# **Original article**

# Effects of a daytime nap on the recognition of neutral and emotional memories

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**Background:** Memory can be facilitated by the emotional strength of encoded information. Offline consolidation during sleep (post-learning sleep, even as little as a nap,) also modulates declarative memory processing. Previous studies have investigated the influence of sleep, especially rapid eye movement (REM) sleep, on emotional memory facilitation in humans. However, the interaction between emotional valence asymmetry and nap-dependent memory consolidation is poorly understood.

**Objective:** To investigate the effects of post-learning nap on the recognition of neutral and emotional memories. **Method:** Ten healthy male participants completed a study session involving 240 emotional (negative and positive stimuli with different arousal magnitudes) and neutral pictures of people, animals, objects, and landscapes. Participants were then immediately tested on the visual recognition performance, in which they need to make recognition judgments on a subset of previously seen ("old") pictures and intermixed unseen ("new") pictures containing similar emotional and semantic contents. Three hours after this initial baseline test, one-half of the participants obtained a 90-minute nap opportunity, recorded with electroencephalography (EEG), whereas the others remained awake. Participants were again tested on the remaining "old" and "new" pictures at 6 hours after learning session.

*Results:* The results revealed a beneficial effect of delayed post-learning nap on the recognition of neutral declarative memory. The extent of neutral memory facilitation was negatively correlated with the amount of stages-2 NREM. Unlike previous studies, the recognition performance for negative emotional items with high arousal condition in the Nap group deteriorated across an offline time rich in stage-2 NREM sleep. However, both groups showed similar decrease in recognition accuracy for positive stimuli.

*Conclusion:* Our findings suggest that declarative memories containing distinct emotional valence and arousal are consolidated differentially during wakefulness and sleep. Under certain conditions, a daytime nap rich in stage-2 NREM sleep may play an important role on these performance differences.

Keywords: EEG, emotion, nap, NREM, recognition memory

A wealth of research evidence demonstrates that memory processing is modulated by emotion [1, 2]. Memory formation can be strongly modulated by the elicitation of emotion at the time of learning [3]. Not only are events that evoke emotions are encoded more strongly, but their memory also appears to persist and even improve over time as the delay between encoding and retrieval increases (from hours to days) [4-6]. In the aspect of sleep and memory processing, diverse studies indicate that sleep, and its varied stages, contribute to latent processes of both declarative and procedural memory consolidation [7-9]. The role of sleep in declarative memory consolidation may depend on more intricate aspects of the information being learned, such as novelty, meaning to extract, and also the affective salience of the material [10].

A number of studies have investigated the influence of sleep on emotional memory consolidation in humans. These studies demonstrated a memory advantage across periods containing sleep in comparison with equivalent periods awake and especially in late-night sleep, a period rich in stage-2 NREM and REM sleep [11, 12]. Furthermore, this emotional memory enhancement has been shown to persist in a follow-up study performed 4 years later [13]. Based on concomitant REM sleepphysiology

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and the neurobiological requirements of emotional memory processing [1, 2], recent work has begun to test a selective REM sleep-dependent hypothesis of human emotional memory consolidation. More recently, it has been shown that only a short period of daytime nap also supports a selective REM sleep-dependent hypothesis of affective memory consolidation in humans [14]. By using a nap paradigm instead of whole night sleep, we demonstrate that sleep and specifically neurophysiology of REM sleep may underlie the consolidation benefits. A selective enhancement of negative emotional memory was observed only across an offline time containing nap. The extent of negative emotional memory facilitation was correlated with the amount of REM sleep and also with right-dominant prefrontal theta power during REM (activity in the frequency range of 4.0–7.0 Hz). These lines of evidence suggest that the enhancement of memories for negative emotional items is associated with specific REM sleep characteristics and is independent of nocturnal hormonal changes. Based on these correlations, researchers have hypothesized that offline time containing sleep, especially REM sleep, may offer a neurobiological state that is particularly amenable to emotional memory consolidation [10, 12, 15].

However, the majority of studies investigating sleep-dependent emotional memory consolidation have principally examined negative and arousing emotional stimuli [16]. Emotions are not necessarily share a unidimensional structure. Emotions have commonly been categorized along two dimensions: arousal (ranging from calm to excited) and valence (ranging from unpleasant to pleasant, with neutral considered an intermediate level [17, 18]. Moreover, evidence suggests that two-dimensionalmodels of emotion influence memory performance via distinct cognitive and neural processes [19, 20].

To date, although a number of reports have investigated the influence of sleep on emotional memory consolidation in humans, no study has to our knowledge investigated the interaction between valence asymmetry and sleep-dependent emotional memory consolidation by using a nap paradigm. Using a visual recognition task, both positive and negative emotional stimuli with different arousal magnitudes are used in the current study to investigate the effect of daytime napping on consolidation of neutral and emotional memories. We hypothesize that affective memories are selectively facilitated by REM sleep, and distinct dimensions of emotional memory are differentially modulated by sleep.

# Material and methods

# Participants

Ten male subjects between the age of 18 and 25 were randomly assigned to either a Nap group (n = 5;mean age 24.0 years [SEM  $\pm$  0.2]) or No-Nap group  $(n = 5; mean age 24.0 years [SEM \pm 0.9])$ . All subjects were right-handed as measured by the Edinburgh Handedness Inventory [21]. None of the subjects had prior history of drug or alcohol abuse, abnormal vision, psychiatric, neurological, or sleep disorders. Subjects maintained a regular sleep schedule 3 days prior to the study and abstained from caffeine, alcohol, and nonexperimental naps throughout the course of the study. Written informed consent was obtained from all subjects prior to the study. All experimental procedures were approved by the human research ethics committee of the Institutional Review Board, Mahidol University.

#### Visual recognition task

The visual recognition task was composed of realistic pictures depicting emotional and neutral scenes obtained from the internet using Google Image. All pictures were resized and converted into a 1024  $\times$ 768 pixel format. A total of 360 pictorial stimuli were compiled. These pictures were divided into 120 negative, 120 neutral, and 120 positive pictures. Each of the valence categories contained a similar proportion of animals, humans, objects, and landscapes, matched in terms of visual stimulus characteristics and semantic contents across sets (including brightness and contrast). To perform complementary analyses on different levels of arousal, this study separated the set of emotional pictures (both positive and negative valences) in two subsets (moderate and high arousal levels) of 60 pictures each. The set of 120 neutral picture stimuli were used to obtain a group of low arousal pictures. Emotional and neutral pictures were intermixed, and the order of presentation was pseudorandom, with no more than 3 stimuli of either emotional or neutral categories being presented in succession. The visual recognition task was designed by using GENTASK program from STIM software version 2.4 (Neurosoft). Participants viewed the pictures on a 17-inch computer screen.

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# Experimental procedure

Both groups performed three experimental sessions: one study session (encoding stage) and two recognition sessions (retrieval stage) (Fig. 1). Before starting each experimental session, the Stanford Sleepiness Scale (SSS) which is a numerical scale 1-7 (1 = being least sleepy, 7 = most sleepy) and the Stress Visual Analog Scale (SVAS) were firstly performed to rate levels of subjective alertness/ sleepiness and stress, respectively. At the study session, both groups viewed 240 pictures: 80 emotionally positive (pleasant valence, 40 high and 40 moderate arousal), 80 negative (unpleasant valence, 40 high and 40 moderate arousal) and 80 neutral (neutral valence, low arousal) picture stimuli. Participants viewed the pictures in 4 trials of 60 pictures, separated by two-minute intertrial intervals. Trials began with the presentation of an initial fixationcrosshair (white cross on black background) for 500 ms, and then the picture stimulus was displayed for 2500 ms, followed by a blank screen (500 ms). Participants were unaware that their memory for the pictures would be subsequently tested (incidental encoding). During two recognition testing sessions, 120 new pictures of similar emotional and semantic contents were intermixed with previously viewed 240 pictures, 1/3 presented during initial baseline test and 2/3 at retest [22]. At the initial baseline test, participants were immediately tested on the visual recognition ability. Participants undertook a surprise recognition memory test in which they viewed 120 picture stimuli in 2 trials of 60 pictures, separated by two-minute intertrial intervals. This session consisted of 81 previously learned pictures ("old" items) from the study session : 27 negative, 27 neutral, and 27 positive pictures, together with intermixed 39 new picture stimuli ("new" items), including 13 pictures not previously seen in each of the valence categories.

Following the initial testing session, at approximately 3 hours later (2:30 PM), participants in the Nap group obtained a 90-min sleep opportunity, recorded with electroencephalography (EEG), whereas those in the No-Nap group remained awake. All nap subjects were never awakened from SWS or REM sleep, as determined using the international criteria of Rechtschaffen and Kales [23], to reduce sleep inertia and the resulting disorientation and confusion experienced [22]. The retest session occurred approximately five and a half hours after the study session (4:30 PM). Both groups viewed a set of 240 pictures in 4 trials of 60 pictures, separated by two-minute intertrial intervals. A set of pictures consisted of 159 "old" items from the study session and 81 "new" items, including an equal amount of pictures in each of the valence categories. Both recognition testing sessions began with the fixationcrosshair (500 ms), followed by the target picture (1000 ms). Next, a respond screen prompted participants to perform an old/new discrimination using a STIM respond pad (Neurosoft), indicating whether they believe the stimuli to be old (from the study session) or new (not seen before). The next trial was not begun until participants make a recognition judgment. During the interval between the two testing sessions, participants were allowed to leave the lab and go about their normal daily activities [14], with the exception of the 90-min sleep opportunity in the Nap group.

# Electroencephalographic recording and sleepstage classification

The set of 31 scalp electrodes with 1 additional ground was placed according to the International 10-20 system [24] at FP1, FP2, FZ, F3, F4, F7, F8, FT7, FC3, FCZ, FC4, FT8, T3, T4, T5, T6, TP7, TP8, C3, CP3, C4, CZ, CPZ, CP4, P3, P4, PZ, O1, O2, and OZ. Both mastoids (A1 and A2) were used as the recording reference (average of both mastoids, ((A1 + A2)/2)). The electrooculogram (EOG) was monitored with 4 electrodes placed on both external canthi (HEOL and HEOR), left supraorbital (VEOU), and infraorbital (VEOL) regions. Electro-Caps are made of an elastic spandex-type fabric with recessed, silver/silver chloride (Ag/AgCl) electrodes attached to the fabric. Electrode impedances were set below 5 k $\Omega$ . The recording system was an Acquire Neuroscan version 4.3 (Neurosoft). The online filter was set to a band pass with the low pass filter at 70 Hz and the high pass filter set at DC. The analog-todigital rate was 500 Hz. Gain was set at 19. A notch filter was set at 50 Hz. Under blinded conditions, sleep stages were scored by a pediatric neurologist using the standard criteria [23]. The EEG data was classified visually, epoch by epoch, as either NREM sleep stages 1-4, REM sleep, awake or movement time. Slowwave sleep (SWS) was calculated as the combination between NREM stages 3 and 4.

# Memory recognition performance and sleep-stage correlations

Recognition memory was assessed immediately after learning and again after the sleep/wake retention period (initial baseline testing and retest, respectively). From participants' judgments in both recognition testing sessions, 4 response categories were possible: correctly identify previously viewed "old" pictures ("hits"), incorrectly identify previously viewed "old" pictures ("misses"), correctly identify unseen "new" pictures ("correct rejections"), and incorrectly identify unseen "new" pictures ("false alarms"). A memory discrimination score ( $P_r$  score) was calculated according to two-high-threshold model (i.e. the difference between the proportions of hit and false alarm rates:  $P_r = P(\text{hits}) P(\text{false alarms}))$  [25]. This measurement has been widely used to assess the recognition accuracy of both neutral and emotional stimuli in visual recognition tests [26-31]. The change in recognition accuracy across an offline time was indexed as the difference in  $P_{\mu}$  scores for items from both testing sessions ( $P_r$  initial test –  $P_r$  retest). To examine the relationship between the specific stages of sleep and the change in memory recognition performance obtained across participants in the Nap group, sleep-stage values including both percentages and durations of each sleep-stage were correlated with the difference in  $P_r$  scores between both testing sessions by using Person's correlation coefficients.

#### **Results**

#### Sleepiness and stress measures

Participants completed three SSS and SVAS before undertaking each experimental session (**Figure 1**). SSS scores (mean ± SEM) for each measure (study, initial test, and retest) were, respectively; Nap =  $3.40 \pm 0.68$ ,  $3.20 \pm 0.37$ ,  $3.80 \pm 0.86$ ; No-Nap =  $4.00 \pm 0.63$ ,  $3.20 \pm 0.49$ ,  $4.00 \pm 0.91$ . SVAS scores (mean ± SEM) for each session (study, initial test, and retest) were, respectively; Nap =  $2.85 \pm 0.42$ ,  $3.40 \pm 0.75$ ,  $3.40 \pm 1.03$ ; No-Nap =  $3.70 \pm 1.22$ ,  $3.60 \pm 1.30$ ,  $4.50 \pm 1.19$ . One-way ANOVAs showed no differences between groups using these subjective ratings at all experimental sessions [Study: SVAS ( $F_{1.8} = 0.42$ , p = 0.54); Initial test: SVAS ( $F_{1.8} = 0.02$ , p = 0.90), SSS ( $F_{1.8} = 0.00$ , p = 1.00); Retest: SVAS ( $F_{1.8} = 0.04$ , p = 0.86), SSS ( $F_{1.8} = 0.00$ , p = 1.00)].

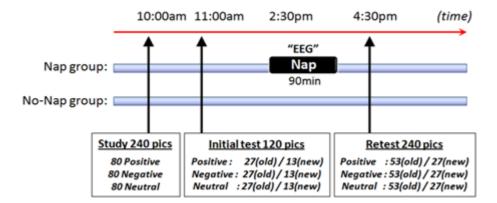


Figure 1. Experimental design. Participants viewed a set of 240 neutral and emotional pictures at the study session. Emotional stimuli (both positive and negative valence) were equally subdivided in two subsets based on different levels of arousal (Moderate and High arousal). During two recognition testing sessions (immediate test and retest), 120 unseen ("new") pictures of similar emotional and semantic contents were intermixed with previously viewed ("old") 240 pictures, 1/3 presented during initial baseline test and 2/3 at retest. Following the initial testing session, the Nap group obtained a 90-min nap opportunity, recorded with electroencephalography (EEG), whereas participants in the No-Nap group remained awake. All groups were then retested on the remaining "old" and "new" picture stimuli.

#### **Recognition memory performance**

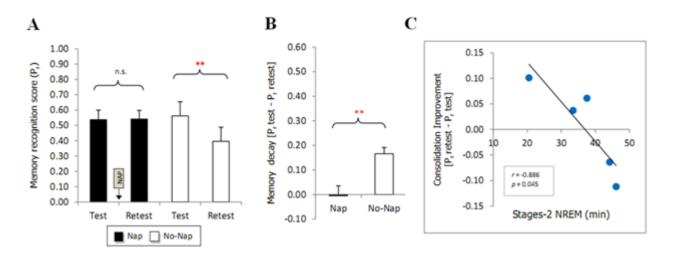
Emotional items were slightly better recognized than neutral ones during the initial test session in both groups (Table 1). We confirmed that both groups performed similarly in all memory types during initial test (all p > 0.53). In the No-Nap group, there was a strong deterioration of memory recognition performance for neutral items during retest compared with items immediately tested after learning (**Figure 2A**) [paired t test, t(4) = 6.63, p = 0.003]. In marked contrast, recognition performance for neutral items in the Nap group was nearly identical between two tests (Figure 2A) [paired t test, t (4) = -0.11, p = 0.91]. The performance difference in recognition of neutral declarative memory between both groups was also evident when quantified as the subtracted difference in recognition memory across an offline

time [( $P_r$  initial test –  $P_r$  retest); Fig. 2B] [*t* test, *t* (8) = -3.62, *p* = 0.007; No-Nap: *M* = 0.17, *S.E.* = 0.02; Nap: *M* = -0.005, *S.E.* = 0.04].

Nap group showed less decrease in recognition accuracy for positive items than No-Nap group, but it was not significant [t test, t (8) = -1.30, p = 0.23; No-Nap: M = 0.14, S.E. = 0.07; Nap: M = 0.04, S.E. = 0.04]. According to complementary analyses of positive pictures per different levels of arousal (moderate vs. high arousal), both groups showed a similar nonsignificant decrease in recognition accuracy for positive stimuli containing high arousal contents [t test, t (8) = -0.258, p = 0.803], however, No-Nap group tended to have lower score than Nap group for positive items with moderate arousal condition (**Table 2**) [t test, t (8) = -1.917, p = 0.092].

**Table 1.** Memory discrimination score ( $P_r$  score) for emotional and neutral items in the Nap and No-Nap group<br/>(mean±SEM)

	Negative		Neutral		Positive	
	Initial test	Retest	Initial test	Retest	Initial test	Retest
Nap group	$0.78 \pm 0.08$	$0.66 \pm 0.06$	$0.53 \pm 0.06$	$0.54 \pm 0.06$	$0.68 \pm 0.04$	$0.64 \pm 0.07$
No-Nap group	$0.70 \pm 0.09$	$0.66 \pm 0.08$	$0.56 \pm 0.09$	$0.39 \pm 0.09$	$0.65 \pm 0.09$	$0.51 \pm 0.14$



**Figure 2.** A) Memory recognition performance ( $P_r$  score) for *neutral items* in the Nap and No-Nap groups at both recognition testing sessions. B) The change in recognition memory across an offline time ( $P_r$  initial test  $P_r$  retest) for the Nap and No-Nap groups. C) Correlation between the amount of change in recognition memory in the Nap group (i.e.,  $P_r$  retest –  $P_r$  initial test) and stages-2 NREM sleep amount (minutes). Person's *r* and significance (*p*) displayed in figures, with statistical value and regression line. Test: initial baseline testing session, Retest: retest session. \*\*p < 0.01; n.s., nonsignificant. Error bars represent standard error of mean.

<b>Table 2.</b> Memory discrimination score $(P_{r})$	r score) for emotional and neutral items with high and moderate arousal level
in the Nap and No-Nap group (me	$ean \pm SEM$ )

		Negative		Positi	ve
		High	Moderate	High	Moderate
Nap group	Initial test	$0.90 \pm 0.04$	0.67±0.12	$0.78 \pm 0.04$	$0.58 \pm 0.06$
	Retest	$0.77 \pm 0.05$	$0.55 \pm 0.08$	$0.68 \pm 0.09$	$0.59 \pm 0.07$
No-Nap group	Initial test	$0.74 \pm 0.08$	$0.67 \pm 0.12$	$0.64 \pm 0.05$	$0.67 \pm 0.14$
	Retest	$0.78 \pm 0.08$	$0.54 \pm 0.09$	$0.50 \pm 0.13$	$0.52 \pm 0.15$

Interestingly, there was an offline deterioration in recognition accuracy for negative items in Nap group [paired t test, t (4) = 3.13, p = 0.03], whereas No-Nap group showed no significant difference in recognition performance between both tests (Fig. 3A) [paired t test, t(4) = 1.18, p = 0.30]. Nevertheless, a difference in recognition accuracy for negative items between both groups fell short of statistical significance when compared as the subtracted difference in recognition memory (Figure 3B) [t test, t(8) = 1.56, p = 0.16; Nap: M = 0.12, S.E. = 0.04; No Nap: M = 0.04, S.E. = 0.03]. Moreover, this lower recognition accuracy for negative items in Nap group was only observed in the high arousal condition (Fig. 3C) [paired t test, t (4) = 2.83, p = 0.05]. The performance difference in recognition memory for this high arousal condition between Nap and No-Nap groups was nearly significant (Fig. 3D) [t test, t (8) = 2.068, *p* = 0.07; Nap: *M* = 0.13, *S.E.* = 0.05; No-Nap: M = -0.04, S.E. = 0.07].

#### Sleep-stage correlations

To examine the relationship between recognition memory performance following nap and sleep stages in the Nap group, sleep-stage data were correlated with the difference in recognition memory across an offline time ( $P_r$  initial test –  $P_r$  retest). The sleepstage amounts of the nap are summarized in Table 3. The extent of neutral memory retention in Nap group was negatively correlated with the amount of stage-2 NREM sleep (r = -0.886, p = 0.045; Figure 2C) obtained across subjects. No other sleep-stages (stage-1 NREM, SWS) or total sleep duration correlated with this retention of neutral declarative memory (all p > 0.05). While we found significant deteriorations in recognition accuracy for negative items in the Nap group [both for total (Figure 3A) and high arousal conditions (Figure 3C)], no significant relationships were evident between these deteriorations and any sleep parameter (Total: all p > 0.16; High arousal: all p > 0.05).

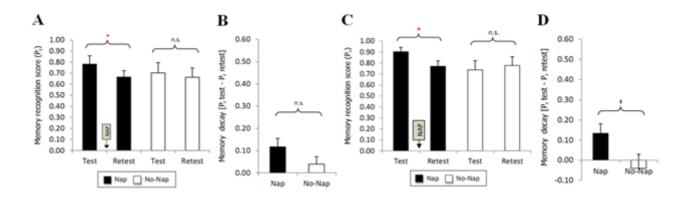


Figure 3. A) Memory recognition performance ( $P_r$  score) for *negative emotional items* in the Nap and No-Nap groups at both recognition testing sessions. B) The change in recognition memory for *negative emotional items* across an offline time ( $P_r$  initial test –  $P_r$  retest) for the Nap and No-Nap groups. C) Complementary analysis of recognition performance for *negative emotional items with high arousal level* and D) the change in recognition memory for these items across time. Test: initial baseline testing session, Retest: retest session. \*p < 0.05; ( $\Box p = 0.07$ ; n.s., nonsignificant. Error bars represent standard error of mean.

	Minutes	% of total nap time	Obtained by % of participants
Total nap time	$44.8 \pm 7.3$		
Stage 1	$4.5 \pm 1.5$	$11.1 \pm 3.8$	100%
Stage 2	$36.4 \pm 4.6$	83.1±5.7	100%
SWS	$3.8 \pm 3.8$	$5.4 \pm 5.4$	20%
REM	0	0	0%

<b>Table 3.</b> Sleep parameters for the Nap group (mean $\pm$ SEM)	Table 3. Sleep	parameters for	the Nap group	$(mean \pm SEM)$
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#### **Discussions**

Using a nap paradigm, we investigated the stabilization effect of sleep on recognition of neutral declarative memory. Subjects taking an early afternoon nap after learning had higher recognition accuracy for neutral items than subjects that remain awake during the baseline-retest interval.

A number of previous studies comparing the recognition performance between emotional- and neutral items after sleep showed no significant difference in the retention of nonemotional declarative memories between sleep and wake groups [11, 14]. However, it should be noted that subjects in these studies sleep immediately after learning rather than sleep at delayed time used in the current study. To this extent, our increasing recognition accuracy for neutral items seen in the Nap group is compatible with the established literature. Alger et al. used a staggered nap paradigm to investigate the effects of delaying sleep onset on retention of nonemotional declarative memory. The authors found a significant negative correlation between the average time spent before napping and decline in recognition performance, in which the 4-hour delayed napping group showed superior retention of neutral declarative memory compared to those who napped immediately after learning and those who remained awake [22]. In the current study, subjects napped approximately 3-hour after to initial testing session. This specific sleeping time window may necessary for the consolidation of neutral episodic memory.

There is now compelling evidence indicating that NREM sleep, especially slow-wave sleep (SWS), promotes the consolidation of nonemotional declarative memories. SWS has been hypothesized to provide the optimal electrophysiological and biochemical state for the processing of hippocampus-dependent declarative memories [8, 32]. Unlike previous studies, our improvement of neutral declarative memory in the Nap group was not correlated with SWS obtained across subjects. Instead, there was an inverse relationship between the recognition accuracy for neutral items and amount of stage-2 NREM. However, the mechanisms underlying this unanticipated association are still unclear.

The current study demonstrated an offline deterioration in negative emotional memory after a period containing sleep relative to an equal amount of time spent awake. Moreover, this lower recognition accuracy for negative items after a daytime nap was only observed in the high arousal condition. However, these significant declines in recognition performance were not observed when compared as the subtracted difference in recognition memory between both groups. Although our study showed no evidence for the facilitation effect of sleep on negative emotional memory, we are not suggesting that emotional memories do not benefit from sleep. A number of studies investigating the influence of sleep on affective memory consolidation in humans found an enhancement of recognition performance for the item containing a negatively emotional component after a period of sleep. It has been proposed that post-learning sleep can modulate emotional memory, making the memory trace more salient with corresponding stronger neuronal connections [29, 33]. Specifically, the extent of emotional memory facilitation was correlated with the amount of REM sleep and together with right-dominant prefrontal theta power during REM [14]. According to the sleep data obtained in previous literature, a short daytime nap should occur at least 90 minutes to obtain REM sleep [32] or even in a shorter time (60 min) during which subjects could enter REM sleep [14]. Although a 90-minute daytime nap was introduced to our study, none of subjects achieved REM sleep. This lacking of REM sleep may be the result of subjects' sleep efficiency. Total nap time of the current study was approximately 45 minutes, which theoretically insufficient to achieve REM sleep. The majority of sleep-stage in our Nap

group was stage-2 NREM sleep which can affect consolidation of emotional memory.

One possible explanation for our deteriorative effect of sleep on negative emotional memory is that reactivations of memory representations during NREM sleep, without subsequent REM sleep, may create transient destabilization of encoded information leading to increased rate of forgetting. It has been suggested that neuronal reactivations of newly encoded hippocampus-dependent memory traces during NREM sleep promote the gradual redistribution of these memory traces from hippocampal to neocortical storage sites for long-term memory [9]. This hippocampal-neocortical reactivation mostly occurs during SWS. Recently, compelling evidence from neuroimaging studies in humans has demonstrated that sleep spindles, a transient oscillatory pattern of 12-16 Hz that occurs during stage-2 NREM sleep, also play an important role in the reactivation of new hippocampal-neocortical memories during sleep [34, 35]. In the aspect of reconsolidation theory, both transient destabilization after reactivation and subsequent reconsolidation of reactivated memories which occur sequentially during sleep are candidate mechanisms underlying performance improvement after sleep [9, 36]. Transient destabilization after reactivation during NREM sleep could promote the redistribution of newly encoded information to longterm storage sites by gradually loosening synaptic connections in hippocampus for the benefit of direct cortico-cortical connections leading to integration of transiently labilized memory into preexisting neocortical networks [36, 37]. Subsequently, REM sleep would contribute to strengthened memory representations by enabling synaptic consolidation during the process of reconsolidation [9, 36]. It has been suggested that an upregulation of plasticity-related immediate early genes (IEGs) activity is associated with REM sleep, and is localized to brain regions involved in prior learning [9, 38, 39].

According to these lines of evidence, our results demonstrated that the lack of subsequent REM sleep affects the consolidation of high arousing negative stimuli. Declarative memories associated with high emotional tone may be susceptible to the destabilization after reactivation of memory traces during NREM than memories lacking affective tone. Without synaptic consolidation occurring in REM sleep, destabilization of high arousing stimuli after reactivation during NREM sleep may persist over time or destabilized nonarousing memories are more capable of turning to reconsolidation relative to emotional memories. Therefore, memories for high arousing negative stimuli would then disrupt after sleep relative to an equal amount of time spent awake, leading to increased rate of forgetting which occurred in Nap group. Conversely, the beneficial effects of sleep on nonarousing stimuli are not necessary to obtain REM sleep. Previous studies have demonstrated the advantages of a daytime nap containing solely NREM sleep on nonarousing declarative memory in various memory tasks [32, 40-42]. Although, this evidence leads to suggest that the synaptic consolidation also occurs during NREM sleep, this process does not appear to benefit emotional memory consolidation in a similar manner to that observed during REM sleep. However, direct evidence of this explanation is scarce at present. While the majority of human studies investigating the consolidation of both arousing and nonarousing stimuli have found the facilitating effect of sleep on declarative memories only for high arousal items, subjects in these studies took longer period of sleep which both NREM and REM could occur sequentially [11,12]. Unlike neutral declarative memories, the optimum benefits of sleep on the consolidation of emotional memories may require both NREM and REM sleep taking place in succession. On the other hand, an offline time without sleep may facilitate the retention of negative emotional memories resulting in performance stabilization observed in the No-Nap group. Previous evidence have demonstrated that memory processing can be facilitated by the emotional strength of encoded information [2]. The influences of emotion on memory retention are known to increase as the time delay between encoding and retrieval expands [4-6]. Remembering arousing stimuli remains the same or improves over time, whereas memories for neutral stimuli decrease over time. The slow consolidation of emotional memories serves an adaptive function. It has been suggested that the combined in uence of amygdala and hippocampal structures as well as neurohormones triggered by arousing stimuli appear to modulate memory strength during this slow consolidation process [2, 43].

An alternative explanation for these differences in negative memory performance between groups could be attributed to subjects' sleep efficiency. In the current study the averaged sleep efficiency of subjects in the Nap group was 50%, whereas subjects' sleep efficiency in a related study that found the benefits of a postlearning nap on the consolidation of negative emotional memory was approximately 90% [14]. Increased sleep fragmentation may disrupt the consolidation of high arousing negative stimuli. By contrast with an offline period during wakefulness, the consolidation of these items during sleep may require a continuous sleep in order to facilitate the retention of negative emotional memories. Interestingly, the deteriorative effect of sleep fragmentation was only vulnerable for high arousing items leading to inefficient consolidation, whereas the consolidation of moderate arousing and nonarousing stimuli during sleep could not be disrupted by sleep fragmentation. It should be noted, however, that such difference in recognizing highly aversive memory between both groups fell short of statistical significance when compared as the subtracted difference in recognition memory. A larger subject sample size and a specific experimental procedure (i.e. comparing memory performance between napping subjects that obtain only NREM sleep and those who achieve both NREM and REM sleep, or NREM sleep fragmentation paradigm) would be required to test these compelling results.

Interestingly, our deteriorative effect of a postlearning nap on emotional memory was restricted to high arousing negative items. No similar result was observed for positive stimuli containing high arousal contents. Both groups showed a slight decrease in recognition accuracy for such stimuli; however, the No-Nap group tended to have lower score than the Nap group for positive items with a moderate arousal condition. One possible reason for this contrasting evidence is that the highly positive pictures used in the current study (i.e. happy families, babies, puppies, old couples, baby animals, and waterfalls) had only relatively moderate arousal impact by comparison with highly negative items (i.e. accidents, physical attacks, tumors, disasters, and wars). As we have seen in our result for negative items with a moderate arousal level, the comparably low emotional impact of our positive stimuli likewise failed to reveal any significant effect of sleep on memory performance. Another explanation is that a daytime nap containing only NREM sleep cannot facilitate the consolidation of positive emotional memory regardless of the different levels of emotional arousal. Further study should use positive stimuli with a higher arousal level to test whether a short period of napping can modulate recognition performance for these items.

## Conclusion

Our study demonstrated that a daytime sleep containing only NREM sleep affects declarative memories with distinct emotional valence and arousal in a distinct ways. Although a postlearning nap could facilitate the retention of neutral declarative memory, recognition performance for negative emotional items with a high arousal condition deteriorated across this offline time.

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#### References

- Cahill L. Neurobiological mechanisms of emotionally in uenced, long-term memory. Prog Brain Res. 2000; 126:29-37.
- 2. McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci. 2004; 27:1-28.
- 3. Phelps EA. Human emotion and memory: Interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol. 2004; 14:198-202.
- Levonian E. Retention over time in relation to arousal during learning: An explanation of discrepant results. Acta Psychol (Amst). 1972; 36:290-321.
- 5. LaBar KS, Phelps EA. Arousal-mediated memory consolidation: role of the medial temporal lobe in humans. Psychol Sci. 1998; 9:490-3.
- Sharot T, Phelps EA. How arousal modulates memory: Disentangling the effects of attention and retention. Cogn Affect Behav Neurosci. 2004; 4: 294-306.
- 7. Walker MP, Stickgold R. Sleep-dependent learning and memory consolidation. Neuron. 2004; 44:121-33.
- Marshall L, Born J. The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn Sci. 2007; 11:442-50.
- 9. <u>Diekelmann S, Orn J. The memory function of sleep.</u> Nat Rev Neurosci. 2010; 11:114-26.
- 10. Walker MP. The role of sleep in cognition and emotion. Ann NY Acad Sci. 2009; 1156:168-97.

- 11. Wagner U, Gais S, Born J. Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. Learning & Memory. 2001; 8:112-9.
- 12. Hu P, Stylos-Allen M, Walker MP. Sleep facilitates consolidation of emotionally arousing declarative memory. Psychol Sci. 2006; 17:891-8.
- 13. Wagner U, Hallschmid M, Rasch B, Born J. Brief sleep after learning keeps emotional memories alive for years. Biol Psychiatry. 2006; 60:788-90.
- Nishida M, Pearsall J, Buckner RL, Walker MP. <u>Prefrontal theta during REM sleep enhances</u> emotional memory. Cereb Cortex. 2009; 19:1158-66.
- Pare D, Collins DR, Pelletier JG. Amygdala oscillations and the consolidation of emotional memories. Trends Cogn Sci. 2002; 6:306-14.
- Walker MP, Van der Helm E. Overnight therapy? The role of sleep in emotional brain processing. Psychol Bull. 2009; 135:731-48.
- Lang PJ, Greenwald MK, Bradley MM, Hamm AO. Looking at pictures: Affective, facial, visceral, and behavioral reactions. Psychophysiology. 1993; 30: 261-73.
- 18. Labar KS, Cabeza R. Cognitive neuroscience of emotional memory. Nat Rev Neurosci. 2006; 7:54-64.
- Kensinger EA. Remembering emotional experiences: The contribution of valence and arousal. Rev Neurosci. 2004; 15:241-51.
- 20. Kensinger EA, Corkin S. <u>Two routes to emotional</u> memory: Distinct neural processes for valence and arousal. Proc Natl Acad Sci USA. 2004; 101:3310-5.
- 21. Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. Neuro-psychologia. 1971; 9:97-113.
- Alger SE, Lau H, Fishbein W. Delayed onset of a daytime nap facilitates retention of declarative memory. PLoS One. 2010; 5:e12131.
- Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subject. US Government Printing Office, National Institute of Health Publication, Washington DC; 1968.
- Jasper HA. The ten-twenty system of the International Federation. Electroencephologr Clin Neurophysiol. 1958; 10:371-5.
- 25. Snodgrass JG, Corwin J. Pragmatics of measuring recognition memory: applications to dementia and amnesia. Exp Psychol Gen. 1988; 117:34-50.
- 26. Sharot T, Delgado MR, Phelps EA. How emotion enhances the feeling of remembering. Nat Neurosci.

2004; 7:1376-80.

- 27. Wagner U, Kashyap N, Diekelmann S, Born J. The impact of post-learning sleep vs. wakefulness on recognition memory for faces with different facial expressions. Neurobiol Learn Mem. 2007; 87:679-87.
- Sterpenich V, Albouy G, Boly M, Vandewalle G, Darsaud A, Balteau E, et al. Sleep-related hippocampo– cortical interplay during emotional memory recollection. PLoS Biol. 2007; 5:e282.
- 29. Sterpenich V, Albouy G, Darsaud A, Schmidt C, Vandewalle G, Dang-Vu TT, et al. Sleep promotes the neural reorganization of remote emotional memories. J Neurosci. 2009; 29:5143-52.
- 30. Schaefer A, Pottage CL, Rickart <u>AJ. Electrophysiological correlates of remembering emotional</u> pictures. Neuroimage. 2011; 54:714-24.
- Glaser E, Mendrek A, Germain M, Lakis N, Lavoie ME. Sex differences in memory of emotional images: A behavioral and electrophysiological investigation. Int J Psychophysiol. 2012; 85:17-26.
- Tucker MA, Fishbein W. Enhancement of declarative memory performance following a daytime nap is contingent on strength of initial task acquisition. Sleep. 2008; 31:197-203.
- Ritchey M, Dolcos F, Cabeza R. Role of amygdala connectivity in the persistence of emotional memories over time: An event related fMRI investigation. Cereb Cortex. 2008; 18:2494-504.
- 34. Andrade KC, Spoormaker VI, Dresler M, Wehrle R, Holsboer F, Samann PG, Czisch M. Sleep spindles and hippocampal functional connectivity in human NREM sleep. J Neurosci. 2011; 31:10331-9.
- 35. Bergmann TO, M lle M, Diedrichs J, Born J, Siebner HR. Sleep spindle-related reactivation of category-specific cortical regions after learning face-scene associations. Neuroimage. 2012; 59:2733-42.
- Rasch B, Born J. Maintaining memories by reactivation. Curr. Opin. Neurobiol. 2007; 17:698-703.
- Diekelmann S, Buchel C, Born J, Rasch B. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. Nat Neurosci. 2011; 14:381-6.
- Ribeiro S, Mello CV, Velho T, Gardner TJ, Jarvis ED, Pavlides C. Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. J Neurosci. 2002; 22: 10914-23.
- 39. Ribeiro S, Shi X, Engelhard M, Zhou Y, Zhang H, Gervasoni D, et al. Novel experience induces persistent

sleep-dependent plasticity in the cortex but not in the hippocampus. Front. Neurosci. 2007; 1:43-55.

- Tucker MA, Hirota Y, Wamsley EJ, Lau H, Chaklader A, Fishbein W. <u>A daytime nap containing solely</u> <u>non-REM sleep enhances declarative but not</u> <u>procedural memory</u>. Neurobiol Learn Mem. 2006; 86: 241-7.
- 41. Lau H, Tucker MA, Fishbein W. Daytime napping:

Effects on human direct associative and relational memory. Neurobiol Learn Mem. 2010; 93:554-60.

- 42. Seeck-Hirschner M, Baier PC, Sever S, Buschbacher A, Aldenhoff JB, Goder R. Effects of daytime naps on procedural and declarative memory in patients with schizophrenia. Psychiatr Res. 2011; 44:42-7.
- 43. McGaugh JL. Memory: A century of consolidation. Science. 2000; 14:248-51.