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The effects of sleep on emotional memory consolidation and emotional reactivity

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Abbreviations

ACh	Acetylcholine
ANOVA	Analysis of Variance
BLA	Basolateral Amygdala
ECG	Electrocardiogram
EEG	Electroencephalography
EMG	Electromyogram
EOG	Electrooculogram
ERP	Event-Related Potential
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma Aminobutyric Acid
HPA Axis	Hypothalamic-Pituitary-Adrenal Axis
IAPS	International Affective Picture System
LC	Locus Coeruleus
LPP	Late Positive Potential
LTD	Long-Term Depression
LTP	Long-Term Potentiation
mPFC	Medial Prefrontal Cortex
NREM	Non Rapid Eye Movement
NE	Norepinephrine
PET	Positron-Emission-Tomography
PFC	Prefrontal Cortex
PTSD	Posttraumatic Stress Disorder
REM	Rapid Eye Movement
REMD	Rapid Eye Movement Sleep Deprivation

S1- S4	Sleep Stage 1- Sleep Stage 4
SAM	Self- Assessment Manikin
SE	Serotonin
SEM	Standard Error of Mean
SWS	Slow Wave Sleep

INTRODUCTION

"We are, after all, our memories. It is our memory that enables us to value everything else we possess. Lacking memory, we would have no ability to be concerned about our hearts, hair, lungs, libido, loved ones, enemies, achievements, failures, incomes or income taxes. Our memory provides us with an autobiographical record and enables us to understand and react appropriately to changing experiences. Memory is the 'glue' of our personal existence." (McGaugh, 2003).

As the quotation emphasizes, the ability to remember is critical for more than the simple recall of facts and events, it is involved in feelings, thinking and behavior and thus determining human identity. Memory contents once acquired are not stored equally well and only some will be remembered for a lifetime. One effective way of making strong memories is repetition or practise. Another way to create long-lasting memories is a high emotional charge of information inducing a personal meaning or significance. The more arousing memory contents are, the better they are remembered later, ranging from slightly improved memory on situations of emotional (dis)comfort up to memory on life-threatening situations that might never be forgotten. Emotionally arousing stimuli elicit feelings varying in threshold, intensity and duration (i.e. emotional reactivity). Similarly to the memory trace which can either decay or become strengthened, the intensity of emotional reactivity to familiar emotional stimuli may change over time. Here, different directions are conceivable: emotional reactivity can decrease, stabilize at a certain level, or even increase (as observed in patients suffering from post-traumatic stress disorder [PTSD]).

A great amount of research has been done to elucidate the mechanisms behind the superior retention of emotional memories. A critical role of sleep in this context seems likely on the background of altered sleep in most affective disorders (e.g. depression, PTSD). First evidence indeed indicates an impact of post-learning periods of sleep for the processing of the two components of emotional memory, i.e. a memory component containing information about the event and an affective component comprising the associated emotional charge which can be measured in the form of emotional reactivity. Recently, researchers have begun to discuss the specific role of different sleep stages like slow wave sleep (SWS) and rapid eye movement (REM) sleep for these two components. Whereas remembering emotionally arousing materials has been shown to benefit particularly from periods of post-learning REM sleep, it is still unknown whether the emotional charge being associated with the learned

material is either enhanced, reduced or not affected by REM sleep. A possible role of SWS in emotional memory processing has not been investigated yet even though an effect of SWS can be presumed on the background of the specific role that SWS plays in the selective consolidation of future-relevant information (which emotional memories are per se). In the present thesis I intended to scrutinize the role of post-learning periods of SWS and REM sleep on the consolidation of emotional memories and its affective charge. The impact of SWS and REM sleep was further elaborated by dissecting their contribution to the processing of context information being associated with an emotional item as well as memory on the emotional item itself. Moreover, since noradrenergic activity has been assumed to be implicated in SWS-dependent emotional memory consolidation, norepinephrine levels were experimentally manipulated during SWS to get insight into possible underlying endocrine mechanism of emotional memory consolidation during sleep.

MEMORY AND EMOTION

Memory

In a very general sense, memory can be defined as the lasting consequence of (learning from) an experience (McGaugh, 2003). However, the kind and extent of this consequence as well as the actual time memories last vary enormously and express themselves in very different neural representations. Many taxonomies have been proposed that classify the phenomenon of memory according to different criteria (e.g. duration that the memory lasts, its stage of formation and brain structures that are involved in its processing).

Duration of memory: short- and long-term memory. From the high amount of information that is acquired every moment by an organism (such as color, smell and sounds of objects, perceived consciously and unconsciously) only few information are stored for the long-term. Whereas a huge amount of information can be held in the sensory memory for a short period of time (milliseconds to a few seconds), the capacity of the short-term memory (STM) is much more restricted and preferentially stores those information that are acquired with selective attention. It lasts from seconds to minutes and is vulnerable to interference. Even less information is permanently stored as long-term memory (LTM) for hours to years. Repetition as well as the significance of learning material are modulators of memory strength that facilitate long-term retention (Anderson, 2000).

Memory stages: encoding, consolidation, retrieval. The ability to remember events, facts or skills requires the successful functioning of each of the three memory stages: encoding (the acquisition of information), consolidation (the stabilisation of these memories) and retrieval (recall or execution of acquired contents). Thus, whenever contents are not available at a later time it could be due to disturbances in any of the stages, e.g. contents are simply not acquired, consolidation is disrupted or the stored contents cannot be accessed. At first, a stimulus that is encoded enters sensory channels to form labile memory traces. Thereafter, these labile memory traces can be strengthened and thereby transformed from short-term memory into long-term memory. As a neuronal correlate, structural and functional changes in synapses and neurons induce long-term potentiation (LTP) which is assumed to underlie memory consolidation (McGaugh, 2000). The brain state is very important for the processing of memories. Whereas encoding and retrieval take place most effectively during wakefulness, memory consolidation benefits from periods of sleep in which neuronal representations of newly acquired memories are reactivated and thereby stabilized.

Memory systems: declarative and non-declarative memory. A current view being supported by evidence from basic research as well as from research on amnesia patients (brain lesion) is the existence of multiple memory systems that can work independently from each other. Consequently, it may happen that amnesic patients show specific memory disturbances for only a few tasks, but may show perfectly intact memory abilities in others depending on the required brain structures to fulfil the task and the locus of their brain damage (Scoville & Milner, 1957). Nowadays, the most common way of subdividing memory systems is in declarative and non-declarative memory (Squire, 1992; Henke, 2010; see Figure 1). The declarative (also explicit) memory system is assumed to depend on intact medio-temporal lobe structures (e.g. the rhinal cortices and the hippocampus) and encompasses mainly consciously learned knowledge about facts and episodes. The memory contents that are stored can either be retrieved in a free recall or recognition procedure (in which memory is tested in presence of the stimulus). Recognition processes in the declarative memory system are commonly subdivided into familiarity (also called ‘item memory’) and recollection (also called ‘source memory’). Whereas familiarity-based recognition is fast-acting, relatively automatic and does not contain any episodic or contextual information of a familiar object, recollection memory is considered taking more time and effort for acquisition and involves temporal and spatial contextual information about an object (Rugg & Curran, 2007; Rugg et al., 2007). Whether both memory processes simply represent differences in quantity (i.e.,

recollection adds contextual details to object recognition, proposed by the single-process model) or if they are independent and qualitatively different processes (dual-process model) is still an issue of debate (Squire & Wixted, 2011; Squire et al., 2011). However, evidence has been raised suggesting differential involvement of brain regions in both memory processes (i.e. a higher involvement of the hippocampus in recollection and perirhinal cortex in familiarity; (Brown & Aggleton, 2001; Eichenbaum, Yonelinas, & Ranganath, 2007). In contrast to the declarative memory system non-declarative (also known as implicit) memory can be acquired unconsciously. Forms of non-declarative learning are skills and habits (also procedural memories) depending on the striatum, priming relying on neocortical structures and classical conditioning which involves the amygdala for emotional responses and the cerebellum for the skeletal musculature (Squire & Zola, 1996). This very broad taxonomy simplifies the complex processes and coherences of memory formation but it can serve as a model stimulating research to test and improve our view on memory. For instance, classical conditioning has been demonstrated to not only rely on cerebellar structures, but also on the hippocampus whose involvement increases with the length of time between the stimuli to be paired (trace conditioning). Even during subliminal priming the hippocampus is recruited whenever stimuli are associated - findings that critically challenge the traditional distinction of memory systems according to consciousness (Henke, 2010). Examples like this implicate that there is no such clear-cut classification of brain structures involved in certain tasks as suggested by simple models, but rather an interplay of all memory systems contributing in a dimensional manner influenced by the kind of contents, conditions, capacities and conscious or non-conscious use of strategies.

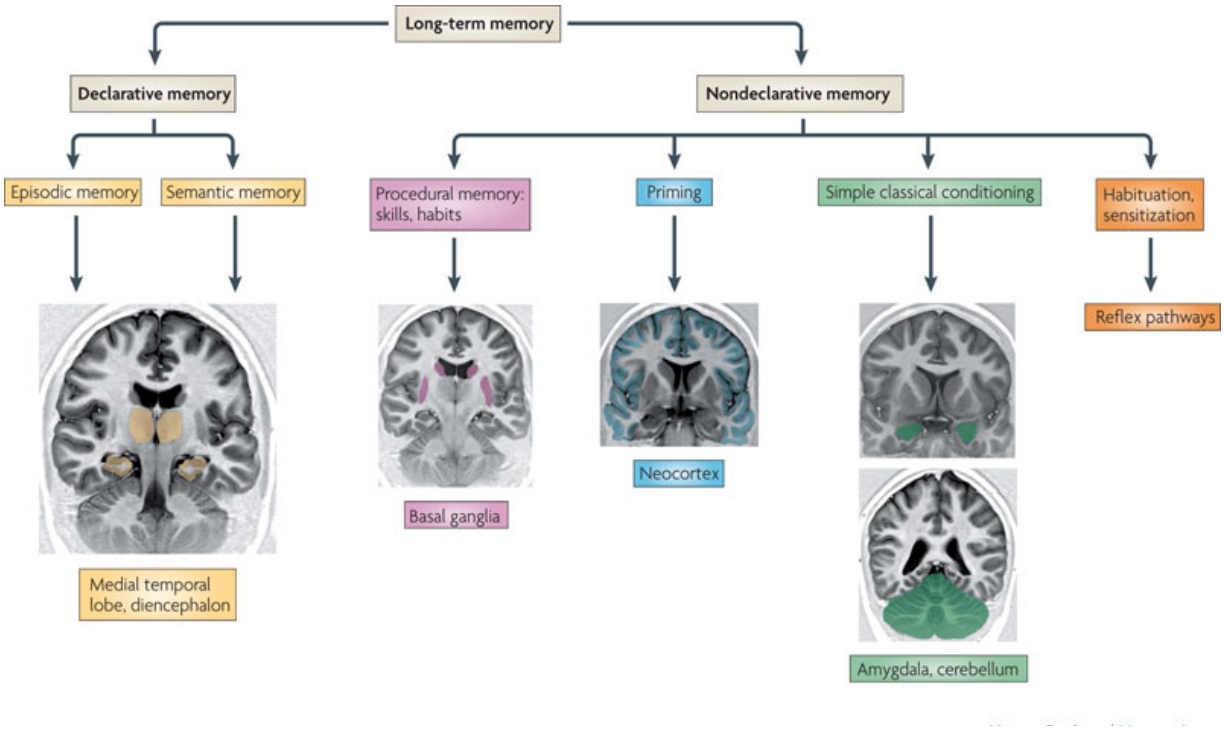

Figure 1. A taxonomy of long-term memory systems together with specific brain structures involved in each system (from Henke, 2010).

Emotions

Emotions can be defined as a set of interrelated subevents which are elicited by a specific situation, person or object. On a functional level emotions characterize the significance (positive or negative) of an event and serve as action dispositions facilitating particular behaviors and reactions (e.g. fight, flight, approach behavior). Mostly emotions can be verbalized and they are accompanied by expressive displays (e.g. postures, gestures, facial and vocal expressions as well as overt behavior like freezing) and bodily responses (e.g. changes in the somatic and autonomic nervous system, endocrine and immune system that modify psychophysiological responses like reflexes, cardiovascular or electrodermal activity) (Lang, 1995; Schelling et al., 2003; Cacioppo, Crites, Berntson, & Coles, 1993). Depending on situational as well as individual influence factors, emotional reactivity (i.e. the threshold, intensity and duration of emotional responsiveness) can vary. In this thesis, mainly the intensity of subjectively perceived emotionality and heart rate change in response to emotional pictorial stimuli is referred to as emotional reactivity. In the following, some of the common classification models of emotion are presented that all go along with different predictions and measurements to assess emotions.

Categorical models/ Basic emotion models. The categorical approaches of emotions define a certain number and nature of different types of emotion as basic emotions that go along with unique evolutionary functions and expressions. Typically, basic emotions encompass anger, joy, fear, disgust, sadness amongst others. Accordingly, each of the defined basic emotions is defined as an affect program, that is elicited by specific stimuli and expressed in an emotion-specific response pattern (e.g. prototypical facial expression, physiological reaction, action tendency) (Grandjean, Sander, & Scherer, 2008). Other emotions are typically considered as blends of basic emotions or complex emotions (Ekman, 1999).

Cognitive appraisal models. The cognitive appraisal models define emotions as complex, multicomponential and dynamic processes. These models aim to explain a huge variety of emotions and emotional expressions and try to account for individual differences in the response to an emotional event (Lazarus, 1991). Most importantly, a multi-dimensional individual appraisal is assumed evaluating relevance, implications, coping strategies and the normative significance of an event, thereby influenced by changes in autonomic responses, action tendencies, motor expressions and subjective feelings (Grandjean et al., 2008).

Dimensional models. The dimensional models of emotions assume that every emotion can be characterized by varying quantitative combinations of two or three emotion dimensions (Russell, 1979; Shallice & Burgess, 1991; Posner, Russell, & Peterson, 2005). These models most often involve the dimensions of valence (from negative to positive, also from unpleasant to pleasant) and arousal (from low to high arousing, also from calm to excited), such as the two-dimensional model of emotions (see Figure 2). Consequently, the dimensional view suggests that there is no unique emotion-specific activation pattern (in the body or brain). Verbal reports of subjective feelings are commonly assessed as measures for arousal and valence. Lang and colleagues developed a 9-point-rating scale (Figure 2, SAM, Self-assessment manikin, (Lang, 1995) for a rating from 1-9 each indicating a degree of excitement (i.e. arousal) and pleasantness (i.e. valence). Other common measures include the assessment of electrodermal (i.e. skin conductance) and cardiovascular (i.e. heart rate change) responses that have been associated with verbal reports of arousal and valence, respectively (Bradley, Codispoti, Cuthbert, & Lang, 2001).

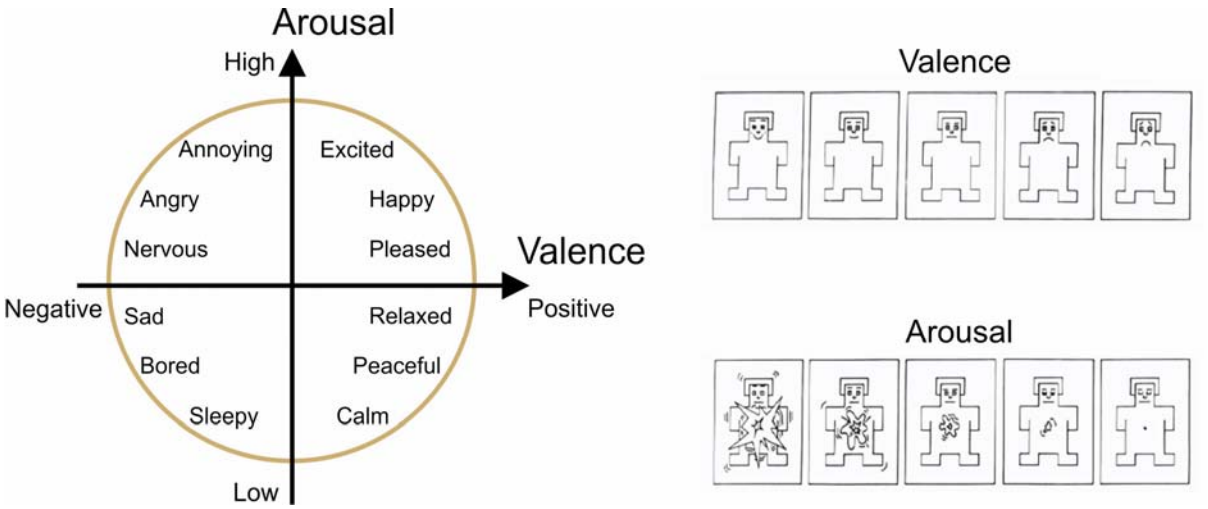

Figure 2. Two- dimensional model of emotions (left) and self-assessment manikin (SAM) rating scales (right) to assess the dimensions of valence and arousal from (Lang, 1995)

Interplay between memory and emotions

Neuroendocrine and –anatomic mechanisms of memory and emotions

Emotions as well as memory are influenced by hormones and neurotransmitters of the same hormonal systems (Erdmann, Ising & Janke, 2000; Wagner & Born, 2000). More specifically, an emotionally arousing stimulus initiates a cascade of neurochemical reactions of the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic nervous system that both are involved in the subjective experience of emotions, bodily responses as well as in the facilitation of long-term retention (Lang, 1995; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Joels, Fernandez, & Roozendaal, 2011; Walker, 2009). When the HPA-axis gets activated hypothalamic nuclei trigger the release of adrenocorticotropic hormone (ACTH) by the liberation of corticotrophin-releasing hormone (CRH) which in turn initiates the secretion of glucocorticoids (GCs) by the adrenal cortex. In addition to the hormones triggered by the HPA axis the sympathetic nervous system exerts its effects via the release of epinephrine by the adrenal medulla (Breedlove, Watson, Rosenzweig, 2010). Next to their direct effects on various target organs, stress hormones can impact brain processes due to their chemical properties to either cross the blood–brain barrier (e.g. GCs) or to activate receptors on the vagus nerve (e.g. epinephrine) that terminates in brainstem nuclei and further projects to noradrenergic pathways (Roozendaal, 2000; McGaugh, 2000). Norepinephrine (NE) is one of the most prominent neurotransmitters implicated in both, the immediate affective and behavioral response to emotionally arousing stimuli and memory consolidation via

noradrenergic signalling pathways in the brain (Cahill, Prins, Weber, & McGaugh, 1994; van Stegeren, 2008). NE unfolds its effects via pre- and postsynaptic α- and β-adrenoceptors that facilitate synaptic plasticity by increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP) which further induces protein synthesis (Joels et al., 2011; Tully & Bolshakov, 2010). Thus, NE can directly facilitate long-term potentiation (LTP), which is currently considered being a neural substrate of memory consolidation (Kandel, 2001). Blockade of adrenoceptors via propranolol abolished the increase in memory enhancement following learning of emotional material (Strange & Dolan, 2004; van Stegeren et al., 2005). The locus coeruleus (LC) as the main source of central NE, is typically activated during emotional and goal-directed behavior (Aston-Jones & Cohen, 2005). The LC innervates the amygdala, a key structure of the limbic system which is implicated in experiencing emotional affect, expressing emotional behavior and promoting long-term retention of the respective stimuli (Kensinger & Schacter, 2006). Importantly, the amygdala is not a locus of memory, but it rather modulates the formation of different kinds of memory via its projections to other brain regions (e.g. hippocampus, neocortex and rhinal cortices; see Figure 3) that are actual storing sites (McGaugh, Cahill, & Roozendaal, 1996; McGaugh, 2000; McGaugh, 2004; McIntyre et al., 2005). Hereby, the extent of amygdala activation during encoding has been revealed to be decisive for later remembering (Cahill et al., 1996; Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000; Hamann, Ely, Grafton, & Kilts, 1999; Cahill et al., 2001; Phelps, 2004). The amygdala has strong connections to the neocortex, especially the prefrontal cortex (PFC) that is assumed to play an important role in evaluation of stimuli and emotion regulation (LaBar & Cabeza, 2006). In an fMRI study, reappraisal of negative scenes coinciding with activation of prefrontal brain regions resulted in decreased subjective negative affect as well as reduced amygdala and orbito-frontal activity (Ochsner, Bunge, Gross, & Gabrieli, 2002). Consequently, PFC-induced amygdala inhibition can influence regulatory cognitive evaluation and behavior which is highly relevant for clinical applications in patients with affective disorders (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; Nomura et al., 2004).

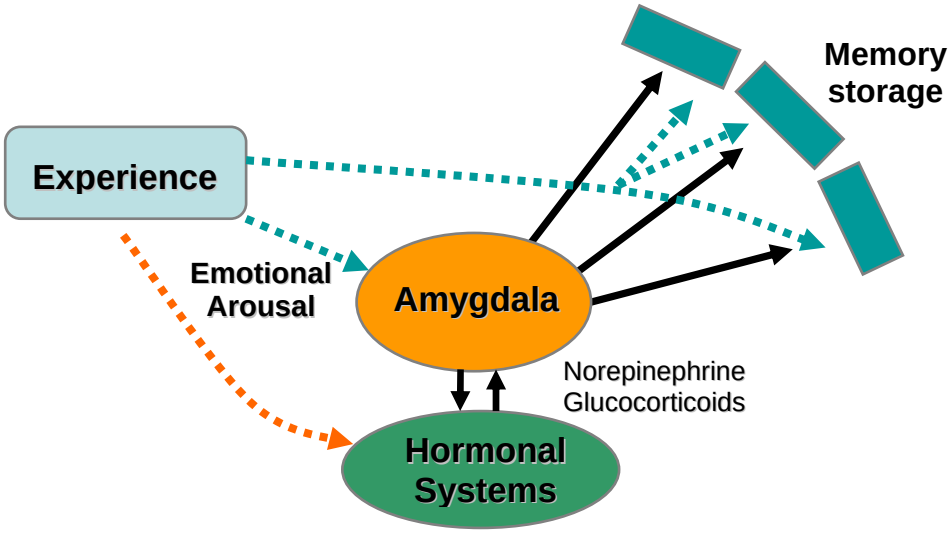

Figure 3. Memory modulation by emotional arousal (adapted from McGaugh, 2000).

Event-related potentials as a measure to dissect memories and emotional reactivity

As described earlier, emotional memories consist of two components encompassing the memory information and the autonomously regulated affective charge. Both components are interrelated with each other, partially use common mechanisms, but might follow different temporal dynamics. Dissecting the affective and the memory component of emotional memories seems to be difficult in light of their simultaneous appearance. There is virtually no way to remember an emotional object or event without being accompanied by an affective tone, as it is hardly possible to experience the affective charge without an impact of its familiarity. However, the late positive potentials (LPPs) of event-related electroencephalogram (EEG) measures have been proven sensitive to the effects of memory accuracy as well as to the affective tone of a memory (Schupp, Flaisch, Stockburger, & Junghöfer, 2006; Johnston, Miller, & Burleson, 1986; Diedrich, Naumann, Maier, & Becker, 1997; Cacioppo et al., 1993). The LPP refers to a complex of overlapping positive potential components and is commonly subdivided into a 300-500 ms and a 500-800 ms post-stimulus interval. More specifically, frontal positivity in the 300-500 ms interval has been associated with memory accuracy judgements (Rugg et al., 1998; Woroch & Gonsalves, 2010; Yu & Rugg, 2010). On the other hand, emotional arousal, evoked by emotional picture presentation, covaried with ERP positivity with largest effects over posterior sites during a late time window of 500-800 ms post-stimulus (Dolcos & Cabeza, 2002; Pollatos, Kirsch, & Schandry, 2005; Rozenkrants, Olofsson, & Polich, 2008). Consequently, electrophysiological components, together with subjective reports and behavioral methods seem to be a suitable

way to gain insight into the simultaneous processing of memory contents and associated emotionality.

Emotional modulation of memory

The two dimensions of emotion, i.e. arousal and valence have been differentially implicated in memory modulation. Hereby, arousal has been identified as the decisive factor engaging amygdala activity and creating strong memories (LaBar et al., 2006). The respective memory enhancement driven by emotional arousal could only be identified in humans with an intact amygdala, independent from an emotional judgement that is not affected by bilateral amygdala damage (Adolphs, Cahill, Schul, & Babinsky, 1997; Cahill, Babinsky, Markowitsch, & McGaugh, 1995). The influence of valence on emotional memory consolidation has been investigated as well, with less consistent findings. Whereas emotion-facilitated retention has been found to be comparable for negative and positive material in some studies (Bradley, Greenwald, Petry, & Lang, 1992; Kensinger et al., 2006), negative were remembered better than positive items in others (Charles, Mather, & Carstensen, 2003) and autobiographical and self-referenced memories tended to be better for positive information (Comblain, D'Argembeau, & Van der Linden, 2005; Kensinger et al., 2006). Emotionally arousing stimuli or information that are acquired under emotionally arousing circumstances, have been shown to be remembered better in terms of quantity (number of events), quality (subjective vividness) and amount of details, sometimes even after long time intervals of many years (Wagner, Hallschmid, Rasch, & Born, 2006; Sterpenich et al., 2009). However, rather than memory accuracy, emotional arousal at the time of acquisition has also been suggested to predominantly enhance the subjective feeling of remembering (Phelps & Sharot, 2008).

Since under natural conditions emotional contents are usually embedded in a certain context, research has started to investigate whether and how the binding of an object and its contextual information is influenced by emotionality. The findings and implications of studies that target the effects of emotional cues on a neutral context are highly contradictory. On the one hand contextual fear conditioning experiments revealed that neutral context information benefit from emotional arousal induced by an aversive stimulus (Huff & Rudy, 2004) with this effect being dependent on hippocampal activation. On the other hand an emotional trade-off has been reported indicating a preferential retention of a central emotional element at the expense of peripheral neutral background information, i.e. an effect commonly known as

weapon focus (Loftus, 1987; Steblay, 1992; Kensinger, Garoff-Eaton & Schacter, 2007). These findings implicate that in this case contextual binding is even impaired. However, the conditions determining whether the impact of emotion on contextual binding is enhancing or impairing remain elusive (Mather, 2007).

SLEEP

Sleep: Stages and Cycles

Sleep is defined as a reversible behavioral state being determined by perceptual disengagement from and unresponsiveness to the environment. The phenomenon of sleep seems to be universal for all living animals, at least for vertebrates (including mammals and birds) and even fruit flies (Allada & Siegel, 2008). On the other hand, sleep deprivation for more than a few hours results in physical and cognitive deficits (Walker, 2008; Hagewoud et al., 2010; Wlodarczyk, Jaskowski, & Nowix, 2002). However, the function of sleep has not been conclusively clarified. Since in the unconscious state of sleep an organism is much more vulnerable for predators or other threats from the environment, Alan Rechtschaffen stated that sleep needs to serve an absolutely vital function, otherwise it would be the biggest mistake evolution ever made. Next to the restorative and energy-preserving functions of sleep accumulating evidence indicates that sleep also plays a fundamental role in memory formation and emotion regulation (Stickgold, 2006; Vandekerckhove & Cluydts, 2010).

Sleep Stages. Human sleep is divided into rapid-eye movement (REM) sleep and non-rapid-eye movement (NREM) sleep, with defined criteria regarding the characteristic patterns of oscillatory electrical brain activity, eye movements and muscle activity. REM sleep refers to sleep periods that are indicated by an electroencephalogram (EEG) with relatively high frequencies and low amplitudes mostly comprising theta activity (4-8 Hz). Additionally, rapid eye movements in the electrooculogram (EOG) and a general low muscle tonus accompany the REM sleep intervals that can last between 5-60 minutes. Respiration and heart rate are increased and irregular compared to the much calmer NREM sleep periods. Furthermore, REM sleep has been associated with vivid dreaming (Aserinsky, 1953; Dement, 1957; Hobson, Stickgold, & Pace-Schott, 1998; Carskadon, 2005).

NREM sleep is subdivided into sleep stage 1 - sleep stage 4 (S1-S4) with sleep depth increasing from S1 to S4. The deeper the sleep stages the lower are the EEG frequencies and

the higher is the amplitude. Autonomic parameters, such as the heart rate, blood pressure and body temperature are decreased. In S1, the transitional phase between wakefulness and sleep, the EEG frequencies slow down compared to alpha (8-12 Hz) and beta (> 13 Hz) activity during wakefulness. With falling asleep, characteristic rolling eye movements are visible in EOG recordings. S2 is determined by the appearance of characteristic events, such as sleep spindles (spatially separated events in form of a spindle with frequencies of 12-14 Hz) or K-Complexes (steep waveforms with an amplitude > 75μV). The sleep stages S3 and S4 that can be aggregated to slow wave sleep (SWS) are typified by a high percentage (> 20% for S3, > 50% for S4) of delta waves (1-4 Hz) with amplitudes > 75μV. The oscillatory pattern can reach even slower activation, such as slow oscillations (< 1 Hz) (Rechtschaffen & Kales, 1968; Stickgold, 2006).

Sleep Cycles. In humans, NREM and REM sleep periods alternate in 3-5 sleep cycles across the night with each cycle lasting about 90 minutes (see Figure 4). Sleep of healthy humans typically follows a characteristic pattern, that is per cycle, an increasing sleep depth from light NREM sleep stages to deep NREM stages after falling asleep followed by a period of REM sleep. Subsequently, the next cycle starting with light NREM sleep stages begins. Typically, the proportion of NREM sleep (and especially SWS) is highest in the early cycles and the proportion of REM sleep rises drastically in the late sleep cycles with increasing sleep duration. The absolute proportion of NREM sleep in a night of sleep is about 75% whereas REM sleep comprises about 25% of sleep. Movement arousals and awakenings frequently occur during the night which mostly are not consciously noticed by the individual. Age-related differences in the amount and proportion of sleep stages express themselves mainly in a reduction of sleep duration and time spent in deep sleep stages across the lifespan. Newborns also usually enter REM periods before NREM periods in their sleep cycles (Carskadon, 2005).

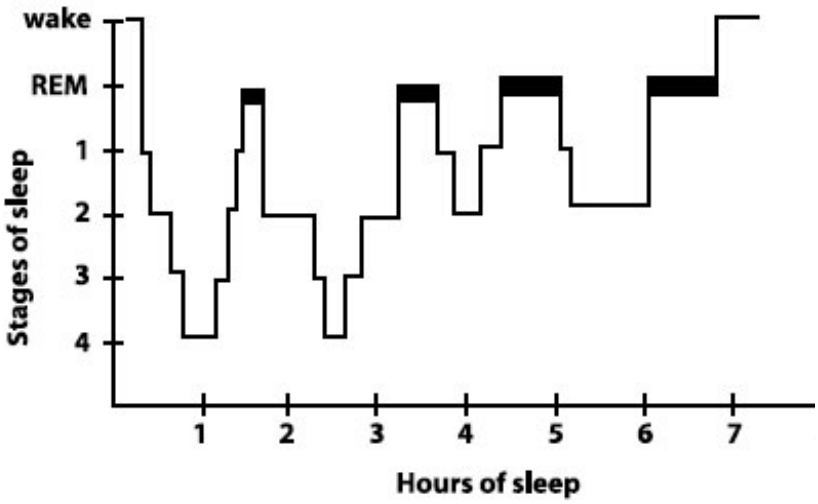

Figure 4. Sleep hypnogram illustrating the prototypical succession of sleep stages across nocturnal human sleep

Neuroendocrinology and -physiology of sleep

Major requirements for humans during wakefulness are attention, interpretation and integration of external and internal stimuli into adequate behavior. At that time the most active brain areas are the ones associated with these behaviors, located in the prefrontal, anterior cingulate, parietal cortices and in the precuneus (Maquet et al., 2000). However, sleep is an active process as well involving the activation of numerous brain regions and being characterized by specific neuroendocrine conditions. Brain activity in NREM and REM sleep fundamentally differs with respect to the brain areas that are activated during these sleep periods. During REM sleep pontine and thalamic areas as well as visual, limbic and paralimbic areas are highly activated, in contrast to the relatively decreased activation of frontal and parietal areas (Braun et al., 1998; Maquet et al., 1996; Nofzinger, Mintun, Wiseman, Kupfer, & Moore, 1997). It is likely that increased activation of the limbic system during REM sleep (including the amygdala as a key structure in emotional processing) contributes to a high extent to the effects of sleep on emotion and memory. NREM sleep mainly displays activation decreases in most brain structures. Among the most deactivated structures during SWS are the thalamus, the brainstem and the basal forebrain. Also basal ganglia, the cerebellum and some cortical areas display reduced blood flow (Maquet et al., 2000). However, deactivation of brain structures assessed by imaging methodology has been suggested to reflect local slow synchronous oscillations, electrophysiological events that have been causally related to declarative memory consolidation (Marshall & Born, 2007).

Further, evidence indicates a contribution of particular neuroendocrine conditions to the process of sleep as well as its functions, e.g. memory and emotion processing. Typically, melatonin is released during darkness and inhibited during the exposure of day light. When falling asleep firing rates of the modulatory neurons in the brain stem containing norepinephrine (NE), serotonin (SE) and acetylcholine (ACh) decrease. However, transient firing bursts of the locus coeruleus (LC, i.e. the main source of brain NE) have been detected after a learning experience during subsequent periods of SWS. The release of growth hormone (GH) and aldosterone are highly related to SWS periods. Cortisol levels reach their nadir during the early SWS-rich night, but highly rise during the second half of the night with the sudden release of corticotropin-releasing hormone (CRH). When entering REM sleep periods noradrenergic LC neurons and neurons in the raphé nuclei (i.e., the main source of SE) decrease their firing rates to a minimum. Simultaneously, cholinergic neurons of the pons increase their firing rates during REM sleep and thereby produce thalamic and cortical activation that is similar to wakefulness (Dujardin, Guerrien, & Leconte, 1990; Jones, 1991).

MEMORY AND EMOTION PROCESSING DURING SLEEP

Memory consolidation during sleep and neurophysiological mechanisms

The process of memory consolidation basically encompasses the transformation of newly acquired labile memory traces into stable memories, sometimes lasting life-long (McGaugh, 2000). Sleep after learning actively benefits the consolidation of many kinds of declarative and non-declarative memories via specific oscillatory electrical and neuroendocrine mechanisms (Diekelmann & Born, 2010; Walker & Stickgold, 2006). In addition to the facilitating effects of sleep on the retention of verbal and pictorial stimuli (Smith, 2001; Marshall et al., 2007), sleep enhances motor-sequence memory (Walker et al., 2003; Robertson, Pascual-Leone, & Miall, 2004) and visual texture discrimination learning (Gais, Plihal, Wagner, & Born, 2000) as well as emotional memories (Hu, Stylos-Allan, & Walker, 2006; Payne, Stickgold, Swanberg, & Kensinger, 2008). Further, the consolidating effects of sleep have been demonstrated to stabilize learned contents making them robust against interference with new contents (Korman, Raz, Flash, & Karni, 2003). The memory enhancing effect of sleep could be demonstrated applying various methodological designs. Sleep-dependent memory benefits could be observed comparing memory retention after a whole night of nocturnal sleep with a corresponding wake retention interval during night or day time

(Diekelmann, Wilhelm, & Born, 2009). Even short naps have been demonstrated to be effective in the stabilization and generalization of newly acquired memories (Korman et al., 2003; Lau, Alger, Fishbein, 2011).

A widely accepted theoretical approach about the particular role of sleep for processes of memory consolidation has been proposed by the system consolidation theory which is based on the principles of the two-stage model of memory (Marr, 1971). According to the two-stage model of memory, two learning systems are distinguished that work at different rates and involve neuronal activity in separate brain sites. More specifically, the model assumes a fast learning system in which new information are temporarily stored and a slow learning system consolidating memories for the long-term. Initially, both learning systems encode new information in parallel whereby the hippocampus holds information for the temporary storage and in the neocortex permanent memory networks are built up. During the consolidation period, information in the temporary store gets repeatedly reactivated which prompts the long-term store to reactivate the memory traces again, together with similar already existing memory traces. The gradual integration of newly acquired memories in the neocortex can be achieved by the repeated strengthening of connections between new and old related representations finally resulting in a restructuring of brain areas that are required for retrieval. The standard theory of system consolidation postulates the specific role of sleep in the processes of reactivation and redistribution of newly acquired memory representations (McClelland, McNaughton, & O'Reilly, 1995; Frankland & Bontempi, 2005). Evidence for a sleep-dependent reactivation of memory representations came from studies showing that the same pattern of neuronal activation that had been detected during encoding of a task is replayed during subsequent periods of sleep (Wilson & McNaughton, 1994). Further, brain areas that were engaged during learning (e.g. the hippocampus in route learning in humans) were activated again during SWS with the degree of reactivation being positively correlated with performance improvement (Peigneux et al., 2004; Huber, Ghilardi, Massimini, & Tononi, 2004). It has been assumed that for the process of reactivation sleep, and here especially SWS, provides optimal conditions including reduced exposure and perception of interfering external stimuli (Wilson et al., 1994; Sutherland & McNaughton, 2000; McNaughton & Wickens, 2003; Peigneux et al., 2004). However, notable evidence has demonstrated that neuronal reactivation also occur during REM sleep (Louie & Wilson, 2001). It can be assumed that by these ‘offline’ repetitions of specific neuronal patterns the associated memory representations become strengthened and integrated into existing neuronal networks.

The current view on the underlying mechanisms of sleep-dependent memory consolidation involves three distinct electrical phenomena. Neocortical slow oscillations (resulting from synchronized firing patterns of neuron ensembles), thalamo-cortical spindles and hippocampal ripples that, together, are assumed to facilitate synaptic plasticity in hippocampal and neocortical neurons. More specifically, slow oscillations temporally synchronize the neuronal activity that is increased during the depolarizing cortical up-states and decreased during hyperpolarizing down-states (Steriade, 2006). The depolarizing up-states are assumed to drive the memory reactivations within the hippocampus which are reflected by hippocampal sharp-wave ripple activity (high frequent EEG activity of 100-300 Hz). Synchronized thalamo-cortical spindle activity repetitively induces synaptic plasticity and has been proposed to facilitate the integration of reactivated memory representations into cortical long-term storing sites (see Figure 5).

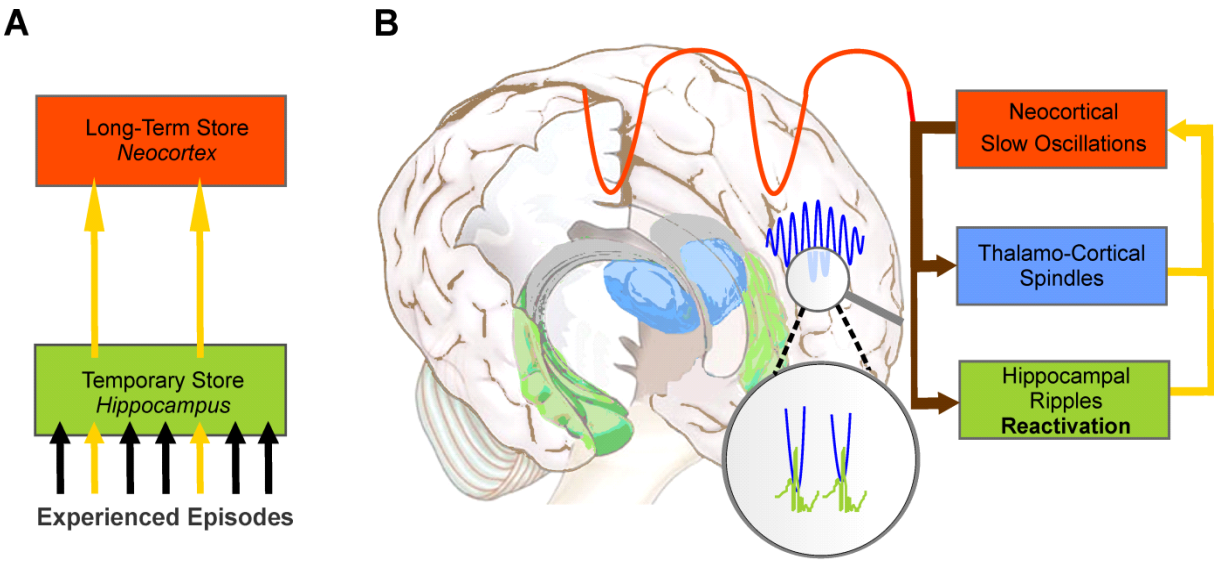

Figure 5. Active system consolidation during sleep. (**A**) Assignment of processes proposed by the two-stage model of memory consolidation to (**B**) Mechanisms occurring in specific brain regions to allow for the integration of temporarily stored information into long term memory in a process of system consolidation (Figure from Born and Wilhelm, 2012).

Given that SWS and REM sleep fundamentally differ according to the portions of dominating oscillatory activity (i.e. delta activity vs. theta activity, respectively) and neuromodulatory activity (e.g. ACh, NE, SE, GC) a differential contribution of sleep stages in processes of memory consolidation can be assumed. However, the exact role of SWS and REM sleep is still a matter of debate. Recent studies revealed greater memory gains for declarative

memories after sleep intervals containing high amounts of SWS whereas procedural and emotional memories benefited more after REM sleep-rich sleep intervals (Plihal & Born, 1997). On the background of these findings, the dual-process hypothesis posits that both sleep stages support different memory systems (Maquet, 2001). The sequential theory, on the other hand, suggested that the succession of periods of SWS and REM sleep may play a critical role for sleep-dependent memory benefits (Giuditta et al., 1995); see Figure 6). In this context, it has been speculated that system consolidation (i.e. the integration of new information into existing neuronal networks) occurs during SWS (Yordanova et al., 2008) whereas synaptic consolidation (i.e. the stabilization of memory representations within localized synaptic networks, (Dudai, 2004) might mainly take place during REM sleep (Diekelmann et al., 2010). It has also been demonstrated that SWS and REM sleep are differentially implicated in the consolidation of different aspects of declarative memories, i.e. memory on the item itself (referred to as item memory) and memory on contextual information that are associated with an item (referred to as source memory). Retention intervals containing high amounts of SWS preferentially facilitated the consolidation of recollection-based source information rather than familiarity-based item memories (Drosopoulos, Wagner, Born, 2005; Daurat, Terrier, Foret, Tiberge, 2007). It can be concluded that relational aspects and the binding of items into a spatiotemporal context, possibly by a stronger engagement of the hippocampus (Staresina & Davachi, 2009; Lehn et al. 2009), benefit more from the above described mechanisms underlying system consolidation occurring preferentially during SWS.

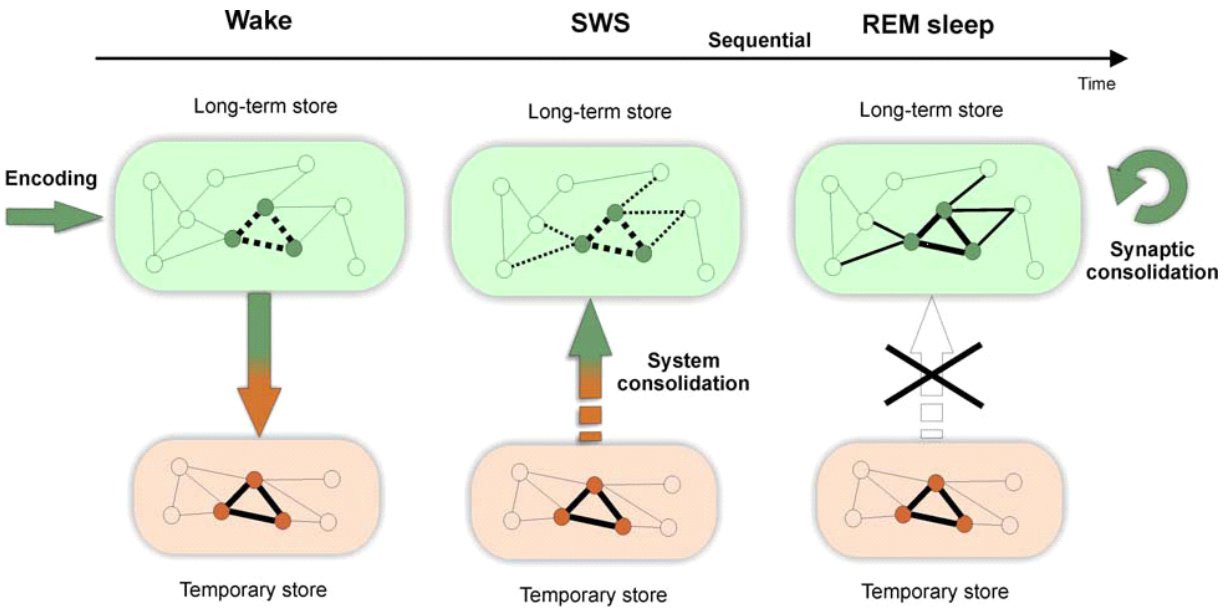

Figure 6. Memory formation during wakefulness and sleep: the sequential contribution of REM and SWS (from Diekelmann et al., 2010).

Effects of sleep on emotional memory consolidation

As for other kinds of memory, sleep appears to affect all stages of emotional memory processing, i.e. encoding, consolidation and retrieval (Walker et al., 2009). Sleep deprivation studies revealed that memory encoding is distinctly impaired resulting in deteriorated retention, at least for emotions associated with high arousal (e.g. anger, happiness) (Van der Helm, Gujar, & Walker, 2010). Brain areas associated with the recognition of emotional contents (e.g. amygdala and medial prefrontal cortex [mPFC]) have been demonstrated being disregulated during encoding in sleep-deprived humans. Here, amygdala reactivity has been found to be highly increased whereas the connectivity between amygdala and mPFC was decreased in response to negative and positive arousing pictures (Yoo, Hu, Gujar, Jolesz, & Walker, 2007; Gujar, Yoo, Hu, & Walker, 2011).

However, growing evidence supports a beneficial role of sleep for memory consolidation of emotional contents as well. The importance of REM sleep for emotional memory consolidation has been suggested several years ago in studies using the method of selective REM sleep deprivation (REMD; Grieser, Greenberg, & Harrison, 1972; Cartwright et al., 1975; Greenberg, Pearlman, Schwartz, & Grossman, 1983). In these studies emotional memory impairments have been attributed to a lack of REM sleep, but comprised profound methodological disadvantages that limit the interpretation. Nevertheless, recent evidence using an improved design (i.e. the split-night design) also confirmed a fundamental impact of REM sleep on emotional memory consolidation. Emotional stories were found to be remembered distinctly better after retention intervals filled with late REM-rich sleep, but not after early SWS-rich sleep compared to respective retention intervals of wakefulness (Wagner, Gais, & Born, 2001). Importantly, retention of the emotional contents was still significantly enhanced for emotional compared to neutral stories four years after encoding, but only when sleep had followed learning and not when subjects had stayed awake (Wagner et al., 2006). Also, a whole night of nocturnal sleep following learning improved retention of emotional material compared to when participants stayed awake for a comparable retention interval during the day (Hu et al., 2006). Importantly, performance improvement of emotional memory has been demonstrated to positively correlate with the amount of REM sleep, REM sleep latency and prefrontal theta activity (Nishida, Pearsall, Buckner, & Walker, 2009). Beside a quantitative enhancement of emotional memories, sleep induces a neural reorganisation of involved brain areas by enhancing connectivity between the amygdala, hippocampus and ventral medial prefrontal areas (Sterpenich et al., 2007; Sterpenich et al., 2009; Payne & Kensinger, 2011). As described earlier, the limbic system including the

amygdala nuclei is highly activated during REM sleep, but not during SWS (Nofzinger, 2005) indicating that REM sleep-dependent amygdala-driven reactivation might be an underlying mechansim for the preferential consolidation of emotional memories during REM sleep (McGaugh, 2004). On a mechanistic level, theta activity might be implicated in the sleep-dependent consolidation benefits of emotional material (Popa, Duvarci, Popescu, Léna, & Paré, 2010). Interestingly, not all information of an emotional scene seems to be equally well consolidated during sleep. Rather, the central element of a scene is remembered better after sleep at the expense of peripheral background details (Payne et al., 2008). However, the specific contribution of sleep stages for the preferential consolidation of the emotional items, but reduced source association, remains unclear. In line with these findings, sleep has been found to especially enhance the gist of information (Diekelmann et al., 2010; Payne et al., 2009) and support the generalization of extinction learning from a specific target to a second stimulus in a fear conditioning paradigm in humans (Pace-Schott et al., 2009). Moreover in a recent study, memories of future relevance have been pointed out to particularly benefit from sleep. Here, the number of word-pairs recalled after a night of sleep was highly increased when subjects knew that a retention test would be performed at a later time and, importantly, retention performance was strongly correlated with slow oscillatory activity during the night after learning (Wilhelm et al., 2011). From these first hints it can be asked whether some aspects of emotional memories (that contain highly relevant information per se) might be also processed during SWS. It can be speculated that contextual source information which has been found to be improved after SWS-rich sleep periods for neutral material (Drosopoulos, et al., 2005) also depends on SWS for emotional material. Additional evidence for this hypothesis derives from animal studies that detected increased phasic activity of noradrenergic neurons during SWS following amygdala-dependent odor learning (Eschenko & Sara, 2008). Furthermore, odor discrimination learning in humans has been related to depend on noradrenergic activity during SWS-rich sleep (Gais, Rasch, Dahmen, Sara, & Born, 2011). Given that odor memory as well as emotional memory both strongly require amygdala activity, emotional memory consolidation might in some aspects also be processed during SWS, thereby possibly involving noradrenergic mechanisms.

Effects of sleep on emotion processing

More than a century ago, psychoanalysis raised the idea that dream sleep might be involved in the emotional processing of memories, possibly exerting a cathartic influence on exuberant

emotions (Freud, 1900; Grenell, 2008). Until today strong empirical evidence for these clinical observations is still lacking. However, a recent model proposed that emotional memories, while in the beginning consisting of a tightly tagged memory and affective component, lose their emotional charge over time. Further, REM sleep was suggested to be critically involved in the separation of both components thereby, on the one hand, supporting the storage of memory information, but on the other hand, simultaneously reducing the associated affective tone (see Figure 7, Walker et al., 2009). The special endocrine conditions during REM sleep (i.e., absence of aminergic, but highly increased cholinergic activation) have been proposed to result in a depotentiation of the associated emotional charge while the memory information is replayed and strengthened.

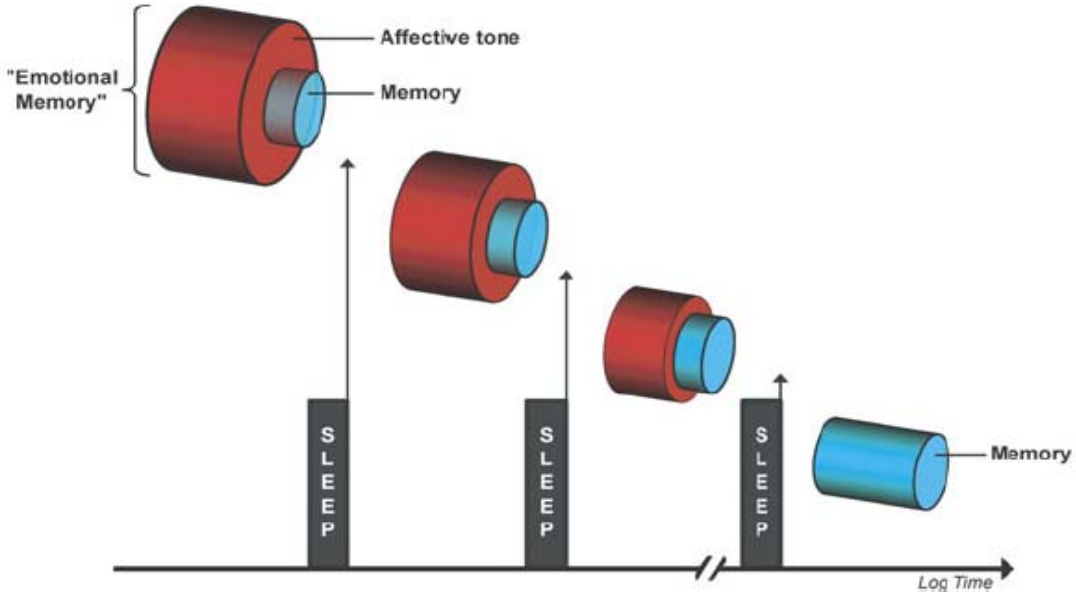

Figure 7. The REM sleep hypothesis (Walker, 2009)

So far, only one study directly addressed the issue of sleep-associated changes in subjectively rated emotional reactivity in response to emotional material. Following the approach of the split night design, subjective ratings of valence and arousal of emotional and neutral pictures (IAPS; Lang, Bradley & Cuthbert, 2008) were assessed before and after intervals of early SWS-rich and late REM-rich sleep as well as before and after respective wake intervals. Negative pictures that had been seen before a REM sleep-rich, but not a SWS-rich or a wake retention interval, were rated more negative in valence compared to new pictures with equal emotional charge. Thus, contrary to the predictions, REM-rich sleep enhanced the subjectively perceived negativity, being confirmed by the subjective ratings of another group

of subjects who slept a whole night and in which this negativity bias was even more pronounced. Additionally, no differences in arousal ratings could be detected for any of the relevant comparisons between the sleep and wake conditions (Wagner, Fischer, & Born, 2002). Another study that compared subjective ratings of emotional pictures one week after encoding revealed no differences, neither in valence nor arousal between familiar and new negative and neutral pictures (Weymar, Löw, Melzig, & Hamm, 2009). Thus, also after a longer time period in which many nights of sleep occurred, no differences in emotional reactivity could be observed, suggesting that the assumption of a sleep-dependent reduction of emotional reactivity might be either wrong or can only be assessed using more sensitive methods.

The impact of sleep on emotional reactivity can be inferred from fMRI studies investigating the activation of brain regions that are specifically associated with emotion processing during encoding and retrieval of emotional stimuli. Clear evidence for a possible role of sleep on emotional reactivity during encoding of emotional stimuli has been provided by measuring brain activity in sleep-deprived subjects. It was shown that negative (Yoo et al., 2007) as well as positive (Gujar et al., 2011) pictures resulted in stronger reactivity of the amygdala and reduced connectivity with the medial prefrontal cortex after a night of sleep deprivation compared to a full night of sleep. Thus, limbic activity is clearly modulated by sleep and contributes decisively to emotional perception and categorisation (Gujar et al., 2011). However, findings on the effects of post-learning sleep on emotional reactivity at retrieval are less consistent. Sleep compared to wakefulness during the night following encoding of negative pictures increased amygdala activity for these pictures at recognition testing in one study (Sterpenich et al., 2009) but not in another (Sterpenich et al., 2007). Further hints for a possible role of sleep in processing of emotional reactivity derive from clinical observations indicating sleep abnormalities in a wide range of psychiatric and neurological mood disorders as highly frequent symptoms. Clinical studies have revealed that the co-occurrence of emotional and sleep disturbances might follow a bi-directional relationship (Van der Helm & Walker, 2009; Walker, 2009; Harvey, Jones, & Schmidt, 2003). Accumulating evidence has been raised in patients suffering from depression or post-traumatic stress disorder (PTSD) displaying abnormalities in various sleep parameters, e.g. increased sleep latency, nocturnal awakenings, REM density and decreased REM sleep latency (Kobayashi, Boarts, & Delahanty, 2007; Berger & Riemann, 2009; Krystal, Thakur, & Roth, 2008). Additionally, sleep deprivation and sleep restriction have been associated with emotional disturbances that occurred in a progressive manner (Dinges et al. 1997).

The split-night design- A method to study the impact of sleep stages on memory and emotion

Sleep is not a unitary phenomenon, but rather a heterogeneous conglomerate of alternating processes during the night. To investigate the role of certain sleep stages for memory consolidation different methodological approaches are possible that all have advantages and disadvantages and might be more or less suitable for specific research questions. Associating the amount or intensity of electrical activity (e.g. slow wave activity during SWS or theta activity during REM) during the sleep episode after learning with performance change at retrieval testing is one possibility to get insight into memory or emotion-relevant processes during sleep. However, correlational designs do not provide information about the direction of influence and designs including a whole night of nocturnal sleep can also not exclude that performance changes result from the interplay of many sleep stages or sequence in which they appeared. Another prominent approach is the deprivation of specific sleep stages (in previous studies mostly SWS or REM sleep were of interest). According to these designs, when sleep deprivation of a certain sleep stage results in decreased performance compared to sleep deprivation of another sleep stage or a non-deprived control, this sleep stage is taken to be of relevance for performance. However, several profound confounds, especially for the method of selective deprivation of REM sleep (REMD) need to be considered for respective interpretations (Born & Gais, 2000; Vertes & Eastman, 2000). As the process of REMD involves sudden interruptions from REM sleep as soon as first signs of this sleep stage occur, simultaneous stressful awakenings and arousal are caused (to a higher extent than awakenings from NREM sleep stages; Cipolli, 1995). Stress caused by REMD can lead to the release of glucocorticoids that are well known to affect all stages of memory formation (Wilhelm, Wagner, & Born, 2011; Kuhlmann, Kirschbaum, & Wolf, 2005; Wolf, Kuhlmann, Buss, Hellhammer, & Kirschbaum, 2004; Lupien et al., 2005) and might be involved in emotional disturbances.

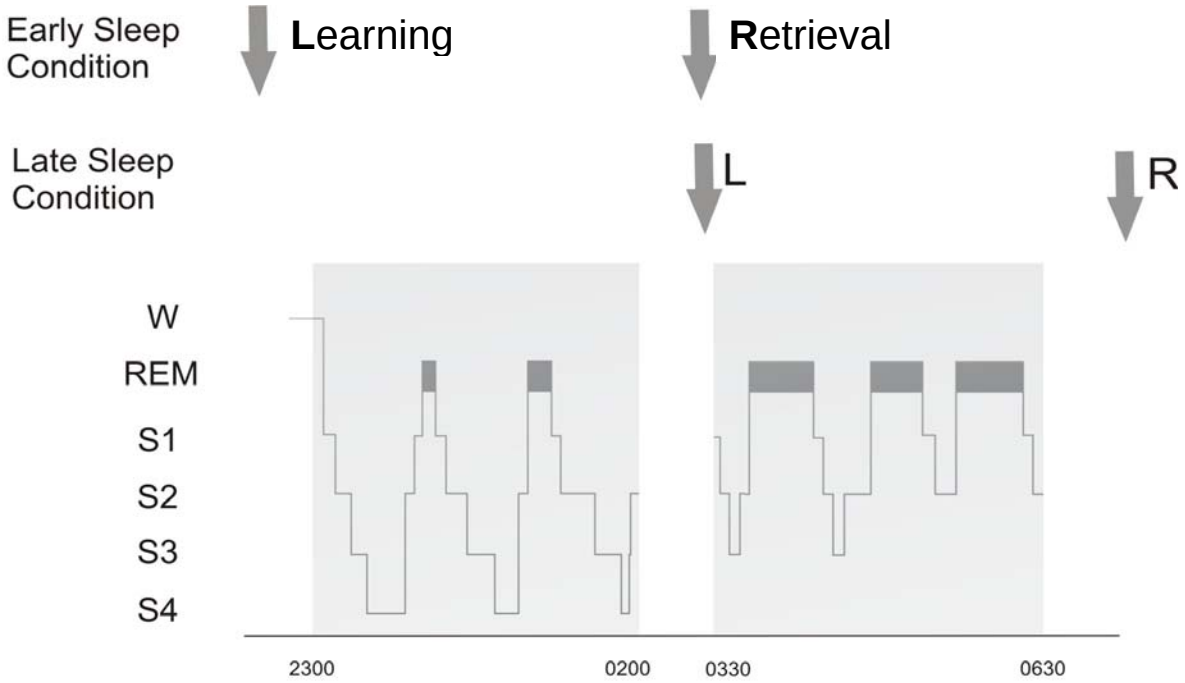

Figure 8. The split-night design. Participants in both, the early and late sleep condition sleep for a 3-hrs interval following learning. Whereas sleep in the early night is dominated by slow-wave sleep (SWS), late sleep contains high amounts of rapid-eye movement (REM) sleep. For this purpose, the time of learning and retrieval differs between conditions.

A design that can control for these confounds is the split-night design, also known as Ekstrand-paradigm (Yaroush, Sullivan, & Ekstrand, 1971; Fowler, Sullivan, & Ekstrand, 1973, Ekstrand, 1977). This approach takes advantage of the natural nocturnal distribution of sleep stages with a high proportion of SWS and minimal amounts of REM sleep occurring in the early hours of sleep and the opposite distribution of these sleep stages in the late hours of sleep (see Figure 8). In this design subjects are assigned to one of two conditions, one in which three hours of early SWS-rich sleep and another where three hours of late REM-rich sleep follow initial stimulus processing. After these sleep periods, subjects are awakened from light NREM sleep stages to keep stress-induced cognitive or emotional disturbances that occur after awakening from REM sleep or deep NREM sleep at a minimum. Changes in memory or emotional reactivity can be compared either between the early and late night condition or to respective wake control conditions in which subjects remain awake in a corresponding three hours early or late retention interval. The comparison to wake control groups has the additional advantage to control for circadian influences, as learning and retrieval performance in experimental and control groups takes places at the same time of the day. However, the amount of sleep deprivation in these groups is different and thus, a differential extent of sleep deprivation might result in a different degree of emotional

responsiveness indicated by amygdala reactivity (Yoo et al., 2007). In general the amount of sleep deprivation and circadian influences can be estimated as relatively small, since subjects are only sleep restricted for a few hours and learning as well as retrieval only differs about 3 - 4 hours between both conditions.

OBJECTIVES AND HYPOTHESES

Three experiments have been conducted to investigate the following aspects of emotional memory processing during sleep: i) the influence of REM sleep and SWS on the simultaneous consolidation of emotional memory information and processing of its affective charge, ii) possible endocrine mechanisms (i.e. noradrenergic activity) underlying emotional memory formation during SWS and iii) whether REM sleep and SWS differentially modulate different aspects of emotional memory, i.e. retention of emotional item memory and associated contextual information (source memory).

Experiment 1. Emotional memory has been shown to benefit from sleep and it has been suggested that the performance enhancement relative to neutral memories is specifically related to REM sleep. First hints point to a modulating influence of REM sleep on emotional reactivity, but the direction of this effect is still a matter of debate. Moreover, the effect of sleep on emotional memory consolidation and emotional reactivity has not been assessed yet in one study. From a theoretical perspective, a specific role in REM sleep has been proposed for the memory enhancement on the one hand and a reduction of emotional reactivity to familiar emotional stimuli on the other hand (Walker, 2009). Experiment 1 aimed to test this hypothesis by a combination of behavioral and event-related EEG measures as well as subjective ratings. Enhanced memory retention and lower subjective ratings in arousal of emotional compared to neutral pictures was hypothesized after a late REM-rich but not after an early SWS-rich sleep retention interval. It was further expected that the late positive potential (LPP) of event-related EEG measures would differentially covary with emotional memory (retention) and emotional reactivity (arousal) across REM-rich and SWS-rich sleep intervals. The 300-500 ms interval at frontal electrode sites that has been associated before with memory accuracy (Rugg et al., 1998) was expected to display distinctly enhanced positivity for correctly remembered emotional pictures compared to new emotional and neutral pictures, specifically after REM-rich sleep. On the other side, positivity in the 500-800 ms interval at parietal sites which has been proven sensitive to emotionality (Dolcos et al.,

2002) was predicted to be reduced for familiar emotional pictures compared to new emotional and neutral pictures after REM-rich sleep.

Experiment 2. Superior retention of emotional memories is causally related to noradrenergic activation during encoding and consolidation of emotionally arousing contents. While norepinephrine (NE) is predominantly released during wakefulness, NE levels during sleep are markedly reduced (McGaugh, 2004; Aston-Jones et al., 2005). Nevertheless, phasic locus coeruleus (LC) activity, which triggers the release of NE in the brain, has been detected particularly during SWS following learning in rats (Eschenko et al., 2008). However, whether SWS plays a role in emotional memory consolidation remained elusive so far. The aim of experiment 2 was therefore to investigate in humans whether the formation of emotional memories is influenced by SWS and whether this effect might specifically depend on noradrenergic activity during post-learning periods of SWS. Hereby, different memory functions were tested, i.e. memory for emotional and neutral pictures and stories as well as for associated context information (i.e. the temporal order of contents within the stories). It was hypothesized that emotional material would be remembered superior compared to neutral memory, but not when noradrenergic release was suppressed by an α2-receptor agonist during a 3 hrs SWS-rich retention interval. Further, a possible influence of NE on processing of emotional reactivity across SWS-rich sleep was explored by the assessment of subjective ratings and the psychophysiological measure of heart rate change in response to emotional and neutral pictures.

Experiment 3. Most studies focusing on the interplay between sleep and emotional memory formation mainly involved recognition-based item memories and revealed a distinct role of REM sleep for their consolidation. However, several findings point to a possible impact of SWS as well, with SWS and REM sleep perhaps targeting different aspects of declarative memory formation. For instance, declarative memory that contains contextual information (i.e. source memory), but not item memory, has been found to be particularly promoted by SWS-rich sleep intervals (Drosopoulos et al., 2005). Additionally, the findings of Experiment 2 suggest that specifically the consolidation of emotional source, but not item information undergoes SWS-dependent processing, involving noradrenergic mechanisms. Experiment 3 aimed to compare the effects of REM sleep and SWS on emotional item and source memory. It was hypothesized that item memory would be distinctly enhanced after late REM-rich sleep intervals for emotional compared to neutral pictures, whereas contextual information would be consolidated during SWS.

Experiment 1 – The role of REM sleep in the processing of emotional memories: Evidence from behavior and event-related potentials

Submitted as: Groch, S.,Wilhelm I., Diekelmann S., Born J. (2011). The role of REM sleep in the processing of emotional memories: Evidence from behavior and event-related potentials.

INTRODUCTION

Emotional contents are well known to be remembered better than neutral contents (McGaugh, 2000). An emotional event elicits a response that includes subjective feelings, behaviors indicating approach or avoidance (like facial expressions), and various physiological responses, all of which to a certain extent can reoccur during later remembering (Lang, 1995; Schelling et al., 2003). The two components of an emotional memory, i.e. the memory information about the experienced event and the affective component, develop over time, sometimes with different dynamics. Dating back to Sigmund Freud's clinical observations (Freud, 1900), a long standing tradition developed proposing that dream sleep as well as the physiologically more accurately defined stage of rapid eye movement (REM) sleep is involved in the emotional processing of memories, possibly exerting a cathartic influence on exuberant emotions. Along this line, a recent model proposed that REM sleep, on the one hand, plays an important role in maintaining the content of an emotional event in memory and, on the other hand, simultaneously reduces its associated affective tag (Van der Helm & Walker, 2009). As an underlying mechanism it was proposed that during REM sleep emotional representations involving the amygdala are reactivated in the absence of noradrenergic activity which mediates the arousal during encoding of an emotional event. However, empirical evidence for this concept is surprisingly scarce.

There is indeed a growing number of studies indicating that REM sleep facilitates the consolidation of emotional memories. However, most of these studies disregarded that aspects of memory content and emotionality may be differentially influenced. Thus, the amount of post-learning REM sleep was revealed to be positively correlated with retention of emotional memories (Nishida, Pearsall, Buckner, & Walker, 2009). In a comparison of effects of early nocturnal retention periods of sleep with predominant slow wave sleep (SWS) and late REM-

rich periods of retention sleep, late REM-rich sleep improved retention of emotional with reference to neutral stories distinctly more than early SWS-rich sleep (Wagner, Gais, & Born, 2001). The enhancing effect of REM-rich sleep on the retention of emotional stories was also significant in comparison with the effects of a corresponding wake retention interval.

So far, only one study directly addressed the issue of sleep-associated changes in the affective responses to aversive emotional and neutral pictures (Wagner, Fischer, & Born, 2002). This study also used the approach of splitting the night into early SWS-rich and late REM-rich periods of retention sleep, and affective responses were assessed by subjective ratings of the pictures' valence (i.e. positive to negative) and arousal (i.e. degree of excitement). Surprisingly, REM-rich retention periods of sleep distinctly increased, rather than decreased, the valence (i.e. rated aversiveness) of the emotional pictures seen before the sleep interval in comparison with new emotional pictures not seen before. The enhancing effect on subjectively experienced aversiveness was even more pronounced after a full night of sleep. These findings are clearly at variance with a reducing effect of REM sleep on the affective tone of emotional memory as postulated by Van der Helm & Walker (2009). However, as the pictures in the study by Wagner and colleagues (Wagner et al., 2002) were also rated before retention sleep, knowledge of this first rating may have confounded the critical rating response after retention sleep. Also, ratings were only affected on the valence dimension, whereas arousal ratings, which are commonly assumed to reflect better the affective tone, remained uninfluenced by sleep in that study. Functional magnetic resonance imaging of amygdala activity presumed to reflect the arousal response to emotional stimuli (Kensinger & Corkin, 2004) did not clarify the picture. One study revealed that post-acquisition sleep, compared with a night of total sleep deprivation, increased activity in the extended amygdala at recognition of emotionally aversive pictures (Sterpenich et al., 2009), but this effect was not observed in another study (Sterpenich et al., 2007).

Overall, it remains an unresolved question whether REM sleep enhances or reduces the affective tone of an emotional memory, and there is indeed no investigation yet targeting the combined role of REM sleep on the consolidation of the content and simultaneous processing of the affective tone of an emotional memory. Event-related potentials (ERPs) and especially the late positive potentials (LPP) of the ERP have been proven sensitive to the effects of memory accuracy as well as to the affective tone of a memory (Schupp, Flaisch, Stockburger, & Junghöfer, 2006). The LPP refers to a complex of overlapping positive potential components in the latency range between 300-800 ms after stimulus onset. LPPs are sensitive to enhancing influences of attention and emotionality, thereby facilitating encoding

at learning as well as recognition at retrieval (Johnston, Miller, & Burleson, 1986; Diedrich, Naumann, Maier, & Becker, 1997; Cacioppo, Crites, Berntson, & Coles, 1993; Schupp et al., 2000). In particular, frontal positivity 300-500 ms post-stimulus has been linked to memory accuracy, as it was consistently found to increase with accuracy of familiarity-based item memory recognition (Rugg et al., 1998; Woroch & Gonsalves, 2010; Yu & Rugg, 2010). On the other hand, ERP positivity peaking 300-600 ms post-stimulus over posterior brain sites was consistently found to be enhanced in response to emotional pictures in comparison with neutral pictures (Schupp et al., 2000; Codispoti, Ferrari, & Bradley, 2007; Krug, Plihal, Fehm, & Born, 2000; Pollatos, Kirsch, & Schandry, 2005). Arousal, evoked by emotional pictures, covaried with ERP positivity with largest effects over posterior sites during a late time window of 500-800 ms post-stimulus (Dolcos & Cabeza, 2002; Pollatos et al., 2005; Rozenkrants, Olofsson, & Polich, 2008).

In the present study we combined behavioral measurements of memory and subjective ratings of valence and arousal with ERP recordings to dissociate effects of post-learning REM sleep versus SWS on the consolidation of the emotional content of a memory and the processing of its affective tone. Subjects memorized neutral and negative emotional pictures taken from the International Affective Picture System (IAPS) before 4-hours retention periods filled with 3 hours of either early SWS-rich or late REM sleep-rich nocturnal sleep. Recognition memory as well as affective ratings were assessed after the sleep periods. ERPs were recorded during both learning and recognition testing. If REM sleep supports specifically the consolidation of the contents of emotional memories, then post-encoding REM sleep, in comparison with SWS, should enhance later recognition of the aversive pictures together with frontal ERP positivity 300-500 ms post-stimulus onset in response to these pictures. If REM sleep in parallel reduces the affective tone, subjectively rated emotional arousal should be decreased after REM-rich periods of sleep and this should be accompanied by a relative decrease in posterior ERP positivity 500-800 ms post-stimulus onset.

METHODS

Participants. Sixteen native German speaking healthy men (mean age: 22.06 years, range 20-26 years) were recruited at the University of Luebeck. All were non-smokers, free of medication and had no history of neurological, psychiatric or endocrine disorders. Participants had followed a normal sleep-wake rhythm (i.e. no shift work, usual sleep time from 2300-

0700h) for at least four weeks before the experiments. Prior to the experiments, subjects were accustomed to sleeping under laboratory conditions during an adaptation night, including wearing EEG caps (Easy Cap GmbH, Herrsching, Germany) with electrodes for polysomnographic recordings. On experimental days participants were required to get up at 0700h and not to consume caffeine or alcohol. The study was approved by the ethics committee of the University of Luebeck and all participants gave written informed consent prior to participation.

Design and procedure. Figure 9A illustrates the study design. The study was conducted according to a within-subject cross-over design with the order of conditions ('early sleep' vs. 'late sleep') balanced across subjects, and an interval of at least two weeks between the subject's two conditions. Subjects reported to the lab at 2100h for the early night condition and at 2200h for the late night condition. Each condition started with the attachment of electrodes. For the early sleep condition, subjects learned the pictures (2200-2230h) immediately before an early 4-hours retention interval including 3 hours of SWS-rich sleep, and were then tested on recognition memory of the pictures. For the late sleep condition, subjects first slept for about 3 hours (starting 2300h) before the learning phase took place 0300-0330h. Then, they slept for another 3 hours filled with REM-rich sleep, before recognition memory was tested in the morning (0715-0800h), after a retention interval of 4 hours in total. Thus, the retention intervals in the two experimental conditions were characterized by either high amounts of SWS (early sleep) or high amounts of REM sleep (late sleep). Lights-off for the early sleep interval was at 2300h, and at 0330h for the late sleep interval, with the start of the 3-hrs sleep period determined by the first signs of sleep stage 2 for more than 1 min. Subjects were awakened from sleep stage 1 or 2 after 3 hours of sleep, and subsequent learning or retrieval testing was timed 45 min thereafter to allow subjects to recover from sleep inertia.

Before each learning and retrieval phase subjects indicated their level of sleepiness on a scale from 1 (active, alert) to 7 (very sleepy) (Stanford Sleepiness Scale, SSS; Hoddes, 1972) and performed on a 5-min version of the Psychomotor Vigilance Test (PVT; Roach, Dawson, & Lamond, 2006). The current mood was assessed once after arrival at the laboratory in the evening by the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988).

Memory task and affective ratings. Four hundred pictures were selected from the International Affective Picture System (IAPS; Lang, Greenwald, Bradley, & Hamm, 1993) and divided into two parallel sets (for the subjects' two conditions) each consisting of 50 neutral low arousing and 50 medium to high arousing emotionally negative pictures for the learning phase, supplemented with 50 new pictures for both categories at retrieval testing. Normative valence (scale from negative [1], neutral [5] to positive [9]) and arousal (scale from not arousing at all [1] to very arousing [9]) ratings were comparable for the four sets (all $p > 0.99$): [mean ± SEM across all sets] valence ratings: negative, 3.07 ± 0.60; neutral, 5.03 ± 0.35; arousal ratings: negative, 5.89 ± 0.37; neutral, 3.11 ± 0.33. Additional 6 negative and 6 neutral pictures were presented before and after the target picture sets to allow subjects to accustom to the learning and retrieval procedure and to prevent primacy and recency effects regarding memory performance.

During learning, pictures were presented in pseudo-randomized order following predefined criteria (e.g., a maximum of two subsequent pictures with the same valence), with each participant receiving the same order. Preceding each picture, a fixation cross appeared for 500 ms. Each picture was presented for 1000 ms to allow subjects for conscious processing, but to keep an appropriate level of difficulty to prevent ceiling performance at the later recognition test. Presentation of the next picture followed after a variable inter-stimulus interval of 3500, 4000 or 4500 ms (mean: 4000 ms). Participants were instructed to look at each picture for the entire presentation time and to try to memorize the pictures, as memory for the pictures would be tested later on. A 5-min break was introduced after half of the pictures were presented.

At retrieval, picture recognition memory was tested by presenting the 100 pictures from the learning phase (referred to as 'old' pictures) randomly intermixed with 100 new pictures. Again, pictures appeared for 1000 ms preceded by a 500 ms fixation cross in pseudo-randomized order according to the same criteria as during learning. After the presentation of each picture participants had to indicate with a corresponding key press (i) whether they had seen the picture before ('old') or not ('new'), (ii) how confident they were about their decision on a scale from 1-9 (with 1 = not confident at all, and 9 = absolutely confident) and (iii) how emotional they perceived the picture on the dimensions of valence (1 = very negative, 5 = neutral, 9 = very positive) and arousal (1 = not arousing at all, and 9 = very arousing). The affective ratings were performed with the computer-based version of the Self-Assessment Manikin (SAM) rating system (Bradley & Lang, 1994) and participants were instructed to give ratings on the SAM spontaneously and quickly (see Figure 9B for

illustration of the task). During both learning and recognition of the pictures, ERPs were recorded. For the picture presentation and rating procedures Presentation® Software (Version 14.6) was used. Triggers were set with the onset of each picture for ERP analysis and key presses were logged for behavioral analyses.

Memory performance and affective ratings

Recognition memory. Retention of pictures was assessed by the absolute number of correctly recognized old pictures at retrieval (i.e. hits), separately for emotional and neutral pictures. To correct for response bias, a recognition memory index was calculated (number of hits minus false alarms, with false alarms constituting 'old' judgements to new pictures), again separately for emotional and neutral pictures. The analyses concentrated on responses to stimuli rated with the highest confidence of 9 (for both hits and false alarms). We were not able to differentiate between items that were correctly recognized with high and low confidence since confidence ratings were asymmetrically distributed with most pictures being recognized with highest confidence. Nevertheless, measures of recognition performance were additionally calculated for all pictures, independently of confidence ratings.

To assess ratings of the pictures' valence and arousal, the mean ratings of pictures after the respective retention interval (i.e. early and late sleep) were calculated for hits and correct rejections (i.e. 'new' judgements to new pictures) of emotional and neutral pictures, respectively.

Recordings of event-related potentials and sleep. Electroencephalographic (EEG) activity was continuously recorded with Ag/AgCl electrodes from 19 scalp sites (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2 according to the international 10-20 system) and referenced to linked electrodes attached to the mastoids. Additionally, horizontal and vertical electrooculographic (EOG) and electromyographic activity (EMG) was recorded. All impedances were kept below 5kΩ. Data were amplified by BrainAmp Amplifiers (Brain Products GmbH, Gilching, Germany) and continuously digitized at a rate of 250 Hz. For ERP analyses, all offline data processing was performed with Vision Analyzer 2.0 Software (Brain Products GmbH, Gilching, Germany). Data were filtered between 0.16 - 20 Hz and segmented from 100 ms before picture onset until 100 ms after picture offset. Baseline correction was applied with reference to the 100 ms-interval before picture onset. Artifact rejection included rejection of segments with voltage differences of ≥ 150μV or

voltage steps between adjacent sampling points ≥ 40μV. Ocular correction was performed according to the Gratton & Coles algorithm (Gratton, Coles, & Donchin, 1983).
For each participant ERPs were computed for nine channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4). The mean potential was calculated during two time windows of interest forming the late positive potential (LPP), i.e. an early time window 300-500 ms after stimulus onset and a late time window 500-800 ms after stimulus onset, that have been reported to be most sensitive to memory accuracy and emotional arousal associated with picture recognition (Rugg et al., 1998; Rozenkrants et al., 2008; Weymar, Low, Melzig, & Hamm, 2009; Weymar, Low, Schwabe, & Hamm, 2010). Data were averaged across artifact-free trials separately for the early and late sleep conditions, for learning and retrieval phases, for emotional and neutral pictures and, for the retrieval phase, separately for hits and correct rejections.

Sleep recordings were scored offline according to standardized criteria (Rechtschaffen & Kales, 1968). For each 3-hours sleep interval, total sleep time (TST), and time spent in the different sleep stages (wake; sleep stages 1, 2, 3, 4; SWS, i.e. sum of sleep stage 3 and 4; REM sleep) was calculated in minutes.

Statistical analysis. Data from one subject were discarded from the sleep analyses due to technical problems with polysomnographic recordings during one night. For ERP analyses, the final samples included 11 participants for the learning session and 12 participants for the retrieval session. ERP data had to be excluded due to technical problems (excessive artifacts in recordings, incorrect setting of triggers) in four cases and confusion with button presses in another subject. Data from another subject did not enter ERP analyses of stimuli rated with highest confidence because there were not enough responses (< 9 per category).

Data analysis was generally based on Analyses of Variance (ANOVA) with the repeated measures factors ‘early/late sleep’ and ‘emotionality’ (negative/neutral) for analyses of the learning phase, and the additional factor ‘response type’ (hits/correct rejections) for analyses of the retrieval phase. ERP analyses included additional factors covering the ‘topography’ (Fz, Cz, Pz, etc.) and the post-stimulus ‘time window’ (300-500 ms vs. 500-800 ms) of ERP responses. Analyses of subjective ratings additionally included the factor ‘old/new’. Significant ANOVA effects were followed by post hoc t-tests. The level of significance was set to $p = 0.05$ and Greenhouse-Geisser correction was applied when appropriate.

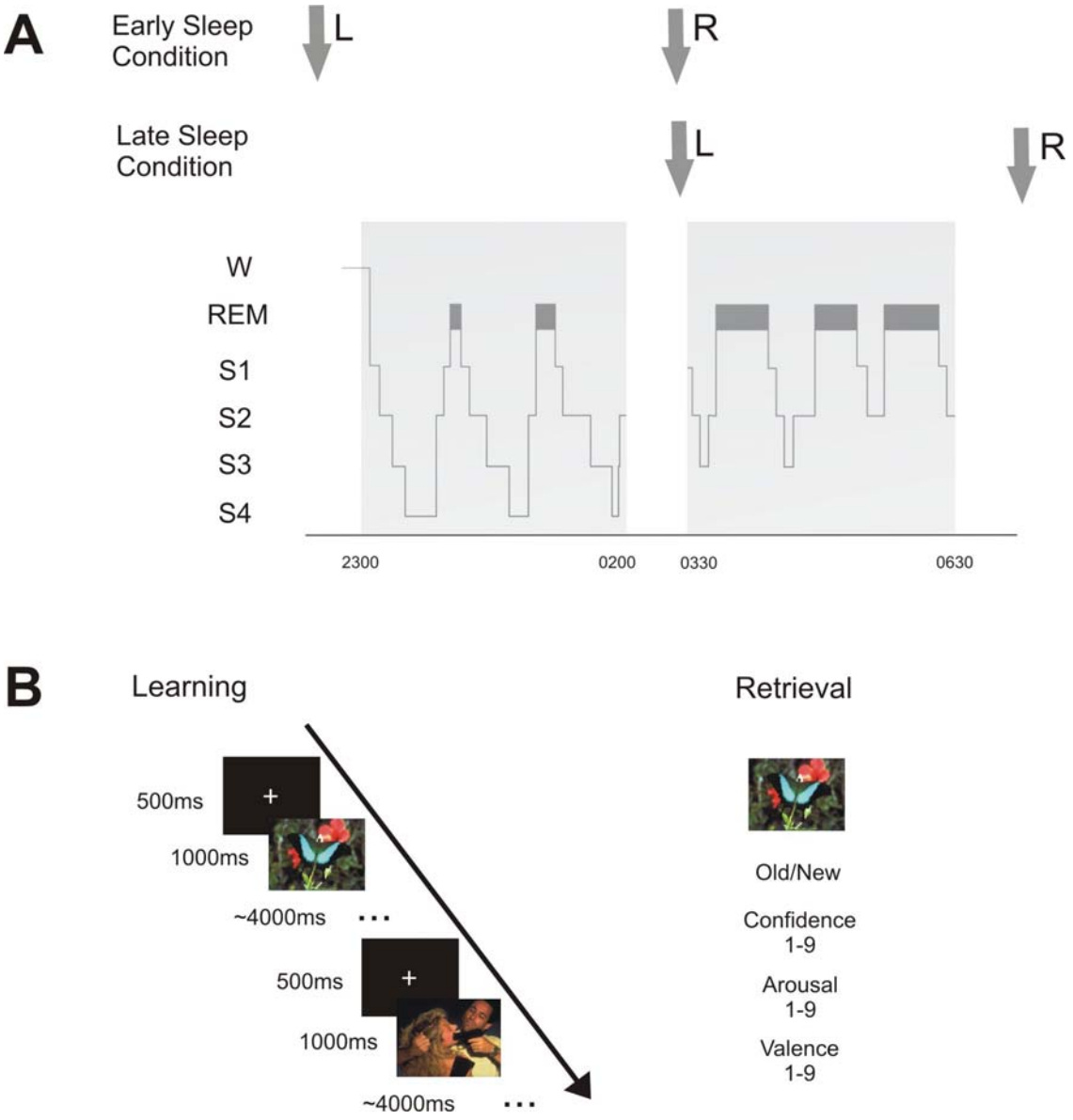

Figure 9. Study design and task. **(A)** Subjects were tested in two conditions (early vs. late sleep) with the order balanced across subjects. In the early sleep condition, subjects learned the pictures 2200-2230h (learning phase - L). A 4-hours retention interval followed, with ~3 hours of sleep containing predominantly SWS. Then, recognition memory for the pictures was tested (0300 h, retrieval phase – R). In the late sleep condition, subjects first slept for 3 hours (lights off at 2300 h) and then learnt the pictures between 0300-0330h, followed by the 4-hours late sleep retention interval with predominant REM sleep. Retrieval took place 0715-0800 h. A typical polysomnogram visualizes the proportion of sleep stages during nocturnal sleep (wake (W), non-rapid eye movement (NREM) sleep stages 1-4 (S1-S4), REM sleep). **(B)** The study task involved emotional and neutral pictures that were presented during learning, preceded by a fixation cross and a variable inter-stimulus interval. At retrieval, recognition memory was assessed by old/new judgement followed by a confidence rating and evaluation of subjectively perceived emotionality regarding valence and arousal of the pictures on 9-point rating scales.

RESULTS

Memory performance and affective ratings

Recognition memory. Rated confidence was highest (9 on a scale of 1-9) in 76% of hits and 16% of false alarms. Consistent with our hypothesis, the recognition index (hits – false alarms) for high confident responses revealed a superior recognition memory for negative over neutral pictures only after the late REM sleep-rich retention interval ($p < 0.01$, for post-hoc t-test comparison of emotional/neutral after late sleep), whereas no such significant difference was detected after early SWS-rich sleep ($p > 0.53$ for respective t-test after early sleep, $F(1,15) = 6.328$, $p = 0.024$, for early/late sleep x emotionality interaction, see Figure 10A). The analysis of hits without correcting for the response bias basically showed the same results ($F(1,15) = 7.748$, $p = 0.014$, for early/late sleep x emotionality; see Table 1 for a summary of recognition data and pairwise statistical comparisons). When responses with low confidence were added, recognition performance did not differ between early and late retention sleep ($p > 0.31$, for 'early/late sleep x emotionality interaction' for analysis of hits and hits – false alarms).

Table 1 Recognition performance and affective ratings

		Early sleep		Late sleep	
		Emotional mean ± SEM	Neutral mean ± SEM	Emotional mean ± SEM	Neutral mean ± SEM
Memory					
High Conf	Hits	32.13 ± 3.34	30.81 ± 3.28	34.94 ± 2.67	29.19 ± 3.40**
	Hits - FA	31.56 ± 3.35	30.38 ± 3.25	34.32 ± 2.58	28.63 ± 3.33**
Total	Hits	42.94 ± 1.80	40.56 ± 2.14	44.25 ± 0.85	41.06 ± 1.53*
	Hits - FA	38.94 ± 2.11	38.50 ± 2.35	39.44 ± 1.49	37.13 ± 2.15
Valence					
	Old (Hits)	3.21 ± 0.18	5.39 ± 0.09	3.20 ± 0.14	5.33 ± 0.08
	New (CR)	3.31 ± 0.14	5.24 ± 0.06	3.26 ± 0.14	5.21 ± 0.05
	Old - New	-0.10 ± 0.07	0.16 ± 0.07	-0.06 ± 0.06	0.12 ± 0.06
Arousal					
	Old (Hits)	4.94 ± 0.49	1.77 ± 0.21	4.84 ± 0.45	1.66 ± 0.23
	New (CR)	4.71 ± 0.45	1.58 ± 0.16	4.62 ± 0.46	1.65 ± 0.22
	Old - New	0.23 ± 0.16	0.19 ± 0.08	0.22 ± 0.11	0.02 ± 0.05

Mean ± SEM recognition accuracy in the retrieval phase after early (SWS-rich) and late (REM-rich) retention sleep is given as absolute numbers of hits as well as hits – false alarms (FA) for responses with highest confidence (High Conf), and also for all responses including low-confident responses (Total). Subjective ratings of valence and arousal are shown as mean (± SEM) ratings for old (hits) and new pictures (CR, correct rejections), as well as for the difference between old and new pictures (old – new; ratings were made on scales ranging from '1' (negative), '5' (neutral) to '9' (positive) for valence, and from '1' (low) to '9' (high) for arousal). For the memory data, significant t-tests between emotional and neutral pictures in the late sleep condition are indicated (there were no significant differences in the early sleep condition). For subjective ratings, t-tests between old (hits) and new (CR, correct rejections) pictures revealed no significant differences for emotional and neutral pictures in the early and late sleep conditions, respectively. * $p < 0.05$; ** $p < 0.01$

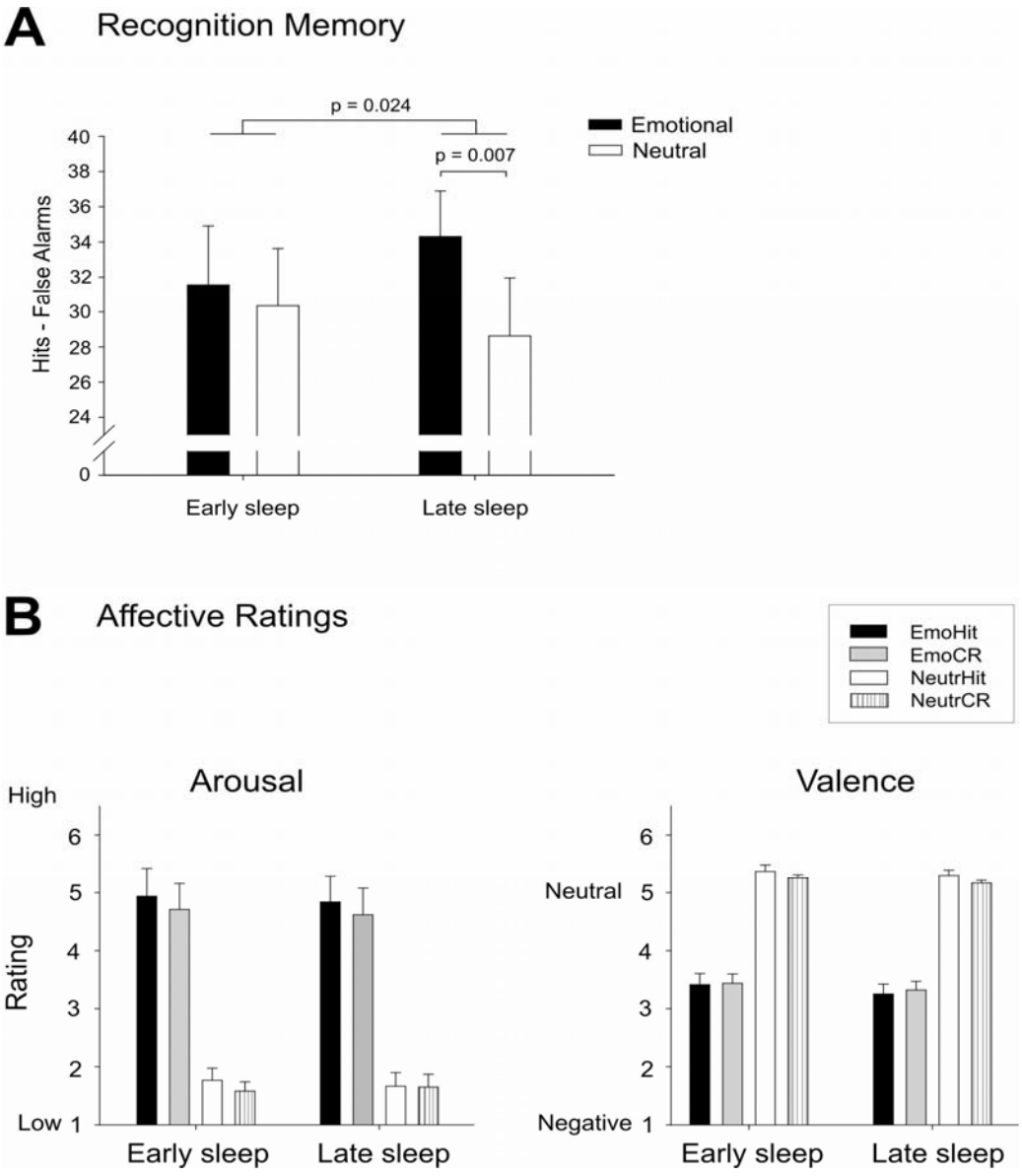

Figure 10. Recognition memory (**A**) and affective ratings of pictures (**B**). At retrieval after late REM-rich sleep, but not after early SWS-rich sleep, negative pictures were distinctly better remembered compared to neutral pictures. In contrast, ratings of arousal and valence

were not differentially affected by early or late sleep. P-values are indicated for significant early/late sleep x emotionality interaction and respective post-hoc comparison between negative and neutral pictures. EmoHit – correctly recognized negative (old) pictures, EmoCR – correctly rejected negative (new) pictures, NeutrHit – correctly recognized neutral (old) pictures, NeutrCR – correctly rejected neutral (new) pictures

Affective ratings. Generally, in the retrieval phase negative emotional pictures were rated as more negative in valence and more arousing than neutral pictures ($p < 0.001$; see Figure 10B and Table 1 for an overview of ratings). However, valence ratings were closely comparable after early and late sleep intervals, for old (hits), new (correct rejections) and the difference of old-new responses (hits – correct rejections; $p > 0.50$ for early/late sleep main effects; $p > 0.60$ for early/late sleep x emotionality interactions). Similarly, the nature of sleep retention intervals did not differentially affect arousal ratings at retrieval testing for none of the respective response types, i.e. old (hits), new (correct rejections) and old-new (hits – correct rejections) ($p > 0.35$ for early/late sleep main effects; $p > 0.25$ for early/late sleep x emotionality interactions).

Event-related potentials (ERPs) and sleep

ERPs. Because analyses of the lateral electrode sites did not add any essential information, for reasons of clarity we present here only results for the midline electrodes Fz, Cz, and Pz. As expected, during learning preceding early and late sleep intervals negative pictures elicited greater ERP positivity than neutral pictures during both time windows of interest, i.e. 300-500 ms and 500-800 ms after stimulus onset ($F(1,10) = 43.69$, $p < 0.001$, for emotionality main effect). Across both time windows, the emotionality effect was distinctly more prominent at central and parietal sites than at Fz ($F(1.23,12.25) = 6.90$, $p = 0.018$, for emotionality x topography). Importantly, ERPs at learning were comparable for the early and late sleep condition for both the 300-500 ms and 500-800 ms time windows (all main and interaction effects: $p > 0.14$).

At retrieval, correctly remembered old pictures (hits) with high confidence, elicited greater positivity compared to correctly rejected new pictures independent of their emotionality ($F(1,10) = 37.49$, $p < 0.001$, for main effect of response type). The effect was clearly more pronounced over anterior than posterior sites ($F(1.61,16.11) = 8.27$, $p < 0.005$, for response type x topography) and concentrated on the early 300-500 ms window ($F(1.71,17.1) = 19.72$, $p < 0.001$, for response type x topography x time window). Like in the learning phase, negative pictures evoked more positive ERP waveforms than neutral pictures

across both time windows ($F(1,10) = 50.08$, $p < 0.001$, for emotionality). However, in contrast to the effect of the response type (i.e. hits vs. correct rejections) the enhancing effect of emotionality on late positivity focused over centro-parietal sites ($F(1.186,11.856) = 9.29$, $p = 0.008$, for emotionality x topography). The emotionality of pictures did not differentially modulate ERPs to hits and correct rejections ($p > 0.40$, for emotionality x response type).

Considering that the effects of response type indicating memory content (independent of emotionality) and of emotionality showed a clear topographical dissociation (with the former effect concentrating over the frontal cortex and the later over centro-parietal cortical areas), we examined the differential effects of early SWS-rich and late REM-rich retention sleep in ANOVAs calculated separately for the frontal, central and parietal electrode site for the early and late time window. ERP positivity did not differ after SWS and REM-rich sleep for the central and parietal electrode sites, neither for emotional vs. neutral nor for correctly recognized vs. correctly rejected stimuli ($p > 0.29$, for relevant early/late sleep interaction effects). However, the nature of retention sleep exerted a clear differential effect on fronto-cortical positivity which focussed on the 300-500 ms post-stimulus interval and critically depended on the emotionality of the pictures. That is, the enhancement in frontal positivity to (correctly recognized) old negative pictures compared to (correctly rejected) new negative pictures was distinctly greater after the late REM-rich than after the early SWS-rich retention interval ($F(1,10) = 7.08$, $p < 0.024$, for early/late sleep x response type x emotionality, see Figure 11 for results of pairwise comparisons and Figure 12 for ERP waveforms). The effect was confirmed by post hoc ANOVAs on the ERPs to negative pictures indicating significantly higher ERP positivity to hits compared to correct rejections after the REM-rich interval compared to recognition testing after the SWS-rich retention interval ($F(1,10) = 5.25$, $p < 0.045$, for early/late sleep x response type interaction). This effect was absent for ERPs to neutral pictures ($p > 0.76$). Post hoc comparisons for frontal ERP positivity 300-500 ms post-stimulus after late REM-rich sleep indicated markedly enhanced positivity for negative emotional compared with neutral hits ($p = 0.001$) as well as for negative hits vs. negative correct rejections ($p < 0.001$; Figure 11). None of these comparisons reached significance in the early sleep condition (all $p > 0.12$). ERP analysis revealed basically the same results when including also responses (hits and correct rejections) made with lower confidence.

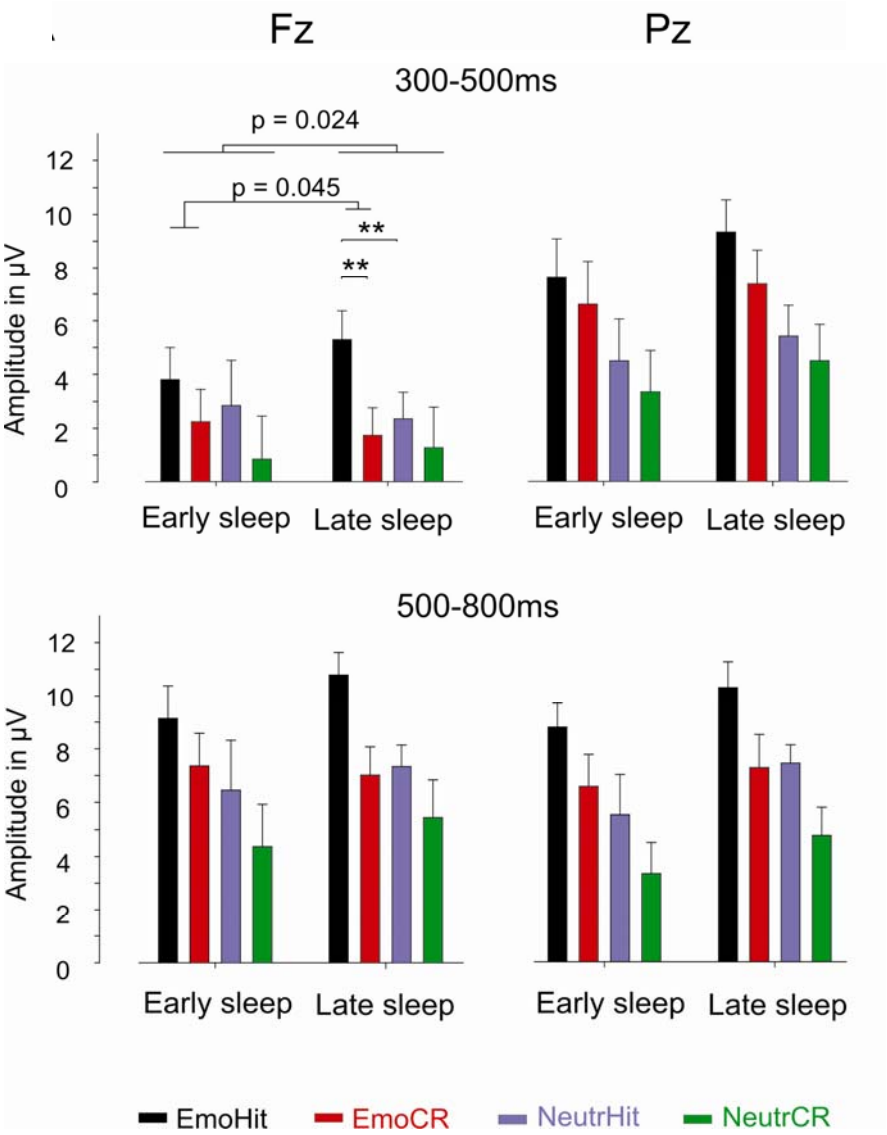

Figure 11. Mean ± SEM ERP positivity over frontal (Fz, left) and parietal (Pz, right) 300-500 ms (upper panel) and 500-800 ms interval after stimulus onset (lower panel) for correctly classified old negative (EmoHits) and neutral pictures (NeutrHits) and correctly rejected new negative (EmoCR) and neutral pictures (NeutrCR) at retrieval testing following either early SWS-rich or late REM-rich retention sleep. P-values are indicated for (i) significant early/late sleep x emotionality interaction and (ii) early/late sleep x response type interaction for emotional pictures. ** $p \leq 0.001$ for respective post-hoc comparison between correctly recognized and correctly rejected negative pictures and correctly recognized negative and neutral pictures in the late sleep condition.

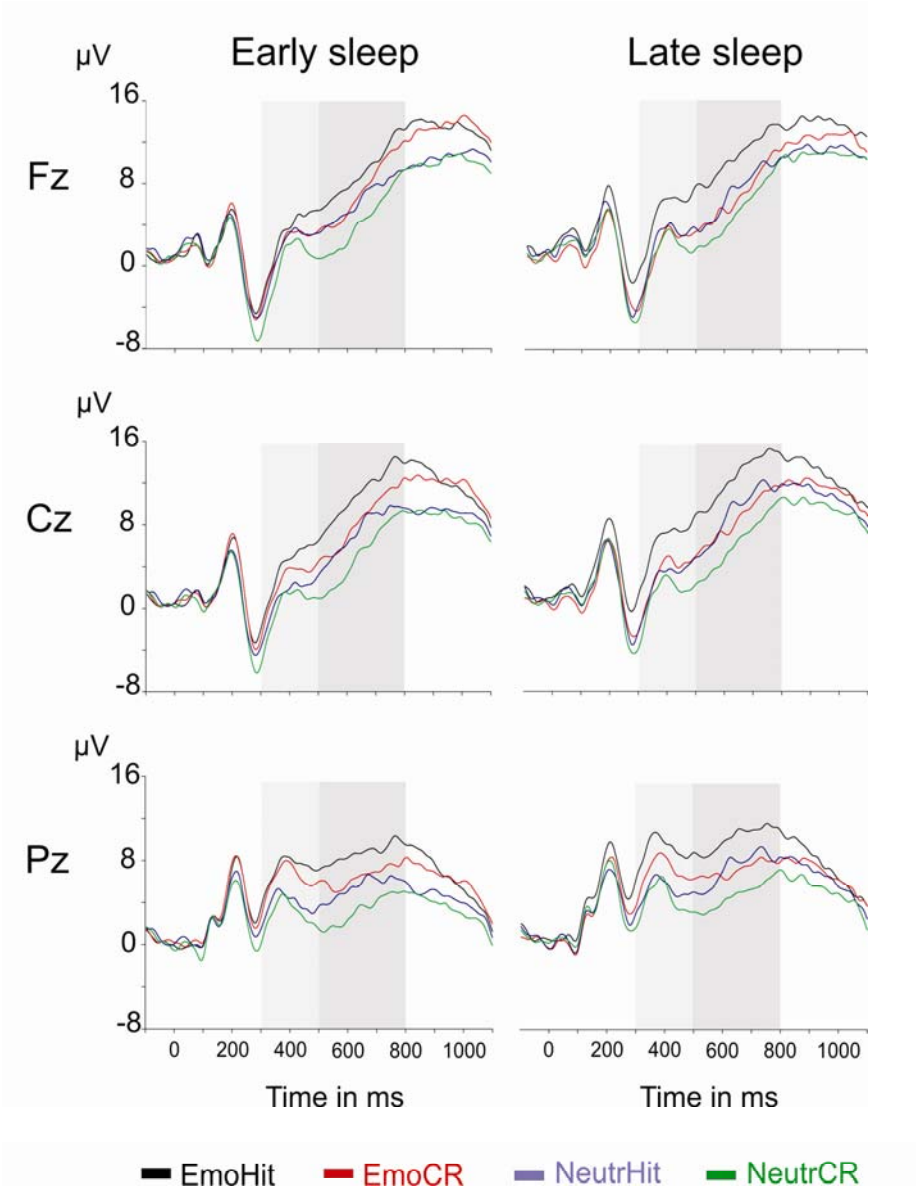

Figure 12. Averaged ERP responses (in μV) in recordings from Fz, Cz and Pz for correctly recognized (old) and correctly rejected (new) negative and neutral pictures during retrieval testing. Shaded areas indicate post-stimulus intervals of interest, i.e. 300-500 ms and 500-800 ms.

Sleep. Sleep data showed the expected prevalence of SWS in the early sleep condition and REM sleep in the late sleep condition ([mean ± SEM] SWS, early sleep: 25.53 ± 3.32%, late sleep: 6.56 ± 1.27; REM, early sleep: 11.15 ± 1.31, late sleep: 26.17 ± 1.29; both $p < 0.01$). None of the other sleep stages differed between conditions (see Table 2, for a summary of sleep results).

Table 2 Sleep parameters

	Time (in min)	
	Early sleep	Late sleep
	mean ± SEM	mean ± SEM
Wake	6.57 ± 2.80	1.47 ± 0.34
Stage 1	19.30 ± 2.80	21.47 ± 2.94
Stage 2	91.53 ± 4.56	103.13 ± 4.64
Stage 3	38.20 ± 6.32	12.20 ± 2.29**
Stage 4	10.20 ± 2.92	0.43 ± 0.43**
SWS	48.40 ± 6.75	12.63 ± 2.44**
REM	20.87 ± 2.43	49.93 ± 2.72**
TST	188.23 ± 1.87	190.60 ± 2.85

Sleep during the early and late sleep retention interval. Sleep parameters are given as mean ± SEM in minutes. SWS = slow wave sleep (sum of sleep stages 3 and 4), REM = rapid eye movement sleep, TST = total sleep time. T-tests were calculated between the early and late sleep condition, ** $p < 0.01$.

Mood, sleepiness and vigilance. Mood measures were well comparable between conditions (PANAS - positive affect: early sleep, 25.19 ± 1.42, late sleep, 26.50 ± 1.98; negative affect: early sleep, 11.81 ± 0.70, late sleep, 11.88 ± 0.58, $p > 0.45$). Also, self-reported sleepiness did not differ between conditions neither at learning (early sleep condition: 3.44 ± 0.38, late sleep 3.56 ± 0.22, $p > 0.70$) nor at retrieval (early sleep 3.31 ± 0.22, late sleep 3.19 ± 0.24, $p > 0.60$). Reaction times on the vigilance task were comparable between the early and late sleep condition at retrieval testing (early sleep: 297.97 ± 8.20 ms vs. late sleep: 294.24 ± 5.34 ms, $p > 0.45$). However, at learning reaction times were slightly faster in the early sleep condition (290.09 ± 5.46 ms) than in the late sleep condition (301.20 ± 5.46 ms, $p < 0.05$).

DISCUSSION

In the present study, behavioral as well as electrophysiological measures consistently revealed an emotional enhancement of picture recognition particularly after a period of late REM-rich retention sleep, but not after early SWS-rich retention sleep. More specifically, the number of negative pictures that were remembered after a 4-hrs retention interval filled with high amounts of REM sleep was significantly enhanced compared to neutral pictures whereas no such emotional facilitation of memory retention was observed when high amounts of SWS had been followed learning. The better recognition of negative than neutral pictures was particularly prominent and robust against response bias influences when only those pictures were selected that received highest confidence ratings, possibly by increasing the signal-to-noise ratio. Whereas REM-rich retention sleep strengthened recognition of emotional compared with neutral pictures, it did not affect the ratings of arousal and valence performed for each picture after the recognition response which were comparable after the two retention sleep conditions. Analyses of ERPs revealed changes in the late positive potential (LPP) consistent with the view that REM-rich sleep strengthens recognition memory for emotional events in the absence of changes in subjective emotionality of the remembered event. Compared with recognition testing after SWS-rich retention sleep, recognition performance after REM-rich sleep was associated with significantly increased positivity over the frontal cortex 300-500 ms post-stimulus onset in response to the 'old' negative pictures with reference to the ERP response to 'new' negative picture. As this fronto-cortical ERP positivity can be considered a correlate of the accuracy of recognition memory for an item (Rugg et al., 1998), which in this case was an emotional picture, we conclude that REM-rich retention sleep enhances the memory for the content of an emotional event. By contrast, the LPP did not differ between the early and late sleep retention conditions over central and parietal posterior sites. In light of findings indicating a close association of posterior ERP positivity, in particular in the 500-800 ms post-stimulus interval, with the emotionally arousing properties of the eliciting event (Dolcos et al., 2002; Pollatos et al., 2005; Rozenkrants et al., 2008), this negative outcome suggests that memory processing during REM sleep does not specifically affect the perceived emotionality associated with a memory representation.

In order to examine the effects of REM sleep on emotional memory processing we used the split-night paradigm (Yaroush, Sullivan, & Ekstrand, 1971; Plihal & Born, 1999; Wagner et al., 2001; Wagner et al., 2002) offering multiple methodological advantages when compared to the method of REM sleep deprivation which was adopted in earlier studies (e.g., for overview see (Vogel, 1975; Vertes & Eastman, 2000). With the method of REM sleep

deprivation, nocturnal sleep is typically disturbed at the first signs of REM sleep thereby reducing the amount of REM sleep but also increasing experienced stress which can in turn influence memory retrieval (Vertes et al., 2000; Siegel, 2001; Born & Gais, 2000). Here, we took advantage of the natural human sleep architecture with prevailing SWS during the first half of the night and high amounts of REM sleep during the second half, when SWS pressure has already been reduced. Since the time of learning and retrieval differed between early and late sleep condition, we cannot completely exclude an impact of circadian factors on learning and retrieval, even though session times differed only about four hours between the two conditions. However, mood and sleepiness ratings before learning and retrieval were well comparable between early and late night conditions. Also, reaction times in the vigilance task did not differ between sleep interval conditions at retrieval, but were somewhat faster before learning in the early night condition. Nevertheless, ERPs during learning did not differ between both conditions indicating a comparable learning baseline.

Our finding that REM-rich sleep preferentially improves recognition of emotional over neutral pictures adds to several recent studies indicating an enhancing effect of this sleep stage on the formation of memory for emotional events. Thus, compared with SWS-rich retention sleep, REM-rich sleep also promoted the preferential consolidation of emotional over neutral stories (Wagner et al., 2001). Other studies revealed a sleep-induced enhancement of memory for emotional pictures which was correlated with time spent in REM sleep and increased signs of theta activity during REM sleep (Nishida et al., 2009; Pare, Collins, & Pelletier, 2002; Popa, Duvarci, Popescu, Léna, & Paré, 2010). The present study is novel in that the amount of REM sleep was manipulated to demonstrate a direct improving influence of this sleep stage on emotional recognition memory.

Although, REM-rich sleep enhanced emotional recognition memory, neither affective ratings of arousal nor of valence changed in parallel, indicating that the two aspects of an emotional memory, i.e. content and affective tone, are represented by dissociable processes. At first glance, this finding is at variance with a foregoing study where REM-rich periods of late retention sleep appeared to aggravate the rated aversiveness of negative pictures seen before retention sleep in comparison with new pictures not seen before (Wagner et al., 2002). However, in that study, subjects also rated the pictures before sleep leaving the possibility that the explicit knowledge of the pre-sleep rating biased the rating of the pictures after sleep. The present study avoided this confound as ratings were assessed only after retention sleep, which thus might probably explain that valence ratings of negative pictures remained unchanged after REM-rich sleep here.

Analyses of ERPs at retrieval revealed a remarkable restriction of the effect of REM-rich retention sleep on fronto-cortical positivity 300-500 ms after stimulus onset. In previous studies, this fronto-cortical positivity has been found to be associated with 'know' rather than 'remember' judgements (Rugg et al., 1998; Rugg & Curran, 2007; Woodruff, Hayama, & Rugg, 2006), suggesting that REM-rich retention sleep in our study primarily enhanced familiarity-based memory rather than recollection-based memory. Although our recognition test did basically not allow discriminating these two aspects, a primary effect on familiarity-based memory is also suggested by the fast learning and retrieval procedures employed in our study, which did not provide any contextual information in addition to the pictures itself, thus most probably inducing familiarity-related retrieval processes (Yonelinas, 2002). Fronto-cortical ERP positivity 300-500 ms post-stimulus being selectively enhanced to correctly recognized negative pictures (in comparison with correct rejections), thus points to a strengthening effect of REM-rich sleep on familiarity-related memory traces for an emotional item. The mechanisms mediating this effect are unclear, but may involve theta activity emerging during this sleep stage coherently in the amygdala and other memory-relevant brain structures, like the medial prefrontal cortex and the hippocampus (Popa et al., 2010).

Despite the selective influence of REM sleep on emotional picture recognition in our study, we do not want to exclude the possibility that under different conditions emotional memory might also be affected by processes occurring during SWS (Groch et al., 2011). Conversely, REM sleep (in addition to SWS) might also be implicated in the consolidation of neutral material. In fact, enhancing effects of sleep on memory for neutral materials often disappear when this material is presented together with emotional materials, suggesting that owing to limited capacities, memory processing during sleep becomes selective (Payne et al., 2008; Wagner et al., 2001; Hu, Stylos-Allan, & Walker, 2006; Wilhelm et al., 2011). Along this line of reasoning, it is possible that the improving effect of REM-rich sleep on familiarity-related recognition memory extends also to neutral stimuli, if these are not presented together with emotional materials.

The memory effect of REM-rich sleep occurred independent from effects on the affective component of the representation, as both affective ratings as well as late centro-parietal ERP positivity, which has commonly been associated with stimulus arousal, remained unchanged. It has been proposed that emotional memory formation during REM sleep goes along with a simultaneous reduction of the emotional affect, as in this sleep stage such memories would be replayed in the absence of noradrenergic activity (Van der Helm et al., 2009). Our findings support this model inasmuch they indicate a disparate influence of REM-

rich sleep on the memory content and its emotional affect, but disagree inasmuch measures of affective value did not indicate any diminution of emotional affect for old negative pictures after REM-rich sleep. It could be that the stronger memory trace for the emotional event at retrieval may have acutely reinstated a stronger emotional response, thus compensating for the presumed reducing effect of REM sleep on emotional affect, although such conceptualization would be difficult to dissect experimentally. Similarly, one could argue that the reduction in affect requires more time to evolve than a 4-hours retention interval as used in our study, especially since the superiority of emotional over neutral memories is also well known to be more pronounced after longer time intervals (Dolcos, LaBar, & Cabeza, 2005; Wagner, Hallschmid, Rasch, & Born, 2006). However, a recent study assessed valence and arousal ratings of emotional pictures one week after encoding and could likewise not detect any differences between old and new pictures (Weymar et al., 2009). Additionally, amygdala activity, which is associated with the arousal dimension of emotions (LaBar & Cabeza, 2006), was even increased for correctly recognized old compared to new emotional pictures after long retention intervals of several months (Dolcos et al., 2005; Payne & Kensinger, 2011). In summary, using behavioral measures of memory, affective ratings, and ERP recordings, we provide evidence that REM-rich sleep, compared with SWS-rich sleep, strengthens emotional over neutral recognition memory but leaves the affective component of emotional memory unspoiled.

Experiment 2 - Contribution of Norepinephrine to emotional memory consolidation during sleep

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INTRODUCTION

Sleep is known to support memory consolidation (Maquet, 2001; Stickgold, 2005). However, the underlying mechanisms are unclear. Declarative memory predominantly benefits from early sleep with high amounts of slow wave sleep (SWS; Peigneux et al., 2001; Diekelmann et al., 2009). During SWS, reactivation of previously learned materials is assumed to strengthen memories by promoting the redistribution of the new representations from hippocampal preferentially to neocortical circuitry for long-term storage (McClelland et al., 1995; Buzsaki, 1998; Diekelmann and Born, 2010). Such processes of sleep-dependent consolidation have been shown to critically depend on characteristic changes in neurotransmitter and hormone release during sleep, e.g., acetylcholine and cortisol (Born et al., 1999; Born and Wagner, 2004; Gais and Born, 2004). Norepinephrine (NE) likewise displays a pronounced sleep-dependent regulation and is well-known to be functionally implicated in memory consolidation, especially of emotional memory (van Stegeren, 2008; McGaugh and Roozendaal, 2008). Compared to wakefulness, NE levels distinctly decrease during SWS and reach minimum concentrations during rapid eye movement (REM) sleep (Aston-Jones and Cohen, 2005; Rasch et al., 2007). The locus coeruleus (LC), which is the main source of cortical NE, projects to multiple brain areas including amygdala, hippocampus and neocortical areas (Young, 1993; Petrovich et al., 2001). Although LC activity rapidly declines after sleep onset, transient firing bursts were detected in LC neurons in rats during SWS (Aston-Jones and Bloom, 1981), which appeared to occur in response to preceding learning-related episodes, possibly in conjunction with reactivation of the learned materials (Eschenko and Sara, 2008). In humans, suppression of NE release by the a2-adrenoceptor agonist clonidine during SWS-rich retention sleep impaired consolidation of odor memories, whereas the noradrenergic reuptake inhibitor reboxetin improved consolidation (Gais et al.,

2011). Like for emotional memories, storage of odors critically involves the amygdala, together with hippocampal function (Gall et al., 1998; Li et al., 2006). Concurrent activation of amygdala and hippocampus via direct and indirect NE pathways appears to be critical for the formation of emotional, episodic and odor memories (Petrovich et al., 2001; Strange and Dolan, 2004).

Yet, the role of NE for consolidating emotional memory during sleep has not been investigated so far. Here, we tested whether suppression of NE impairs memory consolidation during early SWS-rich sleep, particularly of emotional memories. For this purpose, we measured effects of clonidine, infused to generally suppress noradrenergic output from LC, on retention of the contents of and on the temporal order in neutral and emotional stories across 3-h periods of early SWS-rich sleep. Additionally, we assessed retention of emotional and neutral pictures and corresponding subjective ratings as well as heart rate responses.

METHODS

Participants. Fifteen native German speaking healthy men (mean ± SD, age: 22.87 ± 3.42 yrs, range 19-28 yrs, body mass index: 20-25) were recruited at the University of Luebeck. All were non-smokers, free of medication and had no history of neurological, psychiatric or endocrine disorders, and were instructed to follow a normal sleep-wake rhythm for at least four weeks before the experiments, which was ensured by a questionnaire. Prior to the experiments, subjects were accustomed to sleeping under laboratory conditions during an adaptation night, including placement of intravenous catheters. On experimental days they were required to get up at 0700 h and not to consume caffeine or alcohol. The study was approved by the ethics committee of the University of Luebeck and all participants gave written informed consent prior to participation.

Substance administration. Clonidine (112.5 mg Clonidin ratiopharm1, Ratiopharm GmbH, Ulm, Germany) was dissolved in 17 ml saline solution and infused within 10 min at a rate of 100 ml/h. Clonidine is an adrenoceptor agonist that binds with ten times higher affinity to a2- than a1-receptors resulting in a predominant reduction of sympathetic activity, increase of the vagal tone and blockade of the release of norepinephrine. Intravenous administration of clonidine is centrally effective within a few minutes reaching its maximum within 20-30 min. Elimination half-life is 8-15 h. Clonidine has been shown to markedly reduce REM sleep while having no effect on the amount of SWS (Spiegel and Devos, 1980; Gais et al., 2011).

Design and procedure. The study was conducted according to a double-blind, within-subject cross-over design with the order of conditions (placebo vs. clonidine) balanced across subjects and an interval of at least two weeks between the subject's two conditions (Figure 13A). Each condition started with a learning phase (2100-2230 h), followed by a 3-h period of retention sleep during which substances were infused. Memory retrieval was tested the next evening (2030-2130 h) to allow the retest session to start as soon as possible after awakening to keep the influence of partial sleep deprivation low, but simultaneously ensure that most of the substance was washed out. In each condition, participants reported to the laboratory at 1830 h for preparing standard polysomnographical and electrocardiographic (ECG) recordings and for the placement of indwelling venous catheters (one in each forearm) that were connected to long thin tubes to enable substance infusion and blood collection from an adjacent room without the subject's awareness. Additionally, before going to bed a cuff was attached to the upper arm for measuring blood pressure. Before the learning phase (see below), vigilance performance was assessed on a 5-min version of the Psychomotor Vigilance Test (PVT; Roach et al., 2006) and the day's mood was assessed by the Positive and Negative Affect Scale (Watson et al., 1988). Lights were turned off at 2300 h. As soon as polysomnographical recordings showed signs of sleep stage 2 for more than 1 min, placebo (saline solution) or clonidine administration was initiated. Participants were awakened 3 h after sleep onset and stayed awake till retrieval testing in the next evening. Until 0600 h, they were under supervision of the experimenter and instructed for the remaining time until retrieval testing in the evening to stay awake and avoid exhausting physical or mental activities after leaving the laboratory. Wakefulness was ensured by assessing heart rate and physical activity using Actiheart monitors (CamNtech, Cambridge, UK). Before retrieval testing, participants were again presented with the PVT and the PANAS.

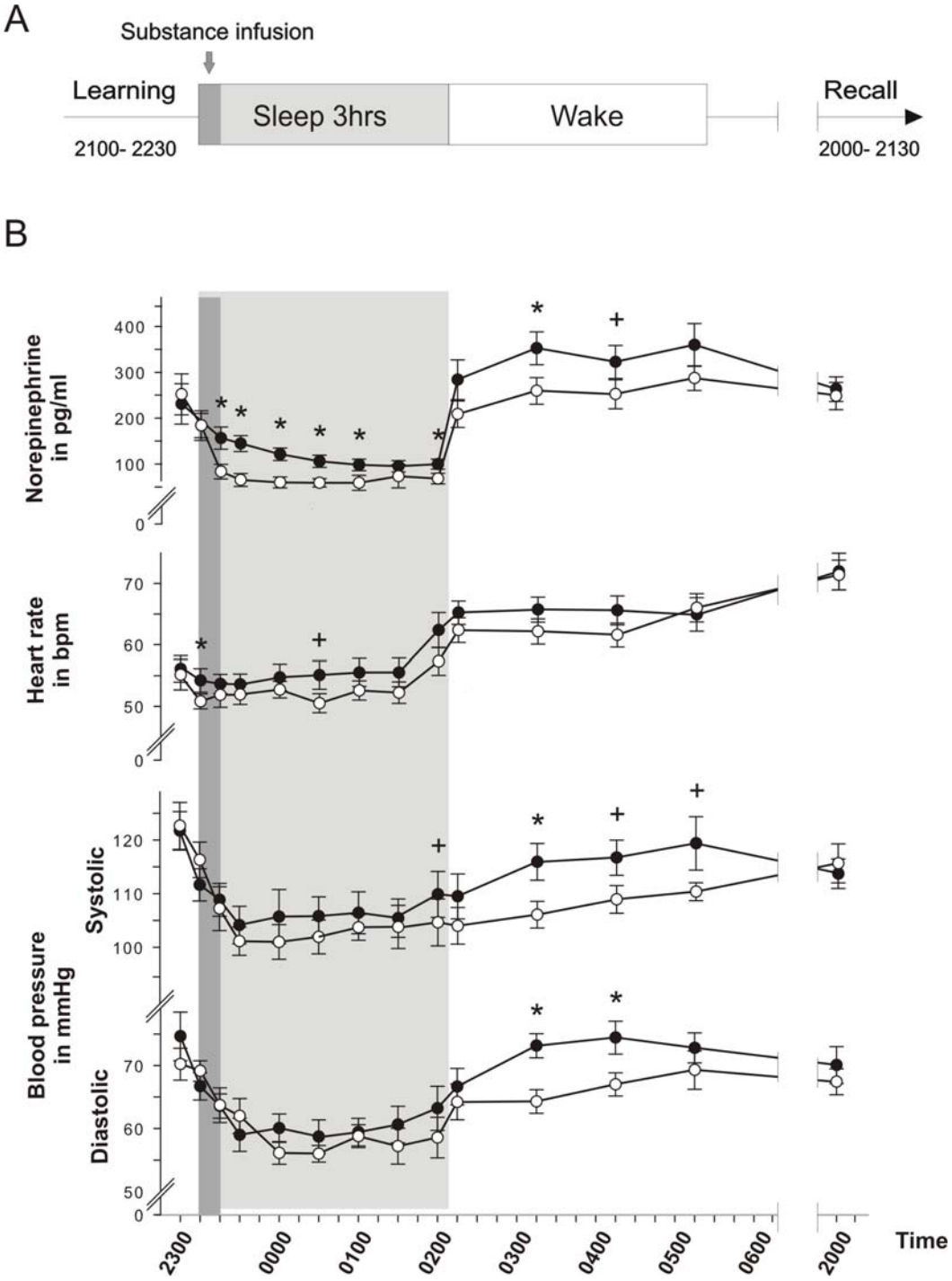

Figure 13. Study design, norepinephrine and cardiovascular measures. **(A)** Subjects were tested in two sessions (clonidine vs. placebo) with the order balanced across subjects. Learning of stories and IAPS pictures started at 2130 h and was followed by a 3 h sleep interval at the beginning of which the substance was infused. After awakening, subjects remained awake until retrieval, which took place the next evening between 2000 h and 2130 h. **(B)** Norepinephrine, heart rate and blood pressure is displayed for placebo (filled circles) and clonidine (open circles) conditions from lights off (ca. 2300 h) until leaving the laboratory (ca. 0600 h). Additionally, all parameters were measured the next evening before retrieval testing. * $p < 0.05$, + $p < 0.10$ for clonidine vs. placebo comparisons at single time point; error bars represent standard error of the mean (SEM).

Memory tasks: stories and pictures. In each treatment condition, participants learned an emotional and a neutral story (described in detail in Schürer-Necker, 1994; Wagner et al., 2001) with the order balanced across subjects. Parallel versions of the stories were used for the subject's two experimental conditions. The topics of the emotional stories were: ''child murder'' and ''paraplegia'', topics of the neutral stories were: ''fashion'' and ''bronze casting''. Subjects were instructed to read each story carefully for 4 min and to memorize as many details as possible. Directly following reading, subjects rated the foregoing story on different dimensions (i.e. comprehensibility, interestingness, emotionality, startle, importance, seriousness, arousal, negativity) on 7-point scales. Afterwards, for an immediate free recall test, subjects had to write down the content of the stories as detailed as possible. During retrieval testing the next day, recall was tested in the same way and, additionally, in a test of memory for the temporal order of the story contents. In this test, participants were presented with 12 words that actually appeared in the stories, each paired with a corresponding synonym. Participants first had to indicate which word of each pair actually occurred in the story (forced-choice recognition) and, then, to put the words chosen in the order as they actually occurred in the stories. There was no time limit for responding on the retrieval tests. To test memory for pictures 120 neutral and 120 negative emotional pictures were selected from the International Affective Picture System (IAPS; Lang et al., 1993). This pool was divided into two parallel sets (for the subjects' two conditions) each consisting of 40 neutral and 40 emotional pictures for the learning phase, supplemented with 20 new neutral and 20 new emotional pictures for retrieval testing. Normative valence and arousal ratings were comparable for both sets: [mean ± SEM] valence ratings: emotional, 2.98 ± 0.88, $p > 0.95$; neutral, 5.08 ± 0.69, $p > 0.85$; arousal ratings: emotional, 5.73 ± 0.69, $p > 0.95$; neutral, 3.18 ± 0.70, $p > 0.95$. Pictures were presented in pseudorandomized order (i.e., a maximum of three subsequent pictures with the same valence), with each participant receiving the same order. Each picture was presented for 6 s, followed by the computer-based version of the Self-Assessment Manikin (SAM) rating system with 9-point scales for valence and arousal (Bradley and Lang, 1994). Participants were instructed to look at each picture for the entire presentation time and to give ratings on the SAM spontaneously and quickly immediately after the presentation was finished. Presentation of the next picture followed with a variable interstimulus interval of 2-10 s (mean 6 s) to avoid anticipatory effects as to the timing of picture presentation, which can influence heart rate responses (e.g., Andreassi et al., 1969). A 5-min break was made after half of the pictures were presented. After presentation of all pictures, participants were asked to write down descriptions of the remembered pictures as

detailed as necessary to allow unambiguous identification (immediate free recall test). At retrieval testing, free recall was tested in the same manner, followed by a recognition test in which the 40 old and 20 new pictures were presented in random order and subjects had to decide whether they had seen a picture during learning ('old') or not ('new') by pressing a corresponding key. Again, after each picture presentation, subjects rated the picture on the SAM. During learning and recognition of the pictures, the heart rate response was recorded (see below).

Cardiovascular measures, blood sampling and hormonal analysis. Heart rate responses to pictures were recorded via two chest electrodes using a S75-04 Bioamplifier (Coulbourn Instruments, Whitehall, PA, USA). R-waves were manually detected and heart rate in beats/min was calculated. Additional measurements of heart rate, blood pressure as well as blood samples were taken repeatedly during sleep and the following wake period (refer to Figure 13B for exact time points). NE was assessed in EDTA-plasma by standard high-performance liquid chromatography with electrochemical detection (Recipe Chemicals + Instruments, Munich, Germany) with intra-assay and inter-assay coefficients of variation (CV) < 6.7% and < 5.3%, respectively. Cortisol concentrations were determined in serum via Immulite (DPC Biermann, Bad Nauheim, Germany, intra- and inter-assay CV < 10%).

Data reduction and statistical analysis. Retention of pictures and stories was assessed by the percentage of correctly recalled pictures/content words at retrieval relative to recall performance at the immediate recall during learning. A recognition memory index was calculated for recognition of pictures (Pr = hits – false alarms). For recognition of stories the sum of correctly recognized words was determined. Recall of temporal order was determined by a deviation score, i.e., the distance of a remembered sequence position of a content word from its actual position in the story. Thus, lower scores indicate better temporal order memory. To assess ratings of the pictures' valence and arousal, the difference between mean ratings of the same pictures presented twice, before and after the retention interval as well as of old vs. new pictures at retrieval testing were calculated, respectively. Additionally, the difference in ratings of the old pictures before and after the retention interval was determined. This report concentrates on the 'old vs. new' difference in ratings at retrieval testing which we considered the more valid measure of clonidine effects on the consolidation of emotional memories. Unlike the 'old vs. new' difference which is based on ratings made in the very same context conditions, the 'pre/post' difference in ratings of the old pictures is confounded

by several non-specific factors (e.g., fatigue due to sleep restriction in the preceding night) that clearly differed for the learning and retrieval phase and could interact with the effects of clonidine. In fact, the 'pre/post' comparisons proved less sensitive to the effects of clonidine: Although they basically confirmed our results on the difference between old and new pictures (see Table 3), they were distinctly more variable and not significant. For heart rate responses to pictures the heart rate change in beats/min was calculated by subtracting the lowest heart rate during the 6 s after picture onset from the 1-s baseline value before picture onset (see Bradley et al., 2001). Like for the ratings, 'old/new' as well as 'pre/post' retention interval comparisons were analyzed. The final sample included 15 men, after data from six subjects were excluded from analysis because at retrieval testing NE levels after clonidine had not yet recovered baseline levels (i.e., levels more than 95 pg/ml below corresponding levels in the placebo condition). Data from four further subjects were excluded because of very low amounts of SWS (less then 10 min in 3 h) in at least one condition, which happened in the placebo condition in two subjects, in the clonidine condition in one subject and in both conditions in one subject. The total sample for the final sleep analyses included 14 subjects because data of one further subject had to be discarded from analysis due to technical problems with polysomnographic recording during one night. Sleep was scored according to standardized criteria and the time spent in the different sleep stages was calculated in minutes and as percentage of total sleep time (TST). Data analysis was generally based on Analyses of Variances (ANOVA) with the repeated measures factors 'clonidine/placebo' and 'emotional/neutral', followed by post hoc t-tests. Analyses of subjective ratings and heart rate responses additionally included the factors 'old/new' and 'pre/post', and analyses of cardiovascular and hormone parameters included a 'time' factor. Greenhouse-Geisser correction was applied when appropriate.

RESULTS

Stories. Ratings confirmed that emotional stories were indeed perceived as more comprehensible, interesting, emotional, startling, important, serious, arousing and negative ($p < 0.01$). Free recall of the stories in terms of remembered content words was generally better for emotional than neutral stories, both at learning and retrieval testing ($p < 0.001$, for 'emotional/neutral'). Additionally, free recall of content words immediately after learning was well comparable between conditions ([mean ± SEM] emotional placebo, 41.40 ± 3.29, clonidine, 43.70 ± 3.35; neutral placebo, 26.53 ± 3.29, clonidine, 23.57 ± 2.25; $p > 0.90$, for

'clonidine/placebo', $p > 0.25$, for 'clonidine/placebo' x 'emotional/neutral'). Retention, i.e., the percentage of emotional or neutral content words recalled at retrieval testing was not affected by clonidine ($p > 0.70$ for respective 'clonidine/placebo' main effects and 'clonidine/placebo' x 'emotional/neutral' interaction, Figure 14A). Also, forced-choice recognition of content words was similar after clonidine (number of recognized words out of 12, neutral: 8.00 ± 0.52, emotional: 8.13 ± 0.51) and placebo (8.07 ± 0.36 and 8.67 ± 0.59, respectively, $p > 0.11$ for 'respective clonidine/placebo' effects). However, clonidine had a clear differential effect on memory for the temporal order in the stories depending on their emotionality ($p < 0.05$, for 'clonidine/placebo' x 'emotional/neutral', Figure 14B). Whereas under placebo, retention of the order of content words in the emotional story was distinctly better than in the neutral story (neutral: 38.67 ± 3.76 vs. emotional: 27.60 ± 3.19, $p < 0.01$), this emotional enhancement was completely abolished when clonidine was administered during the consolidation period (neutral: 33.33 ± 3.27 vs. emotional: 36.07 ± 4.17, $p > 0.50$). However, the post hoc comparison for the temporal order of emotional stories between placebo and clonidine condition failed to reach significance ($p = 0.122$).

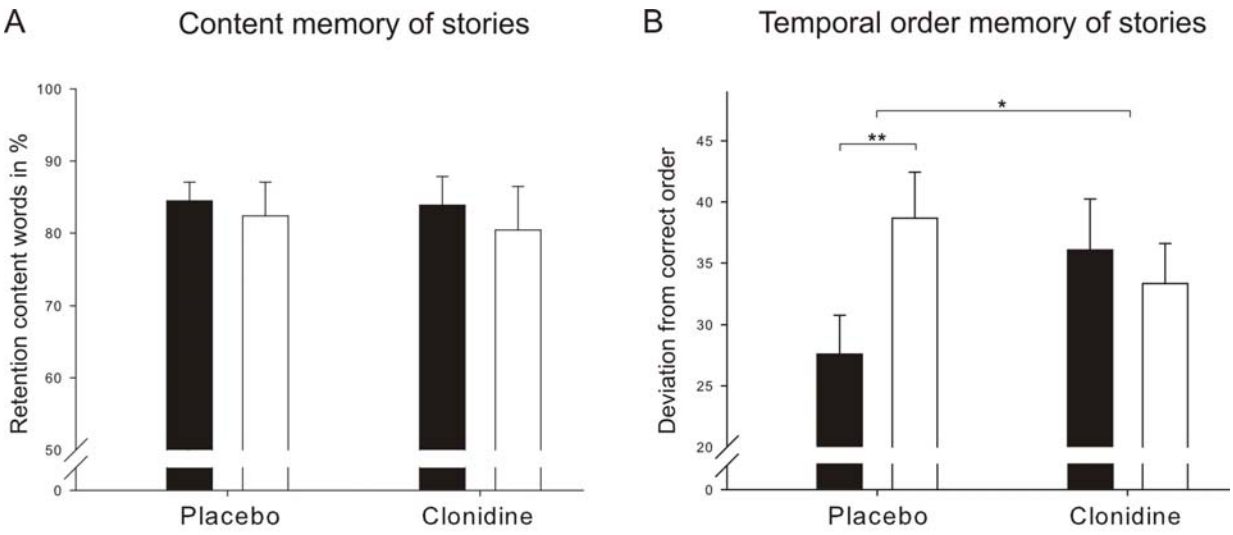

Figure 14. Memory for content words and temporal order of stories. (**A**) Clonidine administration did not influence retention of content words of the stories. (**B**) Retention of the temporal order of events was superior in emotional stories compared to the temporal order of neutral stories after placebo, but not after clonidine. Bar color: white = neutral, black = emotional, * $p < 0.05$, for 'clonidine/placebo' x 'emotional/neutral' interaction, ** $p < 0.01$, for post-hoc comparison; error bars represent standard error of the mean (SEM)

Pictures. Free recall and recognition. Overall, free recall of IAPS pictures was better for emotional ([mean ± SEM] placebo: 24.86 ± 2.25, clonidine: 20.29 ± 1.84) than neutral (placebo: 19.50 ± 2.29, clonidine: 17.71 ± 1.66) pictures at learning ($p < 0.01$, for 'emotional/neutral') as well as at retrieval testing ($p < 0.01$, for 'emotional/neutral'). Free recall at learning did not significantly differ between the placebo and clonidine conditions ($p > 0.085$, for 'clonidine/placebo'). To control for individual difference in baseline performance, retention performance in free recall was measured as performance change at retrieval relative to individual performance at learning. At retrieval testing, both free recall as recognition of pictures were closely comparable for the clonidine and placebo condition ($p > 0.11$, for all 'clonidine/placebo' main and interaction effects, see Table 3 for means ± SEMs). Considering that on the recognition task the imbalance between the number of old and new pictures can produce a response bias in favour of a high false alarm rate, we additionally calculated the signal detection measure C. As expected from previous work (e.g., Weymar et al., 2009), we found a more liberal response criterion for emotional compared to neutral pictures ([mean ± SEM] emotional placebo: -0.38 ± 0.13; emotional clonidine: -0.53 ± 0.08; neutral placebo: -0.14 ± 0.11; neutral clonidine: -0.25 ± 0.12, $p < 0.01$, for 'emotional/neutral'). However importantly, the response bias did not differ between placebo and clonidine conditions ($p > 0.13$, for 'clonidine/placebo'; $p > 0.80$, for 'clonidine/placebo x emotional/neutral').

Subjective ratings and heart rate responses. Emotional pictures were generally rated as more negative and arousing than neutral pictures ($p < 0.001$; see Table 3, for overview of ratings). Moreover, at retrieval old pictures were less negative and less arousing than new pictures ($p < 0.01$ for 'old/new' main effects). Clonidine significantly modified ratings of old pictures. In the placebo condition, the decrease in negative valence of old pictures was present only for emotional pictures. After clonidine, both neutral and emotional old pictures showed decreases in valence which, although modest and per se not significant, were of comparable size ($p = 0.026$, for 'clonidine/placebo' x 'emotional/neutral' x 'old/new' interaction). Accordingly, separate ANOVAs for both treatment groups indicated significance in the placebo condition for the 'old/new' x 'emotional/neutral' interaction ($p < 0.05$) and for post hoc comparison of old and new emotional pictures ($p = 0.008$). No such effect was found after clonidine ($p > 0.59$). Additionally, the diminished arousal ratings for old compared with new pictures was consistently observed only in the placebo condition ($p = 0.014$ and $p = 0.069$, respectively) but not after clonidine (both $p > 0.29$; $p = 0.075$ for 'old/new' x

'clonidine/placebo'). Heart rate decelerations were on average stronger to emotional than neutral pictures, and, only for emotional pictures, showed a distinct decline across the retention interval ($p < 0.01$, for main effect of 'emotional/neutral'), especially if compared to the responses to new emotional pictures ($p < 0.001$ for 'emotional/neutral' x 'old/new' interaction, Table 3). However, heart rate responses were overall very variable and not consistently affected by clonidine ($p > 0.26$ for all effects).

Table 3 Pictures: Memory, subjective ratings and heart rate response

	Emotional		Neutral	
	Placebo	Clonidine	Placebo	Clonidine
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Memory				
Free Recall	88.93 ± 4.43	92.05 ± 4.44	91.43 ± 4.09	89.90 ± 5.73
Recognition	37.33 ± 0.60	37.27 ± 0.54	38.20 ± 0.38	36.80 ± 0.75
Valence				
Old	3.80 ± 0.18	3.91 ± 0.18	5.32 ± 0.07	5.45 ± 0.09
New	3.52 ± 0.19	3.77 ± 0.20*	5.26 ± 0.06	5.29 ± 0.07
Old - New	0.28 ± 0.09	0.13 ± 0.06	0.06 ± 0.08	0.16 ± 0.04
Arousal				
Old	4.83 ± 0.57	4.88 ± 0.50	3.07 ± 0.40	3.11 ± 0.40
New	5.13 ± 0.55	4.91 ± 0.53	3.26 ± 0.42	3.20 ± 0.39
Old - New	-0.29 ± 0.10	-0.04 ± 0.11*	-0.19 ± 0.10	-0.09 ± 0.08
Heart rate				
Old	-2.58 ± 0.36	-2.56 ± 0.28	-2.24 ± 0.45	-2.97 ± 0.68
New	-5.41 ± 0.69	-4.96 ± 0.61	-2.76 ± 0.39	-2.26 ± 0.48

Free recall is given in mean percentage (± SEM) of delayed retrieved pictures related to immediate recall; Recognition is presented as recognition index (P_r=hits-false alarms); values for valence and arousal rating were rated between '1' and '9'; heart rate response is given in change in beats/min, * $p<0.05$

Sleep, hormones, cardiovascular activity, vigilance and mood. Sleep data showed the expected prevalence of SWS in both the clonidine and placebo conditions (Table 4). REM sleep occurred only in minor amounts, and these were further diminished after clonidine ($p < 0.01$). None of the other sleep stages was affected by clonidine administration. Further, we correlated time spent in the different sleep stages with retention measures of pictures, stories, and subjective ratings, which revealed no significant correlations. Plasma NE levels as well as heart rate and blood pressure decreased during retention sleep ($p < 0.05$), with this decrease being more pronounced during infusion of clonidine than placebo ($p < 0.05$, for respective 'clonidine/placebo' x 'time' interaction, Figure 14B). Importantly, none of these measures differed between the treatment conditions at learning, and all recovered levels were comparable between placebo and clonidine conditions before actual retrieval testing ($p > 0.30$, for all comparisons). Clonidine did not significantly affect cortisol concentrations ($p > 0.30$). Reaction times on the vigilance task were well comparable between the placebo and clonidine conditions before learning (mean ± SEM placebo: 280.83 ± 8.47, clonidine: 271.21 ± 6.94, $p > 0.13$, for 'clonidine/placebo') and before retrieval testing (placebo: 287.75 ± 11.23 ms vs. clonidine: 287.77 ± 11.58 ms, $p > 0.90$). Also, mood measures were comparable between conditions at learning (positive affect: placebo, 22.60 ± 1.48, clonidine, 23.60 ± 1.92; negative affect: placebo, 11.60 ± 0.53, clonidine, 12.33 ± 0.81, both $p > 0.40$) and retrieval (positive affect: placebo, 22.53 ± 1.36, clonidine, 23.87 ± 1.73; negative affect: placebo, 12.13 ± 0.78, clonidine, 11.40 ± 0.39, both $p > 0.30$).

Table 4 Sleep parameters

	Absolute time (in min)		Percentage of time	
	Placebo	Clonidine	Placebo	Clonidine
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Wake	4.18 ± 0.67	2.71 ± 0.68	2.22 ± 0.35	1.48 ± 0.38
Stage 1	24.64 ± 4.03	18.36 ± 2.48	13.29 ± 2.16	9.21 ± 1.47
Stage 2	100.75 ± 4.63	110.86 ± 4.21	54.49 ± 2.43	55.81 ± 4.53
Stage 3	32.04 ± 3.69	38.00 ± 3.61	17.41 ± 2.05	9.27 ± 2.14
Stage 4	12.25 ± 4.94	12.68 ± 4.78	6.61 ± 2.65	6.99 ± 2.62
SWS	44.29 ± 6.09	50.68 ± 5.27	24.02 ± 3.34	26.26 ± 3.29
REM	10.43 ± 2.55	1.14 ± 1.07**	5.64 ± 1.38	0.56 ± 0.53**
TST	184.89 ± 1.62	184.11 ± 1.83		
Sleep latency	15.12 ± 2.32	22.43 ± 3.36		
SWS latency	29.96 ± 4.08	29.39 ± 5.84		

Sleep parameters are given in mean (± SEM) of absolute time in minutes and percentage of total sleep time; SWS = slow wave sleep (sum of sleep stages 3 and 4), REM = rapid eye movement sleep, TST = total sleep time; t-tests were calculated between the placebo and clonidine condition, for absolute and percentage of time, respectively, **: $p<0.01$

DISCUSSION

We found that post-learning administration of clonidine during a 3-h period of early SWS-rich sleep had no effect on the retention of emotional or neutral pictures and content words of emotional or neutral stories. However, clonidine impaired memory for the temporal order specifically in emotional stories. In our study we aimed to manipulate NE activity via clonidine administration that mainly activates the a2-autoreceptor in postsynaptic LC neurons and thereby globally suppresses LC noradrenergic output to the forebrain. With a much lower affinity clonidine also binds to a1-receptors. Thus, it cannot be excluded that the findings on memory reported here reflect in part a1-receptor activation. However, blood measures confirmed a marked reduction of NE during the sleep interval. Therefore, we believe that NE suppression is the factor most likely explaining our findings.

Our study was based on the idea that the consolidation of amygdala-dependent emotional memory might in some aspects critically depend on SWS. The idea arose from

rodent studies that identified increased LC burst activity during SWS after amygdala-dependent odor learning (Eschenko and Sara, 2008; note, since in rodents unlike in humans, normally SWS and NREM sleep 2 are not discriminated, LC burst activity, as well as respective effects of clonidine observed here, might likewise be associated with stage 2 sleep). Apart from the occurrence of LC burst activity in rat SWS, it was further demonstrated in humans that increasing NE activity during early SWS-rich retention sleep enhanced retention of odor memory whereas decreasing NE activity, conversely, impaired retention of odor memories (Gais et al., 2011). Given that formation of emotional memory, like odor memory, essentially involves amygdalar function (Buchanan et al., 2003; Phelps, 2004; LaBar and Cabeza, 2006) the present study hypothesized a general contribution of SWS-associated NE activity to amygdala-dependent memory consolidation. This view is only in part confirmed by our finding that the emotion-induced superiority of memory for temporal order was impaired following blockade of NE release by clonidine during an early sleep interval. Whereas under placebo conditions temporal order for emotional stories was enhanced, clonidine blocked the enhancement which resulted in a comparable retention of neutral and emotional temporal order memory in the clonidine condition. Of note, NE suppression diminished the contrast in temporal order memory between emotional and neutral stories, making them more similar, whereas the decrease in temporal order memory for the emotional texts per se was not significant, a pattern that well fits with the view that LC-related NE activity generally acts to enhance signal-to-noise ratio between representations (Foote et al., 1975; Sara, 1985). Also consistent with our hypothesis of a specific impact on emotional aspects of memory consolidation, blocking NE activity during SWS prevented the specific dynamics in perceived valence and arousal of the emotional pictures observed under placebo conditions. In the placebo condition, old emotional pictures, in comparison with new pictures, were rated as less negative and arousing. Blocking NE activity during SWS removed this inhibition of negative emotionality. In a recent review by Walker and Van der Helm (2009), REM sleep, rather than SWS, was proposed to be involved in reducing the affective tone of emotional memories. However, in our study the reduction of the affective tone, reflected by the difference in old vs. new arousal ratings, was not correlated with the amounts of REM sleep, which overall were probably too small to reveal such effect. However, our results show also that the emotional tone alone of the learnt material is not sufficient to make memory consolidation during sleep sensitive to the effect of NE activity. Only memory for the temporal order of the emotional story was impaired after clonidine, whereas memory for content words of the emotional stories and for emotional pictures was not. As the test for

memory of the temporal order was only applied at retrieval testing, but not at learning, the related findings after clonidine might be regarded as less robust. However, given that measures of vigilance and mood, and also recall of item memory, were well comparable during learning and retrieval, it seems justified to ascribe the effect to the substance administered which was the only difference between the two conditions. The most important difference between retention of content words and of their temporal order is that temporal order memory requires building chronological associations of the information after encoding. There is indeed evidence that different aspects of declarative memory are represented in different regions of the medial temporal lobe system. Whereas item memory, as reflected by content words of a story and the main contents of pictures, predominantly involves the perirhinal cortex, the relational aspects of an episodic memory binding an experienced event into a spatial and temporal order are considered key functions of the hippocampus proper and the parahippocampal cortex (Davachi et al., 2003; Dragoi and Buzsaki, 2006; Ekstrom and Bookheimer, 2007; Lehn et al., 2009). In addition, the NE pathways linking the basolateral amygdala with the hippocampus proper appear to be those critically involved in the formation of emotional, episodic and olfactory memory (Pikkarainen et al., 1999; Pitkanen et al., 2000; Petrovich et al., 2001). On this background, it can be speculated that NE activity during SWS facilitates consolidation only of those memories that involve both, an amygdalar component and a strong hippocampal component. This view is also consistent with the finding that sleep especially promotes the temporal order in a hippocampus-dependent memory (Drosopoulos et al., 2007).

Although the 3-h early sleep design with retention testing 23 h after learning has several limitations, e.g., partial sleep deprivation at retesting, this design has clear advantages for the purpose of our study. Human nocturnal sleep typically contains high portions of SWS and only very small amounts of REM sleep during the beginning of the night. Hypothesizing that noradrenergic LC firing during SWS plays a critical role in emotional memory consolidation, we aimed at suppressing NE specifically during early sleep with large amounts of SWS. At the same time we intended to keep the influences of REM sleep on emotional memory at a minimum. Also because clonidine is known to have REM suppressive effects, administration of clonidine over the entire nocturnal sleep period including the late REM-rich part of sleep, would have distinctly increased the difference in REM sleep portions between the treatment conditions, making it increasingly difficult to disentangle effects of NE suppression from those of REM sleep per se. Nevertheless, considering previous work that demonstrated a particular importance of REM sleep for the consolidation of emotional aspects

in a memory (Wagner et al., 2001, 2002; Payne et al., 2008; Nishida et al., 2009) it cannot be excluded that clonidine affected memory consolidation by modulating REM sleep. As we concentrated on retention periods with high amounts of SWS and the portion of REM sleep was generally very small both in the placebo and clonidine condition, any valid assessment of REM sleep contributions was prevented. Due to the relatively long half-life of clonidine the retention interval was extended until most of the substance was washed out to reduce the impact of clonidine on memory performance at retrieval testing. Additionally, to safely exclude any effects of differential NE levels at retrieval testing, subjects with large NE differences between the clonidine and placebo condition at retrieval were excluded from final analyses, making it highly unlikely that NE levels at retrieval affected our results.

Another limitation of our design concerns the lack of a wake control group preventing clear-cut conclusions as to whether the observed effects of clonidine are specific to sleep-dependent consolidation. However, this study was strongly based on a foregoing study (Gais et al., 2011) showing that the consolidation of odor memories is indeed sleep-dependent and can be disrupted by clonidine induced suppression of NE activity. Here, in a closely comparable design we aimed at extending those data on another amygdala-dependent task to further the evidence for contributions of SWS to emotional memory consolidation. Interestingly, as we did not reveal any diminishing effects of NE suppression during SWS-rich early sleep on the consolidation of emotional contents, our findings diverge from those in awake subjects suggesting that NE activity via β-adrenoceptor activation enhances consolidation of emotional contents (Liang et al., 1990; Cahill et al., 1994; van Stegeren et al., 1998; Ferry et al., 1999). Thus, it remains to future studies to target specifically the different roles NE activity appears to play for consolidating emotional contents during wakefulness and sleep. It could be also argued that after sleeping only 3 h, subjects were sleep deprived at retrieval, which is known to increase amygdala reactivity in response to negative pictures (Yoo et al., 2007). However, if such bias occurred it should have affected retrieval of emotional memory in both, placebo and clonidine conditions to a similar extent. Thus, despite some limitations in our experimental design, it seems justified to conclude from our data an essential contribution of SWS-related NE activity to the consolidation of amygdalar-dependent emotional aspects of memories that involve strong interactions with hippocampal function.

Experiment 3 – Dissecting the contributions of slow-wave sleep and rapid-eye movement sleep to emotional item and source memory

In preparation as: Groch, S.,Wilhelm I., Born J. (2011). Dissecting the contributions of SWS and REM sleep to emotional item and source memory.

INTRODUCTION

A large body of evidence indicates that emotional memories are remembered better than neutral memories (McGaugh, 2000). The emotion-induced improvement of long-term memory has been linked to hormonal actions (e.g. norepinephrine) that can facilitate long-term potentiation (LTP, McGaugh 2004). These enhancing effects of emotional arousal, elicited by an emotional stimulus, can increase memory of the object itself (i.e. familiarity-based item memory) as well as of the context the object is presented in, i.e. source memory as the location of an object (Mather & Nesmith, 2008) or the spatial context in contextual fear conditioning designs (McIntyre, Power, Roozendaal, & McGaugh, 2003). However, in contrast to these data, demonstrating the enhancing effects of emotional arousal, there is also notable evidence for an arousal-induced impairment of contextual memory formation (Mather, 2007; Nashiro & Mather, 2011). In accordance with this inhibitory effect of emotional arousal, a central, emotional element in the foreground of a scene is preferentially remembered at the expense of peripheral background information (an effect commonly known as weapon focus) suggesting an emotion-induced impairment of contextual binding (Loftus 1987; Steblay 1992).

Recent evidence indicates that sleep is critically involved in this dissociative processing of emotional items and associated contextual information. Post-learning sleep compared with a respective wake period improved retention of the central emotional element of a picture, but impaired retention of its simultaneously presented neutral background (Payne, Stickgold, Swanberg, & Kensinger, 2008). The authors argued that sleep appeared to specifically benefit the emotionally arousing core of the scene at the expense of peripheral

background information. This finding is in line with numerous studies demonstrating that sleep does not uniformly benefit all encoded memories, but selectively strengthens those that are relevant for the future with this process of memory selection being associated with SWS (Wilhelm et al., 2011; Diekelmann & Born, 2010; Born and Wilhelm, 2012). Moreover, slow-wave sleep (SWS) was shown to preferentially benefit the consolidation of neutral contextual memories (Drosopoulos 2005), but whether SWS also underlies the consolidation of emotional context information has not been investigated so far.

On the contrary, in several studies REM sleep has been shown to play a predominant role in the consolidation of emotional (mostly recognition-based item) memories. More specifically, oscillatory activity (i.e. theta activity 4-8 Hz) occurring during REM-sleep has been identified to correlate with emotional memory gains after sleep (Nishida 2009; Popa 2010). Furthermore, findings from studies using the approach of the split-night design demonstrated a superior retention of emotional compared to neutral material, specifically after a 3-hrs post-learning interval filled with REM-rich sleep, but not after SWS-rich sleep or wakefulness (Wagner 2001; Groch, S., Wilhelm, I., Diekelmann, S., Born, J., unpublished). The effects of REM sleep on emotional memories were attributed to increased amygdala and parahippocampal activation (Maquet et al., 1996; Nofzinger, Mintun, Wiseman, Kupfer, & Moore, 1997; Miyauchi, Misaki, Kan, Fukunaga, & Koike, 2009) and to the specific endocrine conditions during this sleep stage, i.e. increased cholinergic and absence of aminergic activity (Van der Helm, 2009).

In the present study, we aimed to elucidate the role of REM sleep and SWS in memory consolidation of emotional and neutral pictures and their associated context information, i.e., the location of the picture on a computer screen preceded by a colored frame. Thereby the contextual information contained two different qualities, i.e. the picture location (inherent to the object and encoded simultaneously) vs. a colored frame that had to be associated and was encoded prior to the object. Memory formation for both aspects of this context information is assumed to depend on hippocampal activation since one of the main functions of the hippocampus is the binding of an item into a context (Staresina et al., 2009). Here, we hypothesized that SWS is involved in the consolidation of hippocampus-dependent contextual source information of emotionally charged items whereas REM-sleep, in line with previous studies, improves emotional item memories.

METHODS

Participants

Eighteen native, German speaking, healthy participants (6 men and 12 woman, mean age: 21.27 yrs, range 18-26 yrs) were recruited at the University of Luebeck. All were non-smokers, free of medication, had no history of neurological, psychiatric or endocrine disorders, and followed a normal sleep-wake rhythm (i.e. no shift work, usual sleep time from 2300-0700h) for at least four weeks before the experiments. Prior to the experiments, subjects were accustomed to sleeping under laboratory conditions during an adaptation night, including attachment of electrodes for polysomnographic recordings. On experimental days they were required to get up at 0700h and not to consume caffeine or alcohol. The study was approved by the ethics committee of the University of Luebeck and all participants gave written informed consent prior to participation.

Design and procedure

Figure 15A illustrates the study design. The study was conducted according to a within-subject cross-over design with the order of conditions (‘early sleep’ vs. ‘late sleep’) balanced across subjects, and an interval of at least two weeks between the subjects’ two conditions. Subjects reported to the lab at 2100h for the early night condition and 2200h for the late night condition. Each condition started with the attachment of electrodes. For the early sleep condition, subjects learned the task (2200 - 2215h) and were tested on half of the picture set in an immediate retrieval test (2220 - 2240h) before an early 4-hrs retention interval containing a 3-hrs sleep period rich in SWS. After awakening, participants were tested on the other half of the picture set for delayed picture recognition, the location where they had appeared and the color of the frame that preceded each picture. For the late sleep condition, subjects first slept for about 3hrs (starting 2300h) before the learning (0300-0315h) and immediate retrieval phase (0320-0340h) took place. Following a further 4-hrs retention interval, including a 3-hrs sleep period rich in REM-sleep, delayed recognition memory, picture-location and frame-to-picture association were tested in the morning (0800-0845h) for the other half of the picture set. Thus, the retention intervals in the two experimental conditions were characterized by either high amounts of SWS (early sleep) or high amounts of REM sleep (late sleep). Lights-off for the early sleep interval was at 2300h, and 0400h for the late sleep interval with the start of the 3-hrs sleep period being determined by the first signs of sleep stage 2 for more than 1 min. Subjects were awakened from sleep stage 1 or 2 after 3 hrs of sleep and subsequent learning or retrieval testing was timed 45 min thereafter to allow subjects to

recover from sleep inertia. Before each learning and retrieval phase subjects indicated their level of sleepiness on a scale from 1 (active, alert) to 7 (very sleepy) (Stanford Sleepiness Scale, SSS; (Hoddes, 1972) and performed on a 5-min version of the Psychomotor Vigilance Test (PVT; (Roach, Dawson, & Lamond, 2006). The current mood was assessed once after arrival at the laboratory in the evening by the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988).

Memory task

For the learning phase four hundred pictures were selected from the International Affective Picture System (IAPS; Lang, Greenwald, Bradley, & Hamm, 1993) and divided into two parallel sets of 96 pictures, each set including 48 neutral low arousing and 48 medium to high arousing negative pictures (for the subjects' two conditions) respectively. Each of these sets was again subdivided into two parallel sets for immediate and delayed recognition testing (each containing 24 neutral and 24 emotional pictures). For immediate and delayed recognition testing, neutral and emotional picture sets were supplemented with 24 new pictures each. Normative valence (scale from negative [1], neutral [5] to positive [9]) and arousal (scale from not arousing at all [1] to very arousing [9]) ratings were comparable for the eight sets (all $p > 0.99$): [mean ± SD across all sets] valence ratings: negative, 3.11 ± 1.60; neutral, 5.01 ± 1.22; arousal ratings: negative, 5.81 ± 2.15; neutral, 3.17 ± 1.92. To accustom subjects to the learning procedure and prevent primacy and recency effects regarding memory performance, four additional pictures were presented before and after the target picture set and were not included in the analyses. These buffer pictures consisted of two negative and two neutral pictures. During learning, all pictures were presented in pseudorandomized order following predefined criteria (e.g., a maximum of two subsequent pictures with the same valence), with each participant receiving the same order. Pictures were presented in one of four possible target locations (up left, up right, down left, down right monitor screen quadrants). Additionally, preceding each picture, a colored frame in one of four colors (blue, green, red, or yellow) was presented. Each trial started with a fixation cross that appeared for 3000 ms, followed by the colored frame for 1500 ms and an empty screen for 500 ms. Subsequent picture presentation duration was set to 1500 ms. Presentation of the next trial followed after a variable interstimulus interval of 3500, 4000 or 4500 ms (mean: 4000 ms). Participants were instructed to associate the frame color with the subsequent picture and to memorize the pictures, their location on the screen and the color of the frame preceding each picture, because memory for the pictures, frame color and picture location would be tested

thereafter. Following a 5-min break after the completion of the learning phase, immediate memory for object recognition was tested by presenting half of the 96 pictures from the learning phase (referred to as 'old' pictures) randomly intermixed with 48 new pictures. Again, each of these pictures appeared in the middle of the screen in pseudorandomized order according to the same criteria as during learning. Participants had to indicate with a corresponding key press whether they had seen the picture before ('old') or not ('new'). When participants indicated that it was an old picture, they had to additionally state in which location the picture had appeared and which color the preceding frame had been during learning. Delayed recognition took place after the 4hrs retention interval in a similar manner with the other half of the 96 old pictures randomly intermixed with 48 different new pictures. There was no time limitation for immediate and delayed recognition testing, thus participants were allowed to take as much time as necessary to complete the task (see Figure 15B for illustration of memory task).

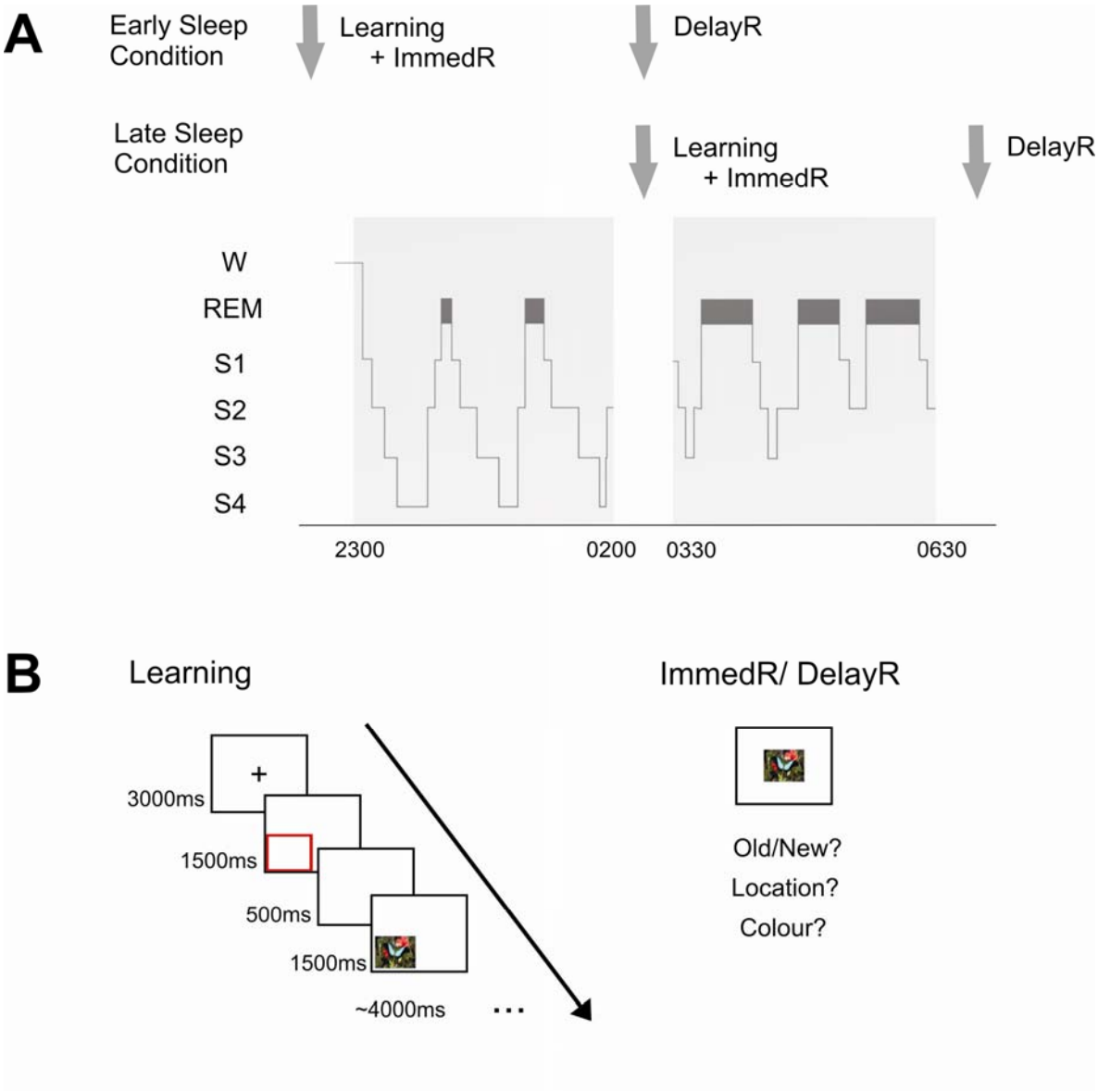

Figure 15. Study design and task. **(A)** Subjects were tested in two conditions (early vs. late sleep) with the order balanced across subjects. In the early sleep condition, subjects learned between 2200-2230h which was followed by an immediate retrieval testing (ImmedR). After a subsequent 4-hours retention interval, with ~3 hours of sleep containing predominantly SWS, delayed retrieval (DelayR) was tested at 0300h. In the late sleep condition, subjects first slept for 3 hours (lights off at 2300 h) were woken up and learnt between 0300-0330h, followed by an immediate retrieval test before the 4-hours late retention interval filled with high amounts of REM sleep. Delayed retrieval took place between 0715-0800 h. A typical polysomnogram visualizes the proportion of sleep stages during nocturnal sleep (wake (W), non-rapid eye movement (NREM) sleep stages 1-4 (S1-S4), REM sleep). **(B)** The study task involved emotional and neutral pictures that were presented at 1 of 4 locations (monitor quadrants) and were preceded by 1 of 4 colored frames (blue, green, red, yellow) during learning and a variable inter-stimulus interval. At both, immediate and delayed retrieval, recognition memory for pictures was assessed by old/new judgements, and subjects had to indicate picture – location association as well as frame color association memory by a respective button press (1 of 4 buttons).

Sleep Recordings

Electroencephalographic (EEG) activity was continuously recorded with Ag/AgCl electrodes from 5 scalp sites (Fz, C3, Cz, C4, Pz) and referenced to linked electrodes attached to the mastoids. Additionally, horizontal and vertical electrooculographic (EOG) and electromyographic activity (EMG) was recorded. Data were amplified by BrainAmp Amplifiers (Brain Products GmbH, Gilching, Germany) and continuously digitized at a rate of 250 Hz. Sleep recordings were scored offline according to standardized criteria (Rechtschaffen & Kales, 1968). For each 3-hrs sleep interval, total sleep time, and time spent in the different sleep stages (wake; sleep stages 1, 2, 3, 4; SWS, i.e. sum of sleep stage 3 and 4; REM sleep) was calculated in minutes and as percentage of total sleep time (TST).

Data reduction and statistical analysis

Retention of pictures was quantified as the percentage of correctly recognized pictures at delayed retrieval, relative to recognition performance at the immediate recognition which was set to 100%, separately for negative and neutral pictures. A recognition memory index was calculated for recognition of pictures to correct for response bias (P_r = hits – false alarms). Similarly, the source memory measures (i.e. picture-location and frame-to-picture association memory) were calculated by relating delayed retrieval to immediate retrieval. Importantly, participants were only asked for their memory on the location and the frame color of the pictures, when they had correctly identified the picture as 'old', thus limiting the absolute number of possible answers for source memory to the absolute number of correctly recognized pictures.

Data analysis was generally based on Analyses of Variance (ANOVA) with the repeated measures factors 'early/late sleep' and 'emotionality' (negative/neutral) for analyses of the immediate and delayed retrieval sessions. Significant ANOVA effects were followed by post hoc t-tests. The level of significance was set to $p = 0.05$ and Greenhouse-Geisser correction was applied when appropriate.

RESULTS

Recognition memory, picture-location association and frame-to-picture association

See Table 5 for a summary of recognition data and pair-wise statistical comparisons. At immediate retrieval, the absolute number of corrected recognition (hits – false alarms) neither

differed between early vs. late sleep conditions, nor for emotional vs. neutral pictures ($p > 0.14$ for respective main and interaction effects). At delayed retrieval, consistent with our hypothesis, corrected recognition revealed superior retention for negative over neutral pictures only after the late REM sleep-rich retention interval ($p < 0.05$). No such difference was detected after early SWS-rich sleep ($p > 0.45$; $F(1,17) = 5.017$, $p = 0.039$, for early/late sleep x emotionality interaction, see Figure 16A). Relating recognition performance at delayed testing to immediate retrieval basically revealed the same results although the early/late sleep interaction was only marginally significant ($p = 0.084$). Post-hoc comparisons confirmed the marked difference between emotional and neutral picture recognition after late sleep ($p = 0.021$), but not after early sleep ($p > 0.12$).

For picture-location memory, generally better retention was observed before and after early sleep (both $p < 0.033$, for main effects early/late sleep). Relative retention (i.e. performance at delayed retrieval relative to baseline performance at immediate retrieval) of picture locations did not differ for emotional vs. neutral and for early vs. late sleep ($p > 0.34$, for respective main and interaction effects, see Figure 16B). Frame-to-picture associations were remembered better for neutral than emotional pictures at immediate retrieval as well as at delayed retrieval ($p < 0.05$, for both main effects 'emotional/neutral'). Retention of the frame-to-picture associations did not differ between early and late sleep at immediate retrieval ($p > 0.17$, for 'early/late sleep' main effect and 'emotional/neutral x early/late sleep' interaction effect). However, at delayed retrieval after the early sleep interval colors of frames were generally better remembered than after late sleep ($p = 0.027$, for early/late sleep main effect), a difference that was mostly explained by the strong memory for neutral associations. Frame-to-picture associations were differentially affected by the nature of retention sleep and emotionality ($p = 0.05$, for 'emotional/neutral x early/late sleep' interaction effect). Respective post-hoc comparisons indicated a pronounced retention superiority of frame-to-picture associations for neutral compared to emotional pictures after early sleep ($p = 0.001$), but no such difference after late sleep ($p > 0.11$). Relative retention of associated frame colors was in complete agreement with the absolute number of remembered frame-to-picture associations (see Figure 16 C for emotional/neutral x early/late sleep interaction and post-hoc comparisons).

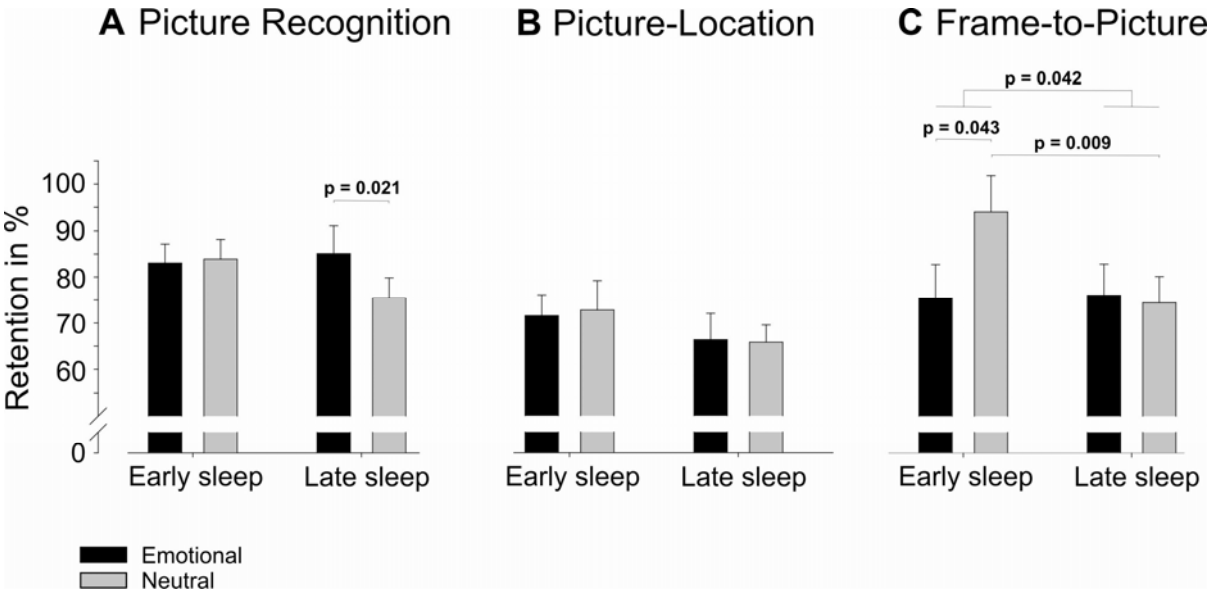

Figure 16. Memory performance for (**A**) picture recognition, (**B**) picture-location association and (**C**) frame-to-picture association. Picture recognition at delayed retrieval was distinctly enhanced for negative compared to neutral pictures after late REM-rich sleep, but not after early SWS-rich sleep. Picture-location association was not differentially affected by early or late sleep. In contrast, frame-to-picture association was specifically enhanced for neutral pictures, but inhibited for emotional pictures only after early SWS-rich sleep and not after late REM-rich sleep. P-values are indicated for respective post-hoc comparisons (i.e. emotional vs. neutral for the sleep conditions and for early vs. late sleep for neutral frame associations). Relative retention at delayed retrieval is indicated with immediate retrieval performance set to 100%.

Table 5 Memory performance: recognition, picture-location, frame-to-picture association

		Early sleep		Late sleep	
		Emotional mean ± SEM	Neutral mean ± SEM	Emotional mean ± SEM	Neutral mean ± SEM
Object Recognition					
Hits- FA	ImmedR	20.50 ± 0.8	21.33 ± 0.5	20.22 ± 0.7	20.28 ± 0.8
	DelayR	17.17 ± 1.0	17.89 ± 1.0	17.28 ± 1.2	15.56 ± 1.2*,+
	%DelayR	83.06 ± 4.1	83.88 ± 4.3	85.08 ± 5.9	75.42 ± 4.4*
Picture - Location					
Hits	ImmedR	13.89 ± 0.9	14.17 ± 1.1	12.94 ± 0.9	12.67 ± 1.1
	DelayR	10.11 ± 1.0	10.00 ± 1.0	8.50 ± 0.9	8.44 ± 0.9
	%DelayR	71.53 ± 4.5	72.75 ± 6.4	66.33 ± 5.7	65.78 ± 3.7
Frame - Association					
Hits	ImmedR	11.94 ± 1.1	13.22 ± 1.3	10.67 ± 0.9	12.17 ± 1.1
	DelayR	8.94 ± 1.1	11.72 ± 1.2**	8.00 ± 1.0	9.17 ± 1.1++
	%DelayR	75.46 ± 7.3	94.04 ± 7.8*	76.09 ± 6.8	74.48 ± 5.7++

Mean ± SEM number of corrected item recognition (hits – false alarms) and number of hits for picture-location and frame-association memory for the immediate (ImmedR) and delayed retrieval phase (DelayR) before and after early (SWS-rich) and late (REM-rich) retention sleep. Additionally, retention at delayed retrieval is given as percentage of immediate retrieval which was set to 100%. Separate ANOVAs were calculated for ImmedR, DelayR and % DelayR, followed by post-hoc comparisons when significant. * $p < 0.05$ and ** $p \leq 0.01$, indicate significant t-tests between emotional and neutral, + $p < 0.05$ and ++ $p \leq 0.01$, indicate significant t-tests between early and late sleep condition for neutral pictures, picture-location and frame-association memory, respectively.

Sleep

Sleep data showed the expected prevalence of SWS in the early sleep condition and of REM sleep in the late sleep condition ([mean ± SEM] SWS; early sleep: 38.26 ± 4.08%, late sleep: 12.24 ± 1.98%; REM; early sleep: 8.54 ± 1.37%, late sleep: 29.14 ± 1.56%; both $p < 0.01$). None of the other sleep stages differed between conditions (see Table 6 for a summary of sleep results).

Table 6 Sleep parameters

	Absolute time (in min)		Percentage of time	
	Early sleep mean ± SEM	Late sleep mean ± SEM	Early sleep mean ± SEM	Late sleep mean ± SEM
Sleep parameters				
Wake	1.47 ± 0.48	0.56 ± 0.15	0.77 ± 0.25	0.29 ± 0.08
Stage 1	13.72 ± 2.18	16.61 ± 1.51	7.37 ± 1.18	8.93 ± 0.79
Stage 2	82.67 ± 5.56	90.11 ± 3.24	44.32 ± 2.96	48.53 ± 1.62
Stage 3	41.22 ± 3.88	19.06 ± 2.80**	21.96 ± 1.97	10.32 ± 1.52**
Stage 4	30.33 ± 5.87	3.53 ± 1.80**	16.31 ± 3.18	1.92 ± 0.97**
SWS	71.56 ± 7.71	22.58 ± 3.63**	38.26 ± 4.08	12.24 ± 1.98**
REM	15.92 ± 2.52	54.06 ± 2.90**	8.54 ± 1.37	29.14 ± 1.56**
TST	186.72 ± 2.21	185.50 ± 1.07		

Sleep parameters are given in mean (± SEM) of absolute time in minutes and percentage of total sleep time; SWS = slow wave sleep (sum of sleep stages 3 and 4), REM = rapid eye movement sleep, TST = total sleep time; t-tests were calculated between the early and late sleep condition, for absolute time and percentage of TST, respectively, **: $p < 0.01$

Mood, sleepiness and vigilance

Mood measures were well comparable between conditions (PANAS - positive affect: early sleep, 25.94 ± 1.97, late sleep, 23.67 ± 1.63; negative affect: early sleep, 11.72 ± 0.44, late sleep, 11.61 ± 0.47; all $p > 0.17$). Moreover, self-reported sleepiness did not differ between conditions at learning (early sleep condition: 3.28 ± 0.30, late sleep 3.83 ± 0.27, $p > 0.07$), but participants reported to be less tired at retrieval after late sleep (early sleep 3.44 ± 0.31, late sleep 2.72 ± 0.30; $p < 0.05$). Reaction times on the vigilance task were comparable between the early and late sleep condition at retrieval testing (early sleep: 303.09 ± 6.30 ms vs. late sleep 307.38 ± 6.90 ms, $p > 0.39$). However, at learning reaction times were faster in the early sleep condition (296.74 ± 6.02) than in the late sleep condition (310.81 ± 6.13; $p < 0.01$).

DISCUSSION

In the present study, we investigated the impact of rapid eye movement (REM) sleep and slow wave sleep (SWS) on memory consolidation of emotional pictures and associated contextual information. REM sleep was predicted to enhance emotional item memory, and SWS to be

involved in the consolidation of contextual source information associated with an emotional item. In keeping with previous studies (Wagner et al., 2001; Groch, S., Wilhelm, I., Diekelmann, S., Born, J., unpublished) we found a specific enhancement of emotional picture recognition, only after a period of late REM-rich retention sleep, not after early SWS-rich retention sleep. More specifically, the number of negative pictures that was remembered after a 4-hours retention interval, filled with high amounts of REM sleep, was significantly enhanced compared with neutral pictures whereas no such facilitation of emotional memory recognition was observed when learning had been followed by periods of SWS-rich sleep. Interestingly, we indeed found evidence for a selective influence of SWS on the retention of contextual source information (associated frame color), depending on the emotional nature of the memorized picture. Memory retention of the frame color of the pictures was distinctly enhanced for neutral pictures after early SWS-rich retention sleep. In stark contrast during the same retention sleep period, no such increased associative binding was found for the frame colors to emotional pictures.

The facilitated consolidation of contextual information for neutral pictures, specifically during SWS-rich sleep is in accordance with the system consolidation theory, which posits that memory contents acquired during the day are reactivated during SWS to be strengthened for permanent storage. Importantly, our results indicate that there is no such SWS-induced reinforcement for the consolidation of context information associated with an emotional item. This differential effect of SWS can only be explained by the difference in emotionality of the central object. It already has been shown that a night of nocturnal sleep enhanced memory for emotional objects in the foreground of a picture, but decreased retention of their neutral peripheral backgrounds (Payne et al., 2008). Our results extend these findings by specifying the sleep stages underlying emotional item memory improvement on the one hand and lacking enhancement of contextual memory consolidation on the other. First, our results confirm the beneficial consolidation of REM sleep for emotional pictures (i.e. item memory) which is in well agreement with previous findings (Wagner et al., 2001; Nishida et al., 2009; Groch, S. Wilhelm, I. Diekelmann, S., Born, J. unpublished). This effect might possibly be related to increased activity of the amygdala and parahippocampal areas during REM sleep (Maquet et al., 1996; Nofzinger et al., 1997; Miyauchi et al., 2009). Second, during SWS, we found a blockade of frame-to-picture memory (i.e. source memory) for emotional relative to neutral pictures. It can be speculated that during SWS a selection process takes place that determines neutral context information being associated with an

emotional item as relatively irrelevant and, thereby, are not subjected to sleep-dependent consolidation processes.

Taken together, the suppression of contextual binding during SWS when the central element is emotional, but in the same night of sleep memory on this central element is enhanced during REM sleep might serve a specific function. Probably, when contextual information do not add to the predictability of an emotional event or are perceived as belonging to the same emotional object, these associations might be considered by the sleeping brain as not relevant enough to be remembered for the long term. In contrast, the emotional object in the centre, no matter in which context it appears, is enhanced which could facilitate processes of adaption and generalization for future situations (see also Payne et al., 2008). In our study, no difference was detected for picture-location association that differs to the frame-association mainly by its integrating nature of simultaneous binding of an object and the location where it appears (Kahneman, Treisman, & Gibbs, 1992). However, regarding picture-location memory, we could not confirm our expectation that SWS compared to REM sleep impacts contextual memory formation, neither for neutral nor for emotional pictures. Even though previous findings suggest that any kind of contextual binding, i.e. the location of an object and spatio-temporally separated object associations engages hippocampal activity during successful encoding, the extent of hippocampal engagement increases with a greater temporal gap between object and associative feature (Staresina et al., 2009). We can only speculate that the lacking enhancing effect of SWS on location memory is due to the relatively small hippocampal dependency of the task we used (Uncapher, Otten, & Rugg, 2006; Sommer, Rose, Gläscher, Wolbers, & Büchel, 2005; Cansino, Maquet, Dolan, & Rugg, 2002). Possibly during SWS specifically those contents are processed that depend to a high degree on hippocampal activation (Marshall, Helgadottir, Mölle, & Born, 2006). Moreover, we cannot exclude the possibility that the different memory systems that process item and source memory information interact in our task, which includes the simultaneous encoding of three kinds of information, rendering the investigation of pure item and pure source memory impossible. However, even when memory on item and source information could have influenced each other, still the retrieval task was constructed to test the different kinds of memory separately from each other. Furthermore, besides many advantages of the split-night design that we used in this study to manipulate the amount of REM sleep or SWS following learning, some confounds cannot be completely eliminated. Though, only four hours separated from each other, learning and retrieval for the early and late sleep conditions take place at a different time, i.e. are prone to circadian confounds including differences in the

neuroendocrine conditions, alertness and emotional reactivity. However, we believe that results from a circadian control (i.e. a group of subjects being awake during the retention interval at the same time of day) would be even more difficult to interpret due to differences in sleep deprivation and amygdala reactivity to emotional material (Yoo, Hu, Gujar, Jolesz, & Walker, 2007). That is why we intended to account for possible circadian influences by comparing baseline measures, i.e. the immediate recall and assessment of vigilance and reaction times. Importantly, baseline measures for the recognition of pictures and picture-frame color association were comparable for early and late sleep conditions as were self-rated sleepiness during learning and reaction times at retrieval. However, before late sleep subjects were somewhat slower in their reaction times and after late sleep subjects felt less tired than after early sleep. Nevertheless, we believe that these differences in self-rated sleepiness and reaction time differences of about 15 ms are unlikely to account for the differential effects of picture recognition and source memory. Picture-location memory was encoded better before early sleep, but also relative measures using the individual baseline levels as reference did not reveal a specific impact of SWS or REM sleep on its retention.

In summary, here we demonstrate for the first time that emotional item and source memories are differentially processed during slow-wave sleep-rich and REM-rich sleep retention intervals. Whereas REM sleep primarily enhanced emotional item memories, SWS specifically facilitated contextual binding of frame-to-picture association for neutral, but not emotional pictures. Two possible hypotheses need to be tested in the future: 1. Whether SWS is not involved in memory of contextual information for emotional contents or 2. whether SWS only supports the binding of relevant associations which would include associations providing relevant or predictive information about the appearance of the emotional object. Since SWS has been proven to be involved in selective memory processing on the one hand and REM sleep to facilitate emotional memories on the other hand, here we show that different aspects of emotional memory might be processed during these specific sleep-stages. In the present investigation, memory of emotional source information has been found to be suppressed during SWS, which could be due to the loss of relevance of the association task, because it is overshadowed by a strong emotionally arousing stimulus. Subsequently, during REM sleep the consolidation of emotional item memory was distinctly enhanced confirming previous findings. With the task and design we used, here, we provide first evidence for a lacking influence of SWS on source memory information of emotional objects, but at the same time propose ‘relevance’ as a possible modulating factor that should be investigated in future studies.

CONCLUSION AND GENERAL DISCUSSION

The three experiments that were conducted in the context of this thesis aimed to elucidate the effects of sleep on emotional memory formation and on emotional reactivity. One major purpose was to dissect the particular role of rapid-eye movement (REM) sleep and slow-wave sleep (SWS) in the processing of the two aspects of emotional memories, i.e. the memory component encompassing the declarative information about an object or event and an affective component containing information about the emotional tone that is elicited by the object or event. With respect to the memory component, I also differentiated between the effects of sleep on retention of the emotional item itself (i.e. item memory) and contextual information of the emotional item (i.e. source memory). The following findings from the three experiments add to the current knowledge on sleep-dependent processing of emotional memories: i) REM-sleep was confirmed to promote item memory specifically for emotional compared to neutral material assessed in both, a free recall and a recognition procedure (**Experiment 1 and Experiment 3**). Further, ii) no simultaneous decrease in perceived emotionality takes place during either an immediate 3-hrs REM-rich or SWS-rich sleep interval which clearly competes against a current model on the function of REM-sleep (**Experiment 1 and Experiment 2**). Next, the studies revealed evidence for iii) the processing of some aspects of emotional memory, i.e. the temporal context during SWS (**Experiment 2**). More specifically, iv) norepinephrine (NE) was found to be engaged in the formation of emotional temporal order memory, but not in simple recall of emotional memory per se and the processing of emotionality during SWS (**Experiment 2**). And finally, first hints speak for v) a particular role of SWS-rich sleep for suppressing the consolidation of neutral context information when associated with an emotional item (e.g. a weapon that had to be associated with a blue colored frame) whereas emotional item memory (i.e. the weapon) simultaneously benefits from REM-rich sleep (**Experiment 3**).

The impact of REM sleep and SWS on different aspects of emotional memory consolidation: item vs. source information

According to the dual process theory, REM sleep and NREM sleep serve different functions in the process of memory consolidation. During SWS, the deepest NREM sleep stage, processes of reactivation and integration of newly acquired contents into existing neuronal

networks are assumed to take place, thereby allowing for qualitative changes and selection processes (e.g. extraction of a gist, Diekelmann et al., 2010). On the other hand, electrical activity (such as theta activity) mainly occurring during REM sleep is assumed to be involved in synaptic consolidation processes, thereby quantitatively strengthening the synaptic connections that were activated during daytime (Maquet, 2001; Diekelmann et al., 2010). Moreover, SWS has been shown to play a distinct role for consolidating declarative memories whereas REM sleep has been proposed to benefit procedural and emotional memory consolidation. Here, evidence is provided for a differential contribution of REM sleep and SWS for the consolidation of emotional item and source memory.

Declarative memory is commonly divided into two different subsystems: Recollection (i.e. source memory) refers to remembering a stimulus including detailed spatiotemporal context information whereas familiarity-based remembering (i.e. item memory) refers to simply knowing that the stimulus has been previously presented (Yonelinas, 2002; Rugg et al., 2007). **In Experiment 1 and Experiment 3** REM sleep has been identified as the critical sleep stage for the superior retention of emotional compared to neutral pictorial stimuli. As the fast learning and retrieval procedure implemented in the study designs used without providing any contextual information in addition to the pictures itself, e.g. source or color, most likely learning and retrieval processes of familiarity have been induced. These REM sleep-related memory benefits for emotional material have been confirmed by behavioral and event-related EEG measures and are in agreement with previous findings (Wagner et al., 2001; Nishida et al., 2009; Walker et al., 2009). The specific activation of enhanced positivity at frontal sites between 300-500 ms post-stimulus interval for correctly recognized old emotional stimuli compared to new or neutral stimuli **(Experiment 1)** has been found to be associated with familiarity instead of recollection processes in earlier studies (Rugg et al., 1998; Rugg et al., 2007; Woodruff, Hayama, & Rugg, 2006). These findings are in agreement with previous studies reporting sleep-related improvement of familiarity-based recognition memory of emotional pictures (Hu et al., 2006; Payne et al., 2008; Payne et al., 2011). Adding to the existing literature, the results emphasize the specific relevance of REM sleep for the recognition of familiarity-based emotional memories.

A further research question aimed at investigating a possible role of SWS in the processing of contextual information when these are associated with an emotionally arousing stimulus. There is abundant evidence for processes acting during SWS that promote the consolidation of hippocampus-dependent contextual information (i.e. source memory), at least for neutral contents (Drosopoulos et al., 2005). Contextual binding, i.e. binding of an object

into a temporal or spatial context, is considered a key function of the hippocampus (Staresina & Davachi, 2009) and has been demonstrated to benefit from SWS for neutral associative memory (Eichenbaum, 2004; Rasch & Born, 2007; Tubridy & Davachi, 2011). In **Experiment 2** memory of neutral and emotional pictures and content words of stories (i.e. item memory) and memory on the temporal order of content words within the stories (i.e. source memory) has been tested. In the experimental condition noradrenergic release was suppressed during a 3 hrs-early sleep interval following learning. Whereas NE inhibition did not affect memory of emotional or neutral item memory, temporal order memory was impaired specifically for emotional material. From these findings it can be concluded that processing of the temporal context of emotional material occurs during SWS and more specifically, that the consolidation of emotional source, but not item memories depends on the presence of NE during early SWS. Importantly, the selective impairment of emotional temporal order memory after NE suppression, but not neutral or emotional item memory, might reflect a specific contribution of SWS-dependent noradrenergic mechanisms to the consolidation of memory contents that require certain brain regions. Item and contextual source information distinctly differ in their dependence on brain regions, i.e. item memory requires the perirhinal cortex whereas binding several items into a temporal context requires hippocampal activation (Lehn et al., 2009). As noradrenergic activation specifically affected temporal order memory of emotional contents, it can be assumed that NE activation during SWS plays a role for consolidation processes requiring the combined activation of the hippocampus and the amygdala. Therefore, phasic noradrenergic activity during SWS might reflect a process of reactivation following amygdala- and hippocampus-dependent learning facilitating its retention (Eschenko et al., 2008; Gais et al., 2011).

The speculation that SWS and REM sleep play a specific role for distinct features of emotional memory consolidation is a principal hypothesis underlying all studies in this thesis. This double dissociation (i.e. SWS benefits contextual integration; REM sleep benefits item memory) could be mediated by the differential involvement of brain structures with emotional familiarity-based memory depending on the amygdala and rhinal cortices and emotional recollection-based memory relying on the amygdala and hippocampus. Further, this notion is supported by evidence suggesting facilitated hippocampal activation during SWS but suppressed hippocampal activity during REM sleep, probably via neuroendocrine modulatory influences (Hasselmo, 1999). One function of SWS might be the selection of information to be consolidated into long-term memory (Wilhelm et al., 2011). Subsequent REM sleep, on the other hand, might facilitate the quantitative enhancement of already selected information.

However, in **Experiment 3** emotional and neutral recognition memory was supplemented by a context association task (i.e. a central emotional or neutral object appearing at a specific location together with a temporally separated presented colored frame). Again, REM-rich sleep enhanced memory specifically for the central emotional pictorial information (i.e. item memory), but SWS-rich sleep was involved in contextual memory (color of the frame that was presented before the picture) only when the central information was neutral. The same contextual information has been consolidated strikingly less when the central information was emotional, suggesting a suppression of the respective neutral context information during SWS. At first sight, these finding seem to compete with the findings of **Experiment 2** demonstrating an at least partial involvement of SWS-rich sleep in the formation of the superiority of hippocampus-dependent emotional compared to neutral contextual memory. However, in contrast to the temporal order of single items, altogether forming a coherent story and composing one emotional event, the picture-color-frame association in **Experiment 3**, as well as the neutral backgrounds in the study by (Payne et al., 2008), do not contain any informational value for the emotional object. As discussed in a recent review, emotional arousal might be able to facilitate both, impairment and enhancement of memory binding (Mather, 2007; Nashiro & Mather, 2011). Probably, the predictability of emotional information (as in fear conditioning learning paradigms) or perception of contextual information as belonging to the emotional object itself might modulate whether SWS promotes these contextual information. On a functional level, both of these outcomes, i.e. enhanced binding and impaired binding, would make sense for an organism in a complex environment offering a variety of more or less informational cues. Sleep might selectively enhance memory for the most important information, i.e. either an emotional object together with contextual details if these details increase the predictability of its appearance or only the emotional object itself if the contextual details do not add to the object's predictability. The facilitation of emotional item information during sleep could promote the applicability of emotional memory contents to situations that are different from learning by enhancing memory of the stimulus itself but reducing memory of specific contextual details. Thereby, SWS might be involved in the selection of information according to its relevance and REM sleep might subsequently preferentially enhance those relevant over irrelevant information. This extension of the current view on emotional memory consolidation includes different aspects of emotional memories and is in accordance with the sequential model of memory consolidation, proposing an importance of the typical succession of sleep stages during human sleep.

The impact of REM sleep and SWS on the processing of emotional reactivity

According to a current model by Matthew Walker (Walker, 2009) about the processing of the two components of emotional memories, i.e. a memory component containing the information about an object or event and an affective component reflecting the autonomic charge and subjectively perceived emotionality, both components should simultaneously vary across sleep intervals (Van der Helm et al., 2009). Hereby, the memory trace has been predicted to be stabilized during REM sleep, specifically for emotional and less for neutral information. On the other hand, the model hypothesized that emotional reactivity would be reduced during sleep. Both of these outcomes could be explained by reactivation of memory contents that has been suggested after studies in rats that detected similar neuronal activation patterns during learning and again offline during sleep (Wilson et al., 1994). By reactivating the learned information, disproportional in favor for the most memorable (here the emotional) ones, the memory traces would become strengthened. At the same time this neuronal replay could result in a kind of internal re-exposure to emotional contents and thereby facilitate habituation resulting in decreased emotional reactivity.

The model described above is challenged by the findings of the present experiments. Emotional reactivity has been tested with subjective ratings of valence and arousal (**Experiment 1 and Experiment 2**) and event-related EEG measures (**Experiment 1**) or psychophysiological measures, i.e. heart rate change (**Experiment 2**). The 500-800 ms interval of the event-related potentials (ERP) at parietal sites has been associated in previous studies with emotionality (Dolcos et al., 2002). However, no difference in mean activity of the ERP-amplitude in this interval as well as in subjective ratings of valence and arousal in response to old familiar and new emotional or neutral pictures has been detected. Thus, contrary to the hypothesis, a reduction in emotional reactivity could be found neither after REM-rich nor SWS-rich 3-hrs sleep interval. As the existing literature suggests, the effects of emotion may increase over time and the 3-hrs interval might be considered too short (LaBar et al., 2006). However, another study that assessed subjective ratings of familiar pictures encoded one week before and new pictures did likewise not find any differences in subjectively perceived emotional arousal and valence, confirming a lack of reduction in emotional reactivity (Weymar et al., 2009). Wagner and colleagues even found an increase in negativity ratings in valence and no change in arousal ratings after REM-rich sleep and a whole night of nocturnal sleep (Wagner et al., 2002). In **Experiment 2**, explorative analyses were performed to test a possible role of noradrenergic activation during early SWS-rich sleep

on processing of emotional reactivity. No difference could be observed in heart rate change and subjective ratings for pre/post and old/new comparisons of emotional and neutral pictures when noradrenergic release was suppressed. However, it cannot be excluded that NE plays a role in sleep-dependent emotional processing, but if so, than most probably during REM sleep as a previous study implicated REM sleep, rather than SWS in emotional reactivity (Wagner et al., 2002). Taken together, the evidence for an impact of sleep on emotional reactivity is rather scarce and contradictory. Results of different approaches suggest increased, decreased or unchanged emotional reactivity after sleep and it remains to future studies to systematically investigate the circumstances and kinds of emotions that are processed during sleep.

Future perspectives

In summary, the findings presented in this thesis suggest that there is a fundamental role of sleep in the consolidation of emotional memories whereas an effect of sleep on processing of emotional reactivity was not found. A number of new research questions emerged from the presented findings. Regarding the memory component of emotional memories, it would be of highest relevance to test how and under which circumstances emotional arousal can affect memory binding. Here, relevance and predictability seem to be promising factors that might modulate the role of sleep in contextual binding. As sleep, and especially SWS, has been shown to be implicated in consolidation of future relevant memories, it can be speculated that SWS-dependent contextual details of relevance might be enhanced for emotional stimuli, but impaired in case of irrelevance. By experimentally boosting the reactivation of emotional item and source memories either during SWS or REM sleep the role of these sleep stages in emotional memory consolidation could be further scrutinized thereby elucidating possible underlying mechanisms. More specifically, material with a central emotional or neutral object embedded in relevant or irrelevant context information could be paired with an odor cue that would be presented during the respective sleep stage of interest (odor task as implemented in (Rasch, Büchel, Gais, & Born, 2007). Following the present conceptual framework, familiarity-based item recognition would be expected to benefit from being reactivated during REM sleep whereas contextual binding (of relevant, hippocampus-dependent context information) should be promoted by reactivation during SWS (see Figure 17 for working model). Furthermore, investigating the role of emotion- and memory-related neurotransmitters and hormones would shed light on the underlying mechanisms of sleep-

dependent processing of emotional information, e.g. the role of norepinephrine during REM sleep. It can be assumed that reduced norepinephrine levels impair the emotional enhancement of familiarity-based item memories during REM-rich sleep.

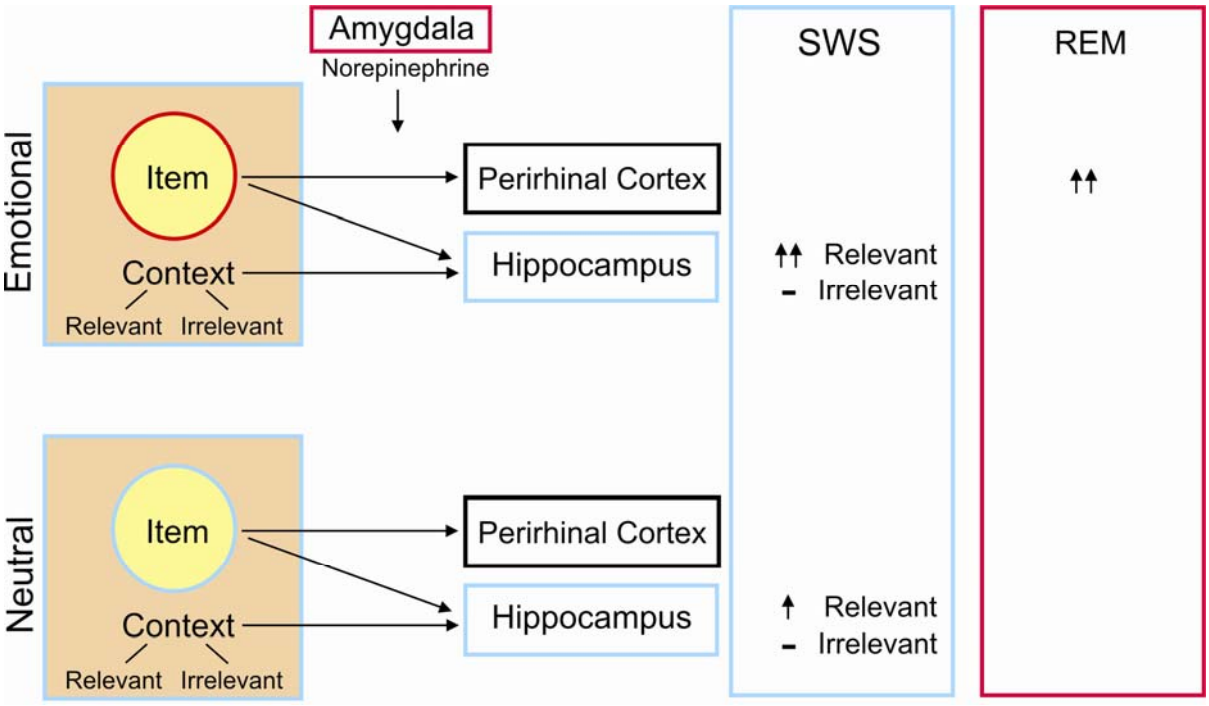

Figure 17. Working model on a possible relationship between the emotional charge of a stimulus and binding into its contextual information. Here, the current knowledge on the engagement of neurophysiological structures in source memory (i.e. hippocampus) and item memory (i.e. rhinal cortices) and its preferential processing during sleep stages (i.e. SWS and REM sleep, respectively) is integrated. Furthermore, depending on the relevance of contextual information for the appearance of the emotional stimulus in this model opposing outcomes are predicted.

Regarding emotional reactivity, the literature seems to be inconsistent at present and systematic approaches need to be developed to investigate the influence of sleep (stages) on different kinds of emotions, dimensions and the role of possible modulating factors. Also, the development of sensitive and reliable psychophysiological measures to assess different levels and degrees of emotionality would fundamentally improve emotion research. Although the usage of photos of objects or scenes provides many advantages with respect to standardisation, this method is rather artificial and impossible to induce certain kinds and degrees of emotions. Probably, the use of virtual reality techniques in combination with psychophysiological and neuroimaging methods bears a great research potential in this

context. In future fMRI studies, the engagement of the amygdala and hippocampus while presenting emotional stimuli (possibly in the context of a virtual reality) before and after retention intervals of sleep should be tested. Whereas retention of emotional memories is believed to become independent of the amygdala over time, amygdala reactivity should remain to be associated with emotional reactivity. Thus, less amygdala activity, but stronger hippocampal, rhinal or neocortical activity would be expected after sleep to confirm a stabilization of the memory trace, but amygdala activity should covary with the degree in emotional arousal.

Abstract

Retention of emotional memories is superior to neutral memories with this effect being facilitated by post-learning periods of sleep. Emotional memories encompass the declarative memory information about an object or event as well as an associated affective tone that at the time of acquisition causally contributed to its long-lasting manifestation in memory. In the present thesis, the particular role of post-learning periods of REM and SWS for consolidating emotional memories and its affective tone has been investigated. Possible neurophysiological mechanisms underlying sleep-dependent emotional memory consolidation were of further interest, thereby focusing on the role of noradrenergic activation during SWS.

To test the effects of SWS and REM sleep on emotional memory, subjects learned emotional and neutral memory material before retention periods of either SWS-rich or REM-rich sleep and were tested immediately thereafter. In parallel, emotional reactivity was measured in response to emotional stimuli as an indicator of their perceived affective tone. As assessed by behavioral, subjective and event-related measures, REM sleep has been found to specifically promote emotional picture recognition memory whereas emotional reactivity remained unchanged (Experiment 1). Experiment 2 revealed that SWS also contributes to the consolidation of emotional context information thereby critically depending on noradrenergic activation during this sleep stage. More specifically, the suppression of noradrenergic release did not affect emotional and neutral item memory but it impaired the consolidation of temporal context memory of emotional stories. Based on these findings, in Experiment 3 the influence of SWS and REM sleep was compared for emotional item and two kinds of contextual source memories. Whereas REM-rich sleep again promoted emotional picture recognition (i.e. item memory), memory for contextual associations (i.e. frame-to-picture associations) was enhanced for neutral, but not emotional pictures after SWS-rich sleep.

Taken together, a facilitating role of REM sleep for emotional item memory could be confirmed but emotional reactivity has not been found to be simultaneously reduced by REM sleep. Here, a contribution of SWS in the processing of emotional context memories has been identified for the first time, thereby critically involving noradrenergic activation during this sleep stage. In contrast, the ability to remember neutral context information associated with emotional objects has been found to be suppressed during SWS. The opposing findings on the role of SWS in the processing of emotional contextual information might originate from differences in the relation between emotional item and associated source information. Future studies need to discriminate possible modulating factors (e.g. relevance of the context for item

processing) that determine whether SWS is beneficial for emotional context information or not.

Zusammenfassung

Emotionale Gedächtnisinhalte werden besser erinnert als neutrale Inhalte. In vorangegangenen Studien konnte gezeigt werden, dass sich ein dem Lernen anschließendes Schlafintervall förderlich auf diesen Effekt auswirkt. Emotionales Gedächtnis besteht zum Einen aus einer deklarativen Gedächtnisinformation über ein Objekt oder Ereignis und zum Anderen aus einer damit assoziierten, affektiven Tönung. In der vorliegenden Doktorarbeit wurde der Einfluss von REM Schlaf (engl. rapid eye movement) und Tiefschlaf auf die Konsolidierung emotionaler Gedächtnisinhalte und deren affektiver Tönung untersucht. Von Interesse waren außerdem mögliche neurophysiologische Mechanismen, die der schlafabhängigen, emotionalen Gedächtnisbildung zu Grunde liegen. Hierbei stand der Einfluss noradrenerger Aktivität während tiefschlafreicher Schlafperioden im Mittelpunkt.

Um die Wirkung von Tiefschlaf und REM Schlaf auf das emotionale Gedächtnis zu untersuchen, lernten Versuchspersonen emotionales and neutrales Material vor einem Behaltensintervall, das entweder durch einen hohen Anteil an Tiefschlaf oder REM Schlaf gekennzeichnet war, und wurden anschließend bezüglich ihrer Erinnerungsleistung getestet. Als Indikator für die wahrgenommene affektive Tönung, wurde parallel dazu die emotionale Reaktivität auf die gelernten emotionalen Stimuli gemessen. Mithilfe von behavioralen und ereigniskorrelierten Maßen, konnte eine verbesserte Wiedererkennung emotionaler Bilder nach REM Schlaf gefunden werden Die emotionale Reaktivität war jedoch weder nach einem REM- noch nach einem tiefschlafreichen Schlafintervall verändert (Experiment 1). In Experiment 2 wurde darüber hinaus eine Beteiligung des Tiefschlafes an der Konsolidierung emotionaler, kontextueller Informationen herausgestellt, welche entscheidend von noradrenerger Aktivierung während dieser Schlafphase abhängig war. Die Unterdrückung der Ausschüttung von Noradrenalin während eines tiefschlafreichen Schlafintervalls beeinflusste nicht die Konsolidierung der Inhalte einer emotionalen oder neutralen Geschichte (Itemgedächtnis), verschlechterte jedoch die Erinnerung an die zeitliche Reihenfolge der Inhalte innerhalb einer emotionalen Geschichte (d.h. kontextuelles Gedächtnis). Auf diesen Ergebnissen aufbauend, wurde in Experiment 3 der Einfluss von Tiefschlaf und REM Schlaf auf emotionales und neutrales Item- und kontextuelles Gedächtnis verglichen. Während REM-reicher Schlaf nach dem Lernen abermals die Wiedererkennung emotionaler Bilder

(d.h. Itemgedächtnis) verbesserte, wurde nach tiefschlafreichem Schlaf die Erinnerung an kontextuelle Informationen, die mit neutralen nicht aber emotionalen Bildern assoziiert wurden (ein farbiger Rahmen gepaart mit einem Bild), verbessert.

Zusammenfassend, lässt sich aufgrund der Studienergebnisse ein begünstigender Einfluss von REM Schlaf auf die Konsolidierung emotionaler Iteminformationen bestätigen. REM Schlaf führte jedoch nicht gleichzeitig zu einer Reduktion der emotionalen Reaktivität in Bezug auf bekannte emotionale Bilder. In der vorliegenden Arbeit wurde erstmals eine Beteiligung von Tiefschlaf an der Bildung von emotionalem, kontextuellen Gedächtnis (Erinnerung an die inhaltliche Struktur einer Geschichte) aufgezeigt. Dieser Effekt wurde durch die noradrenerge Aktivierung während dieser Schlafphase unterstützt. Im Gegensatz dazu konnte gezeigt werden, dass nach einem tiefschlafreichen Schlafintervall die Erinnerung an kontextuelle Informationen verschlechtert war, wenn diese mit einem emotionalen im Vergleich zu einem neutralen Objekt gepaart wurde. Diese gegensätzlichen Befunde zur Rolle von Tiefschlaf bei der Konsolidierung emotionaler, kontextueller Informationen können möglicherweise auf Unterschiede in der Beziehung zwischen emotionalem Item und den jeweils assoziierten Kontextinformationen zurückgeführt werden. Zukünftige Studien sollten den Einfluss möglicher modulierender Faktoren (z.B. die Relevanz des Kontextes für die Verarbeitung von Iteminformationen) aufdecken.

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Publications

Original Articles

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Poster Presentations

Groch, S., Wilhelm, I., Diekelmann, S., Sayk, F., Gais, S., Born, J. (2009). Einfluss von Noradrenalin auf die Konsolidierung emotionaler Gedächtnisinhalte während frühem Schlaf. Tagung „Psychophysiologie und Methodik (APM)“, Leipzig, Juni 2009.

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