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Sleep contributes to the strengthening of some memories over others, depending on hippocampal activity at learning.

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Memory consolidation benefits from sleep. Besides strengthening some memory traces, another crucial, albeit overlooked, function of memory is also to erase irrelevant information. Directed forgetting is an experimental approach consisting in presenting "to be remembered" and "to be forgotten" information, that allows selectively decreasing or increasing the strength of individual memory traces according to the instruction provided at learning. This paradigm was used in combination with fMRI to determine, in Humans, what specifically triggers at encoding sleep-dependent compared to time-dependent consolidation. Our data indicate that relevant items which subjects strived to memorize are consolidated during sleep to a greater extend than items that participants did not intend to learn. This process appears to depend on a differential activation of the hippocampus at encoding, which acts as a signal for the offline reprocessing of relevant memories during post-learning sleep episodes.

Introduction

Ample evidence indicates that sleep favors the consolidation of newly acquired information in memory (Born et al., 2006). Unlike memory consolidation, forgetting is often considered as a nuisance or a default of memory functioning. Yet, forgetting can also be a positive and intentional act, crucial for a proper functioning of memory enabling to update or to erase irrelevant information. From 1890, the psychologist and philosopher William James wrote that "*in the practical use of our intellect, forgetting is as important a function as recollecting*" (James, 1892, p679). Nearly one century later, Crick and Mitchinson (1983) proposed that sleep might contribute to the forgetting of the weakest or irrelevant memory traces.

Even if the mechanism by which recently acquired memory traces are consolidated during sleep is increasingly better understood (Diekelmann and Born, 2010), some issues remain unsolved. Thus, combined with functional MRI (fMRI), we used a directed forgetting

paradigm, known to selectively decrease or reinforce individual memory traces, to causally determine how the quality of the initial memory trace at encoding influences the off-line reprocessing of recently acquired memories during sleep. During an encoding fMRI session, young healthy participants learned a series of words. Each word was followed by an instruction indicating whether the item had "to be remembered" (TBR item) or "to be forgotten" (TBF item). It was stressed that the subsequent memory test would only be based on TBR words. Participants were subsequently pseudo-randomly assigned to one of two groups in which they were either allowed to have regular sleep (RS group) or were totally sleep deprived (TSD group) during the post-learning night. Three days after encoding, during another fMRI session, memory for TBR and TBF items was probed using a recognition task during which subjects had to categorize each word presented as previously encountered (whatever the instruction at encoding) or new (Figure 1). We hypothesized that RS participants would recognize more TBR than TBF items. Given that previous studies using recognition tasks reported only moderate or no beneficial effect of sleep on memory performance (Diekelmann et al., 2009), the recognition rate for TBR items was expected to be equal or marginally smaller in TSD than RS participants. In contrast, sleep deprivation should increase memory errors (Diekelmann et al., 2008) and favour the recognition of TBF items. As sleep-dependent memory consolidation is assumed to imply a replay of hippocampal activity (Buzsáki, 1996) leading to the progressive transfer of the memory burden from hippocampo-neocortical to predominantly neocortical long-term stores (Squire and Zola-Morgan, 1991; Frankland & Bontempi, 2005), we expected, in RS participants, larger hippocampal responses during retrieval of TBR as compared to TBF items and also for TBR items that were later consolidated during sleep compared to forgotten ones.

Materials and Methods

Subjects

Twenty six right-handed volunteers (11 males, 15 females, mean age: 23.1 ± 2.7 years) gave their written informed consent to participate in this study approved by the Ethics Committee of the Faculty of Medicine of the University of Liège. None reported any history of trauma or medical, psychiatric or sleep disorders, nor disturbances of their sleep-wake cycle during the last six weeks. Structural MRI was normal on visual inspection. Self-report questionnaires assessed sleep quality (Buysse et al., 1989) and circadian typology (Horne and Östberg, 1976).

Volunteers followed a constant sleep schedule (according to their own sleep/wake schedule ± 1h) 3 days before the first visit and kept this schedule for 3 more days, until their second visit. After encoding, participants were pseudo-randomly assigned to one of the two following groups (Figure 1). In the sleep group (n = 14, 6 males, 8 females; Regular Sleep [RS] group), subjects were allowed to sleep at home following their regular habits for the three post-learning nights. In the sleep-deprived group (n =12, 5 males, 7 females; Total Sleep Deprivation [TSD] group), subjects remained awake in the laboratory during the first post-learning night. During this night, participants' physical activity was maintained as low as possible. Subjects remained most of the time in a sitting position, played quiet games or watched movies under constant supervision by the experimenters. Food intake was standardized across subjects, and luminance exposure was kept below 8 lux. At 8.00 am, subjects were allowed to leave the laboratory. They were instructed to follow their usual daytime activities and to abstain from napping during the day. All subjects slept as usual at home on the second and third post-learning nights. Sleep quality for each night from before the learning session to before the testing session was assessed using a standardized questionnaire (Ellis et al., 1981).

Stimuli and procedure

Stimuli were six-letter words selected from the Brulex French lexical database (Content et al., 1990). During the learning phase, subjects saw one-by-one a series of one hundred words. Fifty were categorized as "To-be-remembered" (TBR) items and the other as "To-be-forgotten" (TBF) items. TBR and TBF words were counterbalanced across subjects. Lexical frequency was controlled so that there were no difference between TBF and TBR items (TBR = 697.5 ± 849.7, TBF = 697.7 ± 681.1, F = 0.0001, p>0.99). Fifty control items (series of six crosses) were also presented in the encoding phase. The three types of items (TBR, TBF, and crosses) were pseudo-randomly organized so that there was no more than three items of same type consecutively.

Figure 1 illustrates the experimental procedure. The learning phase took place on day 1 and began by the presentation of a white fixation cross, on the centre of the screen, during 800 ms and followed by a black screen for 200 ms. Then subjects saw a target item during 1 s and were instructed to mentally read the word presented. Then, an instruction "To Remember" or "To Forget" was displayed, in yellow, for 3 s. If a Remember instruction was given, participants were instructed to encode the item. If a Forget instruction was presented, subjects were asked to engage suppression processes not to encode this particular word. Control items were presented according to the same procedure and the instruction was replaced by a succession of crosses. In order to be sure that subjects understood the instructions, an example with 3 items was proposed before encoding, outside the scanner.

The recognition task was conducted on day 4, after two recovery nights. The 100 target stimuli were mixed with 100 distractors of equal lexical frequency (target items: 697.6 ± 766.1, distractor items: 698.6 ± 606.3, F<0.001, p>0.99). Subjects first saw a white fixation cross displayed in the centre of the screen for 500 ms, followed by the presentation of a target or a distractor item during 5 s. Subjects had to decide, by pressing keys and in no more than 5

s, if they had already seen the word or not, regardless the instruction given at learning. When control items were presented, subjects had to press one button or the other.

After retrieval, subjects filled out a debriefing questionnaire in which they had to explain the strategies used to memorize TBR items and forget TBF words. More precisely, they had to estimate on a 5-point scale their use of various strategies (mental imagery, rehearsal of one or several words, association with personal events or memories…). A score of 1 indicated that they never used the strategy and 5 that they always used it. They could also indicate the use of a different strategy than those proposed by experimenters. Repetition of words during the 3-day interval between encoding and retrieval was also quantified using a 4-point scale (from never to more than 10 times). Actually, no subject intensively rehearsed encoded items during the retention interval. Consequently, no item in any subject was removed from the analyses (Supplemental Results).

fMRI data acquisition and analyses

Whole-brain functional T2*-weighted MRI data were acquired using a 3T scanner (Siemens, *Allegra*, Erlangen, Germany) using a gradient-echo planar imaging (EPI) sequence (32 transverse slices with 30% gap, voxel size 3.4x3.4x3 mm^3, TR = 2130 ms, TE = 40 ms, flip angle = 90°, field of view (FoV) = 220x220 mm^2). For anatomical reference, a structural MR scan was acquired for each subject (T1-weighted 3D MP-RAGE sequence, TR = 1960 ms, TE = 4.43 ms, TI = 1100 ms, FoV = 230x173 mm^2, matrix size = 256x192x176, voxel size = 0.9x0.9x0.9 mm^3). Head movements were minimized by restraining the subject's head using a vacuum cushion. Stimuli were displayed on a screen positioned at the rear of the scanner, which the subject could comfortably see through a mirror mounted on the standard head coil.

Functional volumes were pre-processed and analyzed using SPM5 (www.fil.ion.ucl.ac.uk). The three initial volumes of each session were discarded to avoid T1 saturation effects. Volumes were realigned using iterative rigid body transformations that minimize the residual sum of square between the first and subsequent images. They were spatially normalized to the MNI EPI template, and spatially smoothed with a 8 mm FWHM Gaussian kernel.

Data were processed using two-step analysis, taking into account the intra-individual and inter-individual variance respectively. For each subject, changes in brain regional responses were estimated at each voxel, using a general linear model.

During the encoding session, 4 trial types were modeled: TBR item recognized as "old" at the retrieval session (TBR-hits), TBR items not recognized during retrieval (TBR-misses), TBF items subsequently retrieved (TBF-hits) and TBF items not retrieved (TBF-misses). During the retrieval session, 6 trial types were modeled: TBR-hits, TBR-misses, TBF-hits, TBF-misses, correct rejections (distractor items considered as new) and false alarms (new items categorized as previously encountered). For each trial type, a given item was modeled as a delta function representing its onset. The ensuing vector was convolved with the canonical hemodynamic response function, and used as a regressor in the individual design matrix. Movement parameters estimated during realignment (translations in *x*, *y* and *z* directions and rotations around *x*, *y* and *z* axes) and constant vector were also included in the matrix as a variable of no interest. High pass filter was implemented using a cut off period of 128 s in order to remove the low-frequency drifts from the time series. Serial autocorrelations were estimated with a restricted maximum likelihood algorithm using an autoregressive model of order 1 (+ white noise).

For the encoding session, linear contrasts estimated (1) the effect of instruction on the processing of items at encoding (TBR > TBF; TBF > TBR); (2) the effect of successful

encoding or unsuccessful active forgetting at encoding (TBR-hits > TBR-misses; TBF-hits > TBR-misses). For the retrieval session, the linear contrasts performed estimated the effect of successful retrieval (TBR-hits > TBR-misses) and of unsuccessful forgetting (TBF-hits > TBF-misses). The individual summary statistical images were spatially smoothed with a 6 mm FWHM Gaussian kernel and used in a second-level, random-effect analysis, to account for inter-subject variance in each contrast of interest. This analysis consisted in one-sample *t*-tests testing for the effect of interest in each group and in two-sample *t*-tests comparing the responses between the two groups. Inclusive or exclusive masks were created with SPM maps thresholded at $p<0.001$ and $p<0.05$ respectively. Corrections for multiple testing were applied where mentioned by using either the family-wise error correction over the whole brain (FWE) or over small spherical volumes of interest (SVC; radius 10 mm) around *a priori* locations of structures of interest taken from the literature.

Results

Behavioral results

Behavioral data of one subject in the TSD group was not included in the analyses due to an abnormal proportion of false alarms. Nevertheless, adding or removing data of this subject did not change the sense of results concerning TBR-hits and TBF-hits. Proportions of "old" responses to TBR, TBF and new items were compared using t-tests. This analysis revealed that TSD participants recognized as much TBR items as did RS participants (mean values (± SEM) RS group: 0.71 ± 0.03; TSD group: 0.76 ± 0.03, $t(23)= 1.32$, $p>0.099$), but recognized more TBF items (RS group: 0.42 ± 0.03; TSD group: 0.58 ± 0.03, $t(23)= -3.38$, $p<0.001$) and made more false alarms (RS group : 0.25 ± 0.04; TSD group: 0.44 ± 0.06, $t(23)= 2.68$, $p<0.01$). These results could not be accounted for by persisting effects of sleep deprivation during recognition. Indeed, median reaction times in a psychomotor vigilance task

(adapted from Dinges et al., 1985), in which simple reaction times spaced by variable intervals (2-9 s) are measured over a period of 10 min, did not differ between sessions (encoding/recognition) and groups (Encoding: RS (mean ± SD) = 262.9 ± 21.8 ms; TSD = 271.5 ± 24.1 ms; Recognition: RS = 264.8 ± 18.1 ms; TSD = 276.8 ± 28.4 ms; all p values > 0.14).

However, as the global increase of "old" responses in the TSD group could indicate the existence of a response bias, data were further analyzed using the discrimination score (d') and the response criterion (C), measures derived from the signal detection theory (Macmillan and Creelman, 1991). As d' and C for TBR and TBF items are not totally independent measures (both taking into account FAs), statistical analyses were conducted using separate *t*-tests for TBR and TBF items. Recognition accuracy was equivalent between groups for TBR (RS group (mean ± SEM): 1.33 ± 0.7; TSD group: 1.1 ± 0.68; $t(23) = -0.79$, $p>0.22$) and TBF items (RS group: 0.52 ± 0.33; TSD group: 0.49 ± 0.26; $t(23) = -0.21$, $p>0.42$). However, similar analyses on response criteria for TBR and TBF items yielded significant group differences (mean C ± SEM: TBR items RS group: 0.1 ± 0.2; TSD group: -0.21 ± 0.34, $t(23) = -2.76$, $p<0.01$; TBF items: RS group: 0.48 ± 0.33; TSD group: 0.06 ± 0.3, $t(23) = -3.31$, $p<0.01$), indicating that TSD participants had a more lenient response criterion than participants in the RS group.

Similar analyses conducted on mean response times failed to reveal any effect of group ($p>0.3$). Distinguishing between the different types of items and possible responses (TBR old, TBR new, TBF old, TBF new, New items categorized as previously encountered or not), statistical analyses revealed similar results (all p values > 0.25). Thus, our data indicate that memory performance differed between TSD and RS participants (for TBF items and false alarms) without any significant difference neither in recognition accuracy nor in response times. Together with the results of the psychomotor vigilance task, these data exclude the

possibility that this difference in memory performance was merely due to an effect of tiredness in TSD participants.

The use of various strategies was estimated by means of a 5-point scale (ranging from 1: this strategy was never used by the participant, to 5: this strategy was always used). To memorize TBR items, participants used mainly a rehearsal strategy of one (mean score ± SD: 4.19 ± 1.06) or several (3.61 ± 1.10) items, created association between items to form a short story or a sentence (3.61 ± 1.3), and tried to associate the words to memories or personal events (3.58 ± 1.42). Mental imagery was less often used by the participants (2.38 ± 1.1). When a forgetting instruction was displayed, participants mainly rehearsed the TBR items presented before (3.58 ± 1.36) and/or tried to think to nothing in particular (2.92 ± 1.41). As for TBR items, mental imagery of TBF items was little used by participants (2.19 ± 1.36).

Finally, statistical analyses were also conducted to compare mental repetitions between groups (using Mann-Whithney U tests) and to compare each type of item (TBR / TBF) within each group (using Wilcoxon tests). These analyses indicate that mental repetitions did not differ between TSD and RS participants for TBR and TBF items (all p values > 0.1). As expected, and consistent with the analysis of the strategies used to memorize or forget words, TBR items were more repeated during the retention interval. More precisely, within the RS group, there was a significant difference between TBR and TBF items regarding the first point of the scale (“item never repeated during the 3-day interval”; 80.4 % of TBF were never repeated vs 59.7% of TBR items, $p<0.001$) and for the second point (“repeated between 1 and 5 times”; TBF: 17.4%; TBR: 29.1%, $p<0.01$). As for the TSD group, there was also a significant difference between TBR and TBF items for the first point of the scale (TBF: 84%, TBR: 68.8%, $p<0.05$) and for the third point (« repeated between 5 and 10 times”; TBF: 1.2%; TBR: 7.7%, $p<0.05$). It is worthy to note that there is no between

group difference concerning the fourth point of the scale ("repeated more than 10 times") which could have be a potential confound in fMRI analyses.

fMRI results

Compared to TBF items and regardless of their status at retrieval (recognized or forgotten), encoding of TBR items activated, in both groups, a set of brain regions including frontal areas, the thalamus and putamen, and the left posterior hippocampus (all p values <0.05, corrected for multiple comparisons; Supplemental Table 1, Figure 2). The reverse contrast (TBF>TBR) revealed mainly frontal and posterior cortical activations but none of them survived correction for multiple comparisons.

Then, we compared brain activity associated, in both groups, to the encoding of TBR items that were later consolidated (TBR-hits) compared to TBR items that were forgotten (TBR-misses). In the RS group, this contrast revealed only higher activity in the left hippocampus ($p^{svc(10mm)}<0.05$). Interestingly, parameter estimates indicated that hippocampal response in RS participants was larger for TBR-hits (mean ± SEM: 0.76 ± 0.32, arbitrary units) compared to TBR-misses (0.35 ± 0.29), but also for TBF-hits (0.67 ± 0.33) compared to TBF-misses (0.44 ± 0.21). The same analysis in the TSD group failed to reveal any significant response. In order to determine whether hippocampal activation at encoding specifically triggers sleep-dependent consolidation and not time-dependent consolidation, we masked the contrast in the RS group by that in the TSD group (exclusive masking, $p<0.05$), revealing that the hippocampal response was indeed significant in RS but not in TSD participants ($p^{svc(10mm)}<0.05$; Figure 2). The reverse contrast (contrast in TSD subjects with exclusive masking by the contrast in RS participants) failed to reveal any significant response.

Similar analyses were conducted to compare TBF-hits to TBF-misses. In RS group (but not in TSD, exclusive masking $p<0.05$), this analysis did not reveal any significant

response. In contrast, thalamic responses were bilaterally significant ([10 -30 4], [-6 -30 6], p FWE corrected <0.05) in TSD but not in RS participants (exclusive masking, p<0.05).

fMRI data acquired during the recognition session were analyzed to assess a different processing of TBR and TBF items during sleep and nocturnal wakefulness. We compared the brain activity associated with correct item recognition (hits) to that of forgotten items (misses), separately for TBR and TBF items. In the RS group, but not in the TSD one (exclusive masking, p<0.05), TBR-hits elicited larger activity than TBR-misses in a large neural network including frontal and posterior cortical areas (precuneus, lingual gyrus, superior parietal lobule) as well as in the amygdala, putamen and cerebellum (Supplementary Table 2). In the TSD group (but not in the RS one, exclusive masking p<0.05), no activation survived correction for multiple comparisons. Similar analyses for TBF items (TBF-hits > TBF-misses) revealed significant differential responses in RS but not in TSD subjects (exclusive masking, p<0.05), in the superior temporal gyrus, in frontal areas (medial frontal and anterior cingulate gyri) as well as in the thalamus (all p values<0.05, corrected for multiple comparisons; Supplementary Table 3). The reverse contrast (TSD>RS, exclusive masking at p<0.05) failed to reveal any significant response.

Finally and importantly, we wondered whether sleep lead to a similar processing of TBR-hits and TBF-hits. To do so, we masked, in each group separately, the contrast "TBR-hits *vs* correct rejections" by the contrast "TBF-hits *vs* correct rejections" (inclusive masking, p<0.001). In RS participants, this analysis revealed common response to recognition for both types of items (compared to correct rejections) in the anterior cingulate cortex and insula, the parahippocampal gyrus, the inferior frontal gyrus, various posterior cortical areas (lingual gyrus, precuneus, calcarine region), the vermis as well as the putamen and caudate nucleus (all p values <0.05, corrected for multiple comparisons; Figure 3, Supplementary Table 4).

The same analysis with TSD participants failed to reveal any significant response surviving corrections for multiple comparisons.

Discussion

This study contributes to better understand the specific conditions in which sleep-dependent memory consolidation occurs. On a behavioral standpoint, recognition accuracy for TBR items was equivalent between groups, as previously reported in studies using recognition tasks (Diekelmann et al., 2009). Interestingly, TSD participants recognized significantly more TBF items than subjects in the RS group, indicating, at a behavioral level, that the status of engrams after encoding varies between TBR and TBF, in such a way that if sleep subsequently occurs, the two types of memories are processed differently. However, it is worth noticing that TSD participants had also a more lenient response criterion than subjects in the RS group that could influence recognition performance.

In order to understand what triggers sleep-dependent memory consolidation, fMRI data obtained during encoding were analyzed. These data revealed higher hippocampal activity for TBR than for TBF items and more interestingly, larger responses in a non-exactly overlapping area for TBR-hits compared to TBR-misses (Figure 2). More interestingly, these hippocampal responses to TBR items during encoding were observed in the RS group and not in TSD participants (exclusive masking). This finding suggests that hippocampal activation at learning specifically triggers sleep-dependent compared to time-dependent memory consolidation. Thus, during sleep, items that subjects strived to memorize and were associated with stronger hippocampal activity are consolidated to a greater extend than items which participants did not attempt to learn. These results extend previous reports demonstrating that medial temporal activations at encoding predict subsequent remembering (Wagner et al., 1998; Eichenbaum et al., 2007). These results are also consistent with a recent study about

false memories indicating that lists of items that did not produce false memories differed from those who did by larger hippocampal responses at encoding (Darsaud et al., 2011). Collectively, these findings indicate that hippocampal activity during encoding crucially influences the offline processing of information during post-learning sleep and the later production of accurate, illusory or unwanted memories.

Sleep-dependent memory consolidation is deemed relying on the coordinated replay of specific firing sequences between the hippocampus and the neocortex (Born et al., 2006). As already reported for motor sequence consolidation (Albouy et al., 2008) or false memories (Darsaud et al., 2011), our data suggest that large hippocampal responses at encoding might tag the neural populations in which sequence replay would preferentially occur during subsequent sleep, leading to their retrieval at retest irrespective of whether they were initially to be remembered or forgotten.

The analysis of fMRI data obtained during the recognition task indicates that sleep and lack of sleep during the first post-learning night lead to a different processing of TBR and TBF items. Indeed, in the RS group but not in the TSD group (exclusive masking), correct recognition of TBR and TBF items was associated with significant responses in a set of neocortical regions including frontal areas (notably the anterior cingulate cortex), temporal, parietal and occipital areas while TSD participants did not exhibit larger responses than in RS subjects (exclusive masking) whatever the contrast considered (Supplemental Tables 2 and 3). The activation of neocortical areas such as the anterior cingulate cortex has been previously observed during the accurate retrieval of declarative memories at the waking state in rodents (Maviel et al., 2004) and humans (Takashima et al., 2006). Finally, TBR and TBF items correctly recognized, which elicited high hippocampal activity at encoding, undergo a similar processing during post-learning sleep as revealed by a common retrieval network for both types of items in RS participants. Interestingly, the same analysis performed in TSD participants did not revealed

any common pattern of activation for TBR and TBF items (compared to correct rejections). These results suggest that sleep, contrary to the simple passage of time, promotes the binding of the elements constituting a memory (item and associated contextual information) across various neocortical areas. This large network could also reflect the establishment of multiple traces within neocortical areas, a key mechanism subserving memory consolidation (Frankland and Bontempi, 2005). By contrast, remaining awake a night long might hinder such a reorganization of memory traces within the brain and possibly reflects an earlier stage of memory consolidation.

These results shed new light on the role of sleep in memory consolidation, providing evidence that during sleep, relevant items which subjects memorized are consolidated to a greater degree than irrelevant information which participants do not intend to learn. This process, which hinders consolidation of irrelevant information in favour of pertinent items, is contingent upon a differential hippocampal activity at encoding between memories to strengthen and those to erase or weaken. Our results indicate that brain activity at learning is crucial to determine the fate of relevant and irrelevant memories during subsequent sleep episodes.

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Figure legends

Figure 1: Experimental protocol. On day 1 (encoding phase, left panel), subjects saw one-by-one a series of words. They were instructed to read mentally the word presented. Each word was followed by the instruction "To remember" or "To forget". Immediately after learning, they were either allowed regular sleep (RS group) or totally sleep-deprived (TSD group) on the first post-learning night. They were all retested after two recovery nights using a recognition task (right panel) during which they had to determine, for each word presented, if they had previously seen it or not, regardless the instruction given at learning. fMRI data were acquired during both encoding and recognition.

Figure 2. Higher responses at encoding for TBR compared to TBF items (green) and for TBR-hits compared to TBR-misses in RS but not in TSD participants (exclusive masking, red). Responses are displayed on a sagittal section of the MNI template at $p<0.001$ uncorrected.

Figure 3. Common brain activations at retrieval for TBR-hits and TBF-hits in the RS group (compared to correct rejections). Activation are displayed at $p<0.05$ (FWE corrected) on sections of the MNI template. 1. Anterior cingulate cortex; 2. Calcarine region; 3. Insula; 4. Inferior frontal gyrus; 5. Caudate nucleus; 6. Lenticular nucleus.

Figure 1

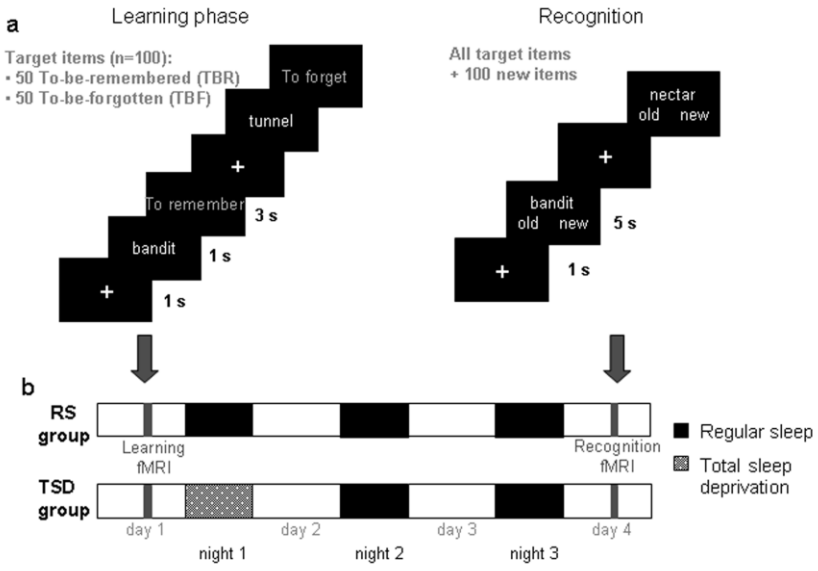
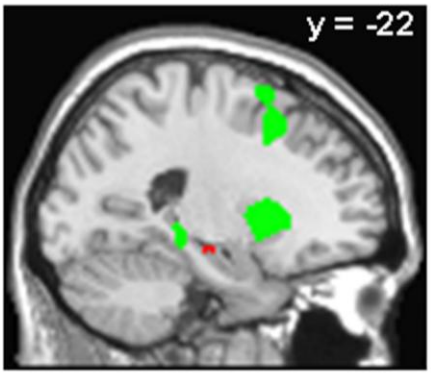

a
Learning phase
Recognition
Target items (n=100):
• 50 To-be-remembered (TBR)
• 50 To-be-forgotten (TBF)
All target items
+ 100 new items
To forget
tunnel
To remember
3 s
bandit
1 s
1 s
nectar
old new
bandit
old new
5 s
1 s
b
RS group
TSD group
Learning fMRI
Recognition fMRI
Regular sleep
Total sleep deprivation
day 1
day 2
day 3
day 4
night 1
night 2
night 3

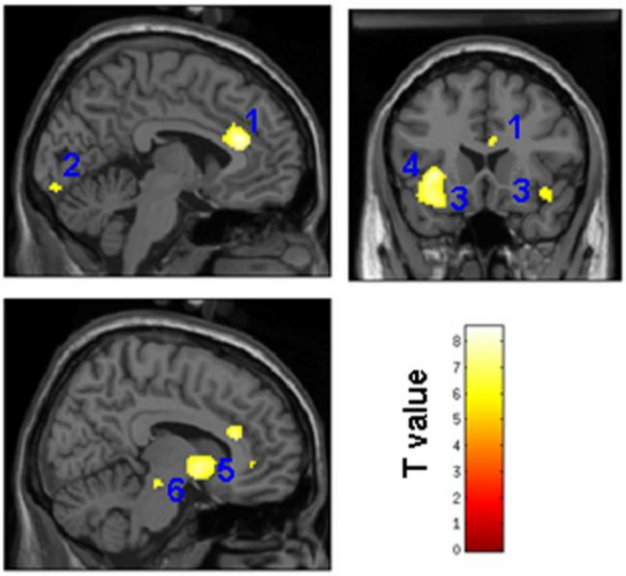
Figure 2



TBR > TBF

TBR-hits > TBR-misses in RS (but not in TSD, exclusive masking at p<0.05) group.

Figure 3



Supplemental information online for

Sleep contributes to the strengthening of some memories over others, depending on hippocampal activity at learning.

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Supplementary results

Alertness

Alertness was objectively measured right before fMRI sessions using an adapted Psychomotor Vigilance Task (Dinges et al., 1985), in which simple reaction times spaced by variable intervals (2-9 s) are measured over a period of 10 min. Median reaction times did not differ between groups in the two sessions (Encoding: RS (mean ± SD) = 262.9 ± 21.8 ms; TSD = 271.5 ± 24.1 ms; Recognition: RS = 264.8 ± 18.1 ms; TSD = 276.8 ± 28.4 ms; all p values > 0.14). A similar analysis conducted on the mean of the 10% slowest reaction times failed to reveal any significant effect of group (RS *vs* TSD) or session (encoding *vs* retrieval), nor interaction between these factors (all p values > 0.44).

Behavioural results

Behavioral data of one subject in the TSD group was not included in the analyses due to an abnormal proportion of false alarms. Nevertheless, adding or removing data of this subject did not change the sense of results concerning TBR-hits and TBF-hits.

Response times during the recognition task were analyzed by means of Student t tests. A first analysis conducted on mean response times failed to reveal any significant effect of group ($p>0.3$). Distinguishing between the different types of items and possible responses (TBR old, TBR new, TBF old, TBF new, New items categorized as previously encountered or not), statistical analyses revealed similar results (all p values > 0.25). To sum up, our data indicate

that memory performance differed between TSD and RS participants (for TBF items and false alarms) without any significant difference neither in recognition accuracy nor in response times. Together with the results of the psychomotor vigilance task, these data exclude the possibility that this difference in memory performance was merely due to an effect of tiredness in TSD participants.

Post-experiment questionnaire

The use of various strategies was estimated by means of a 5-point scale (ranging from 1: this strategy was never used by the participant, to 5: this strategy was always used). To memorize TBR items, participants used mainly a rehearsal strategy of one (mean score ± SD: 4.19 ± 1.06) or several (3.61 ± 1.10) items, created association between items to form a short story or a sentence (3.61 ± 1.3), and tried to associate the words to memories or personal events (3.58 ± 1.42). Mental imagery was less often used by the participants (2.38 ± 1.1).

When a forgetting instruction was displayed, participants mainly rehearsed the TBR items presented before (3.58 ± 1.36) and/or tried to think to nothing in particular (2.92 ± 1.41). As for TBR items, mental imagery of TBF items was little used by participants (2.19 ± 1.36).

Statistical analyses were also conducted to compare mental repetitions between groups (using Mann-Whithney U tests) and to compare each type of item (TBR / TBF) within each group (using Wilcoxon tests). These analyses indicate that mental repetitions did not differ between TSD and RS participants for TBR and TBF items (all p values > 0.1). As expected, and consistent with the analysis of the strategies used to memorize TBR items and forget TBF ones, TBR items were more repeated during the retention interval. More precisely, within the RS group, there was a significant difference between TBR and TBF items regarding the first point of the scale (« item never repeated during the 3-day interval ») (80.4 % of TBF were never repeated vs 59.7% of TBR items, $p<0.001$) and for the second point «(« repeated

between one and five times ») (TBF: 17.4%; TBR: 29.1%, $p<0.01$). As for the TSD group, there was also a significant difference between TBR and TBF items for the first point of the scale (TBF: 84%, TBR: 68.8%, $p<0.05$) and for the third point (« repeated between 5 and 10 times") (TBF: 1.2%,;TBR: 7.7%, $p<0.05$). It is worthy to note that there is no between group differences concerning the fourth point of the scale ("repeated more than 10 times") which could have be a potential confound in fMRI analyses.

Coordinates of interest.

The directed forgetting paradigm is a multi-compound task involving a large range of cognitive processes during encoding and recognition. Consequently, locations of interest were defined on the basis of specific cognitive processes supposed to intervene during the encoding and retrieval phases respectively. The following a priori locations of interest were used for small volume corrections, based on published coordinates (or contralateral coordinates) in the literature for cognitive processes similar to that involved in our encoding and recognition tasks and/or on studies assessing the effect of sleep and sleep deprivation on memory.

Effect of the "Remember" or "Forget" instruction on encoding-related brain activity.

- Remember instruction

Left inferior prefrontal cortex [-48 26 9] and [-44 18 -7] (Reber et al., 2002) ; Anterior cingulate cortex [-6 21 33] (Reber et al., 2002) ; Medial superior frontal gyrus [-9 9 59] (Reber et al., 2002); Posterior hippocampus [-30 -33 -9] (Kuhl et al., 2010); Middle temporal gyrus [-26 35 16] (Wylie et al., 2008); Insula [30 7 16] (Wylie et al., 2008).

- Forget instruction

Superior medial frontal gyrus [-6 67 12] (Wylie et al., 2008); Middle frontal gyrus [-34 27 60] (Wylie et al., 2008); Middle cingulate gyrus [18 -33 40] (Wylie et al., 2008); Middle, superior temporal gyrus [58 -65 24] (Wylie et al., 2008); Middle temporal gyrus [66 -17 -8] (Wylie et al., 2008); Parahippocampal gyrus [-14 -5 -20] and [18 -25 -20] (Wylie et al., 2008).

Encoding data : TBR-hits vs TBR-misses

Hippocampus [26 -16 -22] (Gais et al., 2007).

Recognition data: TBR-hits > TBR-misses

Superior frontal gyrus [-10 58 32] (Cansino et al., 2002); Middle frontal gyrus [36 50 8] (Spaniol et al., 2009); Inferior frontal gyrus [-44 42 0] (Spaniol et al., 2009); Anterior cingulate gyrus [-6 36 30] (Spaniol et al., 2009); Superior parietal lobule [-34 -60 44] (Spaniol et al., 2009); Amygdale [16 1 -21] (Cansino et al., 2002); Lingual gyrus [4 -74 -6] (Cansino et al., 2002); Precuneus [-6 -78 42] (Lundstrom et al., 2005); Cerebellum [-21 -60 -20] (Cansino et al., 2002) et [-22 -56 -26] (Gais et al., 2007); Supramarginal gyrus [48 -54 22] (Darsaud et al., 2011).

Recognition data: TBF-hits > TBF-misses

Medial frontal gyrus [-9 42 24] (Henson et al., 1999) ; Anterior cingulate gyrus [-3 21 39] (Henson et al., 2005) ; Cuneus/precuneus [15 -58 17] (Daselaar et al., 2006) ; Thalamus [-9 -12 6] (Montaldi et al., 2006).

Recognition data: common network for recognition of TBR-hits and TBF-hits (compared to Correct Rejections)

Inferior frontal gyrus [-32 20 -6] and [32 22 -14] (Spaniol et al., 2009); Caudate nucleus [10 10 -4] (Spaniol et al., 2009); Parahippocampal gyrus [-12 -36 4] (Spaniol et al., 2009); Hippocampus [26 -16 -22] (Gais et al., 2007) and [36 -20 -20] (Sterpenich et al., 2007).

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Supplementary Table 1: Brain areas associated, in both groups, to the encoding of TBR and TBF items, regardless of their status at retrieval.

		MNI coordinates (mm)				
Side	**Anatomical region**	**x**	**y**	**z**	**Z score**	**p value**
TBR > TBF						
Left	Putamen	-16	10	6	5.21	0.005*
Left	Thalamus	-4	-18	10	4.74	0.035*
Left	Precentral gyrus	-48	6	14	4.80	0.027*
Left	Middle frontal gyrus (BA 46)	-48	32	14	3.96	0.003**
Left	Inferior frontal gyrus (BA 44)	-48	16	12	3.84	0.004**
Left	Inferior frontal gyrus (BA 45)	-52	18	16	3.75	0.006**
Left	Superior frontal gyrus (BA 6)	-10	12	66	3.59	0.010**
Left	Posterior hippocampus	-26	-34	-8	3.4	0.017**
Left	Anterior cingulate cortex (BA 32)	-6	28	32	3.35	0.020**
Left	Inferior frontal gyrus (BA 47)	-54	18	-6	3.11	0.037**
TBF > TBR						
No suprathreshold clusters						

Coordinates x, y, z (mm) are given in standard stereotactic MNI space. All regions listed are statistically at p FWE corrected <0.05 (*) and $p^{svc(10mm)}$ <0.05 (**) based on *a priori* coordinates from the literature (see Supplemental Information).

Supplementary Table 2: Brain areas that exhibited greater activity for the recognition of TBR items, compared to forgotten items (TBR-hits > TBR-misses).

	MNI coordinates				
Anatomical region	**x**	**y**	**z**	**Z score**	**p value**
RS > TSD (exclusive masking at p<0.05)					
Precentral gyrus	-44	6	44	4.89	0.013*
Putamen	14	6	-12	4.65	0.034*
Inferior frontal gyrus (BA 47)	-44	42	0	4.08	0.001**
Anterior cingulate gyrus	-12	36	26	4.02	0.002**
Superior parietal lobule (BA 7)	-28	-58	50	3.94	0.002**
Precuneus (BA 7)	-6	-72	42	3.56	0.008**
Lingual gyrus (BA 18)	-4	-86	-10	3.39	0.013**
Superior frontal gyrus (BA 10)	-26	60	6	2.39	0.017**
Cerebellum	-16	-52	-26	3.25	0.019**
Middle frontal gyrus (BA 10)	-34	56	4	2.98	0.038**
Amygdala	-20	-4	-20	2.92	0.044**
Superior frontal gyrus (BA 8)	-8	52	42	2.88	0.048**
TSD > RS (exclusive masking at p<0.05)					
No suprathreshold clusters					

Coordinates x, y, z (mm) are given in standard stereotactic MNI space. *: p FWE corrected <0.05; **: p<0.05 after correction for multiple comparisons on small volumes of interest reported in the literature (see Supplemental Information).

Supplementary Table 3: Brain activations associated to the recognition of TBF items, compared to forgotten items (TBF-hits > TBF-misses).

		MNI coordinates (mm)				
Side	**Anatomical region**	**x**	**y**	**z**	**Z score**	**p value**
RS > TSD						
Left	Superior temporal gyrus (pole)	-42	22	-18	4.69	0.030*
Left	Medial frontal gyrus (BA 9)	-12	34	32	3.62	0.007**
Left	Anterior cingulate gyrus (BA 32)	-4	26	36	2.94	0.043**
Left	Thalamus	-10	-12	16	2.92	0.046**
TSD > RS						
	No suprathreshold clusters.					

Coordinates x, y, z (mm) are given in standard stereotactic MNI space. All regions listed are statistically significant at the p FWE corrected <0.05 (*) and p svc(10mm) <0.05 (**) based on a priori coordinates from the literature (see Supplemental Information).