleep deprivation on cognitive brain functioning exists, there is a lack of research on emotional processing following sleep deprivation. This is surprising considering the commonly reported subjective complaints in affective functioning such as irritability and behavioral volatility. The aim of this study was to investigate the impact of sleep deprivation on the ability to recognize the intensity of human facial emotions. Thirty-seven healthy participants, (21 females) aged 18–25 y, were randomly assigned to the sleep control (SC: $n = 17$) or total sleep deprivation group (TSD: $n = 20$). Participants performed an emotional face recognition task, in which they evaluated 3 different affective face categories: Sad, Happy, and Angry, each ranging in a gradient from neutral to increasingly emotional. In the TSD group, the task was performed once under conditions of sleep deprivation, and twice under sleep-rested conditions following different durations of sleep recovery. In the SC group, the task was performed twice under sleep-rested conditions, controlling for repeatability. In the TSD group, when sleep-deprived, there was a marked and significant blunting in the recognition of Angry and Happy affective expressions in the moderate (but not extreme) emotional intensity range; differences that were most reliable and significant in female participants. No change in the recognition of Sad expressions was observed. These recognition deficits were, however, ameliorated following one night of recovery sleep. No changes in task performance were observed in the SC group. Therefore, sleep deprivation selectively impairs the accurate judgment of human facial emotions, especially threat relevant (Anger) and reward relevant (Happy) categories, an effect observed most significantly in females. Such findings suggest that sleep loss impairs discrete affective neural systems, disrupting the identification of salient affective social cues.

3.2 Background

The impact of sleep deprivation on cognitive brain function has received increasing research interest over the past half century, following the discovery of unique sleep stages(Aserinsky & Kleitman, 1953). This is perhaps not surprising considering the societal and medical ramifications of the deficits caused by sleep deprivation, which commonly include impaired sustained attention together with deficits in learning and memory(Drummond & Brown, 2001; Durmer & Dinges, 2005; Walker & Stickgold, 2006).

However, the impact of sleep deprivation on emotional processing has received considerably less research interest(Walker, 2009), despite common subjective complaints of associated irritability and reported behavioral volatility(Baldwin & Daugherty, 2004; Zohar, et al., 2005). This association is also pertinent considering that nearly all psychiatric and neurological mood disorders express co-occurring abnormalities of sleep, suggesting a potential intimate inter-dependence between sleep and affective brain function. Moreover, the implication

of such a relationship becomes particularly germane in the context of interpersonal as well as professional interactions that are predicated on the basis of emotional judgments and decisions(Adolphs, 2003), especially when considering the on-going erosion of obtained sleep time across many age ranges (National Sleep Foundation poll 2007: http://www.sleepfoundation.org/).

To our knowledge, there have been few empirical reports investigating the impact of sleep loss on tasks of emotion processing, with the majority of studies utilizing subjective rating scales. Using a sleep restriction paradigm in combination with questionnaire mood scales, Dinges et al.(Dinges, et al., 1997) reported a progressive increase in emotional disturbance across a seven-day period. In addition, subjective descriptions in participants' daily journals indicated increasing complaints of emotional difficulties. Zohar et al.(Zohar, et al., 2005) examined the effects of sleep disruption on subjective emotional reactions to daytime work events in working medical residents. Sleep loss was shown to increase dysphoria and amplify negative emotional consequences of disruptive daytime events while blunting the positive benefit associated with rewarding or goal-enhancing activities.

Using more objective tasks, several studies have now investigated the effects of sleep and sleep disruption on emotional processing. Pallesen and colleagues reported that the speed and accuracy for rating drawings of emotional facial expressions both deteriorate (slow and less accurate, respectively) following one night of sleep deprivation(Pallesen, et al., 2004). Complementing these findings, Wagner et al.(Wagner, et al., 2007) have demonstrated that the speed of recognizing emotional facial expressions presented prior to sleep significantly improves the next day; a learning benefit that was positively correlated with the amount of intervening REM sleep. Another study by Wagner et al.(Wagner, Fischer, & Born, 2002) examined the effect of early versus late night sleep on the ratings of negative emotional pictures. Following sleep (especially late night) valence (representing pleasant or unpleasant) ratings of previously seen negative pictures were found to increase. Furthermore, Lara-Carrasco et al.(Lara-Carrasco, Nielsen, Solomonova, Levrier, & Popova, 2009) have reported that late night REM sleep deprivation decreases participants' subjective reactivity to previously seen negative emotional pictures.

At a physiological level, using functional MRI (fMRI), Yoo et al.(Yoo, Gujar, et al., 2007b) have demonstrated that one night of sleep deprivation significantly amplifies the human amygdala reaction to increasingly negative and aversive picture stimuli (e.g. amputated body parts or weapons). Furthermore, this amygdala potentiation was associated with a loss of top-down medial prefrontal connectivity, yet an increased coupling with autonomic activating regions of the locus coeruleus. Most recently, Franzen et al. have demonstrated significantly larger pupillary responses to negative emotional pictures compared to positive or neutral stimuli in participants who were sleep deprived(Franzen, Buysse, Dahl, Thompson, & Siegle, 2009), further suggesting an autonomic physiology dysregulation caused by sleep deprivation.

While these studies collectively indicate that sleep loss disrupts aspects of emotional processing, it remains unclear how sleep deprivation impacts the recognition of unique and different types of emotions, which rely on dissociable neural systems(Vuilleumier & Pourtois, 2007). Furthermore, following sleep deprivation, it is unknown whether such impairments can be reversed by recovery sleep, and if so, how quickly. Using a face recognition task, here we examine the impact of one night of total sleep deprivation and subsequent sleep recovery on the ability to identify specific facial emotions.

3.3 Methods

Participants

Thirty-seven healthy participants (21 females) aged 18-25 (mean 19.7 [s.d. ± 0.91]) were randomly assigned to the sleep control (n = 17, 10 females) or sleep deprivation group (n = 20, 11 females). Participants abstained from caffeine and alcohol for the 72 hr prior to and during the study, and kept a normal sleep-wake rhythm with average sleep duration (7-9 hr per night, morning wake time between 06:00-09:00 hr) for the same amount of time. Compliance to this sleep schedule was verified by sleep logs. Exclusion criteria were a history of neurologic, psychiatric or sleep disorders, past history of drug abuse, and current use of anti-depressant or hypnotic medications.

Experimental design

The core experimental protocol is described in Fig.1A, with participants performing an emotional face recognition task (details below) once at 16:00 hr (±30min) on Day 2 and once at 16:00 hr (±30min) on Day 3. The experimental manipulation, differentiating the two conditions, occurred across the night before the first test session (Test1). Specifically, participants in the sleep control group were awake across Day 1 and slept normally at home across Night 1 (obtaining a mean of 7.9 hr [s.d. ± 1.6] based on sleep log diaries), before returning for the first test session on Day 2. Participants in the sleep deprivation condition were similarly awake across Day 1, but were subsequently kept awake in the sleep laboratory under full supervision across Night 1 and across Day 2, accumulating a mean of 30.9 hr (s.d. ± 1.4) of total sleep deprivation prior to Test1. During this time, subject activities were limited to internet, short walks, reading and playing board games, providing a standardized regiment of waking activity across the deprivation period.

Following Test1, both groups returned home to sleep the following night (Night 2), and repeated the same task a second time on Day 3 (Test2). This offered an evaluation of recovery sleep on task performance in the deprivation group, and an evaluation of test performance consistency and repeatability in the control group. Across this recovery night (Night 2), participants in the deprivation group obtained a mean of 13.5 hr (s.d. ± 3.2) sleep, while those in the control group obtained a mean of 8.1 hr (s.d. ± 1.3), based on sleep log diaries. Prior to each of these two tests, all participants completed the Stanford Sleepiness Scale; a standard measure of subjective alertness ranging across a 7-point scale (1 being most alert(Hoddes, Zarcone, Smythe, Philips, & Dement, 1973)). As expected, participants in the sleep deprivation group indicated significantly higher levels of sleepiness (lower scores) at Test1 (mean 5.4 s.d. ± 0.9) than following the recovery night of sleep at Test2 (mean 2.0 s.d. ± 0.9; $t(19) = 2.09$, $p < 0.001$). In contrast, the control group did not differ in their sleepiness ratings on Test1 (mean 2.4 s.d. ± 1.1) compared to Test2 (mean 2.8 s.d. ± 1.6; $t(16) = 2.12$, $p = 0.37$).

Although there is now substantive evidence for the restoration of certain brain and body functions following total sleep deprivation, ranging from 1-14 days recovery(Durmer & Dinges, 2005; Everson, et al., 1989), there is currently less knowledge on recovery rates for the restitution of mood and emotional brain reactivity following acute sleep deprivation. Therefore, in the event that a single night of recovery sleep may not have been sufficient to restore task performance, we also tested participants in the SD group a third time (Test3; not shown in Fig.1) following a more conservative recovery period of 3 weeks (mean 22.9 [s.d. ± 5.1] days).

Task

The experimental task involved evaluating three different affective face categories: Sad, Angry and Happy. Black and white photographs of the same male individual expressing fearful, happy, sad, angry and neutral faces were taken from the Ekman *Pictures of facial affect* set (http://www.paulekman.com/researchproducts.html), a validated stimulus set of human emotional expressions. Each of the three emotional pictures were morphed to the neutral face of the same individual using a series of eight equal gradient steps using a face-morph software tool *(Morph 2.5),* resulting in ten separate images for each of the four emotions; incrementally increasing in their relative degree of affect intensity (Figure 1B). Each emotion was presented in a separate block of trials, and within this block, the ten pictures were presented in a random order on a 14.1" laptop computer screen (central screen location, at half-screen height) at a standard distance from the participant. Subjects were required to make an emotional-strength rating for each face slide on a scale from 1-4, with 1 being most neutral and 4 most emotional, using a standard computer keyboard. For example, for the emotion Happy, the scale was as follows:

1 - Definitely neutral
2 - More neutral than happy
3 - More happy than neutral
4 - Definitely happy

For each block, subjects were first informed which emotional category they would be viewing – Sad, Angry or Happy. Next, subjects were acquainted with the range of emotional gradient by having the ten face slides appear in random order on the screen, one at a time and for 1s intervals, without requiring any response. This allowed subjects to appreciate and become familiarized with the full spectrum of emotion intensity prior to rating. Following this familiarization phase, an instructional screen notified subjects that they would now be rating each of the individual faces for a specified emotional category. Each of these blocks contained 10 face-picture trials, and each trial consisted of a 2s face stimulus presentation, followed by the response screen instructing subjects to rate the face using the above described 1-4 ranking system. Following the keyboard response, the next trial began. After rating all ten pictures, subjects repeated the same process for the remaining three emotional categories. The second session of testing was identical to the first, including the re-presentation of the same individual, with the exception that at the second session, subjects did not perform the familiarization phase prior to the actual rating phase. The order of presentation for each emotional category was randomized across participants, and, within participants, randomized across each of the two sessions.

Behavioral response data were assessed at two different levels 1) mean ratings across the 10 trials at Test1 and at Test2, for each emotion separately, and 2) a more fine grained analysis evaluating separated responses across the increasing affective gradient of picture trials (per emotion), at Test1 and Test2. For this latter analysis, a sliding window approach was implemented across the picture gradient, averaging the rating scores of two successive picture-trials at a time. Thus, the ten picture trials resulted in a total of nine data points for each subject, containing the average score of trials 1-2, then 2-3, 3-4 and so on up to trial 9-10. Comparisons principally focused on within-subject, between-test measures of performance, due to the known inter-subject variability in emotional reactivity and mood disposition(Davidson, 2004).

Statistical analyses were calculated using between and within group analysis of variance (ANOVA) models, together with post-hoc within and between group comparisons calculated using paired and unpaired two-tailed t-tests, respectively. All analyses were performed using the software program JMP v8.0 (SAS®).

3.4 Results

Emotions combined

Before evaluating each emotion separately, we first combined data across the three affective categories to examine the overall influence of sleep deprivation on emotional face recognition. For the sleep deprivation group, marked differences in affective face judgment emerged. Specifically, under conditions of sleep deprivation at Test1, ratings were significantly decreased (lower) compared with performance following recovery sleep at Test2 (**Fig.2** upper left panel bar graphs; paired ttest, $t(19) = 2.09$, $p < 0.005$). Moreover, when separated across the affective spectrum, it was apparent that this recognition deficit escalated as the emotional gradient increased (**Fig.2** upper left panel line graphs; significance values provided in figure), with the exception of the most extreme picture slides. Therefore, rather than imposing a consistent impairment on the processing of basic face perception, sleep deprivation was associated with a more selective deficit, disrupting the ability to identify expressions of moderate and strong emotional salience, and not neutral affect.

In contrast to the differences in task performance in the deprivation group, in the control group that slept each night prior to testing, there was neither a significant change in the overall mean rating at Test1 compared with Test2 (**Fig.3** upper left panel bar graphs; paired ttest, $t(16) = 2.12$, $p = 0.39$), or when separated using the sliding window analysis across trials; from neutral to increasingly emotional (**Fig.3** upper left panel line graphs; significance values provided in figure). Thus, general emotional recognition performance (across all three affective categories) was stable and consistent at the first (Test1) compared to second (Test2) assessments in participants that slept before both tests. We next evaluated the three emotions separately; *Happy*, *Angry* and *Sad* within the sleep deprivation and sleep control groups.

Emotions separated: Sleep deprivation group

Within the sleep deprivation group, for Test1 and Test2, a repeated measures ANOVA investigating the effects of Test Session (Test1, Test2), Emotion (Happy, Angry, Sad) and their interaction, revealed a main effect of Test Session/Condition (sleep deprived vs. sleep recovered; $F = 7.81$; $p = 0.006$) a trend towards a significant main effect of Emotion ($F = 2.53$; $p = 0.084$), and no Emotion by Test Session interaction ($F = 1.25$, $p = 0.29$). Analyses within each emotion revealed further significant differences within the sleep deprivation group.

Happy faces: Under conditions of sleep deprivation there was a significant impairment in the ability to recognize happy facial expressions, being significantly lower (blunted) compared with performance following sleep at Test2 (**Fig.2** upper right panel bar graphs; paired ttest, $t(19) = 2.09$, $p < 0.01$). Moreover, when separated across the affective spectrum, it was apparent that this recognition deficit emerged in the mid-spectrum range, as the expressions become emotionally ambiguous (Fig.2 upper right panel line graphs; significance values provided in figure).

Angry faces: As with the emotion Happy, a marked and even stronger blunting in emotional recognition for the emotion Angry was observed under conditions of sleep deprivation at Test1 (**Fig.2** lower left panel bar graphs; paired ttest, $t(19) = 2.09$, $p < 0.002$). Furthermore, as the faces became progressively emotional, this impairment became increasingly amplified (**Fig.2** lower left panel line graphs; significance values provided in figure).

Sad Faces: In contrast to the differences found for the facial expressions of Anger and Happy (both emotions of high autonomic arousal(Lang, Greenwald, Bradley, & Hamm, 1993)),

no significant impact of sleep deprivation was observed for the recognition of the emotion Sad (an emotion eliciting lower autonomic activation), with equivalent mean scores at Test1 and Test2 (**Fig.2** lower right panel bar graphs; $t(19) = 2.09$, $p = 0.67$). This was similarly true when separating responses across the affective gradient (**Fig.2** lower right panel line graphs; significance values provided in figure).

Emotions separated: Control group

Within the control group, a repeated measures ANOVA investigating the effects of Test Session, Emotion and their interaction, revealed no effect of Test Session ($F = 0.487$; $p = 0.487$), a main effect of Emotion ($F = 5.28$; $p = 0.007$), and no Emotion by Test Session interaction ($F = 0.238$, $p = 0.79$).

Evaluating the three emotions separately confirmed this lack of significant difference between Test1 and Test2 (paired ttests $t(16) = 2.12$ Happy: $p = 0.41$; Angry: $p = 0.41$; Sad: $p = 0.85$) as depicted in the bar graphs **Fig. 3**, and no significant differences were identified across the emotional gradient for any of the three emotions (**Fig.3** line graphs; significance values provided in figure). Thus, emotional recognition performance remained consistent from Test1 to Test2 in participants that slept before both tests, supporting the observation that differences in the deprivation group could not simply be explained by repeated test exposure.

Sleepiness and emotion recognition

To further investigate whether subjective sleepiness was associated with affective face recognition, correlations were performed between the mean emotion ratings for each of the three categories (Sad, Angry, Happy) and participants Stanford Sleepiness Scale scores, at both Test1 and Test2. Within the sleep deprivation group, none of these six correlations were significant, all being $r^2 \leq 0.38$, $p \geq 0.10$. Similarly, within the control group, all equivalent correlations were $r^2 \leq 0.21$, $p \geq 0.40$, except for the emotion Anger at Test2, which resulted in an $r^2 = 0.55$, $p = 0.03$. However, this correlation did not survive a Bonferroni correction for multiple tests ([α / n tests = 0.05/6], resulting in a critical p-value of < 0.008). Therefore, with the exception of a statistical trend of one emotion category within the control group, at one of the test sessions, the level of subjective sleepiness did not appear to demonstrate a strong, consistent relationship with emotion recognition task performance.

Considerations of sleep deprivation and sleep recovery

An alternate potential interpretation of the differences identified in the sleep deprivation group may pertain to the effects of recovery sleep (Test2), rather than sleep loss (Test1). That is, the differences observed in the deprivation group could be attributable to excess sleep on the recovery night, potentially amplifying emotional recognition at Test2, rather than sleep deprivation impairing emotional recognition at Test1. To investigate this possibility, subjects in the deprivation group returned to the laboratory for further testing approximately three weeks later. Contrary to the hypothesis of recovery sleep amplifying differences observed in the Test1-Test2 comparison, performance at this three week session ("Test3") was similar to that following recovery sleep (Test2) for both the emotions Angry and Happy (**Table 1**), and was even statistically greater for Sad. Furthermore, performance at Test3 continued to show statistically significant, or trends towards significant, differences relative to Test1 (**Table 1**). Therefore, the comparative differences between Test1 (sleep deprived) and Test2 (sleep rested) appear to reflect a relative impairment in emotional processing at Test1 due to sleep deprivation, rather than an

effect due to recovery sleep at Test2. Indeed, Test3 data suggest that performance following first-night recovery at Test2 may still carry some residual impairment from the night of sleep deprivation, since performance scores were consistently, although not significantly, higher at Test3.

Taken together these data suggest that one night of total sleep deprivation disrupts the recognition of some, but not all emotional facial expressions, and within the moderate range of expressed emotion. Specifically, moderate affective expressions associated with high emotional arousal– Anger (threat relevant) and Happy (reward relevant) –demonstrated greater sensitivity to disruption under conditions of sleep loss than those of low arousal strength (Sad).

Gender differences

Within the context of sleep disorders, emerging evidence suggests the presence of gender differences in the rates of depression, with females being particularly sensitive to the interaction of mood disorders and sleep abnormalities(Armitage, 2007; Shaffery, Hoffmann, & Armitage, 2003). Considering this association, in an exploratory analysis, we reexamined our findings on the basis of gender. Consistent with previous clinical reports, female participants in the sleep deprivation condition (n = 11) expressed marked and significant blunting in the recognition of both emotions that were impaired at the overall group level (Anger and Happy, with no significant effect observed for Sad, **Table 2A**). In contrast, these comparisons were non-significant in male participants under conditions of sleep deprivation. It should be noted, however, that mean values were somewhat similar between genders, yet the variance was greater in male participants. No significant gender differences were observed in the sleep rested control group (**Table 2B**). Therefore, the differences in emotion rating in the sleep-deprived participants at an overall group level appear to be one expressed most consistently and significantly in female participants, supporting the hypothesis of gender specific sensitivity to sleep disruption within the domain of affective brain function(Armitage, 2007; Shaffery, et al., 2003).

3.5 Discussion

Using an affective face recognition task, here we demonstrate that a single night of sleep deprivation significantly disrupts the ability of the human brain to accurately identify salient emotional expressions in others, particularly in the moderate intensity range of emotion. In addition, these deficits were specific to some but not all affective categories, being most dramatic for emotions associated with eliciting high autonomic arousal – Angry and Happy – corresponding to greatest threat-relevant and reward-relevant value, respectively. Furthermore, when separated by gender, the influence of sleep deprivation on emotion recognition was observed most reliably and significantly in female participants.

Impact of sleep deprivation: It has been argued that facial expressions are the most biologically and socially significant visual stimuli in the human environment. Facial expressions have the ability to code numerous salient and motivational cues ranging from basic primary emotions to more complex informational signals, such as trustworthiness and even the intent of others(Adolphs, 2003; Vuilleumier & Pourtois, 2007). The critical contribution made by facial recognition to optimal human behavior is exemplified by conditions of abnormal affective processing. For example, neurological patients who suffer from an inability to recognize certain emotions due to lesions in key extended limbic or prefrontal regions display marked social dysfunction, being unable to detect and subsequently be guided by relevant affective cues(Damasio, 2000). Developmental disorders such as autism provide additional evidence for

impaired social interaction due to abnormal face processing, particularly expressions carrying emotional significance(Sasson, et al., 2007).

Here we add to this collection of circumstances in which emotional face recognition is significantly disrupted: the condition of sleep deprivation. However, unlike the disorders described above, which express affective dysregulation due to structural brain abnormalities, the impact of acute sleep deprivation is, presumably, largely functional in nature, indicating there are additional circumstances, beyond substantive pathology, which can result in affective recognition deficits. Considering that i) impairments imposed by sleep deprivation were observed for some but not all emotions, ii) these deficits only became evident as the emotion gradient increased to moderate intensity levels, where facial affect is more ambiguous, and iii) these changes dissipated towards the extremities of the emotion gradient, it would suggest that basic aspects of facial perception (early stage processing) are likely intact under conditions of sleep deprivation, at least in the current task. Rather, the progressive impairment within the mid-emotion spectrum range would suggest processing deficits down-stream of initial perceptual coding regions, being more associated with subsequent emotional evaluation and judgment.

Independent of objective changes in emotion reactivity, several reports to date have described the progressive dysregulation of subjective mood state following accumulated sleep loss(Dinges, et al., 1997), amplifying negative mood states to disruptive daytime experiences, while reducing positive mood benefits associated with goal-enhancing activities(Zohar, et al., 2005). Interestingly, mood and emotion have been conceptualized as representing related yet dissociable processes; the former reflecting an evaluation of internal state, and that is more long lasting, the latter concerned with reactivity to evoking stimuli (external or internal), and are often short lived(Ekman & Davidson, 1994). While no measure of subjective mood was assessed in the current study, understanding how sleep-associated changes in mood may contribute to alterations of emotion, such as face recognition, represents a fertile area for future investigations.

Direction of effect: Based on a recent report indicating that the human amygdala becomes hyper-reactive to increasingly negative stimuli under conditions of sleep deprivation(Yoo, Gujar, et al., 2007b), it is perhaps surprising to find a blunting in emotional face recognition stimuli (including the emotion Angry) in similarly sleep-deprived individuals. However, a number of findings are worth considering in this context.

First, amygdala activity has been more consistently associated with the emotion fear (a category not tested in this study) than anger; hence an amplified reaction to angry or sad faces when sleep deprived is not necessarily predicted. Instead, the emotion Anger has been more consistently associated with activation in regions of the basal ganglia, orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC)(Blair, Morris, Frith, Perrett, & Dolan, 1999). Moreover, neuropsychological studies indicate impaired recognition of Anger following lesions in the ventral basal ganglia(Calder, Keane, Lawrence, & Manes, 2004). Similarly, activation in ACC has been observed during the induction and evaluation of the emotion Happy, together with regions of the ventral prefrontal cortex(Dolan, et al., 1996), yet these regions were not recruited significantly for the emotion Sad.

Second, the involvement of the ACC regions in processing Angry and Happy faces (in addition to fear), but not Sad or neutral faces, may relate to the roles these areas play in the regulation of arousal: Anger and, to a lesser degree, Happiness are considered to involve a higher autonomic state of activation than Sadness(Adolphs, 2002). Thus, ACC (and perhaps OFC) activation during the recognition of highly arousing emotional face stimuli may reflect the subject's own arousal elicited either in response to or in a mirroring of the emotion. If disrupted,

the capability of this medial prefrontal system to accurately code and consequently rate stimuli that elicit stronger autonomic arousal would become impaired.

Consistent with this hypothesis, previous PET studies have demonstrated that regions of the medial prefrontal cortex, including the ACC, are amongst those most significantly impaired in their activity following sleep deprivation(Thomas, et al., 2000, 2003). Furthermore, together with alterations in amygdala activity in response to negative stimuli, significant reductions in associated medial prefrontal functional connectivity have been reported under conditions of sleep deprivation(Yoo, Gujar, et al., 2007b). This susceptibility of the prefrontal lobe to sleep deprivation–including ACC regions involved in the evaluation and modulation of affective states–may offer an explanatory neural basis for the inability of subjects in the current study to accurately judge emotional expressions involving autonomic arousal. Indeed, due to these neural impairments, it is interesting to speculate that the ability of the sleep-deprived brain to either represent or cogently mirror emotions of others would be diminished, which, as a consequence, would negate the appreciation and accurate rating of facial expressions.

The results of the delayed test (Test3; three weeks) also support the possibility that performance following one night of recovery sleep may still carry residual impairment from the sleep deprivation night. While still preliminary, such findings may indicate that the restitution of emotional brain function following acute sleep deprivation requires more than one recovery night to restore to baseline abilities, similar to certain measures of cognition(Durmer & Dinges, 2005).

Gender Differences: There is now a substantial literature indicating that females are at greater risk for developing major depression than males, post-puberty, and that this increased probability may be associated with co-occurring differences in sleep architecture(Armitage, 2007; Shaffery, et al., 2003). Here we demonstrate that, in participants without pre-existing mood disorders, sleep deprivation similarly imposes a more significant and reliable impairment in identifying emotion intensity in female compared to male participants. Such a finding may indicate that healthy young females have a greater degree of sensitivity to the effects of sleep loss in the context of affective dysregulation, consistent with reported trends in clinical mood disorders(Armitage, 2007; Shaffery, et al., 2003).

The underlying neurobiological basis for these gender effects continues to be examined, with the influence of different sex steroids, and their consequential effects on down-stream neurotransmitter concentrations, being particularly relevant(Shaffery, et al., 2003). Furthermore, based on gender differences in neurochemistry, it has been argued that women show a greater homeostatic sleep sensitivity and drive than men, such that small reductions in sleep amount, or even a delay-shift in the onset of sleep, triggers a stronger sleep rebound in females than males, especially for slow-wave sleep(Shaffery, et al., 2003). This same sensitivity may explain the more consistent effect imposed by sleep loss in our females subjects. Indeed, our findings indicate that this sensitivity can similarly be observed in measures of emotional reactivity, in addition to physiological sleep changes, such as homeostatic drive.

Ecological relevance: The current findings offer implications at both societal as well as professional levels. Social interactions are critically guided by, and indeed are predicated on the basis of, accurately recognizing emotional facial expressions. Only through such accurate recognition can cogent social judgments and subsequent actions be made. Furthermore, considering that sleep time continues to show a decrease across many age ranges, the relevance of the current findings become increasingly pertinent (National Sleep Foundation poll 2007: http://www.sleepfoundation.org/). Nowhere are these accurate emotional face judgments (which require reciprocal affective and empathetic evaluations and behaviors) more critical than in many

professions that are associated with sleep curtailment, including emergency and resident medical staff, military personnel, and even new parents. Based on the emerging results indicating an intimate link between sufficient prior sleep and appropriate next-day affective regulation(Walker, 2009), a greater appreciation of the importance of sleep in beneficially modulating emotional reactivity and stability at a social, professional and mental health level appears increasingly necessary.

Study Limitations: Our current findings should be appreciated within the context of a number of important limitations. First, no assessment of participant chronotype was performed in this cohort of young adults. Delayed sleep phases are becoming increasingly common in adolescence and early adulthood, and may be especially so in young males(Crowley, Acebo, & Carskadon, 2007; Roenneberg, et al., 2007; Wittmann, Dinich, Merrow, & Roenneberg, 2006). Such changes may have contributed to the observed gender differences reported here, reflected in greater variance of impairment observed under conditions of sleep deprivation in male participants. Considering chronotype is also known to modulate cognitive performance (e.g.(Schmidt, et al., 2009)), rather than testing all participants at a fixed clock time, as performed in the current study (16:00 hr), an alternative and interesting future methodological approach will be to evaluate subjects based on their respective chronotype schedule.

A second study limitation concerns the lack of measured task motivation and/or interest(Harrison & Horne, 2000; Lisper & Kjellberg, 1972). Decreased motivation, independent of general sleepiness, may represent an additional influencing factor in the current study, such that if the task were made more engaging, the observed performance decrements in the deprivation group would be obviated. Although we cannot exclude this possibility, we do note, however, two features of our results that suggest a lack of motivation or interest are less parsimonious explanations; i) the emotional blunting in the SD group was principally observed only in one portion of the emotion spectrum (middle sections, where faces were more ambiguous). A general lack of motivation or interest would predict blunting to be observed more ubiquitously across emotion stimulus gradient. Furthermore, the stimuli were presented in a random order within and across subjects, controlling for a possible time-on-task effects, and ii) the emotional blunting was only apparent for the emotion categories of Anger and Happy, and not for Sad. A motivation or interest explanation would perhaps predict ubiquitous impairments across all categories. Therefore, the specificity and selectivity of impairment, both within and also across emotion categories, supports a more discreet affective dysregulation, rather than a more general motivation contribution.

A third study limitation is the lack of objective measures verifying compliance with sleep duration and sleep-wake times (e.g. actigraphy, polysomnography), as well as sleep deprivation in SD group. The addition of physiological verification of sleep-wake parameters in future studies will help improve homogeneity of compliance across the study, potentially reducing variability in task performance across subjects, particularly in the sleep-deprived condition.

A final cautionary consideration is the potential contribution of learning effects. Although the current task was not designed to assess memory, the process of facial recognition and rating has the potential to be affected by prior task exposure. We did not observe learning effects in the control group, evidenced by the lack of significant differences between Test1 and Test2 performance. This would suggest a degree of repeatability that is independent of learning effects, although it does not discount the possibility. However, at Test3 in the sleep deprivation group, relative to Test1 performance (both sleep-rested measures), we did observe a significant difference in one of the three emotion categories – Sad. This may suggest that with multiple task

exposures (e.g. >2), there are potential learning-related contributions to changes in performance for some emotions. Future repeated-measures experiments would substantially benefit from the use of a counter-balanced crossover design, which would control for such learning-related effects, unlike the current study protocol.

Figures

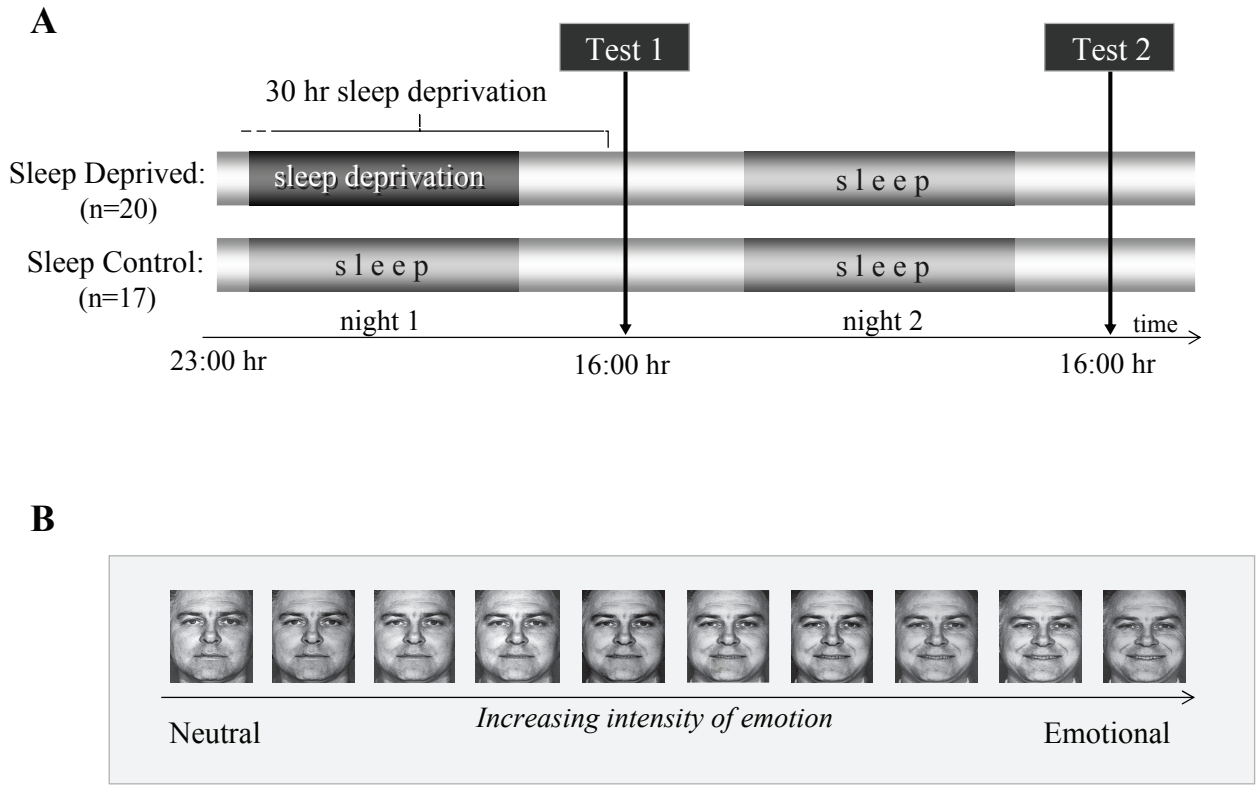

Figure 1. A) Experimental design: Participants in each group performed the emotional face recognition task twice; once on Day 2 (16:00 hr) and once on Day 3 (16:00hr). The experimental manipulation occurred across the night before the first test session (Test1). Participants in the sleep control group were awake across Day 1 and slept normally across Night 1 before Test1 on Day 2. Participants in the sleep deprivation condition were similarly awake across Day 1, but were subsequently kept awake across Night 1, and across Day 2 accumulating 30 hr of total sleep deprivation prior to Test1. Following Test1, both groups had a normal night of sleep (Night 2), and repeated the same task a second time on Day 3 (Test2). B) Example of the emotional face stimuli gradient used for the affective category HAPPY.

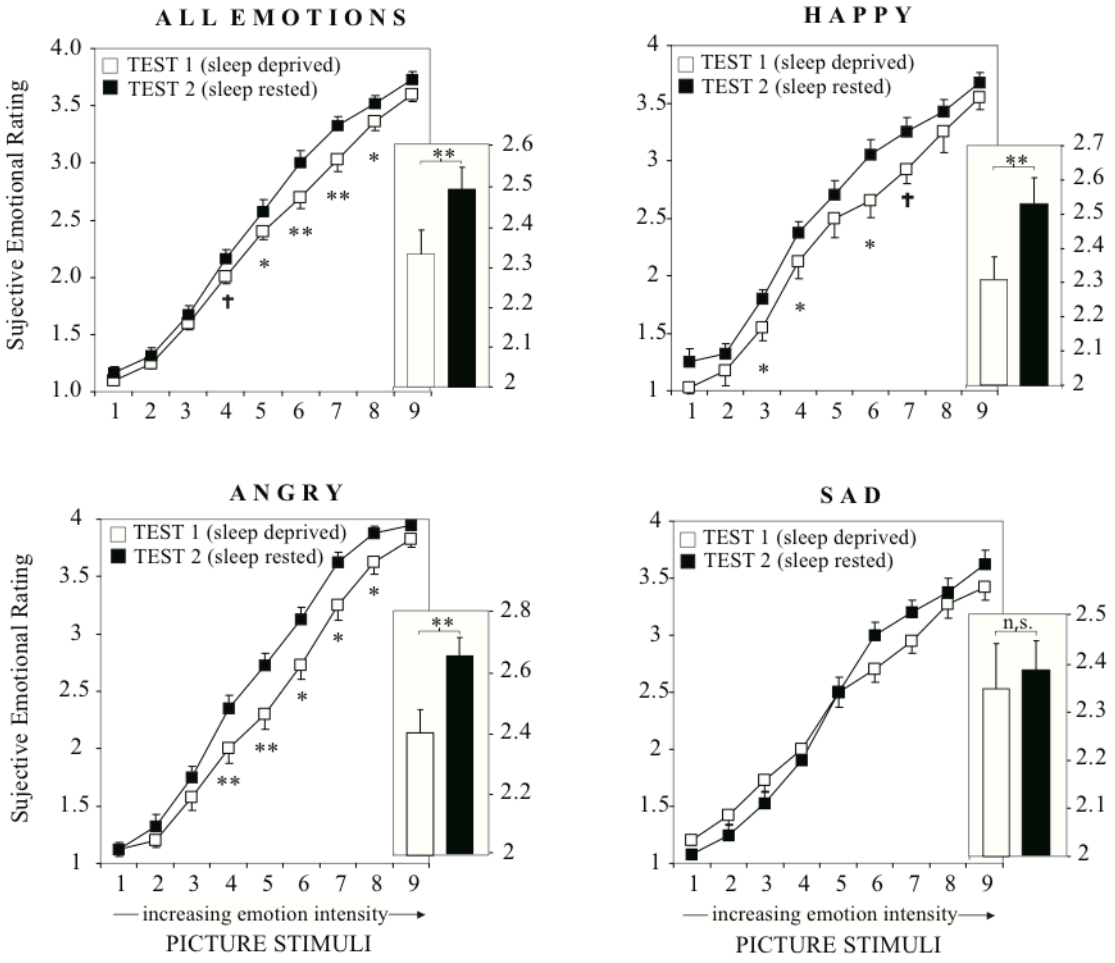

Figure 2. Emotional Face Recognition Performance in the Sleep Deprivation group. Upper left panel: Average rating of face stimuli across all three emotional categories (ANGRY, HAPPY, SAD) combined at Test1 (clear bar, when sleep deprived) and Test2 (filled bar, when sleep-recovered), together with response curves at Test1 (clear squares, when sleep deprived) and Test2 (filled squares, when sleep-recovered) for the rating of the faces stimuli when plotted across the emotional intensity gradient, from low to high (left to right). Remaining panels are mirror data for each of the emotions separately, labeled respectively on each panel.
† $p < 0.08$; * $p < 0.05$; ** $p < 0.01$; n.s. Non-significant. Error bars represent standard error of the mean.

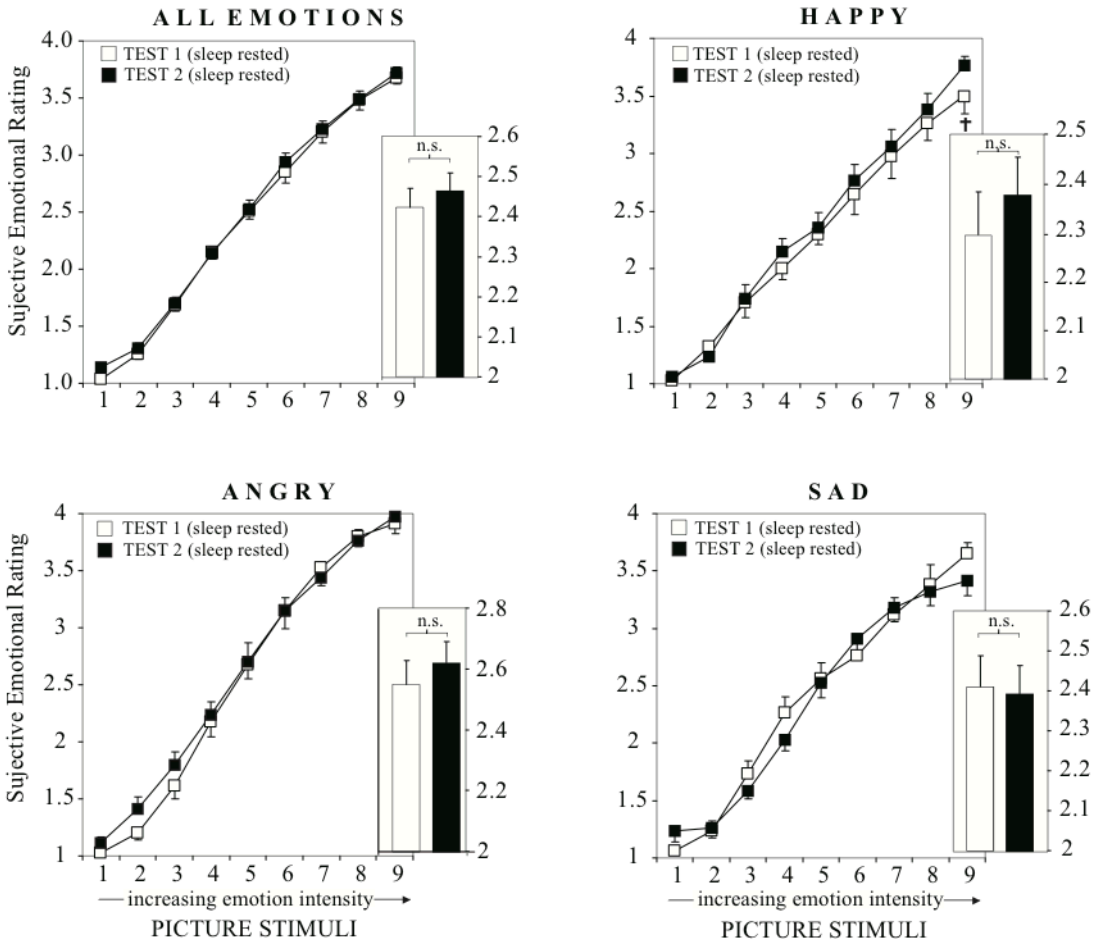

Figure 3. Emotional Face Recognition Performance in the Sleep Rested group. Upper left panel: Average rating of face stimuli across all three emotional categories (ANGRY, HAPPY, SAD) combined at Test1 (clear bar) and Test2 (filled bar) and response curves at Test1 (clear squares) and Test2 (filled squares) for the rating of the faces stimuli when plotted across the emotional intensity gradient, from low to high (left to right). Remaining panels are mirror data for each of the emotions separately, labeled respectively on each panel.
n.s. Non-significant. Error bars represent standard error of the mean.

Tables

Table 1

Average Response Scores (mean ± s.e.m.) in the Sleep Deprivation Group Across Test1, 2 & 3.

Emotion	Test1	Test2	Test3	Test1 vs. Test3	Test2 vs. Test3
Happy	2.31 ± .06	2.53 ± .08	2.48 ± .08	$p < 0.07$	$p = 0.93$
Angry	2.41 ± .08	2.66 ± .06	2.56 ± .06	$p < 0.08$	$p = 0.22$
Sad	2.35 ± .09	2.39 ± .06	2.54 ± .09	$p < 0.01$	$p = 0.04$

Table 2A

Response Scores Sleep Deprivation Group Separated By Gender

Gender	Emotion	Test1	Test2	Test1 vs. Test2
Females	Angry	2.41±0.06	2.64±0.07	$p = 0.01$
	Happy	2.37±0.07	2.56±0.09	$p = 0.03$
	Sad	2.41±0.09	2.46±0.05	$p = 0.63$
Males	Angry	2.4 ±0.15	2.63±0.10	$p = 0.14$
	Happy	2.23±0.12	2.50±0.12	$p = 0.09$
	Sad	2.30±0.18	2.29±0.12	$p = 0.93$

The average response scores (mean± s.e.m.) in the sleep deprivation group, separated by gender. Subjects were sleep deprived during Test 1 and sleep recovered during Test 2. The last column gives p-values for ttests comparing scores from Test 1 versus Test 2.

Table 2B

Response Scores Sleep Control Group Separated By Gender

Gender	Emotion	Test1	Test2	Test1 vs. Test2
Females	Angry	2.63±0.08	2.64±0.07	$p = 0.92$
	Happy	2.21±0.11	2.42±0.09	$p = 0.13$
	Sad	2.44±0.08	2.31±0.11	$p = 0.34$
Males	Angry	2.44±0.15	2.56±0.13	$p = 0.45$
	Happy	2.41±0.12	2.36±0.15	$p = 0.59$
	Sad	2.40±0.14	2.50±0.07	$p = 0.32$

Average response scores (mean ± s.e.m.) in the sleep control group, separated by gender. Subjects were sleep deprived during Test 1 and sleep recovered during Test 2. The last column gives p-values for ttests comparing scores from Test 1 versus Test 2.

Chapter 4. Introduction Neuroimaging Studies

In Chapter 3 I discussed the maladaptive effects of sleep deprivation on emotional behavior (the ability to recognize emotional facial expressions). This study however, does not address what happens during a more naturalistic 24hr period during which we either have a normal night of sleep, or a normal day of wake. In the next two Chapters I went on to explore a more mechanistic account of the function of sleep (as well as wake) in modulating the emotional brain. These two studies rely on neuroimaging methods that measure brain activity, in this case functional magnetic resonance imaging (fMRI), before or after sleep (or a similar period of wakefulness).
In the first study (Chapter 5) fMRI was collected during an emotional task, often referred to as task-related fMRI. In the second study (Chapter 6) fMRI was collected during a resting period, or resting state fMRI. In this last study the analyses were focused on a brain region associated with emotional processing, the amygdala.

The first study was a follow-up study on the Yoo study (discussed on page 16) where participants viewed emotional images after a period of sleep deprivation or after a normal night of sleep. In this study it was found that the amygdala showed more activity after a period of sleep deprivation than after a normal night of sleep.

The study in Chapter 5 used the same task, displaying emotional images, yet now participants were not sleep deprived, but instead were tested twice. Once before sleep and once after sleep (Sleep group), which allowed for an investigation into the changes in amygdala reactivity after a night of sleep (within subjects). The control group (Wake group) was tested in the morning and evening, allowing for an investigation into the changes in amygdala reactivity after a period of wakefulness, across the day.

Similar to the Yoo study, a Psycho-Physiological Interaction analysis (PPI) was performed which is a method used to determine which areas show similar activity patterns in certain context (or condition) versus another context. Specifically, PPI analyses identify voxels in which activity is more related to activity in a seed region of interest (seed ROI) in a given psychological context or condition, such as during attention or in during the viewing of emotional stimuli(O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012).

The seed in this study was the amygdala and our target region of interest was the ventral medial prefrontal cortext (vmPFC), based on its established role in top-down regulatory control of amygdala function (Davidson, 2002; Milad, Vidal-Gonzalez, & Quirk, 2004; Morgan, Romanski, & LeDoux, 1993; Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk, Likhtik, Pelletier, & Pare, 2003; Quirk & Mueller, 2008; Rauch, Shin, & Phelps, 2006; Rosenkranz, Moore, & Grace, 2003; Sierra-Mercado, Corcoran, Lebron-Milad, & Quirk, 2006; Sotres-Bayon & Quirk), and verified functional (Kim, et al.) as well as structural connectivity with the amygdala (Bracht, et al., 2009; Croxson, et al., 2005; Johansen-Berg, et al., 2008). The ventromedial PFC ROI was defined by a 6mm sphere around central coordinates from previously published amygdala functional connectivity studies using emotional reactivity paradigms (Delgado, Nearing, Ledoux, & Phelps, 2008; Dunsmoor, Prince, Murty, Kragel, & LaBar; Milad, et al., 2005; Osuch, Willis, Bluhm, Ursano, & Drevets, 2008; Phelps, et al., 2004; Sterpenich, et al., 2009) (MNI coordinates [x y z]: -2, 32, -6).

One caveat associated with PPI is the necessary deconvolution of the fMRI activity, or the convolution of the psychological condition as described by O'Reilly et al. in a recent paper (O'Reilly, et al., 2012). PPI investigates the interaction between a psychological condition and brain activity (or the fMRI signal in this case). The critical issue is that the psychological context is measured in real time, whereas the brain activity is measured with a lag of about 6s and with temporal blurring, due to convolution with the hemodynamic response function. The analysis requires the two timeseries to be combined, therefore either both need to be convolved with the hedynamic response function (HRF), or both need to be expressed in terms of the underlying neural activity (deconvolved). The study in Chapter 5 analyzed the data using SPM, which uses the latter implementation of PPI. One argument for this method (i.e. deconvolve the activity recorded from the seed ROI) is that since we are interested in interactions on the neural level, PPI should take place on neural-like data, as discussed by Gitelman and colleagues(Gitelman, Penny, Ashburner, & Friston, 2003). One argument against is that there is no deterministic way to deconvolve the HRF if there is no knowledge of its exact shape. O'Reilly et al. argue in their paper that the only way to deconvolve two unknown signals is to use an algorithm that tries to find the most plausible combination of neural signal and HRF to fit to the data(O'Reilly, et al., 2012). The authors write that therefore there is no way of knowing if the deconvolution is really correct, and it may be just as valid to transform the psychological regressor with an assumed HRF. In either case assumptions about the shape of the HRF are important, particularly in the case of event-related designs, in which convolved regressors really reflect the shape of the HRF.

The fMRI study described in Chapter 6 does not investigate task-related brain activity, but instead had participants relax in the scanner, with their eyes open (often referred to as resting state fMRI). Hence, they were not performing any particular task.

Similar to a PPI analysis a resting state analysis also requires a point of focus, or a region of interest (ROI). We focused on the amygdala, based on the study described in Chapter 5 that found differences in these same participants in amygdala reactivity during a task.

The target area we focused on was similarly the medial prefrontal cortex (mPFC), but now more medial than for Chapter 5, based on previous amygdala resting connectivity studies(Gu, et al., 2010). The medial PFC ROI was defined by a 10mm diameter sphere around central coordinates from this previously published amygdala resting connectivity study(Gu, et al., 2010) and studies using emotional reactivity paradigms (MNI coordinates [x y z]: 0, 56, 10)(Heinz, et al., 2005; Modinos, et al., 2012; Phelps, et al., 2004; Sakaki, Niki, & Mather, 2012; Shin, et al., 2004a; Shin, et al., 2005; Williams, et al., 2006), thus covering areas that (i) are densely connected to the amygdala functionally, and (ii) play a crucial role in the processing of emotional stimuli.

The study in Chapter 5 investigated the effect of time of day (circadian effects) by inserting a circadian control task. The resting state study in Chapter 6 however could not have a similar 'resting state control task'. Therefore we focused on chronotype data, which was based on a morning-eveningness questionnaire that measures how much of a morning or evening person the participant is (on a continuous scale). We then examined whether chronotype was associated with the changes in the amygdala resting state network. Future studies could however utilize a nap study to fully rule out any circadian effects on the amygdala resting state network, in addition to the effects of sleep described in Chapter 6. Furthermore, collection of circadian measures such as cortisol measures could additionally aid in elucidating any circadian effects.

Chapter 5. REM-sleep depotentiates amygdala reactivity to previous emotional experiences

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5.1 Overview

Clinical evidence suggests a potentially causal interaction between sleep and affective brain function; nearly all mood disorders display co-occurring sleep abnormalities, commonly involving rapid-eye movement (REM) sleep (Benca, Obermeyer, Thisted, & Gillin, 1992; Benca, et al., 1997; Harvey, 2008; Walker & van der Helm, 2009b). Building on this clinical evidence, recent neurobiological frameworks have hypothesized a benefit of REM sleep in palliatively decreasing next-day brain reactivity to recent waking emotional experiences (Levin & Nielsen, 2007; Walker, 2009). Despite these frameworks, and their predictions, the proposed benefit of REM sleep physiology in depotentiating neural and behavioral responsivity to prior emotional events remains unknown. Combining fMRI and dense EEG sleep recordings in humans, we provide three sets of novel findings: (1) A night of sleep, in contrast to an equivalent time awake, depotentiates amygdala reactivity to previously encountered emotional experiences, associated with re-established regulatory connectivity between amygdala and ventromedial prefrontal cortex, (2) these overnight neural changes are mirrored by marked decreases in behavioral reactivity, demonstrating post-sleep reductions in subjective emotional intensity ratings the next day, and (3) the extent of both these beneficial neural and behavioral reductions are proportional to the extent of adrenergic suppression during REM sleep, indexed by high-frequency EEG activity focused topographically over prefrontal cortex. Together, this collection of findings supports a proposed homeostatic role for REM sleep in the adaptive homeostasis of affective brain function. They further provide neurobiological insights into the underlying mechanisms governing this palliative emotional benefit, mediated by the unique neurophysiological state of reduced central adrenergic activity during REM, expressed in the cerebral signature of high-frequency EEG activity.

5.2 Background

Converging evidence continues to validate a functional role for non-rapid eye movement (NREM) sleep in promoting memory and brain plasticity. In striking contrast, there has been little, if any, empirical data offering a consensus regarding the function of rapid-eye movement (REM) sleep. Clinical evidence suggests a potentially causal interaction between sleep and affective brain function; nearly all mood disorders display co-occurring sleep abnormalities, commonly involving rapid-eye movement (REM) sleep (Benca, et al., 1992; Benca, et al., 1997; Harvey, 2008; Walker & van der Helm, 2009b). Building on this clinical evidence, recent neurobiological frameworks have hypothesized a role for REM sleep and its suppression of central adrenergic activity, in the palliative depotentiation of prior emotional experiences, dissipating emotional strength and associated limbic brain activity. Specifically, the marked suppression of central adrenergic neurotransmitters during REM (commonly implicated in arousal and stress), coupled with activation in amygdala-hippocampal networks that encode salient events, is proposed to (re) process and depotentiate previous affective experiences,

decreasing their emotional intensity (Walker & van der Helm, 2009b). In doing so, REM sleep is hypothesized to adaptively ameliorate the visceral tone originally associated with emotional experiences. In contrast, the failure of such adrenergic reduction during REM sleep has been described in anxiety disorders, indexed by persistent high-frequency electroencephalographic (EEG) activity (>30 Hz) (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, Candy, & Bradley, 1976; Maloney, Cape, Gotman, & Jones, 1997); a candidate factor contributing to hyperarousal and exaggerated amygdala reactivity (Etkin & Wager, 2007; Raskind, et al., 2007; Spoormaker & Montgomery, 2008b; Walker & van der Helm, 2009b). Despite these informed neurobiological frameworks, however, a role for REM sleep and its unique physiology in de-potentiating reactivity to previously encountered emotional experiences remains untested. Furthermore, the neural mechanisms that underlie such an affective benefit similarly remain unexplored.

Building on the specific predictions of these neurobiological frameworks (Levin & Nielsen, 2007; Walker, 2009; Walker & van der Helm, 2009b) and combining functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) sleep recordings, we tested the hypothesis that (1) sleep decreases amygdala and behavioral reactivity in response to previously encountered emotional experiences, associated with reestablished medial prefrontal cortex connectivity and (2) these brain and behavioral sleep benefits are proportional to the extent of decreased central adrenergic levels during rapid-eye movement (REM) sleep, as reflected by reduced gamma (30–40 Hz) EEG activity; a validated proxy indexing reduced central adrenergic activity (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997).

5.3 Methods

In short, 34 healthy adults (age: 18–30 years) were randomly assigned to one of two groups. Each performed two repeat fMRI tests (test 1, test 2), separated by 12 hr containing a night of EEG-recorded sleep (sleep group, n = 18, ten females) or a waking day (wake group, n = 16, nine females; **Figure 1**). During each test, participants viewed and rated the subjective emotional intensity of 150 standardized affective pictures (Lang, 1997) on a 125 scale, corresponding to increasing intensity. Importantly, participants viewed the same stimuli at both test sessions, affording a measure of change in emotional reactivity to previously experienced affective stimuli (test 2 – test 1), following wake or sleep.

Participants additionally performed a circadian control test at the second fMRI session, involving presentation of a novel set of affective stimuli. This control test allowed confirmation that behavioral and fMRI differences in reactivity identified following wake and sleep were independent of time of day.

Participants

Thirty-four healthy participants, age 18-30 years (mean: 21.6, s.d. ± 3.5, 19 Female), were randomly assigned to either the Sleep-group (*n*=18, 10 Female, mean age: 22.2, s.d. ± 3.6) or Wake-group (*n*=16, 9 Females, mean age: 21.0, s.d. ± 3.5). Participants abstained from caffeine and alcohol for the 72hr before and during the entire course of the study and kept a normal sleep-wake rhythm (7–9 h of sleep per night with a sleep onset time before 2:00hr in the morning) for the 5 nights prior to study participation, as verified by sleep logs and actigraphy (a wristwatch movement sensor, sensitive to wake and sleep states). Exclusion criteria, assessed using a prescreening questionnaire, were: a history of sleep disorders, neurologic disorders, closed head

injury, Axis I psychiatric disorders according to the DSM-IV criteria (encompassing mental disorders including depression, anxiety disorders, bipolar disorder, attention deficit disorder and schizophrenia), history of drug abuse and current use of anti-depressant or hypnotic medication. Subjects who reported drinking excessive amounts of caffeine (defined as 3 or more caffeine-containing substances a day such as coffee, tea or caffeine containing soft drinks) were excluded from the study. The study was approved by the local human studies committee, with all participants providing written informed consent.

The two groups did not differ in the average sleep amount obtained 5 nights prior to the study based on objective actigraphy (unpaired *t*-test $P = 0.61$) or subjective sleep logs (unpaired *t*-test $P = 0.74$, **Table 5A**). This was similarly the case when assessing each of the 5 nights separately between the two groups when using either sleep logs or actigraphy (unpaired *t*-tests, all $P > 0.32$), including the last night prior to the study (sleep logs unpaired *t*-test $P = 0.89$ and actigraphy $P = 0.71$). Therefore, participants adhered to the study protocol sleep requirements, and sleep amounts were not different between groups, across any night leading up to the study.

Experimental design

Both groups performed the emotion reactivity test twice inside the fMRI scanner; separated by 12hr, involving the rating and subsequent re-rating of the same standard set of affective picture stimuli (**Figure 1**, main manuscript). Participants in the Sleep-group arrived in the laboratory at 7:30PM (± 30min) to complete the emotional reactivity test (Test1) in the fMRI scanner at 8:30PM (± 30min). Following the scan, participants were prepared for polysomnographic (PSG) sleep recording (details below) in the laboratory, with lights off from 11:30PM-7:30AM (± 30min). The next morning, participants performed the second emotional reactivity test (Test2) in the fMRI scanner at 8:30AM (± 30min). Participants in the Wake-group underwent a similar protocol but the tasks were performed at different times of the day. The first emotional reactivity test (Test1) was performed in the morning at 8:30AM (± 30min), and following a day of standard waking activities outside the laboratory, the second emotional reactivity test (Test2) was performed at 8:30PM (± 30min).

The change in emotional reactivity following a night of sleep (Sleep-group) or a day of wake (Wake-group) was examined by comparing reactivity at Test1 (pre wake or sleep) with reactivity at Test2 (post wake or sleep); [Test2 – Test1]. To assess possible time-of-day differences in emotional reactivity, independent of wake or sleep, an additional circadian control test was performed immediately after Test2 (morning in the Sleep-group, evening in the Wake-group; **Figure 1**). During the circadian control test participants viewed and rated a novel set of emotional images not seen before, matched in terms of arousal and valence to the original set used in Test1 and Test2. Outright time-of-day differences in emotion reactivity were assessed by comparing the two groups on fMRI and behavioral reactivity to this novel circadian control set of stimuli, and which had not passed through either the brain-state of wake or sleep prior to the test.

Task Stimuli: Each emotional reactivity test (Test1, Test2 and the circadian control test) consisted of viewing 150 pictures, selected from the standardized International Affective Picture System (IAPS) (Lang, 1997). Two matched sets of 150 stimuli were counter-balanced as either the experimental set (used in Test1, and represented at Test2, assessing changes in reactivity), or the circadian control set (used to assess time of day differences). Both image sets ranged from low to high arousal (arousal set1: range 2.28–7.12, mean ± s.d. = 5.00 ± 1.01; arousal set 2:

range 2.03–7.35, mean ± s.d. = 4.95 ± 1.29) containing both positive and negative valence (valence set 1: range 1.45–8.28, mean ± s.d. = 5.03 ± 1.95; valence set 2: range 1.46–8.22, mean ± s.d. = 4.92 ± 1.86). Neither set were statistically different from each other in terms of arousal ($P > 0.70$) or valence levels ($P > 0.64$).

Trial timing: Each trial lasted 7700ms, beginning with a black fixation crosshair on a white background (500 ms) followed by the visual-picture presentation (2000 ms), then a response screen (2500 ms), followed by a grey fixation cross on a white background (2700 ms). Images were presented in a pseudorandom order with no more than two pictures of similar valence presented in a row (positive, negative or neutral). Ratings were made through right-handed button responses across a 1-5 emotional scale in accordance with previous studies measuring emotional intensity (Britton, Taylor, Sudheimer, & Liberzon, 2006), with the following instruction presented during the response screen:

Please rate the emotional intensity you feel in response to this picture:

1 = Not at all
2 = Mildly
3 = Moderately
4 = Strongly
5 = Extremely

The total number of responses in the emotion reactivity task for each of the five ratings (1, 2, 3, 4, or 5, corresponding to increasing intensity) were calculated and subsequently converted into a percentage score (percentage of total responses). Changes in emotional reactivity due to sleep and wake were assessed using repeated measures ANOVA models. Due to the averages being constant when using normalized percentages, the main effect of "Test" (Test1, Test2) is not meaningful. Therefore, the primary focus was on the interaction effect.

Task design: Within each participant, Test1 and Test2 were comprised of the same 150 trials separated into five runs of 30 images and presented in the same order, while the order of runs was randomized between participants. The circadian control test also consisted of 150 trials separated into five runs with similar duration.

Experimental design considerations: Although previous studies suggest sleep enhances emotional memory consolidation (Payne, et al., 2008; Wagner, et al., 2001; Wagner, Hallschmid, Rasch, & Born, 2006a; Walker & van der Helm, 2009b), the current study design, with a focus on emotional reactivity, excludes the assessment of memory for three specific reasons. First, is an imbalanced test procedure; during Test2, if participants would have not only rated the emotional intensity (as in Test1), but also had to make a corresponding memory judgment, significant differences in demand characteristics would have been inherent between the two tests. Such a difference would prevent a valid comparison of emotional reactivity (brain and behavioral) between Test1 and Test2. Second, intermixing a memory judgment with emotional intensity ratings on each trial introduces a task-switching confound for Test2 that was not present in Test1. Furthermore, based on previous research (Kanske, Heissler, Schonfelder, Bongers, & Wessa, 2011), such memory testing, representative of a distractor, can inadvertently alter subjective emotional and amygdala reactivity. Finally, a memory test outside the scanner after

Test2 would be uninterpretable. Specifically, such a test would be confounded in part by double encoding (double exposure prior to testing): participants would have been exposed to the stimuli twice: once at Test1 and once at Test2. As a consequence, and compounded by the time delay between Test2 and the test outside the scanner, such a measure of memory could potentially be influenced by encoding, consolidation, reactivation/reconsolidation or a combination of all three, none of which could have been adequately dissociated from each other.

Stanford Sleepiness Scale (SSS)
To assess levels of subjective sleepiness, prior to each emotional ratings task session, all participants completed the validated Stanford Sleepiness Scale (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973); a standard measure of subjective alertness ranging across a 7-point scale, with "1" being most alert.

Functional MRI (fMRI)
Acquisition: Blood oxygenation level–dependent contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens 3 Tesla MRI scanner with a 12-channel head coil. Each image volume consisted of 37 interleaved 3mm slices (96 × 96 matrix; 123 volumes, TR = 2000 ms; TE = 23 ms; voxel size: 2.33 x 2.33 x 3.3mm, FOV = 224mm, 10% distance factor, flip angle = 90°) with GRAPPA (acceleration factor: 2). The first four volumes of each run were discarded to allow for T1 equilibration effects. One high-resolution, T1-weighted structural scan was acquired at the end of the first scanning session (256 × 256 matrix; TR = 1900 ms; TE = 2.52 ms; flip angle = 9°; FOV = 256 by 256 mm; 1 x 1 x 1mm voxels).

Preprocessing: Preprocessing and data analysis were performed using Statistical Parametric Mapping software implemented in Matlab (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Images were slice scan time corrected and motion corrected, and then spatially normalized to the Montreal Neurological Institute template and smoothed using an 8mm full-width-at-half-maximum (FWHM) Gaussian kernel using default parameters in SPM8. For each subject, trial-related activity was assessed by convolving a vector of trial onsets with a canonical hemodynamic response function. The six movement-related covariates (three rigid-body translations and three rotations determined from the realignment preprocessing step) were used as regressors in the design matrix for modeling movement related artifact in the time series. Nonsphericity of the error covariance was accommodated for by a first-order autoregressive (AR1) model, in which the temporal autocorrelation was estimated by pooling over suprathreshold voxels (Friston, et al., 2002).

Contrasts and the GLM: A general linear model (GLM) (Friston, 1995) was specified for each participant to investigate the effects of interest. Contrasts were created at the first level using parametrical modulation (Buchel, Holmes, Rees, & Friston, 1998), integrating the emotional intensity rating of each individual stimulus, allowing for the elucidation of brain activation that co-varied with this regressor (subjective emotional intensity), resulting in a corresponding *t*-statistic for every voxel. Specifically, this method afforded an evaluation of brain reactivity that increased in accordance with participant's increasing emotional intensity ratings – the metric of experimental focus. The resulting contrasts were then taken through to a second level, random effects analysis to assess group-level effects. The *a priori* focus was on the amygdala, based on both the neurobiological frameworks that predict a role for REM sleep in modulating amygdala

reactivity (Levin & Nielsen, 2007; Walker & van der Helm, 2009b), as well as its established involvement in emotional reactivity tasks using similar affective stimuli (Britton, et al., 2006; Curcic-Blake, Swart, & Aleman; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Lane, et al., 1997; Taylor, et al.). Task-specific regions of interest (ROIs) were anatomically defined using left and right amygdalae masks, produced by the Wake Forest University Pick Atlas http://www.fmri.wfubmc.edu/download.htm); a validated method previously used to assess amygdala activity in response to IAPS pictures (Andreano & Cahill; Yoo, Gujar, et al., 2007b). Statistical inferences for the amygdala ROIs are reported using a threshold of $\boldsymbol{P} < 0.05$ FWE, correcting for multiple comparisons (Lieberman & Cunningham, 2009; Poldrack & Mumford, 2009). To assess associations between amygdala activation and sleep physiology parameters, ANCOVA analyses were used, incorporating each participants sleep parameter value as a regressor of interest, similarly reported using a threshold of $\boldsymbol{P} < 0.05$ FWE, correcting for multiple comparisons (Lieberman & Cunningham, 2009; Poldrack & Mumford, 2009). Individual parameter estimates and sleep parameters were plotted to ensure the correlation was not driven by outliers, according to recent recommendations (Poldrack & Mumford, 2009).

Functional connectivity: Functional connectivity was assessed using psycho-physiological interaction analysis, implemented in SPM8, evaluating how regional network activity co-varies in relation to a source region during task performance (Friston, et al., 1997). Within each group, functional connectivity was examined referenced to the seed region of interest (ROI) at the peak locations of the interaction effect in the main analysis: the amygdala (left and right). A GLM was constructed at the first level using three regressors: i) the deconvolved bold signal from the amygdala seed regions, ii) the subjective emotional intensity stimulus ratings (1-5), and iii) the interaction term between the first and the second regressor (Gitelman, et al., 2003). Contrasts for this interaction term revealed brain regions considered to co-vary as a functional network with the source region: the amygdala (Buchel & Friston, 1997). These subsequent connectivity contrasts were then taken through to a second level, random effects analysis. The *a priori* region of interest was on the medial prefrontal cortex and specifically ventral aspects, based on its established role in top-down regulatory control of amygdala function (Davidson, 2002; Milad, et al., 2004; Morgan, et al., 1993; Phelps, et al., 2004; Quirk, et al., 2003; Quirk & Mueller, 2008; Rauch, et al., 2006; Rosenkranz, et al., 2003; Sierra-Mercado, et al., 2006; Sotres-Bayon & Quirk), and verified functional (Kim, et al.) as well as structural connectivity with the amygdala (Bracht, et al., 2009; Croxson, et al., 2005; Johansen-Berg, et al., 2008). The ventromedial PFC ROI was defined by a 6mm sphere around central coordinates from previously published amygdala functional connectivity studies using emotional reactivity paradigms (Delgado, et al., 2008; Dunsmoor, et al.; Milad, et al., 2005; Osuch, et al., 2008; Phelps, et al., 2004; Sterpenich, et al., 2009) (MNI coordinates [x y z]: -2, 32, -6). Statistical inferences for the vmPFC ROI were similarly reported using a threshold of $\boldsymbol{P} < 0.05$ FWE, correcting for multiple comparisons (Lieberman & Cunningham, 2009; Poldrack & Mumford, 2009).

Sleep Recordings: Sleep on the intervening night between Test1 and Test2 in the Sleep-group was monitored in the laboratory with PSG sleep (23:30hr-07:30hr ± 30min) using a Grass Technologies Comet XL system (Astro-Med, Inc., West Warwick, RI). Electroencephalography (EEG) was recorded at 19 standard locations conforming to the International 10-20 System (Klem, Luders, Jasper, & Elger, 1999) (FP1, FP2, F7, F3, FZ, F4, F8, T3, C3, CZ, C4, T4, T5, P3, PZ, P4, T6, O1, O2). Electrooculography was recorded at the right and left outer canthi (right

superior; left inferior). Electromyography was recorded via three electrodes (one mental, two submental). Reference electrodes were recorded at both the left and right mastoid (A1, A2). Data was sampled first at 800Hz by the amplifier, digitized at 400Hz. All data was stored unfiltered (recovered frequency range of 0.1–100Hz), except for a 60Hz notch filter to remove mainline noise. For recording only, each channel was referenced to a forehead scalp derivation.

Sleep scoring: Sleep-staging was performed in accordance with standardized techniques (Rechtschaffen & Kales, 1968), using C3,C4,O1,O2, right and left EOG and EMG channels. EEG and EOG were referenced to the contralateral mastoid, and filtered to 0.3-35 Hz. EMG used a bipolar montage filtered to 10-70Hz and 0.1-12Hz, respectively. Sleep was visually scored in 30-second epochs using the C3-A2 derivation according to standard criteria (Rechtschaffen & Kales, 1968). Sleep architecture values are reported in **Table 5B**, consistent with previous cross-sectional normative values for this age group (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004).

Spectral analyses: All EEG analyses were performed in MATLAB 7.5 (The Mathworks, Natick, MA), including the add-in toolbox EEGLAB (http://sccn.ucsd.edu/eeglab/). Power spectral analysis of sleep EEG was carried out according to previously published methods (Achermann, 2009; Mander, Santhanam, Saletin, & Walker, 2011a; Nishida, et al., 2009; Saletin, Goldstein, & Walker, 2011). Specifically, EEG channels were re-referenced to the average of the left and right mastoid (A1, A2), with high- and low-pass Finite Impulse Response (FIR) filtering at 0.5Hz and 80Hz, respectively. The all-night recording was then visually artifact-rejected in 5-second epochs. Artifact-laden epochs were removed from subsequent analyses. Power spectral density was calculated by use of a Fast Fourier Transform (FFT) on each hamming-windowed 5-second epoch. FFT results were then sorted according to sleep stage and averaged for each respective stage. Power in bands of interest was calculated by averaging across standard EEG frequency band ranges (Nuwer, et al., 1999): delta (0.8 – 4.6Hz), theta (4.6 – 8Hz), alpha (8 – 12Hz), sigma (12 – 15Hz), beta (18 – 30Hz), and gamma (30 – 40Hz), and log-transformed (Buysse, et al., 2008; Dijk, et al., 1995; Gasser, Bacher, & Mocks, 1982; John, et al., 1980; Krystal & Edinger; Krystal, Weiner, & Coffey, 1995; O'Connor, Sorbel, Morzorati, Li, & Christian, 1999; Parsons, Crosby, Perlis, Britt, & Jones, 1997; Uchida, Maloney, & Feinberg, 1992).

Power in the gamma band (**Figure 7**) was the experimental *a priori* focus motivated by 1) the hypothesized benefit of REM physiology on emotional de-potentiation being mediated by the suppression of central adrenergic neurotransmitter levels (Walker, 2009; Walker & van der Helm, 2009b), 2) related, that gamma EEG activity is a validated marker of central adrenergic activity, proportionally increasing and decreasing with corresponding increases and decreases in adrenergic levels (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976). Therefore, gamma EEG activity served as an indirect proxy for adrenergic activity. Beyond the frequency range specificity, gamma activity over prefrontal cortex derivations (average of FP1 and FP2) was targeted based on 1) the known noradrenergic projections from the locus coeruleus to the prefrontal cortex (Gresch, Sved, Zigmond, & Finlay, 1995; Heidbreder & Groenewegen, 2003; Kawahara, Kawahara, & Westerink, 2001), and 2) the association between prefrontal REM activity and emotional information processing (Nishida, et al., 2009). Therefore, the experimental paradigm was specifically designed to test if the extent of reduced prefrontal

adrenergic activity during REM sleep, as indexed by gamma EEG, predicted the extent of the next-day reduction in emotional reactivity at both the central brain and behavioral level.

Statistical analyses: Statistical analyses were carried out using within- and between-group analyses of variance (ANOVA) models; together with planned within-group paired and between-group unpaired two-tailed *t*-tests, with $P < 0.05$ considered significant. In accordance with previous studies relating EEG spectral values to measures of cognition, correlations between behavioral ratings and power values were performed using Spearman's Rho (Geiger, et al.; Hall, et al., 2000b; Newton, et al., 2004a). All analyses were performed using the software program PASW Statistics version 18.

5.4 Results

Differences in Amygdala Reactivity

We first sought to determine the change in emotional brain reactivity following wake or sleep, focusing a priori on the amygdala (Levin & Nielsen, 2007; Walker, 2009; Walker & van der Helm, 2009b). Consistent with the experimental prediction, a significant group (wake, sleep) 3 test (test 1, test 2) interaction was observed in bilateral amygdala, revealing an overnight decrease in reactivity in the sleep group, yet an increase across the day in the wake group (**Figures 2A and 2B**). Moreover, and consonant with the proposed function of top down regulation (Davidson, 2002; Etkin & Wager, 2007; Milad, et al., 2004; Morgan, et al., 1993; Phelps, et al., 2004; Quirk, et al., 2003; Rosenkranz, et al., 2003; Sierra-Mercado, et al., 2006), these overnight reductions in amygdala activity were additionally associated with changes in ventromedial prefrontal cortex (vmPFC) functional connectivity. Specifically, a significant group (wake, sleep) 3 test (test 1, test 2) amygdala connectivity interaction was observed with the vmPFC (**Figures 2C and 2D**), expressing an overnight increase in the sleep group and converse decrease across the day in the wake group. Thus, a night of sleep decreased amygdala reactivity in response to previously encountered emotional stimuli. Furthermore, this overnight dissipation in amygdala activity was further associated with an increase in vmPFC connectivity.

Change in Subjective Emotional Reactivity

Next, we tested the prediction that these overnight decreases in amygdala responsivity were accompanied by a corresponding reduction in subjective emotional intensity ratings, specifically for the most intense emotional responses (5-ratings), where the greatest hypothesized benefit of sleep should occur. As with amygdala activity, a significant group (wake, sleep) 3test (test 1, test 2) interaction was observed in intense emotional ratings ($p < 0.05$; **Figures 3A and 3B**), decreasing in the sleep group ($p < 0.05$) while increasing in the wake group. Indeed, within the sleep group, there was a significant linear shift in the profile of change across the 1–5 ratings ($p < 0.001$), with reductions in the most intense ratings (4s and 5s), and a progressive increase in neutral ratings (1s and 2s; **Figure 3A**). In contrast, no significant linear trend or reductions in extreme emotional ratings (4s and 5s) were observed in the wake group (**Figure 3B**). Therefore, the overnight decrease in amygdala activity following sleep was additionally accompanied by a concomitant reduction in subjective emotional reactivity in response to these previously encountered affective stimuli.

Associations with REM-Sleep Physiology

We finally sought to test the prediction that the overnight decreases in amygdala and behavioral

reactivity in the sleep group were predicted by the extent of reduced REM-sleep gamma EEG activity; a validated marker of decreased central adrenergic activity (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997). Analysis focused on prefrontal EEG activity, based on this region's dense adrenergic innervation (Berridge & Waterhouse, 2003; Heidbreder & Groenewegen, 2003) and established role in emotion regulation (Diekhof, Geier, Falkai, & Gruber, 2011; Quirk & Beer, 2006). Consistent with this prediction, the extent of overnight decrease in both amygdala and behavioral reactivity was significantly correlated with the extent of reduced prefrontal gamma EEG activity during REM (**Figures 4A–4D**), such that those with the lowest levels of REM-gamma (indicative of lowest central adrenergic activity (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997)) expressed the largest overnight decrease in emotion reactivity.

In order to investigate whether other factors played a significant role in our results, three additional analyses were performed at the level of (1) topography: the strength of the predictive relationship between gamma activity and the change in both amygdala and behavioral reactivity decreased from anterior to posterior EEG derivations, (2) frequency: no other frequency band from these same prefrontal EEG derivations correlated with the change in amygdala activity or behavioral reactivity, and (3) brain state: unlike REM-sleep, no significant correlation was found between prefrontal gamma power during non-REM (NREM) sleep and the changes in emotional responsivity.

Initial amygdala reactivity

To confirm that the task produced robust amygdala activation across all participants, independent of group, an amygdala ROI analysis was performed on data from the first emotional reactivity (Test1). Consistent with previous reports using this stimulus set (Bermpohl, et al., 2006; Britton, et al., 2006; Curcic-Blake, et al.; Hariri, et al., 2002; Lane, et al., 1997), the task produced significant reactivity in both left and right amygdala (**Table 1**). Furthermore, no significant differences in the extent of this amygdala activity were observed between the two groups at Test1 (left: $P > 0.17$ right $P < 0.72$ or when combined across left and right: $P > 0.36$).

Circadian control data

Beyond the comparison of Test1 described above, that the findings reported in the main manuscript were not simply a result of differences in basal emotional reactivity at the different times of day between groups was further examined by comparing brain and behavioral reactivity during the circadian control test. This test used a novel stimulus set (see **Methods**), and was performed after Test2 in the morning in the Sleep-group and evening in the Wake-group. No significant differences in amygdala reactivity were identified between the two groups for this circadian control test, neither left nor right. Furthermore, there was no significant difference in behavioral response ratings, with a lack of significant Group (Sleep, Wake) x Emotional Intensity Rating (1-5) interaction ($F_{4,128} = 0.91$, $P = 0.46$), or a main effect of Group ($F_{1,32} = 0.24$, $P = 0.63$). Additionally, for behavioral reactivity, there was no significant difference between the two groups for any of the individual emotional response ratings (1-5; **Figure 5**), including the most intense emotional ratings ('5's, unpaired *t*-test: $P = 0.14$).

To determine whether the effect of REM sleep on brain and behavioral reactivity were specific to emotional stimuli processed overnight during sleep (the original test set), and not a general effect of sleep on reactivity to all emotional stimuli the next day, the association between REM sleep and reactivity to the novel circadian stimuli was also examined. When entered as a

regressor, REM prefrontal gamma activity showed no predictive relationship with amygdala reactivity to novel stimuli in the morning, neither in the left nor right amygdala. Moreover, there was no significant relationship between REM prefrontal gamma and the proportion of intense emotional rating values for the circadian control stimulus set ('5's: Spearman's $\rho = 0.02$, $P = 0.94$), nor any of the other individual emotional intensity ratings (remaining 1-4; all Spearman's $\rho < 0.21$, all $P > 0.23$).

Together, these analyses indicate similar basal reactivity at the neural (amygdala) and behavioral (response-ratings) level at different times of the day in response to the novel emotional stimuli. However, this study cannot fully exclude circadian effects, as they are inherent to the study design used here. A nap study, where sleep is placed in the middle of the day, compared to no nap, would be ideal in order to maintain similar circadian times in both conditions.

Valence and Arousal effects

To investigate whether the differences in amygdala and behavioral reactivity in the Sleep- and Wake-group were specific within the domain of valence (negative or positive), as well as within the domain of arousal (low or high), subset analyses were conducted. Specifically, the experimental 150 stimuli were median split based on the standardized IAPS ratings of valence (75 negative, 75 positive), and again for arousal (75 low arousing, 75 high arousing), with the same analyses reported in the main manuscript repeated for each.

Valence: For the negative valenced stimulus set, a significant Group (Sleep, Wake) x Test (Test1, Test2) interaction was identified in bilateral amygdala ($F = 8.57$, $P = 0.006$), with a significant overnight decrease in amygdala reactivity in the Sleep-group ($P < 0.03$), while the Wake-group demonstrated a non-significant increase across the day (**Figure 6A & B**). Furthermore, a near-significant Group x Test interaction was observed in the most intense emotional ratings ('5's: $P = 0.067$). Paired *t*-test analyses indicated significant overnight decreases in the Sleep-group ($t_{17} = 2.16$, $P = 0.045$, **Table 2A**), while a non-significant increase in the Wake-group was observed ($t_{15} = 0.43$, $P = 0.67$). Moreover, within the Sleep-group, moving across the 1 – 5 ratings, there was a highly significant linear trend ($F_{1,17} = 7.63$, $P = 0.013$) in the profile of overnight change, while no such significant linear trend was observed in the Wake-group.

A similar significant Group (Sleep, Wake) x Test (Test1, Test2) interaction was observed for the positive stimulus set in the right amygdala ($F = 4.37$, $P= 0.045$), revealing an overnight decrease in amygdala reactivity in the Sleep-group, yet increased reactivity in the Wake-group (**Figure 6C & D**). Behaviorally, a significant Group (Sleep, Wake) x Test (Test1, Test2) interaction was identified for the extreme emotional intensity responses ('5's: $P = 0.036$), with a trend for a decrease in the Sleep-group (paired *t*-test: $t_{17} = 1.89$, $P = 0.071$), yet a non-significant increase in Wake-group ($t_{15} = 1.12$, $P = 0.28$, **Table 2A**). Furthermore, within the Sleep-group, there was a highly significant linear shift across the change in the 1-5 ratings ($F_{1,17} = 12.17$, $P = 0.003$), with no such significant linear shift in the Wake-group.

Therefore, as in the main manuscript with all stimulus types combined, decreases in neural and behavioral reactivity were observed following sleep but not wake for both negative and positive valenced stimuli.

Arousal: Focusing on the high arousing stimulus set, a significant Group (Sleep, Wake) x Test (Test1, Test2) interaction was observed in left amygdala ($F = 7.89$, $P = 0.008$), demonstrating a significant overnight decrease in reactivity in the Sleep-group ($P = 0.029$) and a non-significant

increase in the Wake-group (**Figure 62E & F**). A near-significant Group x Test interaction was observed for the most emotionally intense ratings ('5's: $P = 0.065$), with a decrease from Test1 to Test2 observed following sleep (paired t-test: $t_{17} = 2.09$, $P = 0.053$, **Table 2B**), yet an increase following wake ($t_{15} = 0.47$, $P = 0.643$). Furthermore, a highly significant linear trend ($F_{1,17} = 6.77$, $P = 0.019$) in the overnight change across the behavioral ratings (1–5) was observed in the Sleep-group, while no such significant linear trend was found in the Wake-group.

For the low arousing stimulus set, a significant Group (Sleep, Wake) x Test (Test1, Test2) interaction was identified in the right amygdala ($F = 4.66$, $P = 0.038$), revealing a decrease in amygdala reactivity following sleep, while an increase occurred across the day (**Figure 6G & H**). Moreover, a similar significant Group x Test interaction was observed for the most extreme emotional ratings ('5's: $P = 0.030$). Paired *t*-test analyses indicated a significant overnight decrease in the Sleep group ($t_{17} = 2.27$, $P = 0.037$) and a non-significant increase in the Wake-group ($t_{15} = 1.18$, $P = 0.257$, **Table 2B**). Additionally, within the Sleep-group, there was a highly significant linear shift ($F_{1,17} = 15.05$, $P = 0.001$) in the profile of change, while there was no such significant linear shift in the Wake-group.

Thus, the effects of sleep and wake on emotion reactivity reported in the main manuscript were observed for both the high and low arousal stimulus subsets, based on the standardized picture ratings, with amygdala reactivity effects most pronounced for the high arousal stimulus subset**.**

REM sleep physiology associations

Whether the associations between sleep physiology and emotion reactivity reported in the main manuscript were also affected by other factors was examined in three additional analyses:

1) Topography, by assessing the relationship across the head EEG derivations.

Supporting the experimental predictions, the association between gamma activity and the change in amygdala reactivity was most pronounced in the prefrontal (FP1 & FP2) and frontal (FP3 & FP4) EEG derivations, whereas the effect was absent as electrode derivations progressed posterior (C3 & C4; P3 & P4; O1 & O2, **Table 3**). Similarly, at a behavioral level, the decrease in reactivity was significant only for the prefrontal EEG derivations (**Figure 4D**), and progressively decreased moving towards posterior EEG derivations.

2) Frequency, by examining the association between emotional reactivity and prefrontal REM power in the other EEG bands (delta, theta, alpha, sigma and beta) (Nuwer, et al., 1999). No other frequency band from these same prefrontal EEG derivations correlated with the change in amygdala reactivity (neither left nor right). This included a lack of any significant association between amygdala reactivity and prefrontal REM theta EEG activity, a measure previously associated with the selective consolidation (rather than emotional de-potentiation) of affective memory (Nishida, et al., 2009; Popa, Duvarci, Popescu, Lena, & Pare, 2010). Similarly, at a behavioral level, prefrontal REM theta EEG activity was not significantly associated with a decrease in emotional reactivity (Spearman's rho $\rho = 0.29$). Additionally, no other frequency bands were correlated with the extent of reduction in behavioral reactivity (all Spearman's rho $\rho < 0.41$).

3) Brain-state, by examining the association between emotional reactivity and prefrontal gamma in NREM sleep rather than REM sleep. Unlike REM, no significant correlation was found between prefrontal gamma power during NREM and the change in emotional reactivity (Spearman's rho $\rho = 0.20$, $P = 0.42$).

Together, these results suggest that the physiological sleep association reported in the main manuscript were most pronounced during the brain state of REM, the frequency band of gamma, and the topographical location of prefrontal EEG derivations. However, simply because the other correlations did not reach significance does not mean they did not contribute to the main experimental findings discussed in this paper, as it could simply be due to a difference in signal to noise for these measures. Future studies, with an increased sample size and therefore increased power could shed more light on the influence of NREM sleep, topography and different frequency bands on emotional reactivity.

Response times and subjective sleepiness

Objective response times at each test session for each group are provided in **Table 4A**. Examining the most intense ('5') ratings, the *a priori* focus of the study, no significant Group x Test interaction ($F_{1,32} = 2.10$, $P = 0.16$) nor main effect of Group or Test (both $P > 0.30$) was identified. Subjective sleepiness was assessed with the Stanford sleepiness scale, where a between-group, repeated measures ANOVA for the Sleep- and Wake-group revealed a near-significant Group x Test interaction ($F_{1,31} = 4.46$, $P = 0.06$), with no main effects of Test nor Group (both $F_{1,31} < 0.89$, $P > 0.35$). Despite the interaction not reaching significance, post-hoc *t*-tests were performed to further investigate possible between-group differences in sleepiness scores. The two groups did not demonstrate significant differences in sleepiness at Test1 (Sleep-group mean: 2.4 s.e.m. ± 0.2; Wake-group mean: 2.8 s.e.m. ± 0.4) nor at Test2 (Sleep-group mean: 2.7 s.e.m. ± 0.2; Wake-group mean: 2.0 s.e.m. ± 0.3; both $P > 0.26$).

5.5 Discussion

Taken together, these findings describe an overnight depotentiation of neural (amygdala) and behavioral (subjective) responsivity to previously encountered affective stimuli (Levin & Nielsen, 2007; Walker, 2009; Walker & van der Helm, 2009b). Moreover, the success of this depotentiation was predicted by REM sleep gamma EEG activity, a surrogate marker indexing central adrenergic activity (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997). This data can be interpreted within a recently proposed homeostatic model of REM sleep involving the reduction of emotional tone originally associated with prior waking salient experiences, orchestrated by the marked reduction in adrenergic activity during REM sleep (Walker, 2009; Walker & van der Helm, 2009b). Alternatively, or in addition, such findings may be explained by the recognized benefit of REM on emotional memory consolidation (Payne, et al., 2008; Wagner, et al., 2001; Wagner, et al., 2006a; Walker & van der Helm, 2009b), associated with theta EEG activity (Nishida, et al., 2009; Popa, et al., 2010), thereby decreasing postsleep stimulus novelty and hence emotion reactivity. That the changes in neural and behavioral reactivity reported in the current study correlated with REM gamma activity and not theta activity suggests that each component (depotentiation, consolidation), although potential constituents of a broader function of REM (Walker & van der Helm, 2009b), could play different roles. Nevertheless, either mechanism independently, or their combination, may account for our findings and represents a future target for experimental investigation. A future study that includes both an emotional reactivity and an emotional memory component could disentangle the role of REM gamma and REM theta in a more definitive way.

Guided by recent neurobiological models (Walker, 2009; Walker & van der Helm, 2009b), the current study focused on sleep-dependent differences in emotion reactivity within the central nervous system (specifically the brain). However, these models predict similar

downstream adaptive reductions in reactivity within the peripheral nervous system. The consequential impact of such altered central nervous system processing on peripheral nervous system reactivity has potentially important implications, especially considering their respective efferent-afferent interactions known to support symbiotic emotional homeostasis (Critchley, 2005).

These findings also move beyond their experimental boundaries by offering novel insights into the well-documented (but poorly understood) occurrence of sleep abnormalities in mood disorders. Our results afford mechanistic insights into these disorders where amplified emotion reactivity and REM-sleep disruption are highly comorbid. This is especially the case in anxiety disorders (Benca, et al., 1992; Benca, et al., 1997; Harvey, 2008). Of special relevance in this context is the condition of post-traumatic stress disorder (PTSD), characterized by REM abnormalities (Breslau, et al., 2004; Germain, Buysse, & Nofzinger, 2008a; Habukawa, Uchimura, Maeda, Kotorii, & Maeda, 2007; Spoormaker & Montgomery, 2008b; Yetkin, Aydin, & Ozgen, 2010), hyperarousal (Frewen & Lanius, 2006; Krystal & Neumeister, 2009; Nutt, 2000; O'Donnell, Hegadoren, & Coupland, 2004; Strawn & Geracioti, 2008), and exaggerated amygdala reactivity (Bryant, et al., 2008; Shin, et al., 2004b; Williams, et al., 2006). Indeed, the current findings offer a putative neurobiological explanation for the recent pharmacological treatment success involving nighttime suppression of adrenergic activity in PTSD, restoring REM sleep features and improving clinical symptomatology (Raskind, et al., 2007; Raskind, et al., 2003; Shad, Suris, & North, 2011).

Figures

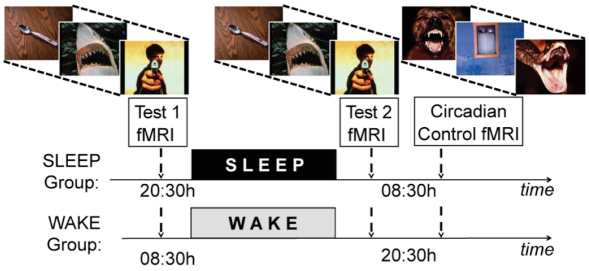

Figure 1 Experimental design: Both groups performed an emotion reactivity test twice inside the fMRI scanner; separated by 12hr, involving the rating and subsequent re-rating of the same standard set of 150 affective picture stimuli (three example images provided). The change in emotional reactivity following sleep (Sleep-group) or wake (Wake-group) was assessed by comparing data at Test1 (pre wake or sleep) with that at Test2 (post wake or sleep); Test2 – Test1. To examine possible time-of-day differences in emotional reactivity, independent of wake or sleep, an additional circadian control test was performed immediately after Test2 (morning in the Sleep-group, evening in the Wake-group). The circadian control test consisted of a novel set of 150 emotional images not seen before, matched in terms of arousal and valence to the original set used in Test1 and Test2 (sets used counterbalanced as either the experimental set or circadian control set).

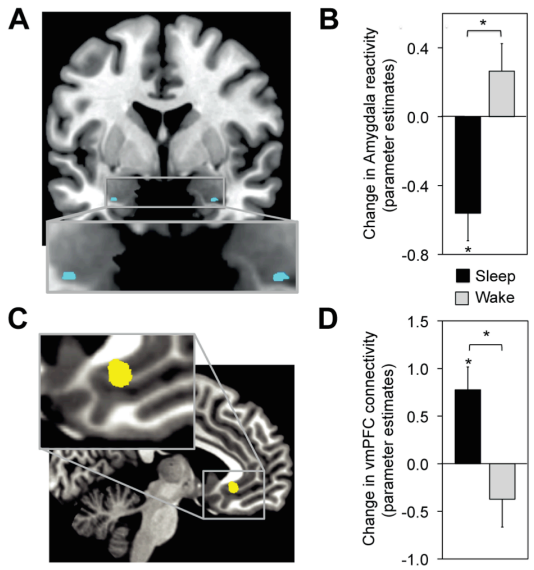

Figure 2 (A,B) fMRI differences in emotion reactivity: Group x Test session interaction in bilateral amygdala (blue), demonstrating a significant decrease in activity from Test1 to Test2 in the Sleep-group yet increase in the Wake-group (peak MNI coordinates [x, y, z]; left: –27, 0, -27; Z-score = 3.07; right: 27, 0, –27; Z-score = 3.14, average of significant voxels depicted in bar graph, a result of this particular interaction ANOVA contrast). (C,D) fMRI differences in functional connectivity: Group x Test session interaction in amygdala-vmPFC connectivity (yellow), demonstrating increased connectivity from Test1 to Test2 in the Sleep-group yet decreased coupling in the Wake-group (peak MNI coordinates [x, y, z]; –6, 30, -7; Z-score = 3.22, average of significant voxels depicted in bar graph, a result of this particular interaction ANOVA contrast). Differences in activation and connectivity using an amygdala ROI and a vMPFC ROI, thresholded at $P < 0.05$ family wise error (FWE) corrected for multiple comparisons. *$P < 0.05$, Error bars: S.E.M.

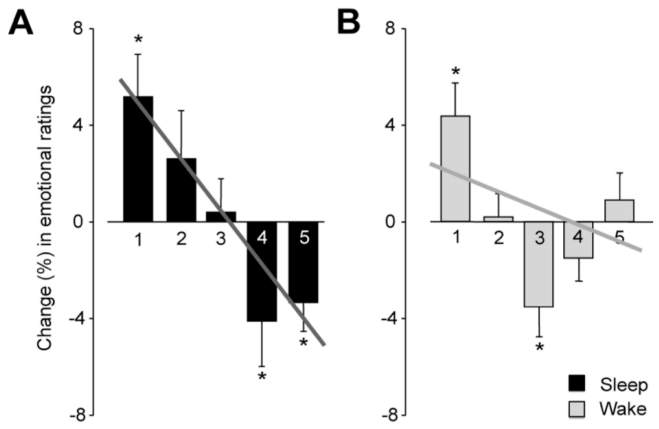

Figure 3 Change in behavioral reactivity between Test1 and Test2 for (A) the Sleep-group, expressing a significant linear shift ($P < 0.001$), and significant decrease in the most intense emotional ratings (4's, 5's) and increase in non-emotional ratings, (B) the Wake-group, resulting in no significant linear profile shift or decrease in the most intense emotional ratings. *$P <$ 0.05, Error bars: S.E.M.

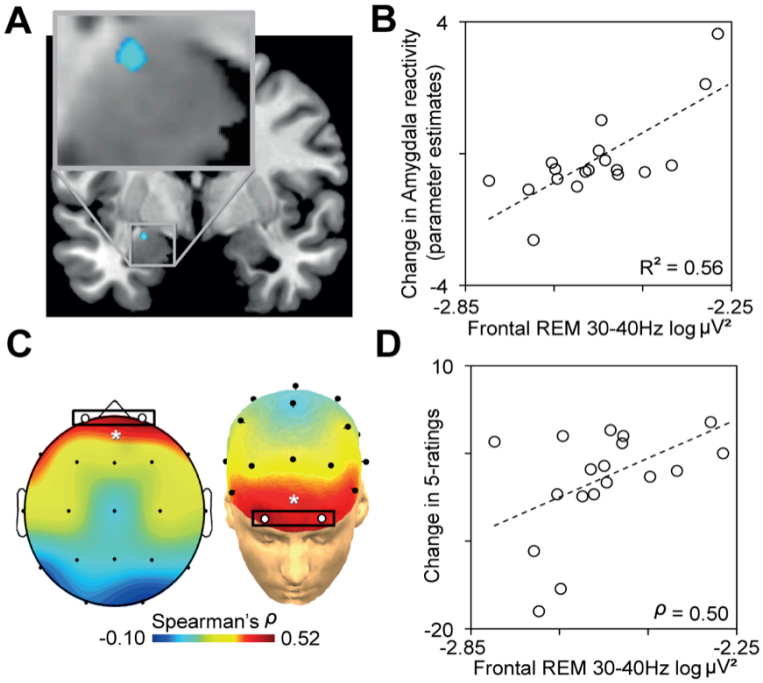

Figure 4 (A) Pearson's correlation between prefrontal EEG gamma power (average of Fp1-Fp2 EEG derivations) and the extent of overnight decrease in amygdala (blue) activity from Test1 to Test2 (peak MNI coordinates [x, y, z]; –22, -7, -17; Z-score = 3.55, average of significant voxels depicted in bar graph, a result of this particular ANCOVA contrast), and (B) corresponding scatter plot of this amygdala-gamma power relationship, with R^2 noted only for descriptive purposes (Poldrack & Mumford, 2009; Vul, 2009), with less gamma activity predicting the degree of overnight decrease in emotional activity. (C) Topographical Spearman's correlation (ρ) plot between REM gamma power and the change in emotional reactivity (5-ratings) demonstrating a significant prefrontal relationship (average of Fp1-Fp2, white circles), and (D) corresponding scatter plot and Spearman's ρ value: the extent of reduced gamma EEG activity over prefrontal cortex was proportional to the overnight decrease in emotional reactivity. *P < 0.05.

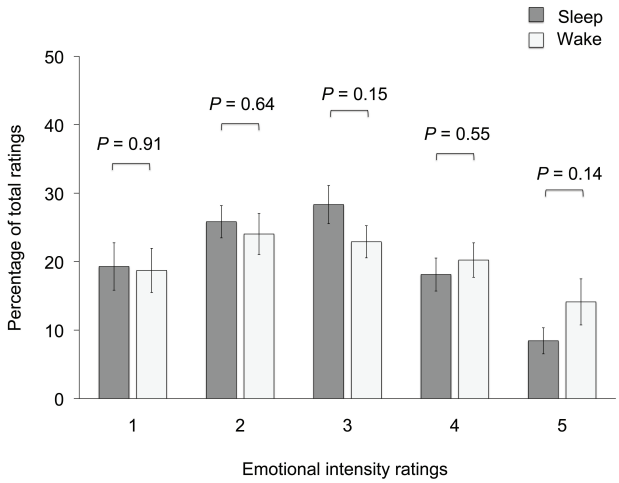

Figure 5: Emotional intensity ratings for the circadian control test in the Sleep-group (dark grey bars; morning) and Wake-group (light grey bars; evening). No between-group differences were observed for any of the 5 intensity ratings (unpaired *t*-tests: all $t < 1.52$, $P > 0.14$).

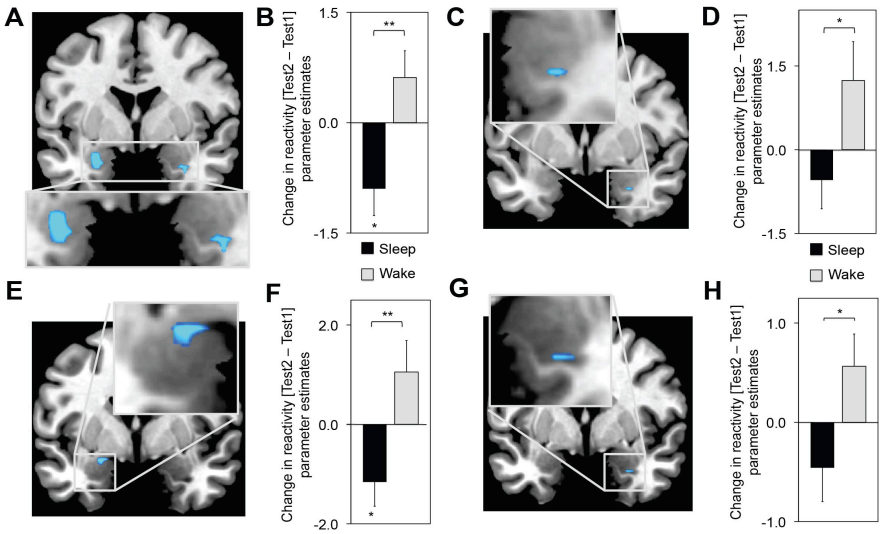

Figure 6: Change in amygdala reactivity between Test1 and Test2 separated by valence and arousal. (**A,B**) Negative valence: Group x Test session interaction in bilateral amygdala (blue), demonstrating a significant overnight decrease in the Sleep-group yet an increase across the day in the Wake-group (peak MNI coordinates [x, y, z]; left: –27, 0, -27; Z-score = 2.76; right: 32, 0, –30; Z-score = 2.75,). (**C,D**) Positive valence: Group x Test session interaction in the right amygdala (blue), demonstrating decreased reactivity following sleep (Sleep-group) and increased reactivity across the day (Wake-group) (peak MNI coordinates [x, y, z]; 29, 2, –27; Z-score = 2.32). Statistical maps thresholded at $P < 0.05$, uncorrected. (**E,F**) High arousal: Group x Test session interaction in the left amygdala (blue), demonstrating a significant decrease in reactivity from Test1 to Test2 in the Sleep-group yet an increase in the Wake-group (peak MNI coordinates [x, y, z]; –22, 0, -14; Z-score = 2.59). (**G,H**) Low arousal: Group x Test session interaction in the right amygdala (blue), demonstrating decreased overnight reactivity in the Sleep-group and increased reactivity across the day in the Wake-group (peak MNI coordinates [x, y, z]; 29, 0, –27; Z-score = 2.22). Statistical maps using amygdala anatomical ROI thresholded at $P < 0.05$ uncorrected. *$P < 0.05$, ** $P < 0.01$. Error bars: S.E.M. All bar graphs average of significant voxels, a result of this particular ANOVA interaction contrast.

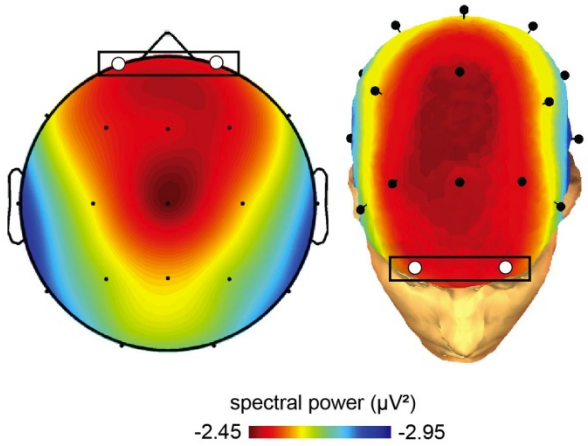

Figure 7: Gamma EEG power (log-transformed) during REM sleep, shown on 2 dimensional (left) and 3 dimensional (right) topographical plots.

Tables

Table 1: Test1 amygdala activity (mean ± SD)

	All participants	Sleep group	Wake group	Unpaired *t*-test
Right amygdala MNI [x y z] 18; 0; -14	0.58 ± 0.16	0.37 ± 0.12	0.81 ± 0.32	$P = 0.18$
Left amygdala MNI [x y z] -20; -5; -14	0.53 ± 0.16	0.47 ± 0.15	0.62 ± 0.33	$P = 0.66$

Table 2A: Percentage (± SEM) emotional intensity ratings for Test1 and Test2 separated by valence.

Emotional Intensity	1	2	3	4	5
Negative stimuli					
Sleep-group					
Test 1	12.3 ± 1.9	19.1 ± 1.9	25.6 ± 2.1	24.5 ± 2.3	18.5 ± 3.1
Test 2	16.0 ± 2.7	23.0 ± 2.8	25.5 ± 2.8	21.5 ± 2.6	14.0 ± 3.2
Wake-group					
Test 1	12.8 ± 1.6	19.7 ± 2.3	25.0 ± 2.3	25.8 ± 1.9	16.7 ± 2.8
Test 2	16.5 ± 1.4	21.6 ± 2.9	19.9 ± 1.8	24.6 ± 2.3	17.4 ± 2.7
Positive stimuli					
Sleep-group					
Test 1	18.5 ± 3.2	33.8 ± 3.0	26.2 ± 2.4	14.9 ± 2.5	6.6 ± 1.8
Test 2	25.5 ± 4.5	34.9 ± 2.5	25.0 ± 3.5	9.8 ± 2.1	4.8 ± 1.5
Wake-group					
Test 1	20.2 ± 2.7	30.1 ± 3.9	26.0 ± 3.0	17.8 ± 3.0	6.0 ± 2.3
Test 2	26.0 ± 3.1	28.5 ± 3.8	22.9 ± 2.5	15.5 ± 3.0	7.2 ± 2.2

Table 2B: Percentage (± SEM) emotional intensity ratings for Test1 and Test2 separated by arousal.

Emotional Intensity	1	2	3	4	5
High arousing stimuli					
Sleep group					
Test 1	5.3 ± 2.2	18.8 ± 2.6	27.8 ± 2.2	27.3 ± 2.6	20.9 ± 3.5
Test 2	9.2 ± 3.3	22.0 ± 2.6	29.3 ± 3.7	23.6 ± 2.7	15.9 ± 3.6
Wake group					
Test 1	7.5 ± 1.4	16.8 ± 2.5	27.1 ± 3.0	30.5 ± 3.0	18.2 ± 3.7
Test 2	10.5 ± 2.1	20.2 ± 3.0	23.7 ± 2.6	26.7 ± 2.7	19.0 ± 3.3
Low arousing stimuli					
Sleep group					
Test 1	25.6 ± 3.1	34.2 ± 2.3	23.9 ± 2.3	12.1 ± 2.4	4.3 ± 1.2
Test 2	32.4 ± 4.1	36.0 ± 2.5	21.1 ± 3.0	7.7 ± 1.8	2.9 ± 1.1
Wake group					
Test 1	25.4 ± 3.0	33.0 ± 3.5	23.9 ± 2.2	13.1 ± 2.0	4.5 ± 1.6
Test 2	32.0 ± 2.6	30.0 ± 2.9	19.1 ± 1.9	13.3 ± 2.3	5.6 ± 1.6

Table 3: Correlation between gamma EEG power and the change in amygdala reactivity

EEG derivations	Location	# Significant voxels	z-score	MNI coordinates
FP1 and FP2	Left amygdala	11	3.55	-22; -7; -17
F3 and F4	Left amygdala	8	3.26	-24; -7; -14
C3 and C4	-	-	-	-
P3 and P4	-	-	-	-
O1 and O2	-	-	-	-

MNI coordinates [x y z] of peak voxel in amygdala. - represents no significant voxels.

Table 4A: Response times in milliseconds (mean ±SEM) for both groups in each of the 5 ratings across Test1 and Test2

Emotional Intensity	1	2	3	4	5
Sleep group					
Test1	2787 ± 70	2930 ± 77	2850 ± 71	2919 ± 81	2695 ± 62
Test2	2716 ± 60	2805 ± 72*	2726 ± 46*	2814 ± 70*	2745 ± 68
Wake group					
Test1	2927 ± 74	3053 ± 59	3023 ± 59	2975 ± 65	2853 ± 63
Test2	2697 ± 55*	2897 ± 43*	2801 ± 48*	2797 ± 61*	2751 ± 51*

*unpaired t-test between Test1 and Test2 $P < 0.05$.

Table 4B: Percentage (%) of emotional intensity ratings per session (mean ± SEM)

Emotional Intensity	1	2	3	4	5
Sleep-group					
Test1	15.4 ± 2.4	26.4 ± 2.3	25.9 ± 1.8	19.7 ± 2.2	12.6 ± 2.3
Test2	20.7 ± 3.4	28.9 ± 2.2	25.2 ± 2.9	15.8 ± 2.1	9.4 ± 2.3
Wake-group					
Test1	16.5 ± 1.8	24.9 ± 2.8	25.5 ± 2.3	21.8 ± 2.2	11.3 ± 2.5
Test2	21.2 ± 2.0	25.0 ± 2.7	21.4 ± 2.0	20.0 ± 2.2	12.3 ± 2.3

Table 5A: Average total sleep time five nights prior to the study based on sleep log and actigraphy data for both groups (mean ± SD)

Night		1	2	3	4	5
Sleep-group	Sleep Log	7.6 ± 1.2	7.2 ± 1.0	7.5 ± 0.8	8.0 ± 0.9	7.7 ± 0.8
	Actiwatch	7.3 ± 0.9	6.7 ± 1.3	7.3 ± 0.8	8.0 ± 1.0	7.7 ± 0.9
Wake-group	Sleep Log	8.0 ± 1.2	7.4 ± 1.3	7.7 ± 1.3	7.7 ± 1.2	7.6 ± 1.1
	Actiwatch	7.7 ± 1.0	7.0 ± 1.3	7.6 ± 1.2	7.6 ± 1.2	7.5 ± 1.2

Table 5B: Polysomnography sleep-stage values for the Sleep-group (mean ± SD)

	Sleep Time (min)	% Sleep Time	Obtained by % of Participants
Total sleep time	452.8 ± 16.2		
Stage 1	29.3 ± 16.7	6.6 ± 3.9	100 %
Stage 2	202.4 ± 30.6	44.8 ± 7.6	100 %
SWS	120.1 ± 36.0	26.4 ± 7.6	100 %
REM	101.0 ± 23.2	22.2 ± 4.7	100 %

Mean duration (in minutes), percent (%) and standard deviation (SD) of total sleep time, percentages of participants obtaining the stage, NREM sleep stages 1 and 2, slow-wave sleep (SWS; NREM stage 3 & stage 4) and rapid eye movement sleep (REM).

Chapter 6. Bistable amygdala connectivity between brainstem and prefrontal cortex determines intra-individual differences in emotional responsivity over time, and sleep.

6.1 Overview

Emotions represent an organizing principle governing human behavior, yet fluctuations in emotion sensitivity occur within a given individual, over time. Increasing evidence describes a role for sleep, and specifically rapid-eye movement (REM) sleep, in emotional brain regulation. Emerging from these findings is a recent framework proposing that such overnight limbic brain changes are mediated by the marked suppression of central adrenergic activity during REM. However, the role of sleep, and specifically REM physiology, in regulating affective resting brain networks remains unknown. Combining functional MRI (fMRI) and EEG sleep physiology, here we investigate the role of REM sleep in recalibrating amygdala resting-state brain connectivity within individuals. We demonstrate the existence of a bistable amygdala network that reliably shifts, within individuals, between phylogenetically older fight-flight brainstem regions and top-down prefrontal cortex nodes across 24hr. Moreover, sleep, and specifically REM-sleep physiology, predicted the shift in bistable amygdala connectivity and with it, participants' unique emotion sensitivity fluctuations. Importantly, demonstrating REM sleep specificity, no such predictions were found for NREM sleep. These findings establish that resting networks of the human brain, at least for the amygdala, are not stable within an individual, but are instead dynamic, showing marked differences following wake and sleep.

6.2 Background

We commonly fluctuate in our emotional sensitivity over time. Music, for example, can evoke intense emotions at one moment, yet the same musical score may leave you indifferent at another point in time. Conversely, changes in innate emotional state can also drive your preference for a particular kind of music one day compared to the next. Such anecdotes suggest that emotional sensitivity is not stable within an individual from one moment to the next, but instead varies in a state-like manner. There is however, a surprising lack of research on what factors drive such *intra*-individual changes in emotional sensitivity across hours or days as most studies focus on *inter*-individual changes. One factor (amongst others) that may contribute to such changes could simply be the passage of time. Indeed, subjective emotional sensitivity *between* individuals can change across several hours(Tucker, Feuerstein, Mende-Siedlecki, Ochsner, & Stern, 2012; van Eekelen, Kerkhof, & van Amsterdam, 2003), although it is unknown whether emotion sensitivity changes *within* individuals across hours. One study investigating the response to daily stressors within the same individuals across 2 weeks, found a re-test correlation of 0.64 for stress-induced increases in anxiety(Cohen, et al., 2000; Eid & Diener, 1999; Uchino, Holt-Lunstad, Bloor, & Campo, 2005). Although such a correlation is high, it also implies that less than two thirds of the

variability is stable. What factors drive the remaining 35% of unexplained variance in intra-individual fluctuations remains unknown. .

One potential factor that could underlie intra-individual changes in emotional sensitivity is a change in neural activity. There is an increasingly well-established literature on brain structures involved in emotional processing. The most prominent neural structure for explaining variability in emotion sensitivity is the amygdala, a brain area essential for learning the emotional significance of a stimulus in the environment and orchestrating the emotional response to it(Phelps, 2006; Phelps & LeDoux, 2005). Another key brain region that has been shown to modulate amygdala output and the accompanying behavioral phenomena during affective processing is the medial prefrontal cortex (mPFC). The mPFC is considered to play an important role in emotion sensitivity, through its anatomical connection with the amygdala, that has an established role in the regulation of emotion sensitivity(Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Fisher, et al., 2009; Kim, et al., 2011). Furthermore, highlighting the role of the mPFC in emotion sensitivity, functional neuroimaging studies on emotional regulation and reappraisal have found increased prefrontal activity and concomitant decreased amygdala activity during successful trials. In addition to the regulatory control of the mPFC, the noradrenergic system, originating mostly in the locus coerules (LC), has a central role in the response to emotional stimuli and stressors. Noradrenergic efferents from the LC have been found to directly innervate limbic structures including the amygdala. The LC is believed to initiate affective responses by stimulating amygdala activity producing hyperarousal(Aston-Jones, et al., 1991; Berntson, Sarter, & Cacioppo, 2003; Liddell, et al., 2005; Samuels & Szabadi, 2008; Valentino & Van Bockstaele, 2008), and shifting brain networks into a highly reactive state in which limbic pathways prevail over prefrontal pathways.

Another possible factor influencing intra-individual changes in emotional state over time is sleep. Emotion sensitivity has been found to increase following sleep loss, while the presence of sleep has been shown to decrease emotion sensitivity. Sleep deprivation results in heightened emotional sensitivity, increased pupil dilation and increased amygdala activity that can be explained by concomitant increases in amygdala-brainstem connectivity and decreases in amygdala-prefrontal coupling. On the other hand, sleep, and specifically rapid-eye movement (REM) sleep, has been proposed to modulate affective brain function(Levin & Nielsen, 2007; Nielsen & Levin, 2007) based on its unique neurochemical and neurophysiological properties(Walker, 2009; Walker & van der Helm, 2009a). Recent studies have supported the notion of REM-sleep modulating the intensity of affective experiences(Rosales-Lagarde, et al., 2012; Spoormaker, Schroter, et al., 2011; Spoormaker, et al., 2010; van der Helm, et al., 2011b), including the extent of high-frequency EEG activity during REM-sleep, a validated proxy indexing the suppression of central adrenergic levels(Berridge & Foote, 1991; Cape & Jones, 1998), presumably due to (re)processing of those experiences during REM-sleep(Cartwright, et al., 2006; Levin & Nielsen, 2009; Nielsen & Levin, 2007; Walker, 2009; Walker & van der Helm, 2009a). While REM physiology has been found to predict *inter*-individual differences in emotional sensitivity, it is unknown whether REM-sleep or a different sleep stage and associated physiology predicts changes within a given individual rather than across individuals.

Here we examine intra-individual changes in both emotion sensitivity and associated amygdala networks across a 12hr time period. Resting-state functional magnetic resonance imaging (rs-fMRI) was the method of choice, based on its established ability to detect intrinsic functional brain networks. Using rs-fMRI combined with EEG-recorded sleep physiology this design tested whether 1) amygdala connectivity with the mPFC and brainstem is modulated by

intervening sleep and wake, 2) intra-individual changes in emotion sensitivity are predicted by changes in amygdala connectivity, and 3) the extent of decreased adrenergic activity during REM-sleep, indexed by reduced gamma EEG activity, predicts the overnight shift in the amygdala network.

6.3 Methods

In short, thirty-three healthy adults (age: 18-30 years) were randomly assigned to one of two groups each undergoing two rs-fMRI scans (Scan1, Scan2), separated by 12hr containing a night of sleep (Sleep-group, *n*=17, 10-females) or a waking day (Wake-group, *n*=16, 9-females) (**Figure 1a**). Immediately after each rs-fMRI scan, subjective emotion sensitivity was assessed using an affective task in which participants viewed and rated the subjective emotional intensity of standardized affective pictures on a 1–5 scale, corresponding to increasing intensity. Two different sets of stimuli were counter-balanced across sessions and participants, whereby stimuli were always novel, not having been previously viewed.

Participants

Thirty-three healthy participants, age 18-30 years (mean: 21.4, s.d. ± 3.3, 19 Female), were randomly assigned to either the Sleep-group (*n*=17, 10 Female, mean age: 21.8, s.d. ± 3.3) or Wake-group (*n*=16, 9 Females, mean age: 21.0, s.d. ± 3.5). Participants abstained from caffeine and alcohol for the 72hr before and during the entire course of the study and kept a normal sleep-wake rhythm (7–9 h of sleep per night with a sleep onset time before 2:00hr in the morning) for the 5 nights prior to study participation, as verified by sleep logs and actigraphy (a wristwatch movement sensor, sensitive to wake and sleep states). Exclusion criteria, assessed using a prescreening questionnaire, were: a history of sleep disorders, neurologic disorders, closed head injury, Axis I psychiatric disorders according to the DSM-IV criteria (encompassing mental disorders including depression, anxiety disorders, bipolar disorder, attention deficit disorder and schizophrenia), history of drug abuse and current use of anti-depressant or hypnotic medication. Subjects who reported drinking excessive amounts of caffeine (defined as 3 or more caffeine-containing substances a day such as coffee, tea or caffeine containing soft drinks) were excluded from the study. The study was approved by the local human studies committee, with all participants providing written informed consent.

The Wake-group and the Sleep-group did not differ in the average sleep amount obtained 5 nights prior to the study based on objective actigraphy (unpaired *t*-test $p = 0.55$) or subjective sleep logs (unpaired *t*-test $p = 0.72$, **Table 1**). This was similarly the case when assessing each of the 5 nights separately between the two groups when using either sleep logs or actigraphy (unpaired *t*-tests, all $p > 0.34$), including the last night prior to the study (sleep logs unpaired *t*-test $p = 0.95$ and actigraphy $p = 0.76$). Therefore, participants adhered to the study protocol sleep requirements, and sleep amounts were not different between groups, across any night leading up to the study.

Experimental design

Both groups underwent two fMRI resting-state scans (Resting-State 1, Resting-State 2) separated by either 12hr across the day (Wake-Group) or 12hr containing a full 8hr night of sleep (Sleep-Group) measured using full-head (19-channel) EEG polysomnography recordings (**Figure 1a**). Following each resting-state scan, participants in both groups performed the emotion responsivity task, involving the rating of a standard set of affective picture stimuli (details

below). Participants in the Sleep-group arrived in the laboratory at 7:30PM (± 30min) to complete the first resting-state scan (Resting-State 1) in the fMRI scanner at 8:30PM (± 30min), followed by the first emotion responsivity task (Emotion Responsivity Task 1). After completion of the task participants were prepared for polysomnographic (PSG) sleep recording (details below) in the laboratory, with lights off from 11:30PM - 7:30AM (± 30min). The next morning, participants underwent the second resting-state scan (Resting-State 2) in the fMRI scanner at 8:30AM (± 30min), similarly followed by the second emotion responsivity task (Emotion Responsivity Task 2). Participants in the Wake-group underwent a similar protocol but the times of the resting-state scans and emotion responsivity tasks were performed in the reverse order. The first scan (Resting-State 1) and emotion responsivity task (Emotion Responsivity Task 1) were performed in the morning at 8:30AM (± 30min), and following a day of standard waking activities outside the laboratory, the second scan (Resting-State 2) and emotion responsivity task (Emotion Responsivity Task 2) were performed at 8:30PM (± 30min).

Task Stimuli: Each emotion responsivity task (Emotion Responsivity Task 1 & 2) consisted of viewing 150 pictures, selected from the standardized International Affective Picture System (IAPS)(Lang, 1997). Two matched sets of 150 stimuli were counter-balanced as either the first or second set. Both image sets ranged from low to high arousal (arousal set 1: range 2.28–7.12, mean ± s.d. = 5.00 ± 1.01; arousal set 2: range 2.03–7.35, mean ± s.d. = 4.95 ± 1.29) containing both positive and negative valence (valence set 1: range 1.45–8.28, mean ± s.d. = 5.03 ± 1.95; valence set 2: range 1.46–8.22, mean ± s.d. = 4.92 ± 1.86). Neither set were statistically different from each other in terms of arousal ($p > 0.70$) or valence levels ($p > 0.64$).

Trial timing: Each trial lasted 7700ms, beginning with a black fixation crosshair on a white background (500 ms) followed by the visual-picture presentation (2000 ms), then a response screen (2500 ms), followed by a grey fixation cross on a white background (2700 ms). Images were presented in a pseudorandom order with no more than two pictures of similar valence presented in a row (positive, negative or neutral). Ratings were made through right-handed button responses across a 1-5 emotional scale in accordance with previous studies measuring emotional intensity(Britton, et al., 2006), with the following instruction presented during the response screen:

Please rate the emotional intensity you feel in response to this picture:

1 = Not at all
2 = Mildly
3 = Moderately
4 = Strongly
5 = Extremely

The average rating across all 150 trials was calculated and the total number of responses for each of the five ratings (1, 2, 3, 4, or 5, corresponding to increasing intensity) and subsequently converted into a percentage score (percentage of total responses). Intra-individual changes in average emotion responsivity across 12hr were assessed by subtracting the average rating in Task 1 from the average rating in Task 2. Intra-individual changes in the distribution of responses across the 5 intensity ratings were similarly assessed by subtracting the percentage for each rating in Task 1 from the percentage for each rating in Task 2.

Stanford Sleepiness Scale (SSS)
To assess levels of subjective sleepiness, prior to each resting-state scan, all participants completed the validated Stanford Sleepiness Scale(Hoddes, Zarcone, Smythe, Phillips, et al., 1973); a standard measure of subjective alertness ranging across a 7-point scale, with "1" being most alert.

Neuroimaging (fMRI)
Acquisition: Blood oxygenation level–dependent contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens 3 Tesla MRI scanner with a 12-channel head coil. Each image volume consisted of 37 interleaved 3mm slices (185 contiguous volumes, 96 × 96 matrix; TR = 2000 ms; TE = 23 ms; voxel size: 2.33 x 2.33 x 3.3mm, FOV = 224mm, 10% distance factor, flip angle = 90°) with GRAPPA (acceleration factor: 2). The first four volumes of each resting-state scan were discarded to allow for T1 equilibration effects. For spatial normalization and localization, a high-resolution, T1-weighted structural scan was acquired at the end of the first scanning session (256 × 256 matrix; TR = 1900 ms; TE = 2.52 ms; flip angle = 9°; FOV = 256 by 256 mm; 1 x 1 x 1mm voxels). During the 6-min long functional scan participants were asked to keep their eyes open, not to fall asleep, think of nothing in particular and let their minds wander, while focusing on the fixation cross projected in black on a white background on a back-projected screen.

Preprocessing: First, scan to scan variance was assessed for quality assurance using tsdiffana and individual scans with supra-threshold shifts (indicating high subject movement) were removed and replaced with the average of surrounding scans, these time-points were subsequently modeled out with dummy repressors (this effected 5 subjects and 6 scans total with a maximum of 5 deleted volumes per scan). After this quality assurance procedure, functional images were slice time corrected and then motion corrected using Statistical Parametric Mapping software implemented in Matlab (SPM8; Wellcome Department of Cognitive Neurology, London, UK). The functional images were spatially co-registered to the anatomical MRI image and then normalized to the Montreal Neurological Institute template and resampled to $2mm^3$ voxels using SPM8. The images were then spatially smoothed using a 6mm full-width-at-half-maximum (FWHM) Gaussian kernel in AFNI. Finally, the time series at each voxel was temporal filtered using a band-pass filter between the frequencies of 0.08 and 0.009-Hz using AFNI in order to isolate the BOLD fluctuations of interest as done in other previous human resting state studies(Fox, et al., 2005; Huang, et al., 2012; Vincent, et al., 2007). Previous studies have found this range to have the greatest power in resting state BOLD signal(Biswal, Yetkin, Haughton, & Hyde, 1995) (Fransson, 2006), the strongest correlations between regions(Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006), and to relate most closely to task-based activations(Fransson, 2006) and underlying structural connectivity(Greicius, Supekar, Menon, & Dougherty, 2009; Vincent, et al., 2007).

For each participant regions within the white matter tracts and the lateral ventricles were defined individually and the time-series from these regions was extracted and entered as a regressor of non-interest for all connectivity analyses in order to help control for non-specific physiological fluctuations throughout the scan. To define these regions, first the anatomical MRI was segmented using SPM8 into gray matter, white matter and cerebrospinal fluid (CSF) maps and normalized to standard MNI space. For the individual specific white matter region, the white

matter map was then iteratively eroded using the function spm_erode.m from the SPM8 toolbox until less than two-thousand 1mm^3 voxels remained in the mask. This procedure results in large centrally located masses within the white matter tracts reducing possible contamination of white-matter signal from neighboring grey-matter regions. For the region in the lateral ventricles, we took the intersection of the individual subject's CSF map with the standardized lateral ventral mask supplied by SPM8 ensuring that all voxels were both within the individual subject's CSF and within the classically defined structure of the lateral ventricles.

Amygdala connectivity analyses: For connectivity analyses, the *a priori* focus ('seed' region) was on the amygdala, based on both the neurobiological frameworks that predict a role for REM-sleep in modulating amygdala activity(Levin & Nielsen, 2007; Walker & van der Helm, 2009b), as well as its established involvement in emotional reactivity tasks using similar affective stimuli(Britton, et al., 2006; Curcic-Blake, et al.; Hariri, et al., 2002; Lane, et al., 1997; Taylor, et al.). An anatomical ROI of the amygdala was derived using the Harvard-Oxford probabilistic atlas distributed with FSL (http://www.fmrib.ox.ac.uk/fsl/), similar to previous amygdala studies(Kim, et al., 2011; van der Zwaag, Da Costa, Zurcher, Adams, & Hadjikhani, 2012). The ROI was defined to only include voxels with a 50% or higher probability of being labeled as the amygdala (left: 1816mm^{3,} right: 2224mm^3), in order to reduce potential confounds of adjacently located structures. All subsequent analyses were performed separately for the left and right amygdala.

For each subject, mean time series data were extracted from the amygdala ROI and used as a predictor (i.e., 'seed')(Biswal, et al., 1995; Fox & Raichle, 2007) in a general linear model (GLM) to identify voxels that were significantly correlated with amygdala activity across time using AFNI. In addition, time series data for 9 covariates of no interest: white matter, CSF (specific regions defined above), global signal, and 6 motion parameters for head movement (three translations and three rotations) were computed and entered in the GLM. The white-matter, CSF and global signal time series were included to reduce the influence of artifacts caused by physiological signal sources (i.e., cardiac and respiratory) on the results(Birn, 2012; Birn, Diamond, Smith, & Bandettini, 2006; Fox & Raichle, 2007; Fox, et al., 2005; Macey, Macey, Kumar, & Harper, 2004; Weissenbacher, et al., 2009) similar to previous studies examining amygdala resting-state connectivity. A global signal regressor was included in order to control for non-specific global changes in brain signal (potentially caused by physiological or external noise) and in order to facilitate within-group and between-group comparisons. Global signal regression has been commonly used when investigating amygdala connectivity networks(Henckens, van Wingen, Joels, & Fernandez, 2011; Kim, et al., 2011; Roy, et al., 2009; Sripada, et al., 2012; Veer, et al., 2012) and has been shown to lead to enhanced detection of system-specific connectivity networks and improve correspondence of functional connectivity networks with underlying anatomical networks(Fox, Zhang, Snyder, & Raichle, 2009). However, it is important to note that global signal regression mathematically necessitates the appearance of anti-correlations in seed-based connectivity analyses(Fox, et al., 2009; Murphy, Birn, Handwerker, Jones, & Bandettini, 2009) and therefore this must be considered when interpreting the anti-correlations reported here. Importantly, while global signal regression does lead to anti-correlations it does not imply any spatial coherence across subjects in the anti-correlations, and when putative non-physiological correlations are induced (e.g. functional connectivity to white-matter noise regions) spatial coherence in anti-correlations does not emerge(Fox, et al., 2009). Finally, additional covariates of no interest were included to account

for noise introduced by parallel imaging acquisition factors. These covariates were derived from a principal components analysis (PCA) of the time series of regions within the eyes and the number of covariates varied across subjects according to the number of components explaining 80 percent variance in the PCA.

Contrast parameter images for the seed region covariate generated at the single subject level were then submitted to a second-level random effects analysis in SPM8. Next, statistical parametric maps were created using an ANOVA model with the two factors of 'Condition' (Sleep-group, Wake-group) and 'Scan session' (first or second). Reported voxel coordinates correspond to standardized MNI space.

Amygdala functional connectivity with the medial prefrontal cortex (mPFC): The *a priori* region of interest was on the medial prefrontal cortex, based on its established role in top-down regulatory control of amygdala function(Davidson, 2002; Milad, et al., 2004; Morgan, et al., 1993; Phelps, et al., 2004; Quirk, et al., 2003; Quirk & Mueller, 2008; Rauch, et al., 2006; Rosenkranz, et al., 2003; Sierra-Mercado, et al., 2006; Sotres-Bayon & Quirk), and verified functional(Kim, et al.) as well as structural connectivity with the amygdala(Bracht, et al., 2009; Croxson, et al., 2005; Johansen-Berg, et al., 2008). The medial PFC ROI was defined by a 10mm diameter sphere around central coordinates from previously published amygdala resting connectivity studies(Gu, et al., 2010) and studies using emotional reactivity paradigms (MNI coordinates [x y z]: 0, 56, 10)(Heinz, et al., 2005; Modinos, et al., 2012; Phelps, et al., 2004; Sakaki, et al., 2012; Shin, et al., 2004a; Shin, et al., 2005; Williams, et al., 2006), thus covering areas that (i) are densely connected to the amygdala both structurally and functionally, and (ii) play a crucial role in the processing of emotional stimuli. The ROI was created using the Wake Forest University Pickatlas toolbox(Maldjian, Laurienti, Kraft, & Burdette, 2003). Statistical inferences for the mPFC ROI were reported using a threshold of $P < 0.05$ FWE, correcting for multiple comparisons(Lieberman & Cunningham, 2009; Poldrack & Mumford, 2009).

Amygdala functional connectivity with the locus coeruleus (LC): The second *a priori* region of interest was the locus coeruleus. Similar to previous amygdala resting state papers(Henckens, et al., 2011; van Marle, Hermans, Qin, & Fernandez, 2010), we implemented a reduced spherical search volume (5-mm radius) around anatomically defined center coordinates for the LC(Astafiev, Snyder, Shulman, & Corbetta, 2010) (MNI coordinates left LC: -4, -38, -24; right LC: 6, -38, -24). The ROI was created using the Wake Forest University Pickatlas toolbox(Maldjian, et al., 2003). To address the fact that the LC is a very small nucleus and hard to image using whole-brain fMRI we ran additional post-hoc analyses investigating the robustness of the LC effect reported in the main manuscript by using a stricter LC ROI. This LC ROI is based on the 1 standard deviation (1SD) probability map of a high resolution study (0.4mm x 0.4mm) of 44 individuals in standard MNI space(Keren, Lozar, Harris, Morgan, & Eckert, 2009). Such a stricter ROI can confirm that the main analyses were not driven by voxels that are likely outside of the LC. Statistical inferences for all LC ROIs were similarly reported using a threshold of $P < 0.05$ FWE, correcting for multiple comparisons(Lieberman & Cunningham, 2009; Poldrack & Mumford, 2009).

Combination of amygdala functional connectivity with both the mPFC and LC: In order to investigate the joint effect of the change in connectivity between both the amygdala-LC and the amygdala-mPFC, the two measures were combined. First, the parameter estimates were extracted

for each individual from the voxels that were significant in the group analysis (the average across all voxels was used, extracted with Marsbar). Then, the change in connectivity for each of the two ROIs was standardized across all participants by subtracting the mean change across all participants and dividing it by the standard deviation across all participants, producing a similar distribution for both measures. Next, the observed changed in amygdala-mPFC connectivity across 12hr was subtracted from the change in amygdala-LC connectivity across the two sessions, creating a combined measure of change in the amygdala network of interest. An increase in amygdala-LC connectivity across the two sessions translates to an increase in the Amygdala-Sensitivity, while an increase in amygdala-PFC connectivity lowers Amygdala-Sensitivity (and vice versa).

To assess associations between amygdala Amygdala-Sensitivity connectivity and intra-individual changes in emotion responsivity and REM-sleep physiology parameters, correlation analyses were performed incorporating each participant's REM-sleep parameter value and their amygdala connectivity parameter (Amygdala-Sensitivity). Individual parameter estimates and sleep parameters were plotted to ensure the correlation was not driven by outliers.

Furthermore, to confirm the LC and mPFC ROI analyses were not driven by outlier voxels, the analyses were repeated not taking the average of the significant voxels, but instead averaging the entire ROI. These different version of the Amygdala-Sensitivity gave similar results to the results described here and are therefore not reported.

Sleep Recordings: Sleep on the intervening night between Resting-state scan 1 and Resting-State scan 2 in the Sleep-group was monitored in the laboratory with PSG sleep (23:30hr-07:30hr ± 30min) using a Grass Technologies Comet XL system (Astro-Med, Inc., West Warwick, RI). Electroencephalography (EEG) was recorded at 19 standard locations conforming to the International 10-20 System(Klem, et al., 1999) (FP1, FP2, F7, F3, FZ, F4, F8, T3, C3, CZ, C4, T4, T5, P3, PZ, P4, T6, O1, O2). Electrooculography was recorded at the right and left outer canthi (right superior; left inferior). Electromyography was recorded via three electrodes (one mental, two submental). Reference electrodes were recorded at both the left and right mastoid (A1, A2). Data was sampled first at 800Hz by the amplifier, digitized at 400Hz. All data was stored unfiltered (recovered frequency range of 0.1–100Hz), except for a 60Hz notch filter to remove mainline noise. For recording only, each channel was referenced to a forehead scalp derivation.

Sleep scoring: Sleep-staging was performed in accordance with standardized techniques(Rechtschaffen & Kales, 1968), using C3,C4,O1,O2, right and left EOG and EMG channels. EEG and EOG were referenced to the contralateral mastoid, and filtered to 0.3-35 Hz. EMG used a bipolar montage filtered to 10-70Hz and 0.1-12Hz, respectively. Sleep was visually scored in 30-second epochs using the C3-A2 derivation according to standard criteria(Rechtschaffen & Kales, 1968). Sleep architecture values are reported in **Table 2**, consistent with previous cross-sectional normative values for this age group(Ohayon, Carskadon, & Guilleminault, 2004).

Spectral analyses: All EEG analyses were performed in MATLAB 7.5 (The Mathworks, Natick, MA), including the add-in toolbox EEGLAB (http://sccn.ucsd.edu/eeglab/). Power spectral analysis of sleep EEG was carried out according to previously published methods(Achermann, 2009; Mander, et al., 2011a; Nishida, et al., 2009; Saletin, et al., 2011). Specifically, EEG

channels were re-referenced to the average of the left and right mastoid (A1, A2), with high- and low-pass Finite Impulse Response (FIR) filtering at 0.5Hz and 80Hz, respectively. The all-night recording was then visually artifact-rejected in 5-second epochs. Artifact-laden epochs were removed from subsequent analyses. Power spectral density was calculated by use of a Fast Fourier Transform (FFT) on each hamming-windowed 5-second epoch. FFT results were then sorted according to sleep stage and averaged for each respective stage. Power in bands of interest was calculated by averaging across standard EEG frequency band ranges(Nuwer, et al., 1999): delta (0.8 – 4.6Hz), theta (4.6 – 8Hz), alpha (8 – 12Hz), sigma (12 – 15Hz), beta (18 – 30Hz), and gamma (30 – 40Hz), and log-transformed(Buysse, et al., 2008; Dijk, et al., 1995; Gasser, et al., 1982; John, et al., 1980; Krystal & Edinger; Krystal, et al., 1995; Meltzer, Negishi, Mayes, & Constable, 2007; O'Connor, et al., 1999; Parsons, et al., 1997; Uchida, et al., 1992) .

Power in the gamma band (**Fig. 2**) was the experimental *a priori* focus motivated by 1) the hypothesized effect of REM physiology on emotional brain networks being mediated by the suppression of central adrenergic neurotransmitter levels(Walker, 2009; Walker & van der Helm, 2009b), 2) related, that gamma EEG activity is a validated marked of central adrenergic activity, proportionally increasing and decreasing with corresponding increases and decreases in adrenergic levels(Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976). Therefore, gamma EEG activity served as an indirect proxy for adrenergic activity. Beyond the frequency range specificity, gamma activity over prefrontal cortex derivations (average of FP1 and FP2) was targeted based 1) on the known noradrenergic projections from the locus coeruleus to the prefrontal cortex(Gresch, et al., 1995; Heidbreder & Groenewegen, 2003; Kawahara, et al., 2001), and 2) the association between prefrontal REM activity and emotional information processing(Nishida, et al., 2009; van der Helm, et al., 2011b). REM-gamma power at other electrode pairs across the head (average of F3 & F4; C3 &C4; T3 & T4; P3 & P4; O1 & O2) were investigated post-hoc and corrected for multiple comparisons using a Bonferroni correction ($p = 0.05/5 = 0.01$).

Statistical analyses: Statistical analyses were carried out using within- and between-group analyses of variance (ANOVA) models; together with planned within-group paired and between-group unpaired two-tailed *t*-tests, with $P < 0.05$ considered significant. In accordance with previous studies relating EEG power spectral values to fMRI parameter estimates, cognition measures and questionnaires, correlations involving EEG power values were performed using Spearman's Rho(Chen, Feng, Zhao, Yin, & Wang, 2008; Feige, Scaal, Hornyak, Gann, & Riemann, 2007; Geiger, et al., 2011; Hall, et al., 2000a; Meltzer, et al., 2007; Newton, et al., 2004b). All analyses were performed using the software program PASW Statistics version 18.

6.4 Results

As expected, participants displayed significant intra-individual changes in emotion responsivity over time, reflected in the change in the average emotion rating at the two different time points spanning the 12hr period (one tailed *t*-test all participants $p < 0.001$; Wake-group $p < 0.001$; Sleep-group $p < 0.001$). Although overall slightly more participants decreased in emotion responsivity (58%) than increased (42%), the extent of change (irrespective of direction) was not significantly different between the Sleep- and Wake-groups (unpaired *t*-test $p = 0.22$, **Figure 1b**), nor was the direction of change (unpaired *t*-test $p = 0.12$) despite the Sleep-group numerically decreasing more (mean ± s.e.m.: -0.18 ± 0.09) than the Wake-group (mean ± s.e.m.: 0.00 ± 0.07).

Therefore, participants in both groups showed significant intra-individual changes in emotion sensitivity across the 12hr interval, with intervening sleep resulting in a numerically but non-significantly higher decrease in sensitivity, in line with previous studies suggesting a possible beneficial role of sleep on emotion sensitivity.

We next sought to determine whether intra-individual changes in emotion sensitivity co-occurred with changes in amygdala connectivity, focusing *a priori* on amygdala connectivity with brainstem and mPFC regions of interest (ROIs), and whether such changes were modulated by intervening sleep and wake. Consistent with the experimental prediction, a significant Group (Wake, Sleep) x Scan (Scan1, Scan2) interaction was observed in both the mPFC and the brainstem region overlapping with the LC. Specifically, an increase in brainstem-amygdala connectivity was observed across the day in the Wake-group, yet an overnight decrease in connectivity in the Sleep-group (**Figure 1c)**, while the opposite pattern was observed for amygdala-mPFC connectivity, degrading across wake while increasing after sleep (**Figure 1d)**. Therefore, amygdala connectivity changed in a bistable manner across a 12hr period, with an intervening waking brain state leading to stronger brainstem-amygdala coupling, while an intervening night of sleep instead enhances amygdala-mPFC connectivity.

Having established intra-individual changes in both behavioral emotion sensitivity and neural amygdala resting connectivity over time, we next sought to determine whether these two features were significantly associated, irrespective of intervening wake or sleep. In order to test the aggregate influence of the two neural changes in amygdala connectivity (brainstem, mPFC), a combined amygdala measure was calculated by subtracting the standardized amygdala-PFC from the amygdala-brainstem connectivity strength changes, reflecting the degree of shift in brainstem versus mPFC dominance: Amygdala-Sensitivity (**Figure 3a**). Therefore, an increase in amygdala-brainstem connectivity results in an increase in Amygdala-Sensitivity, conversely an increase in amygdala-mPFC connectivity lowers it (and vice versa). We found that the shift in Amygdala-Sensitivity across all participants significantly predicted the intra-individual change in behavioral emotion sensitivity, with increases in Amygdala-Sensitivity correlating with increases in the average emotion rating (**Figure 3b**, $r = 0.57$, $p = 0.001$), with the strongest effect for the most intense emotional items. These results indicate that intra-individual changes in the balance of the amygdala network are predictive of intra-individual changes in behavioral emotion sensitivity, irrespective of intervening sleep or wake.

Finally, having established the association between neural and behavioral emotion sensitivity independently of sleep and wake, we next sought to test the prediction that the network changes across a period of sleep were predicted by the physiological quality of that sleep. Specifically, we focused on the extent of reduced REM-gamma EEG activity; a marker of decreased central adrenergic activity, centered on prefrontal EEG activity based on its dense adrenergic innervation and established role in emotion regulation. Consistent with the prediction, the extent of reduced prefrontal REM-gamma was significantly correlated with the extent of overnight shift in Amygdala-Sensitivity (**Figure 3c**). Specifically, those participants with the lowest levels of REM-gamma (a potential indirect reflection of lower central adrenergic activity) expressed the largest decrease in Amygdala-Sensitivity (Spearman's $\rho = 0.54$, $p = 0.025$). Two additional analyses were performed at the level of (1) sleep-state, with a lack of such an association with prefrontal NREM-gamma, and (2) frequency, with no other EEG spectral band demonstrating a similarly significant relationship.

There is a possibility that the intra-individual changes in emotion sensitivity and amygdala networks may be driven by factors other than sleep and wake, such as time of day. If

the changes were driven by circadian time, then the feature of circadian chronotype, which was assessed in each participant, would be expected to show an association with the reported neural and behavioral changes. However, circadian chronotype was not significantly correlated with the change in coupling between the amygdala and brainstem region nor amygdala-mPFC connectivity. Furthermore, the change in the aggregate measure, Amygdala-Sensitivity, was not significantly correlated with chronotype either. While these data do not discount the potential contribution of time of day effects, the lack of association with chronotype, together with the significant relationship between the change in Amygdala-Sensitivity across the night and prefrontal REM gamma activity, a measure that itself is largely independent of circadian phase, suggest that at least a significant portion of the changes in the amygdala network are associated with the brain-states of wake and sleep.

Circadian effects on the amygdala network: As both groups underwent the two resting-state scans at different times of the day (evening and morning or vice versa) they not only passed through different brain states (wake versus sleep) but also different circadian times. To investigate whether circadian chronotype affected the change in amygdala network across the night or across the day, we first examined the correlation between the change in amygdala-LC connectivity and chronotype across all 33 subjects as well as across just the Sleep-group and the Wake-group. We found no effect of chronotype on the change in amygdala-LC connectivity across all subjects ($r = -0.19$, $p = 0.29$), the Sleep-group or Wake-group (both $-0.19 < r > 0.04$, all $p > 0.46$). Secondly, we similarly investigated the effect of chronotype on the change in amygdala-mPFC coupling, with no significant correlation found for all subjects ($r = -0.09$, $p = 0.62$), the Sleep-group or Wake-group (both $-0.34 < r > 0.01$, all $p > 0.18$). These results suggest chronotype did not significantly affect the change in amygdala coupling across the day nor night with the LC and mPFC. The next analyses reported in the manuscript focused on the change in the amygdala network (combining the LC and mPFC coupling with the amygdala) and its relationship to behavior and sleep, therefore the effect of chronotype on the changes in the amygdala network were investigated next (Amygdala-Sensitivity). Chronotype was not significantly correlated with the Amygdala-Sensitivity shift in the Sleep-group ($r = 0.11$, $p = 0.67$), Wake-group ($r = -0.04$, $p = 0.89$) or all subjects combined ($r = -0.06$, $p = 0.74$). Therefore, chronotype did not significantly affect the main results reported in the manuscript.

Circadian effects on emotion responsivity: Considering chronotype could have similarly influenced emotion responsivity, additional analyses were performed to examine the correlation between chronotype and emotion responsivity across all participants (n=33) in the evening and morning. Chronotype was not significantly correlated with average emotion responsivity ratings in the evening ($r = -0.08$, $p = 0.66$) nor morning ($r < 0.005$, $p = 0.98$). Furthermore, chronotype was not significantly correlated either with the change in emotion responsivity across the 12hr in the Sleep-group ($r = 0.25$, $p = 0.33$), Wake-group ($r = -0.07$, $p = 0.71$) nor across all participants ($r = 0.09$, $p = 0.80$).

Valence and Arousal effects:

To investigate whether the relationship between intra-individual changes in emotion sensitivity and the shifts in the amygdala network were specific within the domain of valence (negative or positive), or within the domain of arousal (low or high), subset analyses were conducted. Specifically, the experimental 150 stimuli were median split based on the standardized IAPS

ratings of valence (75 negative, 75 positive), and again for arousal (75 low arousing, 75 high arousing), with the same analyses reported in the main manuscript repeated for each.

Valence: For the negative valenced stimuli a significant positive correlation between the shift in amygdala networks across the 12hr and intra-individual changes in average emotion intensity rating was found ($r = 0.544$, $p = 0.001$, **Fig. 4a**). Therefore, an increase in amygdala LC dominance corresponded with an increase in the average rating of the 75 negative images. A similar pattern was observed for the positive stimulus set, with likewise a significant positive correlation ($r = 0.543$, $p = 0.001$, **Fig. 4b**). Therefore, as in the main manuscript with all stimulus types combined, the shift in the amygdala network across 12hr predicted intra-individual changes in emotion responsivity similarly for only negative or only positive valenced stimuli.

Arousal: Focusing on the high arousing stimuli, a significant positive correlation was found with the shift in the amygdala network across 12hr and the intra-individual changes in average rating ($r = 0.523$, $p = 0.002$, **Fig. 4c**). Furthermore, a similar result was present for just the low arousing stimuli ($r = 0.469$, $p = 0.006$, **Fig. 4d**). Therefore, the changes observed across 12hr in the affective brain network and its influence on intra-individual changes in emotion responsivity reported in the main manuscript were observed for both the high and low arousal stimulus subsets, based on the standardized picture ratings, with increases in the amygdala LC dominance resulting in increased emotion intensity ratings**.**

REM-sleep physiology effects on emotional responsivity: The associations between sleep physiology and the changes in the amygdala network reported in the main manuscript were unique to prefrontal gamma power during REM, which was confirmed in three additional analyses:

1) Topography – examining the association between the amygadala network with EEG derivations across the head. Supporting the experimental predictions, the association between gamma activity and the overnight change in the amygdala network was most pronounced in the prefrontal (FP1 & FP2) and frontal (FP3 & FP4) EEG derivations, whereas the effect was not significant for electrode derivations at more posterior sites (C3 & C4; P3 & P4; O1 & O2, **Supplementary Table 3**).

2) Frequency – assessing the relationship between the shift in the amygdala network and prefrontal REM power in the other *non a priori* frequency bands (delta, theta, alpha, sigma and beta)(Nuwer, et al., 1999). No other frequency band from these same prefrontal EEG derivations correlated with the change in the amygdala network (all Spearman's rho $-0.27 < \rho > 0.22$, all $p > 0.30$).

3) Brain-state – examining the relationship between the shift in the amygdala network and prefrontal gamma in NREM-sleep rather than REM-sleep. Unlike REM, no significant correlation was found between prefrontal gamma power during NREM and the change in the amygdala network (Spearman's rho $\rho = 0.24$, $p = 0.36$). Furthermore, based on previous studies reporting slow wave activity abnormalities in depression, the relationship between slow wave activity (0.8-4.6Hz) in NREM was examined and did not correlate significantly with the change in the amygdala network either (Spearman's rho $\rho = 0.17$, $p = 0.50$).

Together, these results suggest that the association between REM-sleep and the change in the amygdala network reported in the main manuscript was strongest in one brain state (that of REM), frequency band (that of gamma), and topographical location (that of prefrontal EEG derivations). This REM relationship conforms to recently proposed models of REM-sleep information processing(Levin & Nielsen, 2007; Walker, 2009), suggesting that beyond the

strengthening of emotional memory(Walker & van der Helm, 2009b), and the de-potentiation of affective reactivity(Walker & van der Helm, 2009b), REM-sleep resets affective resting-state networks, even in the absence of an emotional stimulus.

Response times & subjective sleepiness: The average response times during each emotion responsivity task for each group are provided in **Table 4**. The groups did not significantly differ in response time during the first or second emotion responsivity task (both $p > 0.14$). Subjective Stanford sleepiness scores before each resting-state scan are reported in **Table 5.** The groups did not significantly differ in sleepiness levels before the first resting-state scan ($p = 0.31$) or the second resting-state scan ($p = 0.13$).

6.5 Discussion

Taken together, these findings demonstrate that the emotional sensitivity of a given individual is not stable across a time period of hours spanning wake and sleep, and furthermore, that these shifts in emotional sensitivity are, in part, associated with underlying neural changes in the functional coupling of the human amygdala. Specifically, time awake across the day significantly decreased connectivity within individuals between the amygdala and the phylogenetically newer mesial PFC region, itself associated with processes of top-down regulatory and cognitive control. In contrast, significant increases in amygdala connectivity developed within individuals, over time awake, in the phylogenetically older arousal related fight/flight brainstem regions proximal to the LC. Moreover, the equivalent time, but containing a night of sleep, resulted in a reverse pattern of these connectivity changes, increasing mPFC coupling, while decreasing amygdala-brainstem coupling. Further, the extent of this amygdala connectivity shift, and associated behavior, was predicted by the decrease in REM gamma EEG activity, a measure previously associated with the extent of central noradrenergic activity (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997).

Previous research has established the prefrontal cortex is especially vulnerable across time awake into a state of sleep deprivation(Chuah, Venkatraman, Dinges, & Chee, 2006; Killgore, 2010; Soshi, et al., 2010). A dissipation of functional integrity helps explain why reaction time, task-switching and divided attention tasks are most impaired in a sleep-deprived state(Drummond & Brown, 2001; Lim & Dinges, 2008; Van Dongen, Belenky, & Krueger, 2011). The current results indicate that prefrontal connectivity at rest with a key emotional area, the amygdala, is similarly impaired after only 12hr of wakefulness. Along with a loss of prefrontal coupling, we observed enhanced connectivity between the brainstem and the amygdala across a day of wake. Such enhanced limbic-brainstem coupling might result from a wake-promoting compensatory mechanism(Aston-Jones, 2005; Berridge, 2008; Berridge, Schmeichel, & Espana, 2012). Such a change in neural activity would be predicted to result in a behavioral phenotype characterized by enhanced emotional responses though dominant adrenergic-amygdala influence. Indeed, we found that the more the amygdala network changed to more dominant brainstem coupling instead of mPFC coupling, the higher the emotional responsivity. In contrast, these changes in the amygdala network were reversed by a night of sleep and thereby returned to more prefrontal dominance. With that shift in the amygdala network came a shift in behavior, associated with a dissipation of emotional responsivity. Such findings can explain why individuals change in their emotional state over time, in addition to other factors such as experience, or simply the passage of time.

While REM-sleep physiology has previously been shown to predict changes between subjects in emotional reactivity(van der Helm, et al., 2011a), it is the same stage of sleep that here predicts intra-individual changes in the affective neural network across 12hr. Though not an a priori focus, we also examined the effect of slow wave activity during NREM sleep on the change in amygdala networks based on earlier studies reporting slow wave activity abnormalities in depression(Plante, Landsness, Peterson, Goldstein, Riedner, et al., 2012; Plante, Landsness, et al., 2012a), but found no significant relationship. This suggests gamma power during REM-sleep, an indirect marker of adrenergic activity is associated with the observed changes in the affective brain network (Berridge & Foote, 1991; Cape & Jones, 1998; Keane, et al., 1976; Maloney, et al., 1997). Although one cannot fully dismiss the possibility of a contribution of circadian factors in either group, that the overnight changes in amygdala connectivity were found to be associated with intervening REM-sleep physiology, suggests REM-sleep is at least partly responsible for some of the observed shift in the amygdala network across a night of sleep. Moreover, REM-sleep itself is a stage of sleep that has been found to be largely independent of circadian time. Furthermore, these results support neurobiological frameworks regarding a role for REM sleep in the optimal homeostasis of affective brain function, potentially by way of modulating network connectivity underlying adaptive emotional states. Nevertheless, future studies that hold circadian time constant in either a 24hr design or nap design will be required to gain a deeper understanding of circadian effects on this amygdala network.

How and why we change in our emotion responsivity from one moment to the next has received little research attention and therefore remained poorly understood, despite the common occurrence of changes in emotional state in daily life. Moreover, affective responsivity to stressors can create aggregated effects that increase vulnerability to problems, including anxiety, depression, and disease (Almeida, 2005; Cacioppo, et al., 1998; Lazarus, 1999; Zautra, 2003). The current study is the first to link intra-individual changes in emotional response to co-occurring bi-stable changes in amygdala resting state connectivity. Furthermore, the brain state of sleep, and specifically REM-sleep physiology, predicted the shift in bistable amygdala connectivity. The present results emphasize the importance of modeling emotional state changes *within* individuals in order to fully capture the phenomenon of affective state and factors influencing it. Finally, these findings could hold more general implications for mood disorders displaying co-morbid sleep abnormalities.

Figures

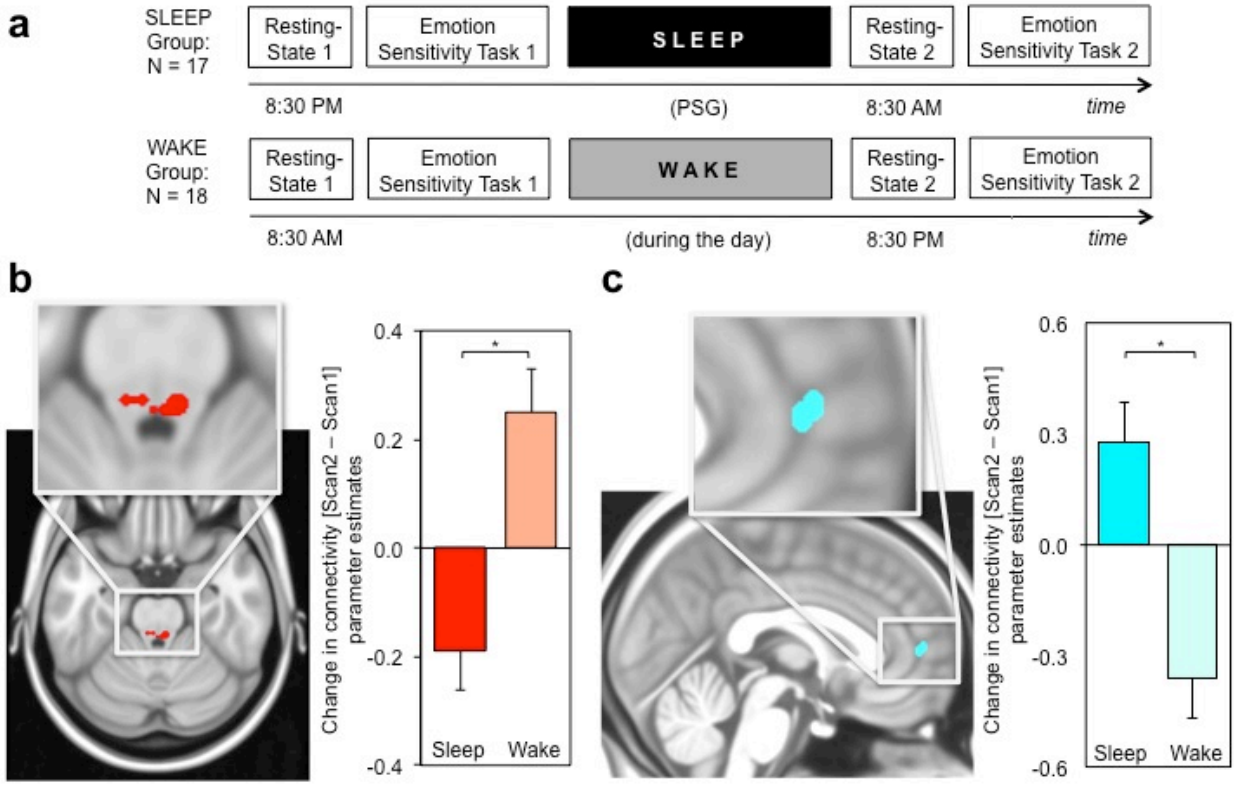

Figure 1. **(a)** Experimental design: Both groups underwent a resting state scan and subsequently performed an emotion sensitivity task twice, separated by 12 hr, inside the fMRI scanner. The task involved the rating of a standard set of affective picture stimuli (150 pictures per set, sets used counterbalanced as either the first set or second set). The change in emotional sensitivity and the change in amygdala resting state networks following sleep (Sleep-group) or wake (Wake-group) was assessed by comparing data from Scan1 and Task1 (pre wake or sleep) with that from Scan2 and Task2 (post wake or sleep). **(b,c)** Differences in amygdala resting state connectivity: Group x Test session interaction in **(b)** the brainstem region overlapping with the LC (red), demonstrating a significant decrease in connectivity from Scan1 to Scan2 in the Sleep-group yet increase in the Wake-group, and **(c)** mPFC connectivity (blue), demonstrating increased connectivity from Scan1 to Scan2 in the Sleep-group yet decreased coupling in the Wake-group. Differences in activation and connectivity thresholded at $P < 0.05$ family wise error (FWE) corrected for multiple comparisons. $*P < 0.05$, Error bars: S.E.M. Bar graphs reflect average of significant voxels, a result of this particular ANOVA interaction contrast

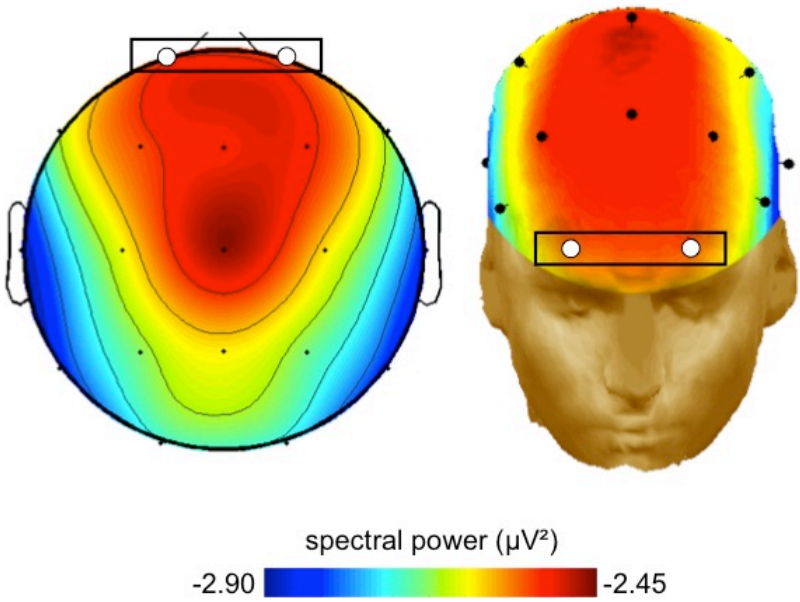

Fig. 2: Gamma EEG power (log-transformed) during REM-sleep in the Sleep-group, shown on 2 dimensional (left) and 3 dimensional (right) topographical plots.

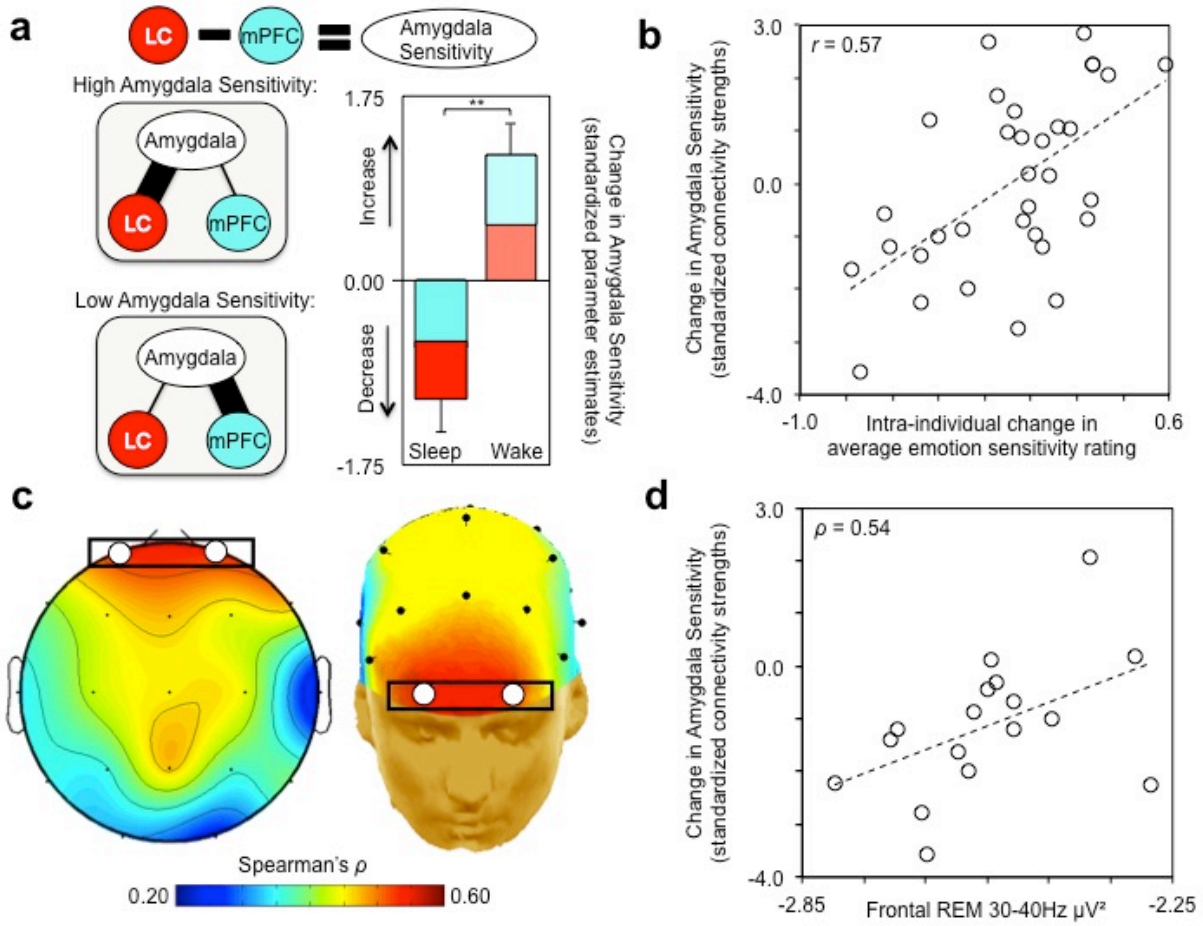

Figure 3. **(a)** Visualization of the Amygdala Sensitivity measure: the combination of the "push—pull" of connectivity with the brainstem and mPFC. The parameter estimates (standardized) are subtracted from those of the LC. The bar graph shows the change in Amygdala Sensitivity from Scan1 to Scan2 in each of the 2 conditions. Error bars represent S.E.M. ** P < 0.00001. **(b)** Pearson's correlation of intra-individual changes in the average emotion sensitivity rating and the intra-individual change in Amygdala Sensitivity across all participants. **(c)** For the participants in the sleep group, a topographical Spearman's correlation (ρ) plot between REM gamma power and the intra-individual changes in Amygdala Sensitivity demonstrating a significant prefrontal relationship (average of Fp1-Fp2, white circles), and **(d)** corresponding scatter plot and Spearman's ρ value: the extent of reduced gamma EEG activity over prefrontal cortex was proportional to the overnight decrease in Amygdala Sensitivity

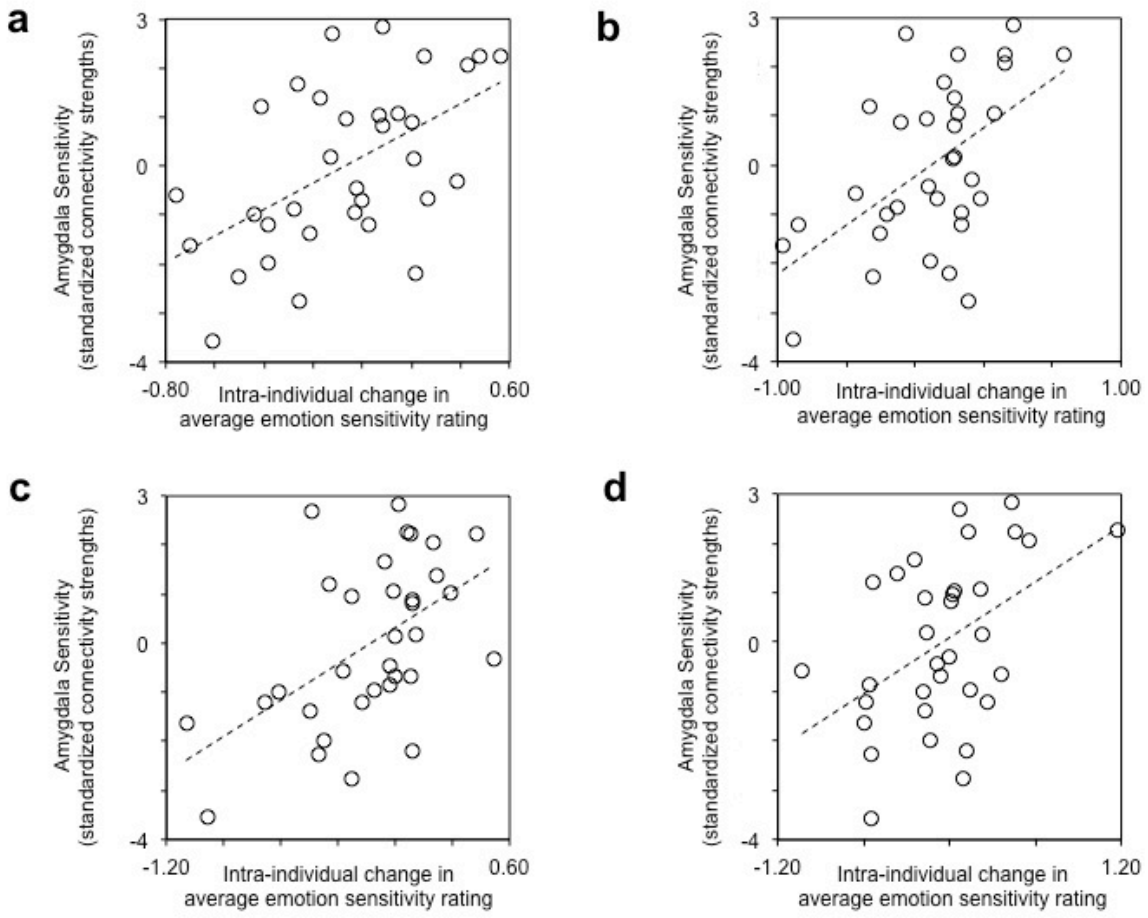

Fig. 4: Correlation plots for the relationship between the change across 12hrs in amygdala resting state networks (Amygdala Sensitivity) and intra-individual changes in average emotion intensity rating split by positively valenced stimuli (r = 0.543, p = 0.001, Panel A), negatively valenced stimuli (r = 0.544, p = 0.001, Panel B), high arousing stimuli (r = 0.526, p = 0.002, Panel C), and low arousing stimuli (r = 0.469, p = 0.0059, Panel D).

Tables

Table 1: Average total sleep time five nights prior to the study based on sleep log and actigraphy data for both groups (mean ± SD)

Night		1	2	3	4	5
Sleep-group	Log	7.6 ± 1.2	7.2 ± 1.0	7.4 ± 0.8	8.0 ± 1.0	7.6 ± 0.8
	Actiwatch	7.3 ± 0.9	6.6 ± 1.3	7.2 ± 0.8	8.0 ± 1.1	7.6 ± 0.9
Wake-group	Log	7.9 ± 1.2	7.4 ± 1.3	7.7 ± 1.3	7.7 ± 1.2	7.6 ± 1.1
	Actiwatch	7.7 ± 0.9	7.0 ± 1.3	7.6 ± 1.2	7.6 ± 1.2	7.5 ± 1.2

Table 2: Polysomnography sleep-stage values for the Sleep-group (mean ± SD)

	Sleep Time (min)	% Sleep Time	Obtained by % of Participants
Total sleep time	455.0 ± 13.5		
Stage 1	28.1 ± 16.4	6.2 ± 3.7	100 %
Stage 2	200.2 ± 30.0	44.1 ± 7.0	100 %
SWS	125.1 ± 30.0	27.4 ± 6.4	100 %
REM	101.7 ± 23.7	22.3 ± 4.8	100 %

Mean duration (in minutes), percent (%) and standard deviation (SD) of total sleep time, percentages of participants obtaining the stage, NREM sleep stages 1 and 2, slow-wave sleep (SWS; NREM stage 3 & stage 4) and rapid eye movement sleep (REM).

Table 3: Spearman's correlation (Rho) between gamma EEG power and the shift in the amygdala network

EEG derivations	ρ	p-value
FP1 and FP2	0.54	0.03
F3 and F4	0.48	0.05
C3 and C4	0.43	0.09
P3 and P4	0.32	0.21
O1 and O2	0.29	0.25

Table 4: Response times in milliseconds (mean ±SEM) for both groups during Task 1 and Task 2

	Task 1	Task 2
Sleep group	2834 ± 66	2792 ± 64
Wake group	2967 ± 59	2840 ± 49

Table 5: Average Stanford sleepiness scale values (mean ± SEM) in both groups prior to each resting-state scan

	Test1	Test2
Sleep-group	2.4 ± 0.2	2.6 ± 0.2
Wake-group	2.8 ± 0.4	2.0 ± 0.3

Chapter 7. Conclusions

Humans spend almost a third of their lives asleep, yet science has still to come up with a definite account of its function. Several lines of research indicate sleep plays an important role in bodily functions such as the immune system(Besedovsky, Lange, & Born, 2012; Gomez-Gonzalez, et al., 2012) and metabolism(Killick, Banks, & Liu, 2012). In addition, a growing body of literature implicates sleep aids brain functions such as decision making(McKenna, Dickinson, Orff, & Drummond, 2007a), executive function(Drummond & Brown, 2001; Durmer & Dinges, 2005), memory processes(Stickgold & Walker, 2007; Walker & Stickgold, 2010) and more abstract concepts such as creativity(Walker, Liston, Hobson, & Stickgold, 2002) and insight(Wagner, Gais, Haider, Verleger, & Born, 2004). Despite the common belief that sleep and emotion are intimately related there is a surprising lack of scientific studies on the role of sleep in emotional processing.

This dissertation served to enhance our understanding of how sleep affects emotional processing at both a theoretical and experimental level. Chapter 2 put forth a theory on the role of sleep, and specifically REM-sleep, in emotional functioning. The studies subsequently presented in this thesis served to investigate this relationship in more detail and test specific predictions flowing from the model utilizing several different methodologies. In this final Chapter I will briefly reiterate the model and summarize the three studies, followed by a discussion of the broader implications of these results.

A theory on the homeostatic function of REM-sleep for affective brain function

Emotions guide much of human behavior, by driving decision making and aiding memory formation. For instance, experiences that are accompanied by intense emotions are selectively promoted by the human brain, by enhancing attention and memory encoding(LaBar & Phelps, 1998; Sharot & Phelps, 2004). A wealth of evidence further suggests that emotional experiences not only persist in our memory over time, but are strengthened compared to neutral experiences (Dolcos, et al., 2005). However, the later recall of these memories tends not to be associated with the same extent of autonomic (re)activation as that elicited at the time of the experience. This suggests that, over time, the emotional tone originally tagging the memory at the time of learning disappears, whereas the information of the experience (the memory) remains (**Figure 4a & b, Chapter 2**).

The REM-sleep hypothesis suggests that this removal of the emotion from the memory preferentially takes place during REM-sleep. In other words, we *sleep to forget* the emotional tone, yet *sleep to remember* the tagged memory of that experience. This model further proposes that if such a process is not completed, the affective charge would persist, potentially resulting in chronic anxiety within autobiographical memory networks.

The brain state of REM-sleep provides an optimal biological milieu for this form of "overnight therapy", based on three features: neuroanatomical, neurophysiological and neurochemical (**Figure 4a, Chapter 2**). First, increased activity within limbic and paralimbic structures during REM-sleep (Nofzinger, 2005) supports the ability for reactivation and hence

(re)processing of affective memories. Second, dominant theta oscillations during REM within subcortical as well as cortical nodes offer large-scale network cooperation for the strengthening of distributed aspects of the emotional memory (e.g. perceptual, contextual). Such activity across related but different anatomical networks can aid the consolidation and integration of that emotional memory. Third, these interactions take place within a brain that is low in aminergic neurochemical concentration (Pace-Schott & Hobson, 2002), particularly noradrenergic input from the locus coeruleus (associated with stress and anxiety responses) and dominated by cholinergic neurochemistry (Itoi & Sugimoto, 2010; Ramos & Arnsten, 2007; Sullivan, et al., 1999; Valentino & Van Bockstaele, 2008). Therefore, REM sleep offers a unique biological state for both consolidation of the informational core of emotional experiences (the memory), yet removing the autonomic arousing charge originally acquired at the time of learning (the emotion).

Specific predictions emerge from this model that were tested in this dissertation. Firstly, the model states that overnight our brain is integrating emotional experiences and putting them into context. In the absence of sleep this process will not take place and emotional processing will therefore be disturbed. With no resetting of the emotional brain for an extended period of time, emotional experiences will not be processed (until sleep occurs). The sleep deprivation study in Chapter 3 supported this notion that the absence of sleep has a direct effect on emotional processing. Using an affective face recognition task, it was demonstrated that a single night of sleep deprivation significantly disrupts the ability of the human brain to accurately identify salient emotional expressions in others, particularly in the moderate intensity range of emotion. These deficits were specific to some but not all affective categories, being most dramatic for emotions eliciting high autonomic arousal— Angry and Happy—corresponding to greatest threat relevant and reward relevant value, respectively.

A second prediction concerns the role of REM-sleep in the consolidation of emotional memories. Specifically, the degree to which the information of emotional experiences are retained, long-term, is predicted to be proportional to the amount of post-encoding REM sleep obtained, how quickly it is achieved (REM latency), as well as the power of theta oscillations during REM. As discussed in more detail in Chapter 2, previous studies have found evidence for all these predictions (Nishida, et al., 2009; Pare, et al., 2002).

The third prediction of the model is that the inverse REM-relationship would hold for the magnitude of emotional depotentiation after sleep. This too appears to be the case as shown by the neuroimaging study discussed in Chapter 4. Participants who had a normal night of sleep displayed a significant overnight decrease in amygdala reactivity in response to re-exposure to the pictures (**Figure 2a&b, Chapter 5**), together with a concomitant increase in amygdala-vmPFC connectivity (**Figure 2c&d, Chapter 5**), whereas the opposite was true for participants who were awake during the day. Furthermore, sleep also resulted in a significant dissipation of subjective emotional intensity ratings, relative to the equivalent period of wake. Additionally, the effect of REM-sleep physiology on emotional reactivity was investigated, with a focus on high-frequency gamma EEG power over the prefrontal cortex as a validated but indirect measure of central adrenergic activity. Consistent with the model's predictions, the success of overnight emotion depotentiation at both a brain (amygdala) and behavioral (intensity ratings) level was predicted by gamma EEG activity. Participants expressing the lowest REM gamma, showed the greatest beneficial overnight reduction in emotion intensity (**Figure 4a through 4d, Chapter 5**). Therefore, the lower the levels of gamma EEG activity, potentially reflecting the degree of beneficial decrease in noradrenergic activity during REM sleep, the greater the decrease in next-

day emotional brain and behavioral reactivity.

Without discounting the contribution of other bioamines (Dahan, et al., 2007) or NREM sleep (Landsness, Goldstein, Peterson, Tononi, & Benca, 2011; Peterson & Benca, 2006; Plante, Landsness, et al., 2012b), it therefore appears that REM sleep and the extent of associated adrenergic reduction, accurately predicts the degree to which sleep dissipates the neural (amygdala) and behavioral strength of emotion from prior affective experiences. Furthermore, this decrease in subcortical limbic reactivity appears to advantageously allow for the next-day reestablishment of amygdala-PFC coupling, offering further regulatory control.

A fourth prediction of the model concerns the broader role of REM-sleep in resetting emotional brain networks in the absence of an emotional stimulus. If emotional neural networks function differently following sleep than following wake, resulting in a behavioral depotentiation of emotional experiences, the affective neural networks could also function differently in the absence of a task, so called 'resting state'. The resting state study discussed in Chapter 5 shows exactly that. First, it demonstrates that the behavioral emotional sensitivity of a given individual is not stable across a time period of hours spanning wake and sleep. Furthermore, these shifts in the behavioral expression of emotional sensitivity are, in part, associated with underlying neural changes in the functional coupling of the human amygdala at rest. Specifically, time awake across the day significantly decreased connectivity within individuals between the amygdala and the phylogenetically newer mesial PFC region, itself associated with processes of top-down regulatory and cognitive control. In contrast, significant increases in amygdala connectivity developed within individuals, over time awake, in the phylogenetically older arousal related fight/flight brainstem regions proximal to the LC. Moreover, the equivalent time, but containing a night of sleep, resulted in a reverse pattern of these resting state connectivity changes, increasing mPFC coupling, while decreasing amygdala-brainstem coupling. Finally, the extent of this amygdala connectivity shift, and associated behavior, was predicted by the decrease in REM gamma EEG activity, a measure previously associated with the extent of central noradrenergic activity.

Implications for psychiatric conditions

The REM-sleep model has important implications for psychiatric conditions. If the process of decoupling emotion from memory is not achieved across the first night following an affective experience, the model predicts a repeat attempt of the affective demodulation on subsequent nights, since the strength of the emotional "tag" associated with the memory would remain high. If this process fails a second time, the same events will continue to repeat across ensuing nights. It is just such a cycle of REM-sleep dreaming (nightmares) that represents a diagnostic key feature of the anxiety condition of PTSD (Lavie, 2001). Notably these patients additionally continue to display hyperarousal reactions to associated trauma cues (Harvey, Jones, & Schmidt, 2003; Pole, 2007), suggesting that the process of separating the affective tone from the emotional experience has not been accomplished, and is hence consistently re-lived during subsequent cued or deliberate waking recollection (**Figure 1a**).

Supporting this possibility, PTSD has been associated with a dysregulation of REM-sleep, together with reports of significantly increased sympathetic autonomic tone (Harvey, et al., 2003; Mellman & Hipolito, 2006). Furthermore, objective sleep disturbances occurring early after trauma exposure, as well as heightened sympathetic vagal tone during REM-sleep, are all associated with an increased risk of meeting criteria for PTSD at subsequent assessments conducted up to 1 year later (Koren, Arnon, Lavie, & Klein, 2002; Mellman, Bustamante, Fins, Pigeon, & Nolan, 2002). Indeed, it has been shown in war veterans that the presence of insomnia

4 months post-deployment is a significant predictor of depression and PTSD symptoms 8 months later (Wright, et al., 2011).

The collection of findings linking (REM) sleep abnormalities to the development of PTSD have led to the possibility that sleep, and particularly REM-sleep, may play an important role in the pathophysiology of PTSD (Germain, Buysse, & Nofzinger, 2008b; Spoormaker & Montgomery, 2008a). Importantly, beyond simple increases or decreases in the total time spent in REM-sleep, qualitative features of REM-sleep may be more accurate and indicative signatures of functional and dysfunctional affective processing. Such features include the structure of REM (e.g. fragmentation), and physiology (e.g. high frequency EEG activity, the latter potentially reflective of qualitative changes in hyper arousal related to central adrenergic activity).

I offer the thesis that it is the pathological persistence of central brain adrenergic activity during REM-sleep in particular, reflected in hyperarousal signals (Harvey, et al., 2003; Pole, 2007; Strawn & Geracioti, 2008), that prevents the capacity of REM-sleep for palliatively decreasing the emotion from the traumatic memory, leaving some patients unable to integrate and importantly depotentiate this stored experience. Moreover, the consequential next-day persistent amygdala hyper-reactivity may further prevent the capacity for a return of adaptive amygdala-PFC connectivity and hence regulation. Conversely, the model predicts that treatments that dissipate adrenergic activity during REM-sleep would have a beneficial clinical outcome in PTSD. It is precisely this benefit that appears to be represented by the recent pharmacological intervention success in PTSD patients. Nocturnal alpha-adrenergic blockade using prazosin in both patients with combat PTSD (Calohan, Peterson, Peskind, & Raskind, 2010; Raskind, et al., 2000; Raskind, et al., 2007; Raskind, et al., 2003; Raskind, et al., 2002) and civilian PTSD (Taylor & Raskind, 2002; Taylor, et al., 2006; Taylor, et al., 2008), has been demonstrated to decrease trauma-dream symptomatology and restore characteristics of REM-sleep. Such findings support a proposed functional role for adrenergic changes during REM-sleep in affective regulation, and in excess, dysregulation.

The REM-sleep model offers a putative underlying neurobiological mechanism explaining this pharmacological treatment success (**Figure 1b**). Specifically, the nighttime blockade of central adrenergic activity during REM-sleep in PTSD dissipates levels back to a potentially critical sub-threshold and normative level, allowing the permissive first stages of emotional dissipation of trauma experiences in REM-sleep, and by doing so, improve clinical symptomatology associated with the trauma memory.

Summary

Evidence to date supports a causal and bi-directional relationship between sleep and emotional brain function. Without sleep, the ability to adequately regulate and express emotions is compromised at both a brain and behavioral level, present for both positive and negative domains of the emotional valence spectrum. In contrast, when sleep is obtained and especially REM-sleep, it instigates a restoration of appropriate emotion recognition, reactivity and associated regulation. Beyond processes of reactivity and recognition, reports further implicate sleep, and REM-sleep most strongly, in the offline modulation of emotional memories and resting state brain networks. Such findings support the proposed model in which REM-sleep is capable of enhancing the memory of prior affective experiences on the one hand, while on the other, dissipating the emotional tone originally associated with such salient experiences at the time of initial exposure. Moreover, this model offers clarifying neurobiological insights underlying the neural mechanisms that contribute to PTSD, as well as explain the recent success

of pharmacological intervention using adrenergic blockers in PTSD.

The research field of sleep and affective brain function is, however, only in its infancy, with much yet to understand. We currently know little about the interplay between peripheral and central nervous system mechanisms leading to abnormalities of emotion processing caused by sleep deprivation. Similarly, the precise combination of sleep factors that reset the balance for optimal next-day emotional brain function is unclear. Is it the stages of sleep, their quantity or quality, their brain oscillations, their neurochemistry? Or the cycling nature of sleep, and/or the timing of sleep? Conversely, why do some emotional events we experience while awake impact our sleep at night, while others do not? Is it their intensity, novelty, salience, valence, temporal proximity to sleep, relationship with past experiences or degree of unresolved understanding? We need to look no further than mood disorders to appreciate this wake-sleep reciprocity that modulates affective states. What does seems clear, however, is that the clinical, professional and public health ramifications of this emerging association between sleep and affective brain function are profound. Moreover, and considering the continued erosion of sleep time throughout industrialized nations, such evidence perhaps should stir emotions in us all.

Figures

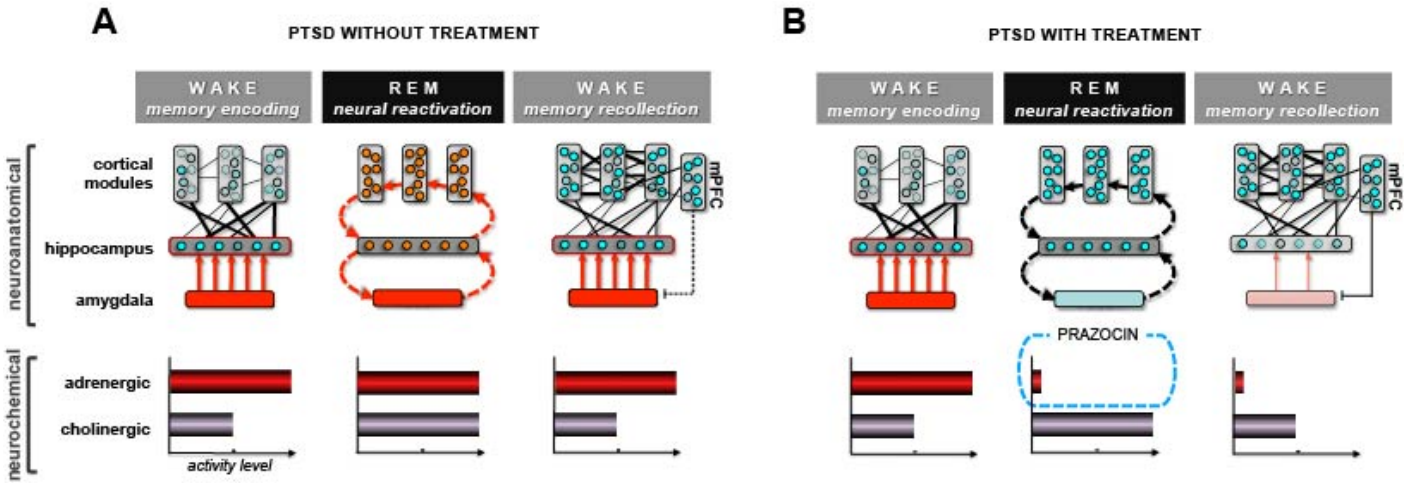

Figure 1. REM-sleep model applied to PTSD. A) REM-sleep in PTSD without treatment, characterized by a pathological persistence of central brain adrenergic activity. Elevated adrenergic activity during REM-sleep prevents the depotentiation of the emotion tone associated with the salient, including traumatic, experiences. B) REM-sleep in PTSD with treatment by Prazosin, allowing for the reduction in adrenergic levels during REM-sleep (note difference in middle panel between a and b). Consequently, there is the restored opportunity for the dissipation of emotion from the prior trauma experiences, preventing hyperarousal reactions during subsequent post-sleep memory recall.

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