

Sleep and Cortisol Interact to Support Memory Consolidation

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Separate lines of research have demonstrated that rises in cortisol can benefit memory consolidation, as can the occurrence of sleep soon after encoding. For the first time, we demonstrate that pre-learning cortisol interacts with sleep to benefit memory consolidation, particularly for negative arousing items. Resting cortisol levels during encoding were positively correlated with subsequent memory, but only following a period of sleep. There was no such relation following a period of wakefulness. Using eye tracking, we further reveal that for negative stimuli, this facilitative effect may arise because cortisol strengthens the relationship between looking time at encoding and subsequent memory. We suggest that elevated cortisol may “tag” attended information as important to remember at the time of encoding, thus enabling sleep-based processes to optimally consolidate salient information in a selective manner. Neuroimaging data suggest that this optimized consolidation leads to a refinement of the neural processes recruited for successful retrieval of negative stimuli, with the retrieval of items attended in the presence of elevated cortisol and consolidated over a night of sleep associated with activity in the amygdala and vmPFC.

Keywords: attention, emotion, fMRI, glucocorticoids, stress

Introduction

The ability to effectively remember information scaffolds all human knowledge. This essential operation occurs not only during encoding and retrieval, but also during the intervening period of memory consolidation, or the time-dependent, largely offline period that allows new memories to become stabilized in the brain. While a plethora of variables can affect the likelihood that information will become stabilized in memory, sleep and the stress hormone cortisol are two of the most influential factors that impact memory consolidation. However, the literatures associated with each of these factors are largely independent (although see [Wagner and Born 2008](#)), and in spite of important links between them in normal aging (e.g., [vanCauter et al. 2000](#)) and various forms of psychopathology that are also associated with memory deficits (e.g., [Otte et al. 2005](#); [Antonijevic 2008](#)), it is not yet understood how sleep and cortisol interact to support memory formation.

Effects of Cortisol on Memory Consolidation

Because many memory-relevant brain regions, including the hippocampus, prefrontal cortex (e.g., [Lupien and LePage 2001](#)), and amygdala (e.g., [Roozendaal 2000](#), [Roozendaal et al. 2009](#)), are rich in cortisol receptors, cortisol exposure can have a marked impact on declarative memory performance. However, the impact of cortisol on memory is complex, and

can depend upon the phase of memory targeted, how cortisol is manipulated, and whether the studied information is emotionally arousing or neutral.

Cortisol or stress exposure, when applied either shortly before or after a learning experience, often selectively modulates the consolidation of emotionally arousing stimuli ([Buchanan and Lovallo 2001](#); [Cahill and Alkire 2003](#); [Cahill et al. 2003](#); [Kuhlmann and Wolf 2006](#); [Payne et al. 2007](#); [Smeets et al. 2008](#); for review, see [Wolf 2009](#)). For example, [Buchanan and Lovallo \(2001\)](#) showed that cortisol administration prior to viewing emotional and neutral pictures resulted in enhanced long-term recall performance of emotional pictures relative to neutral pictures after a 1-week delay. Similarly, [Kuhlmann and Wolf \(2006\)](#) administered cortisol prior to the encoding of emotional and neutral pictures and tested free recall immediately after encoding and again 24 h later, which allowed cortisol's effects on encoding and short-term storage to be differentiated from its effects on consolidation. Although memory performance was unaffected in the immediate test, participants recalled more emotional pictures but fewer neutral pictures following the delay relative to a placebo control group. Exposure to psychosocial stressors, such as the Trier Social Stress Test (TSST), often produces similar effects to cortisol administration. Using the TSST to induce stress and elevate cortisol prior to learning, [Payne et al. \(2007\)](#) found enhanced memory for a narrated slideshow that was emotional in nature, but impaired memory for a closely matched neutral slideshow when tested one week later (see also [Payne et al. 2006](#)). Interestingly, because all of these studies employed retention delays of at least 24 h spanning a period of sleep, they were unable to determine whether sleep is necessary for the beneficial effect of stress on memory consolidation to emerge.

Although there are several potential explanations for cortisol's selective benefit to emotionally arousing information, one is that it helps “tag” this information at encoding in a way that facilitates subsequent memory consolidation. The concept of “emotional tagging” suggests that the encoding of arousing material activates neural mechanisms, likely involving the amygdala and other key memory regions, resulting in long-term plasticity in those synapses marked by the tag ([Richter-Levin and Akirav 2003](#); see also [Morris 2006](#); [Wang and Morris 2010](#)). Working in concert with the arousal generated by the emotional stimuli themselves, elevated cortisol at the time of encoding might help set these tags. Consolidation processes would then select this information for preferential processing in a manner that leads to long-lasting plastic changes. A recent fMRI study provides preliminary support for this idea. [Van Stegeren et al. \(2007\)](#) demonstrated that participants with higher endogenous cortisol levels had markedly stronger

amygdala responses to emotional pictures compared with participants with lower cortisol levels, whereas administration of the noradrenergic antagonist propranolol blocked this cortisol-dependent amygdala activation. Thus, the enhancing effect of cortisol on the consolidation of emotional information likely depends on interactions with arousal-induced noradrenergic activation in the amygdala (Roosendaal et al. 2007), and perhaps on consequent strengthening of connections among the amygdala, hippocampus, and prefrontal regions (van Stegeren 2009).

There are, however, exceptions to the pattern of selective emotional memory consolidation under stress (Abercrombie et al. 2003; Andreano and Cahill 2006; Rimmele et al. 2003), and cortisol's influence on memory for neutral information is even more mixed. It has alternately been shown to facilitate (e.g., Abercrombie et al. 2003; Maheu et al. 2005; Andreano and Cahill 2006; Beckner et al. 2006), have no effect on (e.g., Buchanan and Lovallo 2001; Cahill et al. 2003), and impair (e.g., Kirschbaum et al. 1996; Payne et al. 2006, 2007) memory for neutral stimuli. One reason for these discrepant findings may concern important differences between the hormonal effects of endogenous cortisol (e.g., circadian or stress-related) versus those elicited by exogenous cortisol manipulation (e.g., where cortisol is directly administered). Endogenous cortisol elevation is associated with multiple endocrine alterations, including increased secretion of corticotropin-releasing hormone (CRH; Croiset et al. 2000) and noradrenergic activation (Roosendaal et al. 2006). In contrast, pharmacological glucocorticoid treatment selectively increases cortisol, and inhibits the hypothalamic-pituitary-adrenal (HPA) axis, thus reducing CRH secretion (Stark et al. 2006). As animal models have shown that CRH enhances emotional learning and memory (Roosendaal et al. 2002), this may explain why increasing endogenous cortisol (e.g., Payne et al. 2007) may have different effects on memory than administering cortisol exogenously (e.g., Rimmele et al. 2003). The effects of cortisol on memory may also depend upon dose: Small cortisol elevations may facilitate emotional learning, while large increases may impair it (for review, see Lupien and LePage 2001). Thus, the mnemonic effects of cortisol elevation during extreme stress likely do not parallel the effects of more modest fluctuations in cortisol levels.

To circumvent these complexities, the present study focuses on resting cortisol levels prior to encoding, examining how individual differences in these levels may influence the efficacy of memory formation. Moreover, while prior studies examining the effects of cortisol on memory consolidation have tested memory after a delay of at least 24 h (including a night spent asleep), here we distinguish the effects of resting cortisol levels on memory consolidation over a sleep-filled delay from a wake-filled delay of comparable duration.

Effects of Sleep on Memory Consolidation

Like stress, sleep has a profound impact on declarative memory consolidation. Performance on a variety of tasks is better when a retention delay includes an interval of sleep than when it includes a comparable amount of time spent awake (for review, see Diekelmann and Born 2010; Payne 2011; Stickgold and Walker 2013), and it has been proposed that the neurochemical environment during sleep may optimally support memory consolidation (Stickgold 2005; Diekelmann

and Born 2010). The facilitative effects of sleep exist for neutral stimuli, but can be even greater for negatively emotional stimuli (for review, see Walker 2009; Payne and Kensinger 2010). For example, when participants were presented with scenes composed of a negative or neutral object placed on a neutral background, a day spent awake led to reductions in memory for the entirety of the negative arousing scenes (both the objects and backgrounds), while a night of sleep selectively preserved memory for the negative objects (Payne et al. 2008, 2012). This finding suggests that sleep unbinds scenes, consolidating only the most emotionally salient aspect of the experience (Payne et al. 2008; Payne and Kensinger 2010). A recent fMRI study demonstrated that different retrieval networks were involved in successful recognition of the negative objects, depending on whether participants spent a consolidation interval awake or asleep prior to a recognition task (Payne and Kensinger 2011; see also Sterpenich et al. 2009). A diffuse memory network, including activity in the lateral prefrontal and parietal cortices and medial temporal lobe, was activated more strongly during successful retrieval of negative items following a day of wakefulness relative to a night of sleep. However, after a night of sleep, activation corresponding to successful retrieval of negative items was more refined, and centered on limbic areas including the amygdala, ventromedial prefrontal cortex (vmPFC), and cingulate gyrus (Payne and Kensinger 2011). Similar effects were reported by Sterpenich et al. (2009) when retrieval was tested days or months later, with increased retrieval-related activity within the amygdala and vmPFC if participants had slept soon after encoding the items. Together, these studies suggest that the sleep-based facilitation of emotional memory consolidation is reflected in a restriction and refinement of the neural processes needed for successful retrieval. Although it is clear that sleep preferentially benefits memory for emotional information, it is not known why this happens. An interesting possibility is that, just as cortisol's effects on long-term memory may be dependent on sleep, as mentioned previously, the effects of sleep-based consolidation may be intensified in individuals with higher cortisol levels at the time of encoding.

Present Study: Testing the Interactive Effect of Cortisol and Sleep on Memory Consolidation

In the current study, we investigated whether resting levels of cortisol prior to encoding would influence memory consolidation differently depending on whether the retention delay contained sleep or wakefulness. We hypothesized that higher levels of cortisol at encoding, which would help tag emotional items as being important for subsequent processing, would predict better memory for emotional relative to neutral stimuli, but only when participants slept during the retention interval. Although extensive research has examined the separate effects of cortisol and sleep on memory consolidation, the few studies to investigate their interaction have examined cortisol levels during sleep (Plihal and Born 1999; Born and Wagner 2004; Wagner et al. 2005; Wilhelm et al. 2011). No prior study has examined whether pre-sleep cortisol levels influence the efficacy of subsequent memory consolidation during sleep, an interaction that would be predicted if cortisol helps to tag memories at encoding and thus enable their efficient consolidation over periods of sleep (Stickgold and Walker 2013).

Second, we explored and attempted to characterize the attentional and neural mechanisms underlying the interactive effect of cortisol and sleep on memory by examining 1) whether, using eye tracking, elevated cortisol and time spent looking at items during encoding would interact to predict memory for those items following a sleep-based consolidation delay, and 2) whether elevated cortisol during encoding would promote a stronger relation between looking time at encoding and successful retrieval-related neural activity. Both of these relations would be expected if cortisol helps create a tag for salient information at encoding that is later preferentially consolidated during sleep.

To preview our findings, we first demonstrate that pre-encoding cortisol interacts with sleep to influence memory performance: Resting cortisol levels are strongly related to subsequent emotional (and less so, neutral) memory, but only when sleep (and not wakefulness) occurs during the consolidation interval. This novel finding suggests that cortisol may be a mechanism supporting sleep's benefit to memory consolidation. Consistent with the interpretation that cortisol may enable the creation of a tag for salient stimuli, we further show that higher pre-encoding cortisol increases the likelihood that sleep-based consolidation processes selectively act on the emotional information that receives the most attention during encoding. Finally, we show that elevated cortisol during learning promotes a stronger relation between looking time at encoding and successful retrieval-related activity in the amygdala and vmPFC, but only in those who sleep between encoding and retrieval. These results suggest that when emotional stimuli are the target of optimized consolidation, it is reflected in a more focal pattern of activation observed with fMRI during retrieval.

Materials and Methods

Participants

Participants were 134 right-handed native English speakers with normal or corrected-to-normal vision. The 126 used here (18–34 years old, $M = 22.4$) are those who gave an uncontaminated cortisol sample prior to encoding. They were screened for neurological, psychiatric, and sleep disorders, and for medications affecting the central nervous system or sleep architecture. They also were required to sleep for at least 7 h a night and be in bed by 2:00 AM for the five nights leading up to the study. Informed consent was obtained in a manner approved by the Boston College Institutional Review Board.

Participants were assigned to one of four groups: Sleep, Wake, Morning Short Delay, and Evening Short Delay, which were scheduled simultaneously. We subsequently scheduled an additional Morning Short Delay and Evening Short Delay group, as we wanted to have additional control groups who underwent eye tracking during encoding (see “Conditions” section below for more detail). Although full random assignment was not possible due to class schedules and other scheduling conflicts, we ensured that the groups did not differ in scores on the Morningness-Eveningness Questionnaire (Horne and Ostberg 1976; $F_{3,122} = 0.19$, $P = 0.90$), thus minimizing concerns about time of day preference effects between groups.

Conditions

The two conditions of critical interest in the present study were the Sleep condition (25 participants: 12 females) and the Wake condition (17 participants: 8 females). Participants in these groups were matched on a number of factors, including age ($P = 0.33$), scores on the Beck Depression Inventory (BDI; Beck and Beamesderfer 1974; $P = 0.69$), Beck Anxiety Inventory (BAI; Beck et al. 1988; $P = 0.63$), and

Morningness-Eveningness Questionnaire (MEQ; $P = 0.91$), and the amount of sleep obtained on the night before retrieval ($P = 0.23$). Participants in the Wake condition viewed the stimuli in the morning (7:00–10:00 AM) and were tested 12 h later following a full day of wakefulness; they did not nap between sessions. Participants in the Sleep condition viewed the stimuli in the evening (8:00–10:00 PM) and were tested 12 h later, following a full night of sleep in the laboratory. Sleep amount was statistically equivalent between groups the night before retrieval (Sleep: $M = 7.56$, $SD = 0.73$; Wake: $M = 6.95$, $SD = 1.56$; $t_{41} = 1.21$, $P = 0.23$).

The Morning and Evening Short Delay conditions were included as circadian control groups to ensure that differences between the Sleep and Wake groups were not due to time of day effects, and included two different subsets of participants. The first subset of participants in the Short Delay conditions encoded and retrieved stimuli while undergoing fMRI (Morning fMRI subset: 23 participants: 12 females; Evening fMRI subset: 15 participants: 9 females). A second subset of participants was scheduled in order to allow the collection of eye-tracking data, as occurred in the Sleep and Wake groups; these participants encoded stimuli during eye tracking and retrieved stimuli outside of the scanner (Morning eye-tracking subset: 23 participants: 12 females; Evening eye-tracking subset: 23 participants: 12 females).

Participants in the Morning and Evening Short Delay conditions were also matched on age ($P = 0.43$), BDI scores ($P = 0.27$), BAI scores ($P = 0.48$), MEQ scores ($P = 0.81$), and the amount of sleep obtained on the night before retrieval ($t_{44} = 0.59$, $P = 0.56$). These participants viewed the stimuli between 7:00 and 10:00 AM (Morning Short Delay condition) or 7:00 and 10:00 PM (Evening Short Delay condition) and were tested 20 min after encoding (see supplementary Appendix A for depiction of experimental design).

Cortisol Procedure

Cortisol was assessed via saliva, which provides an index of bioavailable free cortisol (Kudielka and Kirschbaum 2005). To avoid contamination of cortisol samples, participants were required to refrain from physical activity, eating, drinking (anything besides water), smoking, and brushing their teeth during the 2 h prior to encoding, and also to refrain from drinking water for at least 15 min prior to encoding. Only one Morning Short Delay participant failed to follow these instructions, whose data are not included in the analyses due to the contaminated cortisol sample.

Although saliva samples were collected at multiple time points (see supplementary Appendix A for entire experimental design), per our research question, here we analyze only the sample collected prior to encoding for each group. Due to variability in factors such as the time to calibrate eyes to the eye tracker (Sleep, Wake, Short Delay eye-tracking subset), and the time to set participants up in the scanner (Short Delay fMRI subset) prior to encoding, there was some variability in the length of time that elapsed between the cortisol sample and time of encoding. Importantly, however, including sample-to-encoding time as an additional regressor in the subsequently reported analyses did not qualitatively change the results reported in the manuscript (see supplementary Appendix B.1). Additionally, given the diurnal variation in cortisol (e.g., Kahn et al. 1988), with this experimental design (i.e., the Wake group encodes in the morning, while the Sleep group encodes in the evening), it is important to ensure that the amount of time between waking and the pre-encoding cortisol sample did not influence the subsequently reported results. Indeed, it did not (see supplementary Appendix B.2). For a list of participants' exact wake time and pre-encoding cortisol sampling time, please see supplementary Appendix C.

Encoding Procedure

During encoding, participants studied 124 composite scenes for 3 s each. These scenes were composed of either a negative object or a neutral object (62 each) placed on a plausible neutral background. By “plausible,” we mean that either version of the scene could theoretically be observed in real life; for instance, an avenue would be a plausible neutral background for both a taxi cab (neutral) and taxi cab accident (negative). Objects had been previously rated for valence and

arousal (1 = low; 7 = high), with negative objects rated as highly arousing and low in valence (arousal: 5–7; valence <3), and neutral objects rated as non-arousing and neutral in valence (arousal <4; valence: 3–5; Kensinger et al. 2007; Waring and Kensinger 2009). Participants in the present study also gave valence and arousal ratings for the objects at the end of the study; their ratings confirmed that negative objects were highly arousing and low in valence (Arousal: M : 5.33, SD : 1.35; Valence: M : 2.58, SD : 1.37) and that neutral objects were non-arousing and neutral in valence (Arousal: M : 3.85, SD : 1.23; Valence: M : 4.44, SD : 1.14).

To ensure that participants were actively thinking about each scene, participants indicated whether they would approach or back away from the scene if they encountered it in real life (as in Payne and Kensinger 2011). This task was chosen because it requires participants to think about their reactions to the scenes, a type of self-referential processing that is likely to lead to deeper encoding (Symons and Johnson 1997). The Sleep and Wake groups encoded stimuli outside of the scanner while undergoing eye tracking to assess attentional factors at encoding. Encoding occurred in two blocks of 62 images each, with negative and neutral scenes randomly intermixed within each block. We divided the encoding session into two blocks to allow a short break (length determined by each subject; ~10–60 s long) between blocks so that participants could have an opportunity to sit back from the eye tracker and rest their eyes. The Morning Short Delay and Evening Short Delay (eye tracking) control groups underwent this exact same encoding procedure. The Morning Short Delay and Evening Short Delay (fMRI) control groups encoded the stimuli while undergoing fMRI (without eye tracking). They encoded all 124 negative and neutral scenes, also in random order, in a single block (eye gaze was not tracked, so there was no need for a break to rest the eyes). For example stimuli and a visual depiction of the encoding procedure, see supplementary Appendix D.

Eye-tracking Data Collection and Analysis

The eye-tracking apparatus was a SensoMotoric Instruments (SMI) eye-tracker. Participants' eye gaze patterns were tracked at 500 Hz by a SMI iView X Hi-Speed 1250 tracking column. Participants were seated 24 inches away from a 14-inch computer screen at eye level with the center of the computer screen. Prior to the encoding task, each participant's eye gaze was tracked during a 17-point calibration; participants were asked to shift their gaze to 17 points on the computer screen to ensure that the eye tracker was accurately tracking the left pupil within 1° of accuracy in each direction (x and y). Participants were asked to look naturally at the screen as each picture appeared and to look at the fixation cross in between trials.

For eye-tracking data analysis, we computed the percentage of time during which visual fixations, defined as a 50 ms or longer gaze, were within 1° of a predetermined area of interest (AOI; Manor and Gordon 2003). The AOIs were drawn around the negative and neutral objects within the scenes using BeGaze software, which was also used to analyze the data. As recommended by the eye-tracking software manufacturers, the first fixation for each image was excluded from the analysis, as it usually represents the last fixation from the previous trial (i.e., where the fixation cross was located on the previous screen). Eye-tracking data from three participants (one female Sleep participant, one female Morning Short Delay participant, and one male Evening Short Delay participant) were unable to be analyzed, leaving 24 Sleep participants and 22 each of Morning and Evening Short Delay participants included in analyses focusing on eye gaze.

Recognition Procedure

Following the 12-h (Sleep and Wake conditions) or 20-min (Morning and Evening Short Delay conditions) delay, participants performed an unexpected recognition task. They viewed objects and backgrounds, presented separately and one at a time, and indicated whether each was "old" (included in a previously studied scene) or "new" (not previously studied). On the recognition test were 124 old objects (62 negative, 62 neutral), 124 old backgrounds (62 studied with a negative object, 62 studied with a neutral object), 124 new objects (62 negative, 62 neutral), and 124 new backgrounds (by definition, all neutral; for

example stimuli and a visual depiction of the retrieval procedure, see supplementary Appendix D). Analyses in the current study focus on participants' memory for negative and neutral objects.

fMRI Image Acquisition and Preprocessing (For Sleep and Wake Groups)

Data were acquired on a 3.0T Siemens Trio Scanner (Trio, Siemens Ltd., Erlangen, Germany) using a standard 12-channel head coil. The stimuli were projected from a Macintosh MacBook to a color LCD projector that projected onto a screen mounted in the magnet bore. Participants viewed the screen through a mirror located on the head coil.

Anatomical images were acquired using a high-resolution 3D multi-echo magnetization prepared rapid acquisition gradient echo sequence (MEMPRAGE; repetition time = 2200 ms; echo times = 1.64, 3.5, 5.36, 7.22 ms; flip angle = 7°; field of view = 256 × 256 mm; acquisition matrix 256 × 256; number of slices = 176; 1 × 1 × 1 mm resolution). Coplanar and high-resolution T_1 -weighted localizer images were acquired. In addition, a T_1 -weighted inversion recovery echo-planar image was acquired for auto alignment.

Functional images were acquired via a T_2^* -weighted EPI sequence sensitive to the blood oxygen level-dependent (BOLD) signal, with a repetition time of 3000 ms, an echo time of 30 ms, and a flip angle of 85°. Forty-seven interleaved axial-oblique slices (parallel to the line between the anterior and the posterior commissures) were collected in a 3 × 3 × 3 mm matrix.

Preprocessing and data analysis were completed using SPM8 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). Slice time correction was completed, and motion correction was run, using a 6-parameter, rigid-body transformation algorithm by SPM8. The images were normalized to the Montreal Neurological Institute (MNI) template. The resultant voxel size was 3 × 3 × 3 mm, and spatial smoothing was completed at a 6 mm isotropic Gaussian kernel.

Event-Related fMRI Data Analysis

At the first level of analysis for each subject, the regressors of interest were the time points when objects were viewed, broken down by object valence and object memory (hits vs. misses). For each of those regressors, we included the proportion fixation on the object as a parametric modulator. The first-level model also included the following regressors of no interest: Hits and misses to backgrounds were modeled separately, and instances where new items were presented (false alarms and correct rejections) were modeled together. Additionally, a regressor accounting for linear drift was included.

At the second-level group analysis, we conducted one analysis for the Sleep group and one analysis for the Wake group, each examining the parametric relation between looking time at encoding and neural activity during successful retrieval of negative objects (hits). We additionally compared the activity between the two groups using both exclusive and inclusive masking procedures. In these group analyses, each participant's cortisol level at encoding was entered as a regressor. Only regions that consist of at least 9 contiguous voxels, with peak activity at $P < 0.001$, as determined by a Monte Carlo simulation to correct for multiple comparisons at $P < 0.05$ (Slotnick et al. 2003), are reported in the results.

Results

Effects of Cortisol and Sleep on Emotional Memory

We first address the behavioral hypothesis that higher levels of cortisol at encoding will facilitate memory for emotional more than neutral stimuli, and that this effect will be enhanced when the consolidation period includes sleep.

Memory Performance by Valence and Group

The average percentage of successfully remembered objects, organized by Valence and Group, is summarized in Table 1.

Table 1

Mean (SD) hit, false alarm (FA), and corrected recognition (hits minus FA) performance on the memory test as a function of Group and object Valence

	Sleep		Wake		Morning Short Delay		Evening Short Delay	
	Negative	Neutral	Negative	Neutral	Negative	Neutral	Negative	Neutral
Hits	0.79 (0.11)	0.57 (0.15)	0.74 (0.15)	0.55 (0.17)	0.76 (0.14)	0.67 (0.13)	0.82 (0.12)	0.74 (0.13)
False alarms (FA)	0.18 (0.10)	0.13 (0.08)	0.17 (0.10)	0.15 (0.10)	0.16 (0.10)	0.13 (0.09)	0.16 (0.10)	0.13 (0.09)
Corrected recognition	0.60 (0.15)	0.43 (0.14)	0.56 (0.18)	0.40 (0.16)	0.60 (0.15)	0.54 (0.13)	0.65 (0.17)	0.61 (0.17)

A mixed effects analysis of variance, with Valence (within-subjects), Delay length (between-subjects), and Time of Testing (between-subjects) entered as factors of interest, determined that there was a significant main effect of Valence on corrected recognition ($F_{1,76} = 52.873$, $P < 0.001$; $\eta p^2 = 0.41$): Corrected recognition scores (hits minus false alarms) were significantly higher for negative compared with neutral objects. This is consistent with the emotional memory enhancement effect (for reviews, see Hamann 2001; Buchanan and Adolphs 2002). Additionally, there was a Delay length by Valence interaction, such that the emotional memory enhancement effect (i.e., the memory benefit for negative relative to neutral stimuli) was intensified after a long delay (for the Sleep and Wake groups) compared with a short delay (Morning and Evening Short Delay groups); $F_{1,76} = 15.883$, $P < 0.001$; $\eta p^2 = 0.17$. There was no interaction between Delay length and Time of Testing ($F_{1,76} = 0.092$, $P = 0.76$; $\eta p^2 = 0.001$), indicating that the effect of Delay length was comparable regardless of whether participants were tested in the morning (i.e., Morning Short Delay and Sleep groups) or in the evening (i.e., Evening Short Delay and Wake groups). There also was no interaction between Valence, Delay length, and Time of Testing ($F_{1,76} = 0.024$, $P = 0.88$; $\eta p^2 < 0.001$).

Effects of Cortisol on Consolidation: Sleep Versus Wake

We first examined how pre-encoding cortisol influenced memory consolidation for negative and neutral objects across the Sleep- and Wake-filled delays. Multiple regression analyses were conducted with Group (Sleep vs. Wake), Cortisol level, and the Group by Cortisol interaction term as predictors of corrected recognition. The interaction term was created by multiplying the Group (Sleep vs. Wake, dummy-coded) and Cortisol variables. Not unexpectedly due to the circadian rhythm of cortisol (e.g., Kahn et al. 1988), Sleep subjects' mean (SE) raw cortisol level was 0.080 $\mu\text{g/dL}$ (0.009), and Wake subjects' mean (SE) raw cortisol level was 0.528 $\mu\text{g/dL}$ (0.063). All analyses were conducted using participants' raw cortisol values. However, all figures plot standardized cortisol values, to better visually depict how the effect of cortisol on memory differs between the Sleep and Wake groups.

Focusing first on memory for negative objects, there was a main effect of Cortisol on memory (hits minus false alarms), such that higher pre-encoding cortisol levels predicted better memory performance ($t_{41} = 2.18$, $\beta = 2.49$, $P = 0.035$). There was no main effect of Group. The main effect of Cortisol on negative object memory was qualified by a Group by Cortisol interaction ($t_{41} = 2.23$, $\beta = 2.92$, $P = 0.031$): Resting levels of pre-encoding cortisol predicted negative object memory across the Sleep-filled delay ($t_{24} = 2.31$, $\beta = 0.43$, $P = 0.031$), but not across the Wake-filled delay ($t_{16} = 0.40$, $\beta = 0.10$, $P = 0.70$; see Fig. 1A). For neutral object memory, the pattern of results was similar but weaker. There was neither a main effect of Cortisol nor of Group, and the Group by Cortisol interaction term was

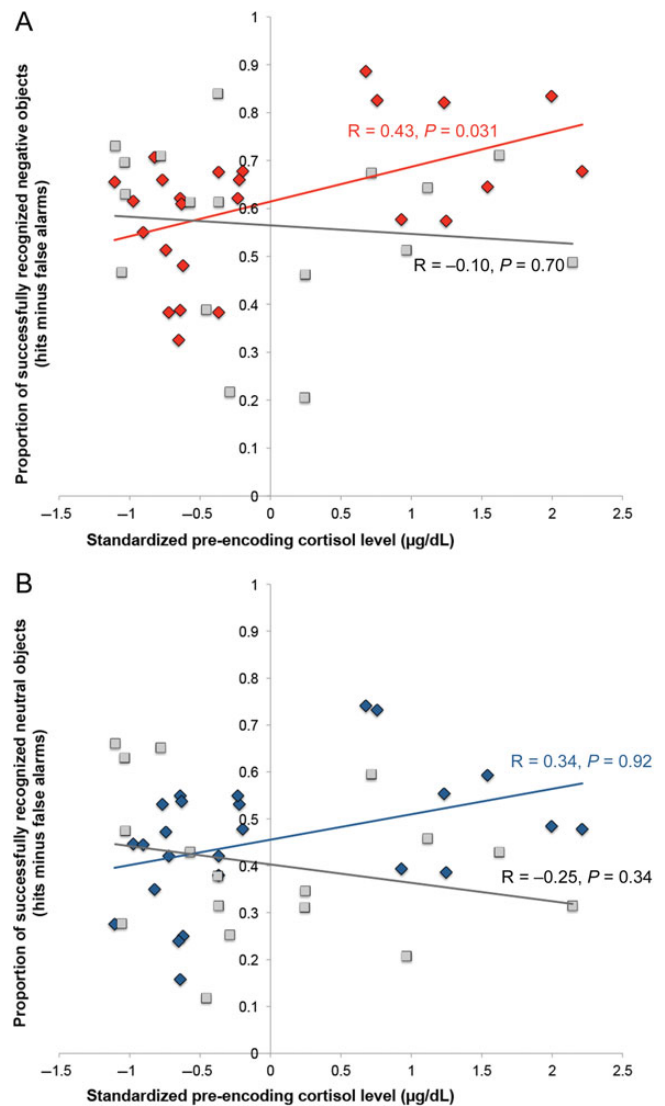


Figure 1. Panel (A) plots the effect of cortisol levels on memory performance for negative objects. There was a strong relation between standardized levels of cortisol (x-axis) and memory performance (y-axis) in the Sleep group (in red) but not the Wake group (in gray). The interaction between Cortisol and Group was significant ($t_{41} = 2.23$, $\beta = 2.92$, $P = 0.031$). Panel (B) plots the effect of cortisol levels on memory performance for neutral objects. There was a marginally significant relation between standardized levels of cortisol (x-axis) and memory performance (y-axis) in the Sleep group (in blue), but not the Wake group (in gray). The interaction between Cortisol and Group was marginally significant ($t_{41} = 1.95$, $\beta = 2.55$, $P = 0.059$). Legend: Sleep [red diamonds (neg), blue diamonds (neu)], Wake [gray squares], Sleep Linear Fit [red line (neg), blue line (neu)], Wake Linear Fit [gray line].

marginally significant ($t_{41} = 1.95$, $\beta = 2.55$, $P = 0.059$): Cortisol marginally predicted neutral object memory in the Sleep group ($t_{24} = 1.76$, $\beta = 0.34$, $P = 0.092$), but not the Wake group

($t_{16} = 0.98$, $\beta = 0.25$, $P = 0.34$; see Fig. 1B). Thus, pre-encoding cortisol leads to a stronger memory trace, but only if sleep occurs during the consolidation interval (i.e., only if consolidation is optimized).

Effects of Cortisol on Consolidation: Eliminating Potential Confounds of Time of Day, Gender, and Menstrual Cycle

In order to make the claim that the enhancing effects of cortisol on memory are linked to the sleep that occurred post-encoding, it is important to address the possibility that circadian effects influenced memory performance. Thus, similar multiple regression analyses investigating the effects of cortisol on memory were run for the Morning and Evening Short Delay groups as those run for the Sleep versus Wake groups (see previous section). Importantly, there was no main effect of Cortisol or Group (Morning vs. Evening Short Delay) on memory for negative objects or neutral objects. Critically, there was no Group by Cortisol interaction for negative objects ($t_{37} = 1.42$, $\beta = 0.33$, $P = 0.17$) or neutral objects ($t_{37} = 1.05$, $\beta = 0.24$, $P = 0.30$), suggesting that time of day did not influence the effects of cortisol on consolidation.

As the activity of the HPA axis, and more specifically, fluctuations in cortisol, vary by gender and menstrual cycle phase (Kirschbaum et al. 1999), we also investigated whether either variable was a significant predictor in any of the analyses on the effects of cortisol on consolidation. There was no main effect of Gender, nor was there a Cortisol by Gender interaction for any group for negative (all P 's > 0.12) or neutral (all P 's > 0.34) object memory. When female participants were grouped according to whether they were in the follicular (days 1–13 of the menstrual cycle) or the luteal phase (day 14 of the cycle until the beginning of the next period) at the time of the experiment, Cycle was not a significant predictor of pre-encoding cortisol level (all P 's > 0.11), nor of corrected recognition of negative (all P 's > 0.26) nor neutral (all P 's > 0.58) objects.

Effects of Cortisol on Consolidation: Interim Summary

Although previous studies have tied pre-learning cortisol to facilitated memory for negative stimuli (e.g., Buchanan and Lovullo 2001; Payne et al. 2007), the current study is the first to demonstrate that the effects of pre-encoding cortisol on memory are significant only when sleep occurs during consolidation.

Effects of Cortisol and Sleep on the Interaction Between Attention and Consolidation

We hypothesized that this facilitative effect may be partly due to cortisol's ability to tag information as relevant at the time of encoding, thereby allowing subsequent prioritization of that information during sleep. Thus, we analyzed the eye-tracking data to examine whether higher resting cortisol at encoding would promote an interaction between encoding-phase and consolidation-phase processes, increasing the likelihood that sleep-based consolidation processes selectively act on the information that receives the most attention during encoding.

Effects of Cortisol and Group on Looking Time to Negative and Neutral Objects

We first examined whether resting cortisol levels were related to attention to negative and neutral objects during encoding, regardless of whether the object was subsequently remembered. A linear regression analysis with Cortisol, Group, and the interaction between Cortisol and Group entered as predictors showed that none of these variables affected the proportion of time participants attended to negative (all P 's > 0.30) or neutral objects (all P 's > 0.12) within the scenes. The proportion of time spent viewing the objects is the amount of time participants' gaze was fixed on the object AOI, divided by the total time participants' gaze was directed anywhere else within the scene (i.e., excluding any time when participants momentarily closed their eyes or looked off screen). Thus, despite some prior evidence that cortisol can lead to an avoidance of looking at angry (van Honk et al. 1998) or threatening faces (Roelofs et al. 2007), cortisol levels did not influence attention allocation at encoding in the present study.

Effects of Cortisol on the Interaction Between Attention and Consolidation: Sleep Versus Wake

We next examined the intriguing possibility that pre-encoding cortisol would influence the link between looking time at encoding and subsequent memory, with cortisol's elevation increasing the likelihood that objects viewed only briefly would later be forgotten and that objects that were the focus of attention for longer would be remembered. We thus analyzed the difference in looking time to objects at encoding (i.e., the proportion of time participants looked at the object relative to the rest of the scene) as a function of later memory (proportion of time during encoding participants looked at subsequent "hits" minus the proportion of time during encoding participants looked at subsequent "misses"). See supplementary Appendix E for a visual depiction of the encoding and retrieval procedure as it relates to the calculation of the difference in looking time as a function of memory (the dependent variable).

As expected, participants spent proportionally more time viewing objects they would later remember (70.3% for negative objects; 59.7% for neutral objects) than objects they would later forget (61.6% for negative objects; 48.5% for neutral objects). We then examined whether the strength of this looking-time effect on subsequent memory would be affected by Cortisol or Group. For negative objects, a multiple regression analysis revealed that Cortisol ($t_{40} = 2.05$, $\beta = 2.67$, $P = 0.047$), but not Group (Sleep vs. Wake; $t_{40} = 1.50$, $\beta = 0.51$, $P = 0.14$) significantly predicted the connection between looking time and subsequent memory. There was also a significant interaction between Cortisol and Group ($t_{40} = 2.04$, $\beta = 2.99$, $P = 0.049$), such that cortisol was a stronger predictor of the link between looking time and subsequent memory for negative objects if sleep rather than wake followed encoding: Cortisol marginally predicted the link between looking time and subsequent memory for negative objects in the Sleep group ($t_{23} = 1.869$, $\beta = 0.37$, $P = 0.075$), but did not predict the link between looking time and subsequent memory for negative objects in the Wake group ($t_{16} = 0.168$, $\beta = 0.043$, $P = 0.87$; see Fig. 2A). For neutral objects, there was no main effect of Cortisol ($t_{40} = 0.99$, $\beta = 1.34$, $P = 0.33$) nor of Group ($t_{40} = 0.94$, $\beta = 0.33$, $P = 0.36$), and the Group by Cortisol interaction was not significant ($t_{40} = 1.09$, $\beta = 1.68$, $P = 0.28$): Cortisol did

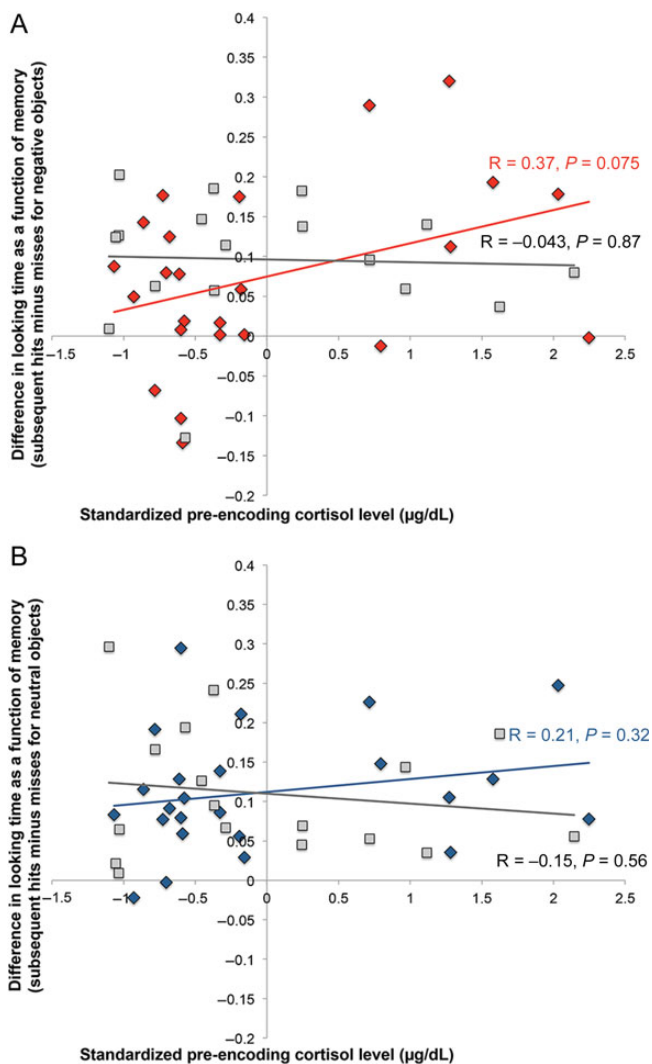


Figure 2. Looking time was calculated as the proportion of total scene viewing time that participants spent looking at the object within the scene. A score was then computed to reflect the difference in looking time between subsequently remembered and subsequently forgotten items, and a linear regression was used to test the effects of cortisol and sleep on this score. Panel (A) plots the results for negative objects. There was a marginally significant relation between standardized levels of cortisol (x-axis) and the difference in looking time (y-axis) in the Sleep group (in red) but not the Wake group (in gray). The interaction between Cortisol and Group was significant ($t_{40} = -2.04, \beta = -2.99, P = 0.049$). Panel (B) plots the effect for neutral objects. There was no effect of standardized levels of cortisol in the Sleep group (in blue) nor the Wake group (in gray), and the interaction between Cortisol and Group was not significant. Legend: Sleep [red diamonds (neg), blue diamonds (neu)], Wake [gray squares], Sleep Linear Fit [red line (neg), blue line (neu)], Wake Linear Fit [gray line].

not predict the link between looking time and subsequent memory for neutral objects in the Sleep group ($t_{23} = 1.026, \beta = 0.21, P = 0.32$) nor in the Wake group ($t_{16} = 0.60, \beta = 0.15, P = 0.56$; See Fig. 2B).

Overall, pre-encoding cortisol appears to affect subsequent memory by promoting the interaction between looking time at encoding and the ability to remember objects later on. However, this effect appears to be specific to negative objects, and critically, it is stronger when sleep directly follows encoding. This novel finding suggests that pre-encoding cortisol modulates the relation between processes implemented during encoding and those exerted during sleep-based consolidation of emotional stimuli.

Effects of Cortisol on the Interaction Between Attention and Consolidation: Eliminating Potential Confounds Associated with Time of Day, Gender, and Menstrual Cycle

As previously described, with a sleep versus wake design, it is important to ensure that any differences found between the Sleep and Wake groups are indeed due to the sleep occurring between encoding and retrieval, as opposed to effects of time of day. Accordingly, we ran similar multiple regression analyses investigating the effects of cortisol on the link between attention and consolidation for the Short Delay groups as those run for the Sleep and Wake groups. There was no main effect of Cortisol or Group (Morning vs. Evening Short Delay) on the relation between looking time and subsequent memory. Critically, there was no Group by Cortisol interaction for negative objects ($t_{43} = 0.44, \beta = 0.093, P = 0.67$) or neutral objects ($t_{43} = 0.81, \beta = 0.18, P = 0.43$), as one might expect if time of day, rather than sleep, was driving the results. Thus, our finding that pre-encoding cortisol promotes the interaction between looking time at encoding and the ability to later remember negative objects can most likely be attributed to sleep, as opposed to the time of testing.

Additionally, we investigated whether the effect of cortisol on the relation between looking time at encoding and subsequent memory could be explained by gender or variations in menstrual cycle. Gender did not affect the link between attention at encoding and subsequent memory (hits minus misses) for negative (all P 's > 0.26) or neutral (all P 's > 0.20) objects, and there also was no Cortisol by Gender interaction for negative (all P 's > 0.27) or neutral (all P 's > 0.09) objects. When female participants were grouped according to whether they were in the follicular or luteal phase at the time of the experiment, Cycle was not a significant predictor of pre-encoding cortisol level (all P 's > 0.23), attention at encoding (negative: all P 's > 0.10, neutral: all P 's > 0.40), or the link between attention at encoding and subsequent memory (negative: all P 's > 0.06, neutral: all P 's > 0.33). Further, including Gender and Cycle as additional regressors did not significantly reduce the predictive strength of the Group by Cortisol interaction.

Effects of Cortisol on the Relation Between Attention at Encoding and Neural Activity During Retrieval (Sleep and Wake Groups)

Our eye-tracking data suggest that cortisol facilitates memory for negative stimuli by promoting the interaction between looking time at encoding and the ability to later remember those stimuli, especially when sleep occurs during the retention interval. These results suggest that the consolidation of these negative stimuli may be optimized when two conditions are met—when the stimuli are viewed for a sufficient period of time during encoding and when sleep occurs during the consolidation interval. If so, we expected to observe differences in the neural regions recruited during the successful retrieval of these optimally consolidated negative stimuli when compared with suboptimally consolidated stimuli (i.e., those not looked at for a sufficiently long time, or those that had only wake during the retention interval). More specifically, we hypothesized that these optimally consolidated negative stimuli would be associated with a refinement in the neural network recruited during their retrieval, as has previously been reported following a period of sleep (e.g., Payne and Kensinger 2011), with

Table 2

Retrieval-related activity in the Sleep group (A) and the Wake group (B): The higher the resting cortisol level, the stronger the parametric relation with looking time to negative objects at encoding

Region	Gyrus	Approximate Brodmann's area (BA)	MNI Coordinates (x, y, z)	TAL coordinates (x, y, z)	Cluster size (no. of voxels)	Activation remained with exclusive masking of wake activity?
A						
Sleep group: Effect of pre-encoding cortisol on the relation between looking time at encoding and successful retrieval-related activity to negative objects						
Frontal lobe	Medial frontal gyrus	8	-12, 30, 42	-12, 31, 37	20	Y
Frontal lobe	Medial frontal gyrus	10	10, 54, 4	10, 52, 1	9	Y
Frontal lobe/cingulate cortex	vmPFC/anterior cingulate	32/10	8, 44, 4	8, 43, 2	26	Y
Temporal lobe	Amygdala*	NA	28, 0, -22	28, -1, -18	11	Y
B						
Wake group: Effect of pre-encoding cortisol on the relation between looking time at encoding and successful retrieval-related activity to negative objects						
Caudate	NA	NA	-14, 10, 20	-14, 11, 18	52	Y
Frontal lobe	Middle frontal gyrus	8	28, 20, 28	28, 21, 25	24	Y
Frontal lobe	Inferior frontal gyrus	47	24, 36, -8	24, 35, -8	10	Y
Insula	NA	13	22, 16, 16	22, 16, 14	28	Y
Temporal lobe	Hippocampus	NA	-36, -32, -2	-36, -31, 0	10	Y
Thalamus/putamen	NA	NA	22, -28, 10	22, -27, 11	12	Y

*Activity in the amygdala is present (within the Sleep group) at the reduced threshold of $P < 0.005$ (9 voxels), while activity in all other regions is present at $P < 0.001$ (9 voxels).

retrieval activity arising mostly in limbic regions including the amygdala and vmPFC.

To test this hypothesis, we examined how pre-encoding cortisol influenced the parametric relation between looking time at encoding and neural activity during subsequent recognition of negative objects. For each subject, we examined the regions that showed retrieval activity that varied parametrically as a function of the looking time devoted to negative objects at encoding. These values were used for a group analysis, including each participant's cortisol level as a regressor. Thus, we were able to investigate the effects of pre-encoding cortisol level on the relation between looking time at encoding and neural activation during successful retrieval of negative and neutral objects. We conducted these analyses separately in the Sleep and the Wake groups and then compared the activity between the two groups by using exclusive masking procedures.

Table 2 reports all significant clusters showing a relation with cortisol within the Sleep group (Table 2A) and the Wake group (Table 2B). There was no overlap in the regions that showed a relation with cortisol in the Sleep and the Wake groups. As hypothesized, the key finding was that activity in the vmPFC ($P < 0.001$) and, at a slightly reduced thresholding level ($P < 0.005$), in the amygdala was observed for those who slept during the consolidation interval, but was absent for those who remained awake (See Table 2A). By contrast, for those who remained awake during the consolidation interval, there was a stronger relation between looking time at encoding and successful retrieval-related activity in a larger set of regions including the inferior frontal gyrus, middle frontal gyrus, and hippocampus (See Table 2B). Thus, when negative objects were attended to in the presence of higher cortisol and subsequently consolidated during sleep, there was a refinement of the processes supporting their successful retrieval, with activity centered on limbic regions.

To confirm these group differences, we used an exclusive masking procedure, thus identifying voxels where effects were not shared between the Sleep group and the Wake group. The activity for the group of interest was thresholded at $P < 0.001$ with a 9-voxel cluster extent. The activity for the group excluded was thresholded at $P < 0.1$ requiring no voxel extent because the more liberal the threshold of an exclusive mask, the more conservative is the masking procedure.

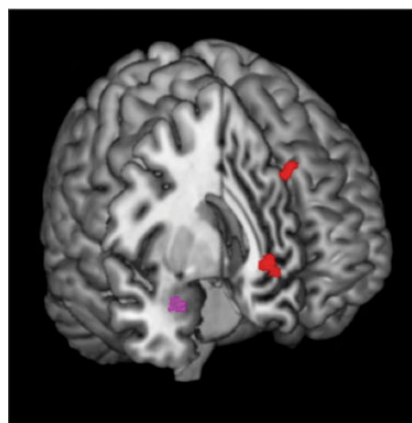


Figure 3. Higher cortisol was associated with a stronger relation between looking time at encoding and activity in limbic regions including the amygdala and vmPFC at retrieval, but only for participants who slept. This figure shows the results of an exclusive masking procedure, revealing activity tracking with cortisol levels in the Sleep group and not in the Wake group. Activity in the medial PFC (in red) was active at $P < 0.001$ and 9 voxels while activity in the amygdala (in pink) was active at $P < 0.005$ and 9 voxels. None of these regions were active in the Wake group, even with a reduced threshold of $P < 0.10$.

Results were unchanged by application of the masking procedure: All activity present in the original analysis remained when the Sleep group-level contrast was exclusively masked with that of the Wake group, or vice versa (See Fig. 3 and right-most column of Table 2). Thus, we can be confident that the finding that higher levels of cortisol are associated with an enhanced relation between looking time at encoding and activity in the amygdala and vmPFC during successful retrieval of negative objects is specific to the Sleep group. To further confirm that activity in the amygdala and vmPFC was specific to the Sleep group, we entered participants' pre-encoding cortisol level as a subject-level regressor. Rather than including all cortisol values in the same regressor column, we entered the Sleep participants' cortisol values into one column (entering a value of zero for all Wake participants) and the Wake participants' cortisol values into a separate column (entering a value of zero for all Sleep participants). When investigating the effect of cortisol on the relation between looking time at encoding

and successful retrieval-related activity for the Sleep group greater than the Wake group, activity in the vmPFC was present at a threshold of $P < 0.005$ and a 9-voxel extent. Activity in the amygdala was present at a threshold of $P < 0.005$ and a reduced (5-voxel) extent.

Discussion

Although previous studies have separately tied sleep (see Diekelmann and Born 2010; Payne 2011; Stickgold and Walker 2013) and the stress hormone cortisol (e.g., Payne et al. 2004; de Quervain et al. 2009; Roozendaal et al. 2009; Wolf 2009; Joëls et al. 2011) to enhanced memory consolidation, the current study provides evidence that they interact to benefit subsequent memory performance. For the first time, we demonstrate that resting cortisol levels prior to learning predict memory performance 12 h later (most strongly for negative but also for neutral stimuli), but only if sleep occurs during the consolidation delay. Moreover, and now specifically for negative stimuli, this interactive effect arises because cortisol intensifies the link between time spent looking at negative images during encoding and subsequent memory for those images post-sleep. The neural consequence of this optimized consolidation is that a smaller set of limbic regions, including the amygdala and vmPFC, supports the retrieval of these items following sleep, rather than recruitment of a more diffuse memory retrieval network following wakefulness. The importance of each of these findings is expanded upon in the sections below.

Cortisol and Sleep Interact to Enhance Memory Consolidation

Prior to this study, it was well established that stress (e.g., Payne et al. 2007) and cortisol administration (e.g., Buchanan and Lovallo 2001; Abercrombie et al. 2003) could enhance emotional memory consolidation. However, because these prior studies utilized delays of 24 h or longer, which necessarily include a period of sleep, it was unknown whether sleep was necessary for the beneficial effect of stress on memory to emerge. Here, we demonstrate that higher resting cortisol levels assessed prior to a consolidation delay predicted enhanced memory, but only if sleep occurred in the consolidation interval. This was not the case if wake occurred during a consolidation interval matched in length to the Sleep group, or across the short delay intervals in the circadian control groups.

These findings are made all the more interesting considering a unique feature of the design. Due to the circadian rhythm of cortisol (e.g., Kahn et al. 1988), all participants in the Sleep group were within a narrow range of relatively low cortisol levels during the evening assessment, and in fact showed markedly less variability in cortisol levels than the Wake group, whose cortisol levels were also much higher. Thus, it appears that even small differences in comparably low resting levels of cortisol are sufficient to influence the efficacy of memory consolidation over periods of sleep.

Although our study examined the influence of pre-sleep cortisol on later memory performance, several studies investigating how cortisol levels during sleep affect memory processing have returned quite different results. For example, Wagner et al. (2005) found that when the cortisol synthesis inhibitor metyrapone was used to suppress cortisol during

sleep, emotional memory was enhanced relative to placebo—suggesting that high cortisol during sleep might curtail sleep-dependent consolidation. Similarly, Wilhelm et al. (2011) showed that cortisol administration during sleep impaired memory for the temporal order of information, while cortisol administration during wakefulness enhanced this ability. Of course, there are a number of potentially important differences between these studies and the present one aside from the time at which cortisol was measured. It is possible that individual differences in resting levels of cortisol affect memory consolidation differently than larger changes in cortisol created by administering or inhibiting cortisol directly. However, it is also possible that higher levels of pre-sleep cortisol are advantageous for memory consolidation in a way that does not remain true over the course of a night, perhaps because elevations in pre-sleep cortisol overlap with and influence the learning experience by creating a tag at encoding that only later benefits from sleep. Clearly, understanding the complex set of interactions between cortisol and sleep on the ultimate success of memory consolidation is an important area for further study.

Cortisol and Sleep Interact to Enhance Consolidation of Negative Stimuli by Increasing the Likelihood That Attended Information is Later Remembered

While prior studies have focused on the influence of cortisol on attention to (van Honk et al. 1998; Roelofs et al. 2007) or memory for (e.g., Buchanan and Lovallo 2001) emotional stimuli, they have not examined the relation between the two. We show here that although nearly all subjects looked longer at objects they would later remember rather than forget (shown in Fig. 2 by the positive “difference in looking time” values for nearly all subjects), elevated cortisol exaggerated the effect, but only for negative objects, and more strongly if subjects slept thereafter. By taking subsequent memory into account, we revealed a novel attention-memory interaction that is modulated by elevated cortisol at encoding—an effect that may stem from additional processing during sleep.

It should be noted that cortisol only marginally predicted the link between looking time at encoding and subsequent negative object memory for the Sleep group ($P = 0.075$), while having a stronger relation to memory performance for these objects ($P = 0.031$). Thus, the eye-tracking findings may not be as convincing as the memory findings, especially when assessing the Sleep group alone. However, the critical finding in both the eye-tracking and memory analyses is the Group by Cortisol interaction. This interaction suggests that cortisol's impact on the link between looking time at encoding and subsequent emotional memory (i.e., elevated cortisol increased the likelihood that objects viewed only briefly would be forgotten and that objects that were the focus of attention for longer would be remembered) was stronger for the Sleep group than for the Wake group ($P = 0.049$), just as cortisol's impact on memory performance was stronger for the Sleep group than for the Wake group ($P = 0.031$).

It is important to note that, in the eye-tracking analyses, cortisol was not directly related to the proportion of time participants attended to negative or neutral objects, suggesting that cortisol does not simply influence the way attention is allocated to the complex scenes during encoding. Although avoidance of negative faces has been noted with higher cortisol levels in

other studies (van Honk et al. 1998; Roelofs et al. 2007), we did not see evidence of negative avoidance here. Differences in stimuli (complex scenes rather than faces), or task goals (being required to process the scenes here, rather than having threatening faces presented as distractors) may explain why cortisol did not affect attention allocation in the present study. The lack of a general effect of cortisol on attention allocation suggests that higher cortisol did not influence how people encoded the memories, but rather interacted with sleep to influence how effectively they formed them.

We suggest that cortisol may aid in the “tagging” (Morris 2006) of salient emotional representations during encoding, which in turn enables sleep to later selectively consolidate those tagged representations. As has been previously suggested, the selective preservation of memory for emotionally salient information may be a form of selective processing that is beneficial for survival (Payne and Kensinger 2010).

The neuroimaging data are consistent with this interpretation. The retrieval of items that were attended for a longer proportion of time in the presence of high cortisol and consolidated over a period of wakefulness relied on a larger number of regions typically associated with episodic retrieval, including the lateral prefrontal cortex and hippocampus. Critically, however, when those items were attended to for a longer proportion of time in the presence of high cortisol and then consolidated over a sleep-filled delay, their retrieval relied instead on a more refined and restricted set of activations within the vmPFC and amygdala. Prior research has suggested that a refinement in neural activity can be associated with increased neural efficiency. For instance, individuals who score higher on intelligence tests can show less brain activity while performing cognitive tasks than other individuals (Haier et al. 1992; for a review, see Neubauer and Fink 2009). Similarly, individuals who are fast performers on a task (Rypma et al. 2006) or who are low in anxiety (Basten et al. 2011) can show lower activity during task performance than their counterparts. Although we acknowledge it as speculation, the present results are consistent with the proposal that neural efficiency is optimized when information is encoded in the presence of high cortisol and then consolidated over a sleep-filled delay. In addition, the vmPFC and amygdala are the same set of circumscribed limbic regions previously revealed to support negative object memory retrieval after a sleep-filled delay when compared with a wake-filled one (Payne and Kensinger 2011). The consistency of these regions between studies is remarkable given that the Payne and Kensinger (2011) study did not assess the role of cortisol. Taken together, these findings suggest potential mechanisms underlying the differences observed in memory performance between groups of individuals who sleep rather than remain awake during a consolidation interval (Payne et al. 2008, 2012). In order for optimized consolidation to occur, it may not be sufficient to sleep during the consolidation interval; it may also be necessary to have sufficiently high levels of pre-sleep cortisol to enable the tagging of stimuli that will later benefit from sleep-based processing.

Limitations and Future Directions

We view this study as an important first step in understanding how pre-encoding cortisol interacts with sleep-based consolidation to influence the ultimate fate of a memory. Although we feel that our results showcase this area as an important one for

future study, we acknowledge several limitations that should be addressed in future work. For example, while there is a precedent for using a single pre-encoding cortisol sample to assess the effects of resting cortisol on cognition (e.g., Oosterlaan et al. 2005; Putman et al. 2004; Takahashi et al. 2004; van Bokoven et al. 2005), future work would benefit from averaging two or more encoding samples together in order to better account for variability. Additionally, future work would benefit from collecting additional cortisol samples throughout the study in order to investigate the relation between memory and post-encoding cortisol, the cortisol awakening response, and the diurnal cortisol slope, all of which are important questions in their own right. Finally, future studies should consider alternative ways to control for effects of the diurnal variation in cortisol. The present study was complicated by the fact that the diurnal cycle of cortisol results in higher cortisol levels for those who encode in the morning (i.e., Wake and Morning Short-Delay groups) than those who encode in the evening (i.e., Sleep and Evening Short-Delay groups). To control for this diurnal variation in cortisol, we included Short Delay or “circadian control” conditions, to show that our observed effects of cortisol on emotional memory were indeed due to sleep rather than time of day. An important next step would be to replicate the present findings using an afternoon nap paradigm, where cortisol levels between a Nap and Wake condition would be statistically equivalent, and thus circadian fluctuations in cortisol would not be a concern.

General Conclusions

Although additional work is needed to clarify the mechanisms involved, the current results underscore a potentially critical role for endogenous cortisol in sleep-based memory consolidation effects. First, cortisol interacts with sleep to enhance, and perhaps even enable, memory consolidation benefits, as such benefits are not observed following a delay containing wakefulness. This suggests that the beneficial effect of cortisol on memory consolidation shown in prior studies, nearly all of which spanned at least a 24-h delay (e.g., Payne et al. 2007), may be dependent on the sleep that occurs during the consolidation interval. Second, this consolidation benefit is strongly modulated by attentional processes at encoding that interact with sleep to selectively consolidate emotionally salient information. When negative items were well attended in the presence of elevated cortisol at encoding, those items were more likely to subsequently benefit from sleep-based consolidation processes and thus be better remembered later on. No such relation was observed following wakefulness, nor was the relation present for neutral items. This latter finding raises the intriguing possibility that it is not sufficient for an item to simply be the focus of attention; instead, another cue to salience, such as the emotional arousal and accompanying noradrenergic activation associated with viewing negative images (Abercrombie et al. 2006; Roozendaal et al. 2006; van Stegeren et al. 2007), may be critical for setting a tag in memory and triggering the interactive effects of cortisol and sleep. Because sleep has been shown both to provide an optimal neurobiological environment for memory consolidation (e.g., Diekelmann and Born 2010) and also to specifically enhance emotional memory (e.g., Wagner et al. 2001; Hu et al. 2006; Wagner et al. 2006; Payne et al. 2008, 2012; Nishida et al. 2009; Payne and Kensinger 2010, 2011; Baran et al. 2012), these findings suggest

that pre-learning cortisol may enable the tagging of information for subsequent consolidation during sleep. The neuroimaging results support this interpretation: Elevated cortisol led to a stronger relation between the time spent looking at negative images at encoding and intensified activity in emotional processing regions (particularly the amygdala and vmPFC) during successful retrieval of those negative images following sleep (but not following wakefulness). This reliance on the amygdala and vmPFC parallels the findings of other studies examining the effects of sleep on emotional memory retrieval (Sterpenich et al. 2009; Payne and Kensinger 2011), suggesting that sufficiently high cortisol at encoding may be needed to enable the shift to reliance on a smaller set of limbic regions at retrieval.

There is still much to be understood regarding how cortisol levels interact with sleep to enhance emotional memory consolidation. Yet the present study makes an important advance by demonstrating that the facilitative effect of pre-learning cortisol on emotional memory following delays of at least 24 h including sleep (e.g., Buchanan and Lovallo 2001; Abercrombie et al. 2003; Payne et al. 2007) may be attributable to interactions with sleep-dependent consolidation processes. Additionally, the present results provide strong evidence for endogenous cortisol in modulating the relation between attentional focusing on emotional information at encoding and the subsequent sleep-based consolidation of that information, perhaps because cortisol tags information for subsequent consolidation. Overall, the present study suggests that individuals who encode emotional information in the presence of elevated cortisol, perhaps whether intentionally encoding (e.g., watching a horror movie) or unintentionally encoding (e.g., if being held at gunpoint), may be more likely to remember such information following sleep compared with those with lower levels while first experiencing it.

Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

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Notes

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References

Abercrombie HC, Kalin NH, Thurow ME, Rosenkranz MA, Davidson RJ. 2003. Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behav Neurosci*. 117:505–516.

Abercrombie HC, Speck NS, Monticelli RM. 2006. Endogenous cortisol elevations are related to memory facilitation only in individuals who are emotionally aroused. *Psychoneuroendocrinology*. 31:187–196.

Andreano JM, Cahill L. 2006. Glucocorticoid release and memory consolidation in men and women. *Psychol Sci*. 17:466–470.

Antoniijevic I. 2008. HPA axis and sleep: identifying subtypes of major depression. *Stress*. 11:15–27.

Baran B, Pace-Schott EF, Ericson C, Spencer RM. 2012. Processing of emotional reactivity and emotional memory over sleep. *J Neurosci*. 18:1035–1042.

Basten U, Stelzel C, Fiebach CJ. 2011. Trait anxiety modulates the neural efficiency of inhibitory control. *J Cogn Neurosci*. 23:3132–3145.

Beck AT, Beamesderfer A. 1974. Assessment of depression: the depression inventory. *Mod Probl Pharmacopsychiatry*. 7:151–169.

Beck AT, Epstein N, Brown G, Steer RA. 1988. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol*. 56:893–897.

Beckner VE, Tucker DM, Delville Y, Mohr DC. 2006. Stress facilitates consolidation of verbal memory for a film but does not affect retrieval. *Behav Neurosci*. 120:518–527.

Born J, Wagner U. 2004. Memory consolidation during sleep: role of cortisol feedback. *Ann NY Acad Sci*. 1032:198–201.

Buchanan TW, Adolphs R. 2002. The role of the human amygdala in emotional modulation of long-term declarative memory. In: Moore S, Oaksford M, editors. *Emotional cognition: from brain to behavior*. London, Benjamins. p. 9–34.

Buchanan TW, Lovallo WR. 2001. Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology*. 26:307–317.

Cahill L, Alkire MT. 2003. Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding. *Neurobiol Learn Mem*. 79:194–198.

Cahill L, Gorski L, Le L. 2003. Enhanced human memory consolidation with post-learning stress: interaction with the degree of arousal at encoding. *Learn Mem*. 10:270–274.

Croiset G, Nijssen M, Kamphuis P. 2000. Role of corticotropin-releasing factor, vasopressin and the autonomic nervous system in learning and memory. *Eur J Pharmacol*. 405:225–234.

de Quervain DJF, Aerni A, Schelling G, Roozendaal B. 2009. Glucocorticoids and the regulation of memory in health and disease. *Front Neuroendocrinol*. 30:358–370.

Diekelmann S, Born J. 2010. The memory function of sleep. *Nat Rev Neurosci*. 11:114–126.

Haier RJ, Siegel B, Tang C, Abel L, Buchsbaum MS. 1992. Intelligence and changes in regional cerebral glucose metabolic rate following learning. *Intelligence*. 16:415–426.

Hamann S. 2001. Cognitive and neural mechanisms of emotional memory. *Trends Cogn Sci*. 5:394–400.

Horne JA, Ostberg O. 1976. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol*. 4:97–110.

Hu P, Stylos-Allan M, Walker MP. 2006. Sleep facilitates consolidation of emotional declarative memory. *Psych Sci*. 17:891–898.

Joëls M, Fernandez G, Roozendaal B. 2011. Stress and emotional memory: a matter of timing. *Trends Cogn Sci*. 15:280–288.

Kahn J, Rubinow DR, Davis CL, Kling M, Post RM. 1988. Salivary cortisol: a practical method for evaluation of adrenal function. *Biol Psychiatry*. 23:335–349.

Kensinger EA, Garoff-Eaton RJ, Schacter DL. 2007. How negative emotion enhances the visual specificity of a memory. *J Cogn Neurosci*. 19:1872–1887.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med*. 61:154–162.

Kirschbaum C, Wolf OT, May M, Wippich W, Hellhammer DH. 1996. Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci*. 58:1475–1483.

Kudielka BM, Kirschbaum C. 2005. Sex differences in HPA axis responses to stress: a review. *Biol Psych*. 69:113–132.

Kuhlmann S, Wolf OT. 2006. Arousal and cortisol interact in modulating memory consolidation in healthy young men. *Behav Neurosci*. 120:217–223.

Lupien SJ, LePage M. 2001. Stress, memory, and the hippocampus: can't live with it, can't live without it. *Behav Brain Res*. 127:137–158.

- Maheu FS, Joobar R, Lupien SJ. 2005. Declarative memory after stress in humans: differential involvement of the beta-adrenergic and corticosteroid systems. *J Clin Endocrinol Metab.* 90:1697–1704.
- Manor BR, Gordon E. 2003. Defining the temporal threshold for ocular fixation in free-viewing visuocognitive tasks. *J Neurosci Methods.* 128:85–93.
- Morris RGM. 2006. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *Eur J Neurosci.* 23:2829–2846.
- Neubauer AC, Fink A. 2009. Intelligence and neural efficiency. *Neurosci Biobehav R.* 33:1004–1023.
- Nishida M, Pearsall J, Buckner RL, Walker MP. 2009. REM sleep, prefrontal theta, and the consolidation of human emotional memory. *Cereb Cortex.* 19:1158–1166.
- Oosterlaan J, Geurts HM, Knol DL, Sergeant JA. 2005. Low basal salivary cortisol is associated with teacher-reported symptoms of conduct disorder. *Psychiat Res.* 134:1–10.
- Otte C, Lenoci M, Metzler T, Yehuda R, Marmar CR, Neylan TC. 2005. Hypothalamic-pituitary-adrenal axis activity and sleep in posttraumatic stress disorder. *Neuropsychopharmacol.* 30:1173–1180.
- Payne JD. 2011. Learning, memory and sleep in humans. *Sleep Med Clin.* 6:15–30.
- Payne JD, Chambers AM, Kensinger EA. 2012. Sleep promotes lasting changes in selective memory for emotional scenes. *Front Integr Neurosci.* 6:1–11.
- Payne JD, Jackson ED, Hoscheidt S, Jacobs JW, Nadel L. 2006. The impact of stress on neutral and emotional aspects of episodic memory. *Memory.* 14:1–16.
- Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L. 2007. Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. *Learn Mem.* 14:861–868.
- Payne JD, Kensinger EA. 2011. Sleep leads to changes in the emotional memory trace: evidence from fMRI. *J Cogn Neurosci.* 23:1285–1297.
- Payne JD, Kensinger EA. 2010. Sleep's role in the consolidation of emotional episodic memories. *Curr Dir Psychol Sci.* 19:290–295.
- Payne JD, Nadel L, Britton WB, Jacobs WJ. 2004. The biopsychology of trauma and memory. In: Reisberg D, Hertel P, editors. *Emotion and memory.* Oxford, Oxford UP.
- Payne JD, Stickgold R, Swanberg K, Kensinger EA. 2008. Sleep preferentially enhances memory for emotional components of scenes. *Psychol Sci.* 19:781–788.
- Plihal W, Born J. 1999. Memory consolidation in human sleep depends on inhibition of glucocorticoid release. *Neuroreport.* 10:2741–2747.
- Putman P, van Honk J, Kessels RPC, Mulder M, Koppeschaar HPF. 2004. Salivary cortisol and short and long-term memory for emotional faces in healthy young women. *Psychoneuroendocrinology.* 29:953–960.
- Richter-Levin G, Akirav I. 2003. Emotional tagging of memory formation—in the search for neural mechanisms. *Brain Res Brain Res Rev.* 43:247–256.
- Rimmele U, Domes G, Mathiak K, Hautzinger M. 2003. Cortisol has different effects on human memory for emotional and neutral stimuli. *Neuroreport.* 14:2485–2488.
- Roelofs K, Bakvis P, Hermans EJ, van Pelt J, van Honk J. 2007. The effects of social stress and cortisol responses on the preconscious selective attention to social threat. *Biol Psychol.* 75:1–7.
- Roosendaal B. 2000. Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology.* 25:213–238.
- Roosendaal B, Barsegyan A, Lee S. 2007. Adrenal stress hormones, amygdala activation, and memory for emotionally arousing experiences. *Prog Brain Res.* 167:79–97.
- Roosendaal B, Brunson KL, Holloway BL, McGaugh JL, Baram TZ. 2002. Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *Proc Natl Acad Sci USA.* 99:12908–13913.
- Roosendaal B, McEwen BS, Chattarji S. 2009. Stress, memory, and the amygdala. *Nat Rev Neurosci.* 10:423–433.
- Roosendaal B, Okuda S, van der Zee EA, McGaugh JL. 2006. Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc Natl Acad Sci USA.* 103:6741–6746.
- Rypma B, Berger JS, Prabhakaran V, Bly BM, Kimberg DY, Biswal BB, D'Esposito M. 2006. Neural correlates of cognitive efficiency. *Neuroimage.* 33:969–979.
- Slotnick SD, Moo LR, Segal JB, Hart J. 2003. Distinct prefrontal cortex activity associated with item memory and source memory for visual shapes. *Cogn Brain Res.* 17:75–82.
- Smeets T, Otgaar H, Candel I, Wolf OT. 2008. True or false? Memory is differentially affected by stress-induced cortisol elevations and sympathetic activity at consolidation and retrieval. *Psychoneuroendocrinology.* 33:1378–1386.
- Stark R, Wolf OT, Tabbert K, Kagerer S, Zimmerman M, Kirsch P. 2006. Influence of the stress hormone cortisol on fear conditioning in humans: evidence for sex differences in the response of the prefrontal cortex. *Neuroimage.* 32:1290–1298.
- Sterpenich V, Albouy G, Darsaud A, Schmidt C, Vandewalle G, Dang Vu TT, Desseilles M, Phillips C, Degueldre C, Balteau E *et al.* 2009. Sleep promotes the neural reorganization of remote emotional memory. *J Neurosci.* 29:5143–5152.
- Stickgold R. 2005. Sleep-dependent memory consolidation. *Nature.* 437:1272–1278.
- Stickgold R, Walker MP. 2013. Sleep-dependent memory triage: evolving generalization through selective processing. *Nat Neurosci.* 16:139–145.
- Symons CS, Johnson BT. 1997. The self-reference effect in memory: a meta-analysis. *Psychol Bull.* 121:371–394.
- Takahashi T, Ikeda K, Ishikawa M, Tsukasaki T, Nakama D, Tanida S, Kameda T. 2004. Social stress-induced cortisol elevation acutely impairs social memory in humans. *Neurosci Lett.* 363:125–130.
- van Bokoven I, van Goozen SHM, van Engeland H, Schaal B, Arseneault L, Séguin JR, Nagin DS, Vitaro F, Tremblay RE. 2005. Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *J Neural Transm.* 112:1083–1096.
- van Cauter E, Leproult R, Laurence P. 2000. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. *JAMA.* 284:861–868.
- van Honk J, Tuiten A, van den Hout M, Koppeschaar H, Thijssen J, de Hann E, Verbaten R. 1998. Baseline salivary cortisol levels and preconscious selective attention for threat: a pilot study. *Psychoneuroendocrinology.* 23:741–747.
- van Stegeren AH. 2009. Imaging stress effects on memory: a review of neuroimaging studies. *Can J Psychiatry.* 54:16–27.
- van Stegeren AH, Wolf OT, Everaerd W, Scheltens P, Barkhof F, Rombouts SA. 2007. Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol Learn Mem.* 87:57–66.
- Wagner U, Born J. 2008. Memory consolidation during sleep: interactive effects of sleep stages and HPA regulation. *Stress.* 11:28–41.
- Wagner U, Degirmenci M, Drosopoulos S, Perras B, Born J. 2005. Effects of cortisol suppression on sleep-associated consolidation of neutral and emotional memory. *Biol Psychiatry.* 58:885–893.
- Wagner U, Gais S, Born J. 2001. Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. *Learn Mem.* 8:112–119.
- Wagner U, Hallschmid M, Rasch B, Born J. 2006. Brief sleep after learning keeps emotional memories alive for years. *Biol Psychiatry.* 60:788–790.
- Walker MP. 2009. Overnight therapy? The role of sleep in emotional brain processing. *Psychol Bull.* 135:731–748.
- Wang S-H, Morris RGM. 2010. Hippocampal-neocortical interactions. *Ann Rev Psychol.* 61:49–79.
- Waring JD, Kensinger EA. 2009. Effects of emotional valence and arousal upon memory trade-offs with aging. *Psychol Aging.* 24:412–422.
- Wilhelm I, Wagner U, Born J. 2011. Opposite effects of cortisol and consolidation of temporal sequence memory during waking and sleep. *J Cogn Neurosci.* 23:3703–3712.
- Wolf OT. 2009. Stress and memory in humans: twelve years of progress? *Brain Res.* 1293:142–154.