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ORIGINAL ARTICLE

Opposite effect of motivated forgetting on sleep spindles during stage 2 and slow wave sleep

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Abstract

Memories selectively benefit from sleep. In addition to the importance of the consolidation of relevant memories, the capacity to forget unwanted memories is also crucial. We investigated the effect of suppressing unwanted memories on electroencephalography activity of subsequent sleep using a motivated forgetting (MF) paradigm as compared with a control non-forgetting task. Subjects were randomly assigned to nap or no-nap groups. We used a modified version of the think/no-think paradigm with dominant number of no-think words cued to be forgotten and included only subjects capable of suppressing unwanted memories by performing an initial subject inclusion experiment. In both groups and conditions, the performance of the subjects in recalling the word pairs learned in the beginning of the day was evaluated in a final recall test. We found that both nap and no-nap groups recalled significantly less no-think words in the MF condition compared to the control condition. Moreover, for the nap group, in the MF compared to the control condition, spindle power and density increased during stage 2 (S2) whereas they decreased during slow wave sleep (SWS). Interestingly, recall performance of no-think words was negatively correlated with spindle power during S2 whereas it was positively correlated with spindle power during SWS. These results indicate that sleep spindles are sensitive to the previous MF experiences and suggest a differential role of sleep spindles during S2 and SWS in memory processing during sleep.

Statement of Significance

Although sleep spindles are present in two different brain states, i.e. stage 2 (S2) and slow wave sleep (SWS), it is poorly understood how the involvement of sleep spindles in memory processing depends on these two non-rapid eye movement sleep stages. Moreover, in addition to memory consolidation, it is important to understand the link between forgetting of unwanted memories and sleep. Here for the first time, to the best of our knowledge, we studied motivated forgetting-dependent changes in spindle activity separately during S2 and SWS. The striking finding was that the retention of to be forgotten words was oppositely correlated with spindle activity during S2 compared to SWS. These results suggest the importance of the macro states for the involvement of sleep spindles in memory processing during sleep.

Key words: motivated forgetting; sleep spindles; stage 2 sleep; slow wave sleep

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Introduction

It is thought that sleep plays a critical role in the consolidation of new memories [1]. There is evidence to suggest that different stages of sleep have differential roles on reprocessing of memories during sleep [2, 3]. A striking feature of cortical activity during slow wave sleep (SWS) is 0.5–2 Hz oscillations known as slow oscillations (SOs). Thalamocortical spindles, which are waxing and waning oscillations (8–12 Hz slow spindles, 12–16 Hz fast spindles), are another spontaneously generated oscillatory phenomenon that are present during stage 2 (S2) as well as SWS and are an electrophysiological hallmark of non-rapid eye movement (NREM) sleep. Growing evidence suggests the involvement of sleep spindles in the consolidation of both declarative and procedural memories during sleep [4–6]. Previous studies showing the correlation between sleep spindles during NREM sleep and declarative memory consolidation mostly focused on either the entire NREM sleep period [7, 8] or only one stage, i.e. S2 [9–14] or SWS [15, 16]. However, few studies compared the correlation between sleep spindles and memory consolidation separately for S2 and SWS [17, 18]. Specifically, it has been shown that although there was a significant positive correlation between declarative memory retention and spindle activity during SWS, there was no significant correlation between memory retention and spindle activity during S2 [17]. In a recent study investigating the causal role of sleep spindles in memory consolidation using transcranial alternating current stimulation, it has been shown that inducing sleep spindles only in S2 did not improve declarative memory [5]. Moreover, the importance of the coupling between SOs, which are dominant during SWS, and sleep spindles in the processing of declarative memory was suggested in several studies [19–23]. It has also been suggested that spindle activity during S2 (transition to rapid eye movement sleep [REM] sleep in animals), when locus coeruleus neurons are silent, may enhance targeted forgetting [24]. These findings suggest the differential contribution of sleep spindles in S2 versus SWS to memory processing during sleep.

In addition to the importance of the consolidation of relevant memories, the capacity to forget unwanted memories is also crucial. Emerging reports suggest that only selective memories benefit from sleep [8, 25–29]. Especially using the directed forgetting paradigm, it has been shown that only memories previously cued to be remembered are facilitated during sleep whereas the retention of to be forgotten items does not change significantly by sleep [7, 30]. Although previous studies showed changes in aspects of postlearning sleep physiology [10, 11, 18, 31, 32], the effect of suppression of unwanted memories on the following sleep is not known.

Here, we investigated the changes in electroencephalography (EEG) activity during sleep, specifically sleep spindles, separately during S2 and SWS after a motivated forgetting (MF) task using a modified version of the think/no-think (TNT) paradigm with a dominant number of no-think words. The TNT protocol was first introduced by Anderson *et al.* [33, 34] to study MF, which refers to down prioritization through active inhibitory control, toward increased forgetting of unwanted memories. The TNT task involves the learning of word pairs (cue and target) to a predetermined level of accuracy. Once learned, the cue is presented and the participant is instructed to either "think" or "not think" of previously associated targets. It has been previously suggested that not all people are capable of successful suppression of unwanted memories [35, 36]. Hence, we included only subjects capable of suppressing unwanted memories in the main experiment by performing an initial subject inclusion experiment. After learning a set of word pairs, subjects engaged in the MF task (MF condition) and in a control non-forgetting task in the control (Ctrl) condition. EEG activity during subsequent sleep was compared between the MF and control conditions, separately during S2 and SWS. We especially sought to determine the correlation between the changes in spindle activity and the retention of no-think words. We further tested the impact of sleep on retention of no-think words by including an additional no-nap group.

Methods

Subjects

Initially 89 volunteers (age range: 19–30 years; 14 females) participated in the inclusion experiment. All participants were right-handed, native Persian speakers, with similar educational backgrounds (undergraduates or MSc students), and had normal or corrected-to-normal vision. They reported normal nocturnal sleep patterns (7–9 hour starting between 10 pm and 12 am) for the week before the experiment. They had not used caffeine, nicotine, or energy drinks on the experimental day and had not performed excessive exercise within the last 24 hours. As assessed by questionnaire, participants had no history of any neurological or psychiatric disorders. All subjects gave written informed consent. The experimental protocols were approved by the ethics committee of the Ferdowsi University of Mashhad. All volunteers were rewarded with either monetary compensation or course credits.

Initial subject inclusion experiment

In order to investigate the neural correlates of forgetting we included only participants who proved to be capable of suppression through MF. The aforementioned characteristic was evaluated through an initial experiment with a task similar to the one introduced by Benoit *et al.* [37]. The task consists of three phases: learning, TNT (MF), and a final recall test (Supplementary Figure S1). In the learning phase, first, participants were exposed to 30 word pairs, 10 in each category: no-think, think and baseline. Selected words were Persian, abstract, emotionally neutral, and comprised four or five letters [38]. They were also controlled for frequency of occurrence in a comprehensive Persian language corpus. In the next phase, for each word pair of the three categories, the first word was presented and subjects were required to report the associated word. The corresponding word pair was subsequently presented, and this sequence of words was repeatedly presented until the subjects had learned 50% of words (the task was terminated and the subject was excluded if after five presentations the participant had not achieved minimum accuracy). At the end of the learning phase, the first word was presented and subjects were instructed to tell their response as soon as they recalled the associated word (baseline recall test). The MF phase followed, in which cue words were randomly and repeatedly presented 16 times each, whereas baseline words were not presented. The color of the cue words defined whether the word pairs belonged

to the think or no-think categories. For the think category (green cue words), subjects were instructed to think of the response word and keep it in mind the entire time that the cue was on the screen. For the no-think category (red cue words), they had to pay full attention to the cue word, but they were instructed to keep the response word out of consciousness for the entire trial. Half an hour after the TNT phase a final recall test was carried out for all items. Recall of each word was accepted only if it was presented by the participants in less than 3.4 seconds [37]. Participants were included in the main experiment only if (1) they had forgotten at least one of the words belonging to the no-think category (which had been remembered on the baseline recall test) and (2) the difference between the number of forgotten words belonging to the no-think category and baseline category was greater or equal to one.

Main experiment

Of 37 subjects participating in the main experiment, 22 subjects were assigned to the nap group and 15 subjects were assigned to the no-nap group, randomly. Subjects assigned to the nap group attended an adaptation session around 1 week prior to the main experiment, which allowed them to become familiar with the experimental setup, procedures, and the EEG recording room (the flowchart of the experimental protocol is shown in Supplementary Figure S2. Two subjects were not able to nap in the adaptation session and hence were excluded from the main experiment.

On the MF experimental day, subjects arrived at the laboratory at approximately 10 am. They watched a movie until 12 pm and then engaged in a word pair associate learning task similar to the learning phase of the initial inclusion experiment explained earlier, but with different word pairs and a different amount of TNT word pairs. Participants first learned 20 word pairs, of which 5 were later randomly assigned to the think and 15 to the no-think category. The main experimental protocol is depicted in Figure 1. Thereafter, they were allowed to rest in the laboratory and had a snack. Then, they engaged in an MF task similar to the TNT phase of the initial subject inclusion experiment (Figure 2A). At 2 pm, subjects were prepared for EEG recording and were instructed to nap for as long as they could, but were awakened at the latest 140 minutes after the recording onset. Finally, the performance of the subjects was tested in a final recall phase. We assessed subjects' sleepiness by Stanford Sleepiness Scale before the task (after learning) as well as before final recall test (the sleepiness scale was 1.2 ± 0.20 and 1.5 ± 0.27 for the control and MF conditions, respectively). During both the baseline and the final recall test subjects were instructed to give their response as soon as they recalled the associated word. All response times were recorded. Only responses given within 5 seconds after presentation of the word were evaluated (the time window was not known to the subjects). The threshold of 5 seconds was chosen so that similar to the inclusion experiment approximately 85% of response times were below the threshold value and so the corresponding words were considered as remembered ones (Supplementary Figure S3).

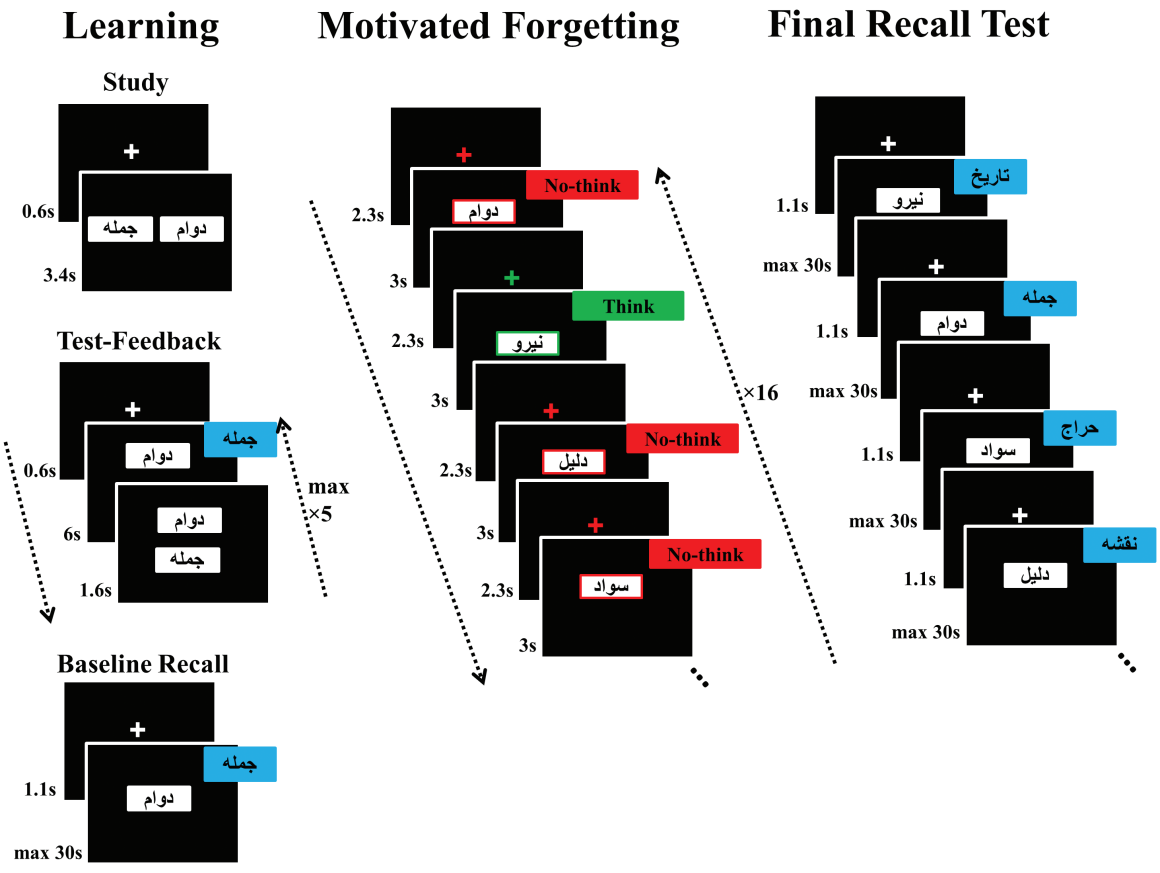

Figure 1. Schema of the main experimental protocol during which participants first learned 20 word pair (learning phase). During the MF phase, subjects were required to suppress the words associated with the red-cued words (15 pairs) and remember the words associated with the green-cued words (5 pairs). A later final recall test evaluated the performance of the participants in the MF task.

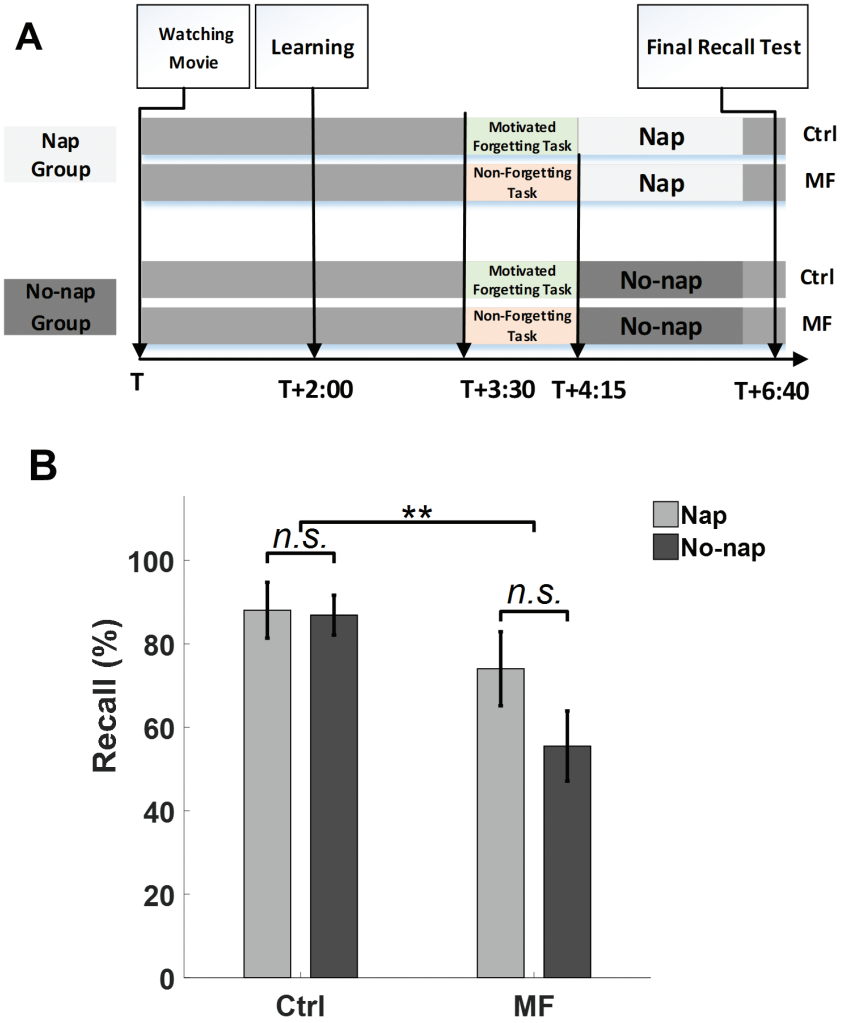

Figure 2. Study design and behavioral data. (A) Time course of the MF and control conditions for both the nap and no-nap groups describing watching movie, learning phase, MF/control non-forgetting task, and final recall test. *T* = 8 am for both control and MF conditions of the no-nap group and *T* = 10 am for both control and MF conditions of the nap group. (B) Recall rate of no-think words in the control (Ctrl) and MF conditions for the no-nap (black, 15 subjects) and nap (gray, 10 subjects) groups. (**p-value = 0.01, n.s. p-value >0.1). Error bars represent standard error of the mean.

Recall rate was defined as the number of recalled no-think words in the final recall test divided by the number of recalled no-think words in the baseline recall test. Thus, a recall rate smaller than 1 indicated that subjects had indeed forgotten the no-think words. In order to ensure that our protocol could successfully induce MF, we conducted a control condition on a different day in which the MF phase was replaced by a task during which subjects only counted the number of dots of the words presented on the screen. The order of MF and control sessions was counterbalanced. The experimental procedure was exactly the same between the control and MF conditions (except the MF task, which was replaced by a non-forgetting task in the control condition, Figure 2A). The length of time elapsed between the MF and control sessions was approximately 10 days (2 subjects were excluded from the nap group because they could not attend the other session sooner than 4 weeks). The data of one subject was discarded because of going to sleep later than the norm (the averaged sleep onset latency [SOL] of the subjects were 4.98 ± 1.41 minutes, means ± SEM, whereas for the excluded subject the SOL was 46 minutes).

For the no-nap group, procedures of the control and MF conditions were the same, except that subjects arrived at the laboratory at approximately 8 am, and after the MF task (MF condition) or the control non-forgetting task (control condition) they watched a movie for approximately 1.5 hours instead of taking a nap (Figure 2A). We started the experiment 2 hours earlier for the no-nap group to decrease the sleepiness of subjects at the time they were watching movie instead of falling asleep.

Sleep EEG recording and preprocessing

EEG data were acquired using a g.USBamp (g.tec Medical Engineering GmbH, Austria) from 28 active electrodes located at F3, F1, FZ, F2, F4, FC5, FC3, FC1, FCz, FC2, FC4, FC6, C5, C3, C1, Cz, C2, C4, C6, CP5, CP3, CP1, CPz, CP2, CP4, CP6, P3, P4, according to the 10–20 system, with a sampling frequency of 512 Hz. Horizontal and vertical electrooculography (EOG) and chin electromyography (EMG) were monitored throughout the nap. A band pass 0.1–60 Hz filter was applied to remove low- and high-frequency artifacts from the EEG signals. A notch 50 Hz filter was used to

remove the powerline noise. Movement artifacts were removed through visual inspection. In addition, channel rejection was performed through visual inspection of the spectral power of all the 28 channels corresponding to each subject. Data of two subjects were discarded because of the high noise levels (the amplitude of more than 30% of data was larger than 400 mV). EEG electrodes FCz, Cz, and PCz along with EOGs and chin EMG were used for sleep staging. Sleep staging were performed by consensus. For each subject, 30-second epochs of sleep EEG were visually scored according to the (American Academy of Sleep Medicine) AASM standard [39] into stages 1, 2, 3, and REM sleep, with S2 corresponding to light NREM sleep, and stage 3 corresponding to SWS. The mean amount of time spent in different sleep stages during the afternoon nap is given in Supplementary Table S1. It has been suggested that the first portion of NREM sleep is linked to the presleep experience [31]. Moreover, there are evidences that the properties of NREM stages change during the time of sleep [40]. Therefore, we performed the analysis over the same time window from the beginning of each sleep stage for control and experiment conditions. As we further sought to investigate the correlation between the recall rate and sleep parameters among the subjects, we considered the same time window over each sleep stage for all the subjects to make their sleep parameters comparable. As the minimum S2 duration for 3 subjects was around 16 minutes and the minimum SWS duration for 1 subject was 18 minutes (Supplementary Table S1), we restricted the analysis to the first 16 and 18 minutes of artifact free S2 and SWS, respectively, to include more subjects (only for 1 subject with a shorter S2 duration, the first 12 minutes of S2 was used). Data from 5 subjects with S2 and SWS durations below the predefined threshold (which was 12 minutes for S2 and 18 minutes for SWS) were discarded.

Spectral analysis

Spectral power analysis was performed using fast Fourier transform and a Hanning window of 20 seconds with 50% overlap. The frequency resolution was 0.03 Hz. For each subject, average power spectra were calculated separately at each electrode location. For investigating the SOs, as well as slow and fast spindles the frequency ranges 0.5–2, 8–12, and 12–16 Hz were selected, respectively [41].

SO and spindles detection

The algorithm used for detecting SO was similar to the one previously used by Klinzing *et al.* [41]. Briefly, the EEG signal was filtered into the bandwidth 0.16–3.5 Hz using finite impulse response (FIR) filters from the EEGLAB toolbox [42]. Next, the positive to negative zero-crossing were determined and the consecutive ones within an interval 0.8–2 seconds were selected. The negative and positive peaks within the aforementioned interval were detected and averaged. The negative peak of SOs was determined if the amplitude of the negative peak was larger than 1.25 times the averaged negative peak. Finally, the amplitude difference between the negative and positive peaks had to be larger than 1.25 times the averaged difference between the negative and positive peaks (averaged over control and MF conditions). For spindle detection a previously introduced approach [41] was used. EEG signals corresponding to S2 and SWS separately were band-pass filtered with FIR filters between 8–12 and 12–16 Hz for detecting slow and fast spindles, respectively using FIR filters from the EEGLAB toolbox [42]. Next, the root mean square (RMS) of the filtered signals was calculated at every point using a moving window of 0.2 seconds and smoothed using a moving window of length 0.2 seconds. After computing the standard deviation (STD) of the filtered signals (averaged over control and MF conditions), a threshold was defined as 1.5 × STD. A spindle was detected if the RMS signal remained above the defined threshold for 0.5–3 seconds. Once the spindles were detected the time point of spindle event was defined as the time of maximum peak. The details of detected events for each subject and stage are presented in Supplementary Table S1.

Statistical analysis

Normality of data distribution was verified by the lillietest, which is an improved approach compared to the Kolmogorov test [43]. A two-factor mixed-design analysis of variance was applied for statistical investigation of the behavioral performance with condition (MF/control) as a within-subject factor and group (nap/no-nap) as a between-subject factor.

We examined the significance of EEG power and changes in spindle density between the two conditions, MF and control, using a two-tailed paired-samples *t*-test (the difference values in the dependent variable between the conditions were mostly (>95%) normally distributed). In addition, the relationship between fast spindle power and recall performance for no-think words was investigated via Spearman correlation. To correct for multiple comparisons during both evaluating the statistical difference between conditions and correlation analysis, a cluster-based permutation procedure similar to a previous article [44] was applied. Clusters were formed over channels by thresholding the *t*-value of paired-samples *t*-test with significant difference in spindle power and density between conditions and the *r*-value of Spearman correlation for significant correlation between spindle power and behavioral data. A spatial cluster was formed as the sum of all *t*-values (*r*-values) exceeding a limit corresponding to $p = 0.05$, over the data points closely related in space. These cluster level statistics were then subjected to a Monte Carlo permutation algorithm [41] (1024 permutations). A similar procedure was performed for each of 1024 random permutations in order to establish the null distribution of cluster values. The final threshold for significance of the summed *t*-value (*r*-value) within clusters (cluster level) was set to $p < 0.05$. The results of paired-samples *t*-test and Spearman correlation over sample electrodes in the clusters verified as significant are also presented in the Result section. The same cluster-based permutation procedure was used to compare spindle properties between SWS and S2 stages.

Results

Recall performance

In the initial inclusion experiment (standard TNT paradigm), of 89 subjects, only 57 subjects (64% of the subjects) succeeded to forget no-think words (see the Methods section). For these subjects, the recall rate of no-think words was significantly lower than the recall rate of think words (paired *t*-test, $p < 0.001$, the recall rate for think and no-think words were 105 ± 3.5% and

53 ± 3.6%, respectively, n = 57). For the other 32 subjects, there was no significant difference between the recall rate of think and no-think words.

In the MF condition of the main experiment, as we specifically sought to investigate the effect of a forgetting task on subsequent sleep, we considered more to be forgotten words (no-think words) compared to the standard TNT task. As eliminating all think words might reduce the tendency to retrieve in response to cues and so decrease the need for inhibition, we included 5 think words as well (25% of the total words). We found that both the nap and no-nap groups recalled significantly less no-think words in the MF condition as compared to the control condition (Figure 2B, $F_{1,23}$ = 7.7, p = 0.01, mixed-design analysis of variance, the recall rate for the nap group in the control and MF conditions were 88.0 ± 6.7% and 74.03 ± 8.9%, respectively, n = 10 and the recall rate for the no-nap group in the control and MF conditions were 86.9 ± 4.8% and 55.5 ± 8.4%, respectively, n = 15), which indicated the efficiency of the protocol in inducing MF. Moreover, there was no significant difference in the recall rate of no-think words between the nap and no-nap groups (Figure 2B, $F_{1,23}$ = 2.2, p = 0.15, mixed-design analysis of variance) and there was no significant group by condition interaction (Figure 2B, $F_{1,23}$ = 1.13, p = 0.3, mixed-design analysis of variance).

In addition, the recall rate of think words was significantly larger compared to no-think words (Supplementary Figure S4, $F_{1,23}$ = 0.25, p = 0.023, mixed-design analysis of variance, the recall rate for the think words in the nap and no-nap groups were 95.7 ± 10.21%, n = 10 and 76.7 ± 9.3%, n = 15, respectively and the recall rate for the no-think words in the nap and no-nap groups 74.03 ± 8.9% and 55.5 ± 8.4%, respectively). There was also no significant difference in the recall rate of think words between the control and MF conditions which could be also expected as there was no difference in the two conditions regarding think words.

Spindle activity during S2 and SWS

We studied the effect of MF on subsequent sleep by comparing the aspects of sleep, especially sleep spindles between the MF and control conditions for the nap group. There were no significant difference between the conditions in total sleep time, time spent in S2, SWS, or REM (Table 1, Paired t-test; $p > 0.1$). We hypothesized that S2 and SWS might contribute differently to the processing of the new memories during sleep. Hence, we performed all the analysis separately for S2 and SWS. Moreover, we restricted all the analysis to the first 18 and 16 minutes (12 minutes for 1 subject, see the Methods section) of SWS and S2, respectively to make the sleep parameters of the subjects with largely variable duration of S2/SWS (Supplementary Table S1) comparable as sleep parameters such as fast spindle power change significantly over the time

Table 1. Polysomnographic sleep parameters

Parameters	Ctrl (min)	MF (min)	p
TST	99.6 (±5.9)	96.1 (±6.7)	0.5
S2	28.6 (±3.2)	28.1 (±3.8)	0.76
SWS	37 (±3.5)	41.1 (±3.7)	0.33
REM	7.8 (±1.8)	3.8 (±2.6)	0.2

TST (total sleep time), S2, SWS, and REM sleep durations. Means (±SEM) are reported. The p-value of the corresponding pairwise comparisons (two-tailed paired-samples t-test) between Ctrl and MF is shown separately for each parameter.

of sleep, especially during SWS (Supplementary Figure S5); the fast spindle power during the first half of SWS was significantly larger compared to the fast spindle power during the second half of SWS (nonparametric cluster-permutation statistics; $p < 0.001$ for the control condition and p = 0.035 for the MF condition, n = 10). There was no significant difference in the fast spindle power between the first and second halves of S2. However, the main results (especially the ones during S2) showing the opposite effect of MF on spindle activity during SWS versus S2, did not change essentially when all artifact-free epochs of S2/SWS were considered (Supplementary Figure S9). We first compared the EEG power at SO, slow and fast spindles frequency ranges (0.5–2, 8–12, and 12–16 Hz, respectively) between the two conditions. During S2, the fast spindle power significantly increased in the MF condition compared to the control condition at the frontocentral region (Figure 3A, nonparametric cluster-permutation statistics; p = 0.03, n = 10). Oppositely, during SWS the fast spindle power significantly decreased in the MF condition compared to the control condition at the left posterior region (Figure 3A, nonparametric cluster-permutation statistics; p = 0.03, n = 10). However, there was no strong difference in SO power or slow spindle power between the two conditions for either sleep stages. Comparing the power spectrum between the MF and control conditions over the FC2 and CP3 electrodes (two electrodes with the strongest effect in the paired-samples t-test before cluster-based permutation analysis) during S2 and SWS, respectively, we showed more clearly that the power at fast spindle frequency range changed significantly between the two conditions with opposite direction for S2 and SWS (Figure 3B, paired t-test; for FC2 during S2 p = 0.017, 13.26 ± 1.32 μV^2 for the control condition versus 16.08 ± 1.96 μV^2 for the MF condition; for CP3 during SWS p = 0.003, 10.28 ± 1.46 μV^2 for the control condition versus 8.9 ± 1.24μV^2 for the MF condition, n = 10). These results suggest a differential role of sleep spindles during S2 and SWS.

In addition to power, we compared the fast spindle density between the MF and control conditions. Consistent with the power spectral analysis, the fast spindle density significantly increased in the MF condition compared to the control condition at the frontocentral region during S2 (Supplementary Figure S6, nonparametric cluster-permutation statistics; p = 0.049, n = 10). Oppositely during SWS, the fast spindle density significantly decreased in the MF condition compared to the control condition at the left posterior region (Supplementary Figure S5, nonparametric cluster-permutation statistics; p = 0.01, n = 10). Comparing the fast spindle density between the MF and control conditions at FC2 and CP3 electrodes during S2 and SWS, respectively, we further showed that fast spindle density changed significantly between the two conditions with opposite direction for S2 and SWS (Figure 3C, paired t-test; for S2 p = 0.04, for the control condition 3.2 ± 0.3 [number per 30 seconds] versus 3.9 ± 0.2 [number per 30 seconds] for the MF condition; for SWS p = 0.005, 4.4 ± 0.2 [number per 30 seconds] for the control condition versus 3.6 ± 0.2 [number per 30 seconds] for the MF condition, n = 10). There were no significant changes in slow spindle or SO densities between the MF and control conditions that withstood multiple comparisons.

Correlation between sleep spindles and recall performance

We next sought to determine whether there was a correlation between the changes in sleep spindle power and the changes in

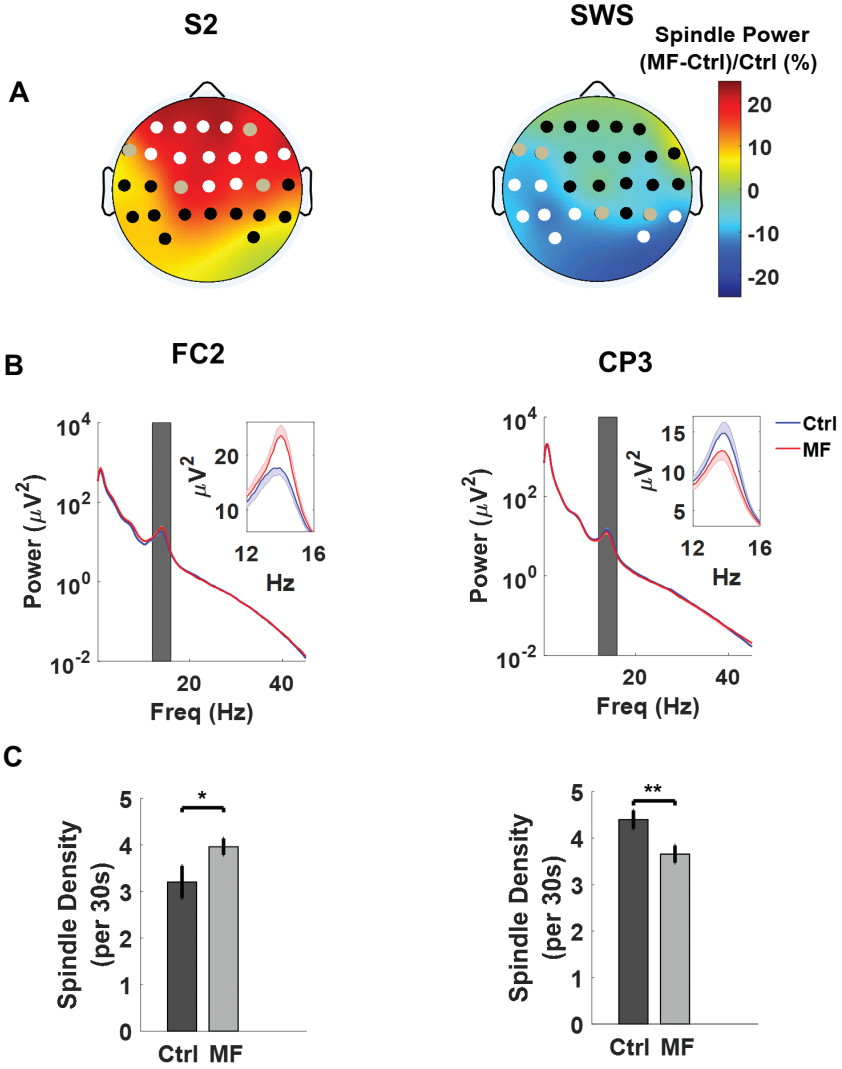

Figure 3. Effect of MF on fast spindle activity during S2 and SWS (10 subjects). (A) Topography distribution of percentage changes in the fast spindle power, (MF–Ctrl)/Ctrl, during S2 (left) and SWS (right) ($p < 0.05$, white electrodes; $0.05 < p < 0.1$, gray electrodes; $p > 0.1$, black electrodes for pairwise comparisons between Ctrl and MF). Significant positive and negative clusters were identified over frontocentral (F3, F1, Fz, F2, FC3, FC1, FCz, FC2, FC4, FC6, Cz, C2) and left posterior (C5, C3, CP5, CP3, CP1, P3) regions during S2 and SWS, respectively (nonparametric cluster-permutation statistics; sample level $p < 0.05$; cluster level $p < 0.05$). (B) Mean spectral power for FC2 during S2 (left) and for CP3 during SWS (right) for Ctrl (blue curve) and MF (red curve) conditions. Shaded areas illustrate the power in fast spindle range with significant difference between the two conditions ($p < 0.05$). (C) Mean fast spindle density for FC2 during S2 (left) and for CP3 during SWS (right) in the Ctrl (black) and MF (gray) conditions ($^*p < 0.05$, $^{**}p < 0.05$). Error bars represent standard error of the mean.

memory performance (MF versus control condition). Interestingly, in line with the opposite changes in spindle activity during S2 versus SWS (explained in the Spindle activity during S2 and SWS section), we found that while during S2, there was a significant negative correlation between the difference in the recall rate (MF–control) and fast spindle power difference (MF–control) in the frontocentral region, during SWS, there was a significant positive correlation between the recall difference and fast spindle power difference in the frontocentral and left posterior regions (Figure 4A, nonparametric cluster-permutation statistics; $p = 0.023$ for S2 cluster, $p = 0.02$, $p = 0.037$ for SWS frontocentral and left posterior clusters, respectively, $n = 10$). In other words, in the MF compared to the control condition, subjects with greater fast spindle activity during S2 were more successful in suppressing the no-think words whereas subjects with less fast spindle activity during SWS recalled less no-think words. The results were also shown for two typical electrodes of each cluster (Figure 4B, Spearman correlation; FC2 for S2 $r = -0.77$, $p = 0.014$, CP3 for SWS $r = 0.68$, $p = 0.035$, see Supplementary Figures S7 and S8 for the results corresponding to other electrodes, $n = 10$). These findings further indicate the opposite link of spindle activity during S2 and SWS to memory processing during sleep.

In addition to fast spindle power, there was a significant positive correlation between recall rate and fast spindle density during SWS over F2, FC3, FC1, FCz, FC2, C2, CP2, CPz electrodes (nonparametric cluster-permutation statistics; $p = 0.014$, $n = 10$). However, there was no significant correlation between recall rate and fast spindle density during S2 that withstood multiple comparisons. There was also no significant correlation between stage2/SWS/REM duration/percentage or slow spindle/SO power/density and recall rate.

Comparison between fast spindles during S2 and SWS

We next compared fast spindle characteristics during S2 versus SWS. Sample epochs of SWS and S2 together with the detected

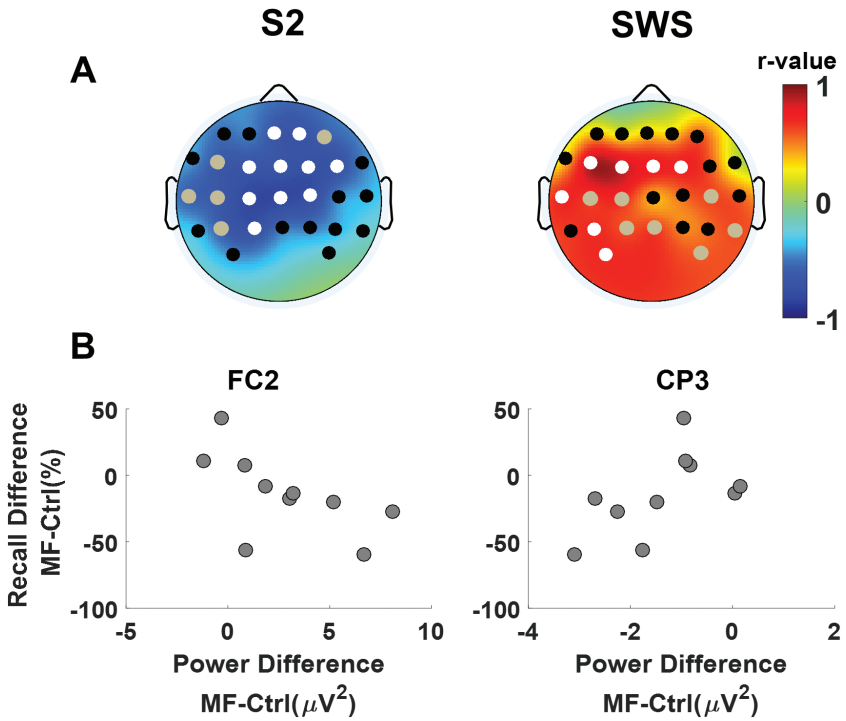

Figure 4. Association between fast spindle power and recall rate during S2 and SWS (10 subjects). (A) Topographic distribution of Spearman correlation coefficients between the difference in the fast spindle power (MF–Ctrl) and the difference in the recall rate (MF–Ctrl) during S2 (left) and SWS (right) ($p < 0.05$, white electrodes; $0.05 < p < 0.1$, gray electrodes; $p > 0.1$, black electrodes). During S2, a frontocentral negative cluster (Fz, F2, FC1, FCz, FC2, FC4, C1, Cz, C2, CP1) and during SWS, two significant positive clusters in frontocentral (FC5, FC3, FCz, FC2) and left posterior (CP3, P3) regions were identified (nonparametric cluster-permutation statistics; sample level $p < 0.05$; cluster level $p < 0.05$). (B) Correlation between the difference in the fast spindle power (MF–Ctrl) and the difference in the recall rate (MF–Ctrl) for FC2 during S2 (left) and CP3 during SWS (right) (S2, FC2, $r = -0.77$, $p = 0.014$, SWS, CP3, $r = 0.68$, $p = 0.035$).

fast spindles are shown in Figure 5A. As could be expected, on average, fast spindles during SWS were nested in oscillations with lower frequencies corresponding to SOs (Figure 5B). We further compared the fast spindle power, density, amplitude, and duration during S2 versus SWS separately for the control and MF conditions (Figure 5C). For both conditions, fast spindle amplitude and duration were significantly larger during S2 compared to SWS (fast spindle amplitude for the control condition, $p = 0.05$ for the left posterior cluster [FC5, FC3, C3, C1, CP3, CP1, P3 electrodes], and $p = 0.06$ for the right posterior cluster [FC6, C6, CP6, Cp4, CP2, P4 electrodes]; for the MF condition cluster, $p < 0.001$ [all electrodes except P4, FC2], nonparametric cluster-permutation statistics, fast spindle duration for the control condition, $p = 0.002$, cluster consisting of all the electrodes, for the MF condition $p < 0.001$, cluster consisting of all the electrodes, $n = 10$). However, fast spindle density was significantly larger during SWS compared to S2 only for the control condition, mostly over frontocentral electrodes (nonparametric cluster-permutation statistics; $p = 0.02$, frontocentral cluster consisting of F3, F1, Fz, F2, F4, FC5, FC3, FC1, FCz, FC2, FC4, FC6, C6 electrodes, $n = 10$). Oppositely, for the MF condition, fast spindle power was significantly lower during SWS compared to S2 over left posterior electrodes (nonparametric cluster-permutation statistics; $p = 0.049$, cluster consisting of C6, C4, C2, CP6, Cp4, CP2, P4 electrodes, $n = 10$). It is important to notice that sleep in the control condition did not correspond to baseline sleep but to sleep after a remembering task (learning a word list), whereas sleep in the MF condition corresponded to sleep after a forgetting task. Growing evidences indicate that fast spindle co-occurring with SOs (which mostly correspond to SWS fast spindles) are particularly important for memory consolidation during sleep [19–23, 45]. Our findings further suggest that while increasing fast spindle activity during SWS is associated with boosting of relevant memory consolidation, increasing fast spindle activity during S2 might enhance erasing of unwanted memories.

Discussion

This study contributes to better understanding of the differential modulation in S2 and SWS of sleep spindles by a foregoing MF experience. In the MF condition, fast spindle activity (power and density) increased significantly during S2 at the frontocentral region, whereas it decreased significantly during SWS at the left posterior region as compared to the control condition. Moreover, the difference in the fast spindle activity as compared to the control condition was correlated negatively with difference in retention during S2, but positively with the difference in retention during SWS. The significant decrease in the recall rate of no-think words in the MF condition compared to the control condition indicated the efficiency of the MF task in inducing forgetting. By comparing the nap group with the no-nap group, we also investigated the role of sleep in processing of unwanted memories, and found no significant difference in the retention of no-think words between the nap and no-nap groups. Although this result should be considered cautiously, as it was obtained by comparison between two different groups with relatively small number of subjects, it is in line with the previous findings indicating that sleep does not enhance the consolidation or forgetting of unwanted memories [7, 30]. Conversely, it has been

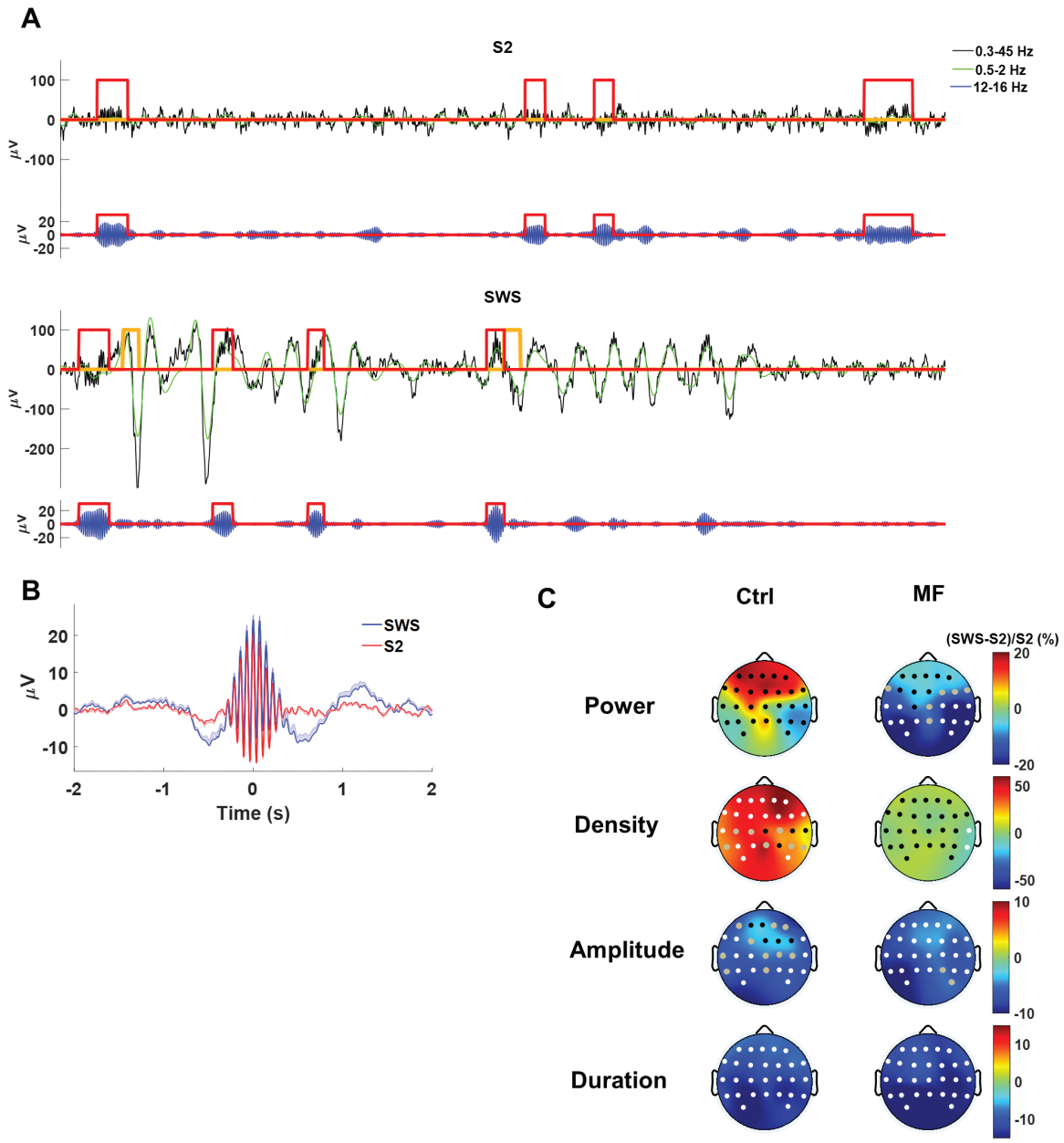

Figure 5. Comparison between fast spindles during S2 and SWS. (A) Sample epochs during S2 (top) and SWS (bottom) over the Cz electrode, together with EEG filtered between 12–16 Hz (blue) and 0.5–2 Hz (green). Detected fast and slow spindle events were shown in red and orange, respectively. (B) Averaged spindle event during S2 (red) and SWS (blue) over the Cz electrode. Averaging was performed with reference to the maximum peak of the detected event (zero time). (C) Comparison between fast spindle power, density, amplitude, and duration during S2 versus SWS in Ctrl (left) and MF (right) conditions ($p < 0.05$, white electrodes; $0.05 < p < 0.1$, gray electrodes; $p > 0.1$, black electrodes).

proposed that sleep, especially REM sleep, may induce systematic forgetting [24, 46, 47]. Specifically, using a directed forgetting task, it has been shown that nocturnal sleep enhanced the forgetting of to be forgotten items [28]. We did not find any significant differences in REM sleep duration (minutes or percentage) between MF and control conditions. However, as the portion of REM sleep is low during nap (REM sleep duration was zero for five subjects, Supplementary Table S1), to reliably investigate the effect of MF on REM sleep, nocturnal sleep instead of nap should be considered. The important role of REM sleep and spindles only during S2 in directed forgetting has been recently suggested [24] because during these two states noradrenergic cells in the locus coeruleus, which are the source of norepinephrine, are suppressed. Absent of norepinephrine is important for reliable forgetting as norepinephrine signaling contributes to enhance long-term potentiation (LTP) and block depotentiation [48]. We found that higher fast spindle activity during S2 was associated with more success in forgetting of no-think words. However, the benefit of spindles during S2 on forgetting might be canceled out by the effect of positive coupling between memory retention and spindle activity during SWS resulting in no overall significant changes in retention of to be forgotten words by NREM sleep.

Growing evidence suggests the importance of sleep spindles for memory processing during sleep. Previous studies showing

associations between sleep spindles and declarative memory consolidation during sleep found a positive correlation between the retention of items and spindle activity [32]. Several of these studies focused on S2 and found that declarative memory consolidation was positively coupled with fast spindle activity during S2 [11, 49]. However, these findings are not necessarily inconsistent with our results showing a negative correlation between retention of no-think words and the spindle activity during S2 as compared to the control condition. First, in all of these studies the consolidation of the relevant memories was investigated while we focused on the recall of no-think words cued to be forgotten, that might be processed differently during sleep as was suggested by several studies [7, 25]. Second, in most of these studied baseline, sleep was not included and so the obtained correlations might be a result of other factors, including general learning capacity [50–52]. A few studies did consider this important factor by calculating the changes in the spindle activity with respect to a control condition, and found a positive correlation between declarative memory consolidation and changes in spindle activity during S2 [9, 11]. In order to exclude the effect of the interindividual baseline differences in sleep spindles, we calculated the difference in spindle activity between the MF and control conditions for investigating the correlation between spindle activity and memory retention. However, as we meant to investigate the effect of MF on subsequent sleep, our control condition consisted of learning word pairs without the MF task. Here, following a MF task, subjects with more spindle activity during S2 compared to the control non-forgetting task recalled less no-think words, i.e. were more successful in forgetting of unwanted memories.

Although most studies focused only on the consolidation of relevant memories, few studies investigated the correlation between sleep spindles and retention of unwanted memories. In a recent study, a positive correlation between spindle amplitude during NREM sleep (including S2 and SWS) and retention of a to-be-forgotten word list was found at midline electrodes [8]. However, another study found a negative correlation between the retention of to be forgotten words and spindle density at a number of frontal electrodes [7], and positive correlation between spindle density and proportion of words cued for remembering at parietal regions. However, the correlations in none of these studies survived correction for multiple comparisons and both studies considered the spindle activity during NREM sleep without distinguishing between S2 and SWS. On the basis of our results, the difference in the relative time spent in each of S2 or SWS might explain the contrary results of these two studies. In fact, averaging the spindle activity over the entire NREM sleep combining S2 and SWS might have resulted in nullification of the correlations or opposite results in different studies with dominant S2 or SWS. More importantly, in both studies, baseline sleep was not included and so the obtained correlations might be affected by the interindividual baseline differences in sleep spindles.

A well-established body of literature supports the importance of the two macro states of NREM sleep, i.e. S2 and SWS, in memory consolidation during sleep [1, 53, 54]. It has been suggested that the thalamocortical system [55]and sleep spindles [17]in these two states have differential properties. Recently, Lustenberger *et al.* [51] investigated the correlation between sleep spindles and overnight memory retention separately for the first and last hour of NREM sleep with dominant S2 and SWS, respectively. They found a negative correlation between fast spindle density during the first hour of NREM sleep and overnight retention and a positive correlation between slow spindle density during the last hour of NREM sleep and overnight retention. However, in order to exclude the individual differences, they calculated the difference in spindle activity between the two same study nights and found a positive correlation between overnight retention and only slow spindle density during the first hour of NREM sleep. Contrary to the evidences showing the importance of SWS in declarative memory consolidation [1], one study showed that SWS awakening had no effect on word pair learning [49]. However, in this study the absolute spindle activity or spindle density did not change significantly in the SWS deprived condition compared to the control condition. Moreover, the spindle activity during S2 and NREM sleep was positively correlated with the declarative memory consolidation for the control condition, but there was no such significant correlation for the SWS deprived condition. Although our findings shed light on the differential involvement of spindles during S2 versus SWS, they are merely based on correlations. In order to further clarify this differential role of sleep spindles on memory processing during sleep, future experimental studies are required in which sleep spindles are selectively induced during S2 or SWS.

Recent studies have revealed the existence of two distinct types of sleep spindles with different topographical distributions and temporal relations to the SO. Frontal slow spindles (9–12 Hz) occur mainly during cortical up to down transition whereas more posterior fast spindles (12–16 Hz) occur mostly in the beginning of the up state [20, 41, 56–58]. Moreover, slow and fast spindles have different pharmacological properties [59] and are involved differently in memory consolidation during sleep [4, 60]. Many studies investigating the correlation between sleep spindles and memory performance did not differentiate between slow and fast spindles. However, a growing number of studies support the association between memory consolidation and fast but not slow spindles [60, 61]. Consistent with these findings we also found significant changes between the control and MF conditions only for fast spindle activity.

In summary, we demonstrate the involvement of sleep spindles in the processing of unwanted memories by considering a novel experimental design. We provide evidence for opposite changes in spindle activity during SWS and S2 during sleep following effort to suppress newly learned words, cued to be forgotten. Interestingly, we showed the importance of the brain macro state of NREM sleep (S2 or SWS) for this involvement. Although there was a negative correlation between recall performance of no-think words and spindle activity during S2, fast spindle activity was positively correlated with the retention of no-think words during SWS. Our findings suggest a differential role of sleep spindles during S2 and SWS in processing unwanted memories.

Supplementary material

Supplementary material is available at *SLEEP* online.

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