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Report

Hippocampus-Dependent Strengthening of Targeted Memories via Reactivation during Sleep in Humans

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Summary

Recent accumulating evidence in animals and humans has shown that memory strengthening occurs, at least partially, during sleep [1, 2] and relies on the covert reactivation of individual memory episodes [3–5]. However, it remains to be determined whether the hippocampus critically promotes memory consolidation via the reactivation of individual memories during sleep. To investigate the hippocampal-dependent nature of this phenomenon in humans, we selected two groups of chronic temporal lobe epileptic (TLE) patients with selective unilateral (TLE+UHS) or bilateral (TLE+BHS) hippocampal sclerosis and a group of matched healthy controls, and we requested them to learn the association of sounds cueing the appearance of words. On the basis of other similar behavioral paradigms in healthy populations [4, 6], sounds that cued only half of the learned memories were presented again during the slow-wave sleep stage (SWS) at night, thus promoting memory reactivation of a select set of encoded episodes. A memory test administered on the subsequent day showed that the strengthening of reactivated memories was observed only in the control subjects and TLE+UHS patients. Importantly, the amount of memory strengthening was predicted by the volume of spared hippocampus. Thus, the greater the structural integrity of the hippocampus, the higher the degree of memory benefit driven by memory reactivation. Finally, sleep-specific neurophysiological responses, such as spindles and slow waves, differed between the sample groups, and the spindle density during SWS predicted the degree of memory benefit observed on day 2. Taken together, these findings demonstrate that the hippocampus plays a crucial role in the consolidation of memories via covert reactivation during sleep.

Results and Discussion

The hippocampus is critical for encoding recent episodic experiences into memory. Eventually, some of the encoded episodes are thought to be stabilized in long-term cortical storage via processes of memory consolidation, thereby enabling the memories to be accessed in the future, independent of the hippocampus [7]. Animal and human studies have provided compelling evidence that sleep, and, more specifically, the slow-wave sleep (SWS) stage, is important for episodic memory consolidation [1–3, 8] and relies on the covert reactivation of individual memory episodes [3–5]. Neuronal reactivation of memory representations during SWS contributes to the facilitating effect of sleep on memory consolidation, presumably by promoting the gradual redistribution of memory traces from hippocampal to neocortical brain regions for long-term storage [9, 10]. Thus, cuing of newly encoded memories during sleep using olfactory or auditory stimuli enhances the strengthening of these memories, demonstrating the important role of reactivation during SWS in the consolidation of memory [3, 4]. However, it remains to be determined whether the hippocampus promotes memory consolidation via individual memory reactivation during nighttime sleep.

In the current study, two groups of chronic temporal lobe epileptic (TLE) patients with selective unilateral (TLE+UHS; n = 7) or bilateral (TLE+BHS; n = 4) hippocampal sclerosis and a healthy control group matched in age, gender, and years of education (n = 9) (Experimental Procedures; Supplemental Experimental Procedures, Figure S1, and Tables S1–S3 available online) were selected to test the hypothesis that the strengthening of individual memories via reactivation during sleep is dependent on the structural integrity of the hippocampus in humans.

To address this question, we used a modified version of a behavioral paradigm that has been previously tested in healthy populations, demonstrating that memory reactivation during sleep, but not during wakeful periods, has robust selective memory strengthening effects [4]. In the current study, the task was structured in two phases, where each phase was separate by 12 hr and by a night's sleep (i.e., 9 p.m. [day 1] and 9 a.m. [day 2]) (Figure 1A; Supplemental Experimental Procedures). Participants were informed of the 2-day structure: on day 1, the task consisted of a learning phase, and memories encoded during this phase would be tested the following day (day 2). By explicitly informing the participants of the retrieval phase before the encoding phase on day 1, we expected to enhance the relevance of the learning material, an aspect that has been shown to be critical in the selective process for which memories benefit during overnight consolidation [11].

In the learning phase of day 1, the participants were trained to associate 28 different sounds that were consistently cued with the appearance of specific words, which were presented in one of the four corners of a PC monitor. The sound-word pairs were not semantically related (for the complete list of pairs, see Table S7). We carefully emphasized to the participants that the goal of the task on day 1 was to achieve a specific degree of learning accuracy (i.e., $\geq$50%) before going to sleep and that the criterion was solely based on the correct association of the sound-word information. The participants were informed that several encoding rounds were available to facilitate accomplishing this learning criterion. At the time

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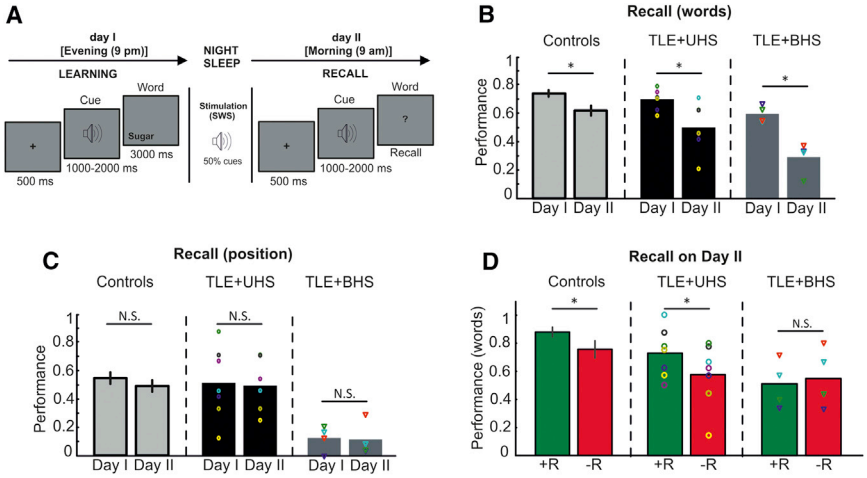

Figure 1. Experimental Design and Memory Performance before Day 1 and after Day 2 Nighttime Sleep

(A) On day 1, the participants were taught to associate a list of sound-word pairs. After all of the pairs were presented, a cue-recall test followed, in which the performance of each participant was individually tracked. During the test, the sound was presented, and the participants attempted to recall the associated word. This procedure was repeated until the subjects achieved a learning criterion of >50% of the sound-word pairs. On day 2, all of the sound-word pairs were retested.

(B) The bars indicate the behavioral memory performance in the ability to recall correct words (hit rate) before (day 1) and after (day 2) one night's sleep, which was separately averaged for the controls and the unilateral (TLE+UHS) and bilateral (TLE+BHS) patients with hippocampal sclerosis.

(C) The bars indicate the average behavioral memory performance of the controls and TLE+UHS and TLE+BHS patients in their ability to correctly recall the location of the word displayed during encoding when the memories were tested before (day 2) and after (day 2) one night's sleep.

(D) The bars indicate the average proportion of the controls and TLE+UHS and TLE+BHS patients, of the correctly recalled words belonging to memories that were reactivated overnight (+R), and of words that were not reactivated (–R) on day 2.

In (B)–(D), each patient is represented using the same color code. Error bars denote the standard SEM. *$p < 0.05$; N.S. denotes nonsignificance. See also Figures S1 and S2.

of encoding, the participants were requested to attend to all of the sound-word pairs in succession. They were permitted to read the words aloud if they considered it to be helpful during the learning process. Learning was assessed using a testing phase that consistently followed each encoding round after a brief resting time of 2–3 min. During this test phase, all of the sounds were presented again, and the participants were asked to recall the associated word. For the TLE patient group, but not the healthy control group, the experimenter initially provided a letter as a cue (the first letter of the paired word) when the participants were unable to recall the word. If the patient could not recall the word after the experimenter provided the initial three letters of the word, then the experimenter would provide the correct word (similar to a feedback-based learning procedure), and the next testing trial started without this last word marked as a hit for the round. Thus, although it is likely that the repeated presentation of the encoding information and provided feedback "artificially" enhanced the ability to learn in TLE patients, this enabled the methodological facilitation of the formation of memories on day 1. To balance the recall difficulty in individuals, the experimenter never provided letter cues to the healthy controls during the encoding rounds. On all of the tests, the participants were also asked to report the position at which the word was displayed on the PC monitor. The participants were informed that the ability to recall the position information was not included in the learning criteria on day 1 but that they should still report this information if they were able to do so. If the participants failed to achieve this learning criterion, then another encoding round began. The experimenter identified the last encoding round as the participant approached the learning criterion on the just-finished encoding round. Thus, the last encoding round served as a measure of presleep memory performance, which could be analyzed against the behavioral memory accuracy on day 2. During this final round, the entire word was never provided as feedback to the patient. All of the participants included in the study achieved the learning criterion in two to three rounds (mean for controls, 2.88; for TLE+UHS, 2.71; and for TLE+BHS, 2.75). In three cases, interference occurred during the first round of encoding (e.g., the task instructions were not sufficiently clear prior to the start of the encoding phase), which impaired the ability of the participants to effectively learn during this first round. In these three cases, a fourth round was implemented. Importantly, during the night, sounds cuing only half of the learned memory pairs were presented during the initial SWS stages, thus promoting memory reactivation of a select set of encoded episodes and equalize, at the individual level, for subsequently reactivated and nonreactivated associations after learning and before sleep (Supplemental Experimental Procedures) [4, 6].

On day 2, 45–60 min after the participants awoke, a memory cued-recall test was readministered. Testing during day 2 was performed at the same location and by the same experimenter of the learning phase on day 1, thereby ensuring that the contextual features between learning and testing would be preserved [12]. The results from the analysis of the behavioral memory performance on day 1 (controls, 0.74 ± 0.07 [mean ± STD]; TLE+UHS, 0.69 ± 0.07; and TLE+BHS, 0.59 ± 0.06) and day 2 (controls, 0.61 ± 0.11; TLE+UHS, 0.50 ± 0.17; and TLE+BHS, 0.29 ± 0.11) (independent of whether the stimuli were cued during sleep) revealed that, overall, the TLE+BHS patients recalled fewer sound-word pairs compared to the control and TLE+UHS groups [main effect of group in ANOVA, $F(2,17) = 9.51$, $p < 0.01$; two-sample t test for controls versus TLE+UHS, $t(14) = 1.62$, $p = 0.13$; controls versus TLE+BHS, $t(11) = 5.32$, $p < 0.01$; and TLE+UHS versus TLE+BHS, $t(9) = 2.56$, $p = 0.03$]. Furthermore, although memory forgetting occurred in all three groups [main effect of day in ANOVA, $F(1,17) = 57.69$, $p < 0.01$] (Figure 1B), there was a trend toward a differential degree of memory forgetting between groups, indicating that the forgetting rates tended to be greater in the TLE+BHS patients [marginal interaction day × group in ANOVA, $F(2,17) = 3.54$, $p = 0.052$; two-sample t test for controls versus TLE+UHS, $t(14) = -1.43$, $p = 0.18$; controls versus TLE+BHS, $t(11) = 2.81$, $p = 0.02$; and TLE+UHS versus TLE+BHS, $t(9) = 1.25$, $p = 0.24$]. Consistent with previous findings, our results showed rapid forgetting in TLE patients [13, 14].

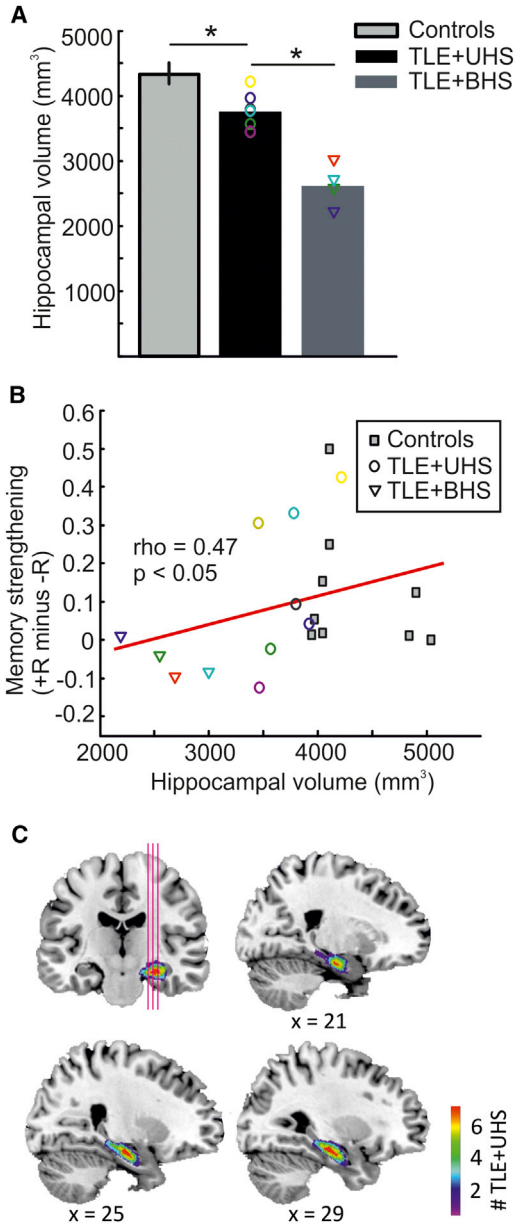

Figure 2. Hippocampal Volume and Memory Strengthening

(A) The average bilateral hippocampal volume in the controls and the average volume of the spared and intact portion of the lesioned hippocampus in TLE+UHS patients and in the TLE+BHS patients. Two-sample t tests (one tailed) revealed that the bilateral hippocampal volume was greater in the control group compared to the TLE+UHS group ($p < 0.001$). In addition, the hippocampi were greater in the TLE+UHS group compared to the TLE+BHS group ($p < 0.001$).

(B) Correlation analysis between the bilateral hippocampal volume and memory strengthening. The solid line represents the regression line.

(C) Anatomical overlap of the sclerotic region of the hippocampus in TLE+UHS patients.

In (A) and (B), each patient is represented using the same color code. Error bars denote the SEM. *$p < 0.05$; N.S. denotes nonsignificance. See also Figure S1 and Table S3.

Furthermore, consistent with the notion of the important role of the hippocampus in spatial memory processing [15], we observed a remarkable impairment in the ability of patients in the TLE+BHS group to report the correct position of the word compared to the TLE+UHS and control groups [main effect of group in ANOVA, $F(2,17) = 10.57$, $p < 0.01$; two-sample t test for controls versus TLE+UHS, $t(14) = 0.19$, $p = 0.85$; controls versus TLE+BHS, $t(11) = 6.21$, $p < 0.01$; and TLE+UHS versus TLE+BHS, $t(9) = 3.42$, $p < 0.01$] (Figure 1C; Supplemental Results). However, there were no apparent behavioral accuracy changes in the ability to correctly report the position of the word between day 1 and day 2 across groups [day × group interaction in ANOVA, $F(2,17) < 1$), an effect that remained consistent despite controlling for the number of wrong answers [ANOVA, $F(2,17) < 1$] (Supplemental Results and Table S4).

Next, we investigated whether the strengthening of individual memories during sleep was effectively mediated by the reactivation of specific memories during SWS in our sample. During the SWS stage, sounds for half of the learned pairs were presented, thereby promoting memory reactivation of a select set of encoded episodes. Memory strengthening driven by memory reactivation was measured as the difference in the proportion of hits for learned pairs that were stimulated overnight compared to nonstimulated learned pairs. These results revealed a trend in the beneficial effect of memory reactivation during SWS observed in the control and TLE+UHS patients, but not in the TLE+BHS patients [a marginal group × memory reactivation effect in the ANOVA, $F(2,17) = 3.46$, $p = 0.055$]. A series of planned one-sample t tests (one tailed) confirmed that reactivation of a selective set of memories during SWS was effective in reducing forgetting rates when tested on the subsequent day (i.e., memory strengthening) in the control ($p = 0.02$) and TLE+UHS ($p = 0.04$) groups, but not in the TLE+BHS ($p = 0.91$) group (Figure 1D). Thus, these findings provide empirical evidence for the role of the hippocampus in strengthening individual memories via reactivation during SWS.

Given that the selective bilateral alteration of the hippocampus severely impairs memory strengthening induced by memory reactivation during SWS, we next evaluated the possibility that the volume of the intact hippocampus was also linked to variations in memory consolidation derived from overnight reactivation. To address this question, we first determined that the volume of the spared hippocampus at the bilateral level differed between groups and that this measure was effectively modulated according to the groups' lesion. Indeed, the volume of the hippocampus significantly differed between groups [ANOVA, $F(2,19) = 28.04$, $p < 0.001$] (Figure 2A). A series of two-sample t tests (one tailed) confirmed that the hippocampal volume was greater in control subjects compared to the TLE+UHS patients [$t(14) = 2.94$, $p = 0.006$]. Moreover, the hippocampal volume of the TLE+UHS patients was greater compared to that of TLE+BHS patients [$t(9) = 6.04$, $p < 0.001$]. Thus, having confirmed that our sample reflected the volumetric differences in the intact hippocampus, we then determined the correlation between each individual's bilateral hippocampal volume and the behavioral measure for memory strengthening (i.e., the difference in the proportion of hits for learned pairs that were stimulated overnight compared to nonstimulated learned pairs). Our analyses showed that the degree of memory benefit observed on day 2 for memories that were reactivated compared to memories that were not reactivated during sleep positively correlated with the volume of the structural integrity of the hippocampus in our sample (rho = 0.47, $p < 0.05$) (Figure 2B). This association between hippocampal volume and memory behavior was statistically marginal despite the correlation of this memory index with

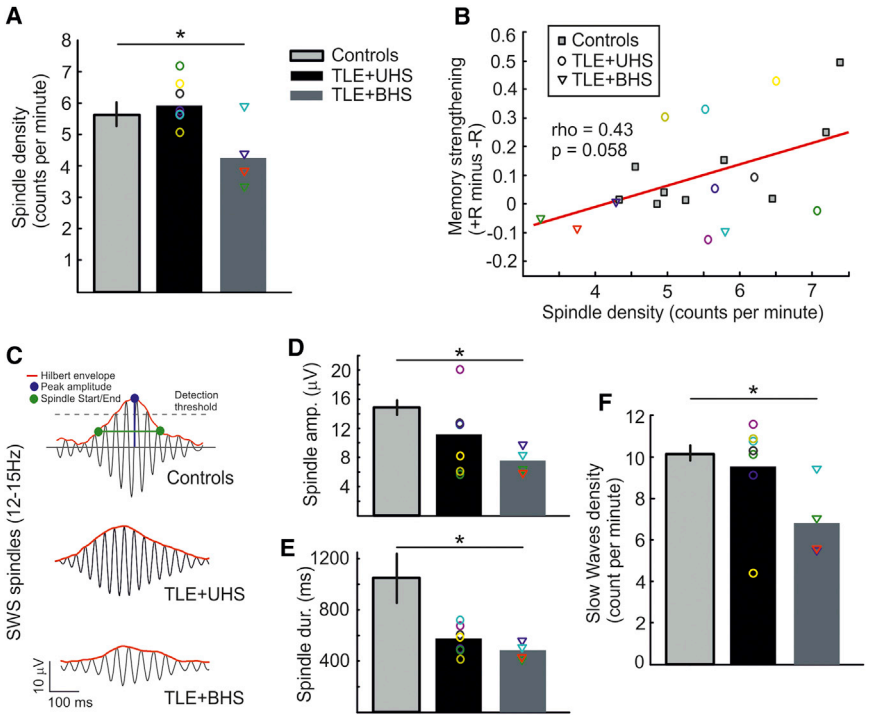

Figure 3. Spindles and Slow Waves during SWS and Memory Strengthening

(A) Average spindle density for each group of participants. A post hoc two-sample t test revealed that the spindle density of the control and TLE+UHS groups differed compared to the density in the TLE+BHS group (however, a trend was observed when compared to the controls, p = 0.069; to TLE+UHS, p = 0.01), but not between the controls and TLE+UHS patients (p = 0.53).

(B) Correlation analysis between spindle density and memory strengthening measured as the difference between the recall accuracy of items reactivated during SWS (+R) and items not reactivated overnight (–R) on day 2.

(C) Individual spindle events were selected on the basis of their power and duration. Raw EEG data were filtered in the spindle range (12–15 Hz). The instantaneous amplitude was extracted using the Hilbert transform (red trace). A detection threshold was set at the mean + 3 SD of the spindle power across SWS sleep (horizontal dotted line). Peaks exceeding this threshold (blue dot) were considered putative spindles. The start/end threshold was set at the mean + 1 SD of the spindle power across SWS sleep (horizontal green line), thereby defining the start and end times (green dots, respectively), which determined the spindle duration. Events with durations between 0.4 s and 4 s were further analyzed (Supplemental Experimental Procedures and Table S4).

(D) Average spindle amplitude for each group (controls versus TLE+UHS, p = 0.087; controls versus TLE+BHS, p < 0.001; and TLE+UHS versus TLE+BHS, p = 0.21).

(E) Average spindle duration for each group (controls versus TLE+UHS, p = 0.051; controls versus TLE+BHS, p < 0.001; and TLE+UHS versus TLE+BHS, p = 0.15).

(F) Average slow-wave duration for each group (see analysis details in the Supplemental Experimental Procedures) (controls versus TLE+UHS, p = 0.52; controls versus TLE+BHS, p = 0.001; and TLE+UHS versus TLE+BHS, p = 0.085).

In (A), (B), and (D)–(F), each patient is represented using the same color code. Error bars denote the SEM. *p < 0.05 (ANOVA); N.S. denotes nonsignificance. See also Figure S1 and Table S5.

the volume of the intact bilateral hippocampus only in the TLE+UHS and TLE+BHS patients (rho = 0.59, p = 0.061). These findings provide important insights into how individual differences in the structural integrity of the hippocampus may reflect the differential degree of functional impairment of memory consolidation in patients with hippocampal lesions.

Furthermore, there was a high degree of anatomical overlap in the sclerotic region of the anterior hippocampus between TLE+UHS (Figure 2C) and TLE+BHS (Figure S1) patients, indicating that the contribution of this hippocampal region to the reactivation of sound-associated memories during sleep may be functionally dissociated from the posterior segments of the hippocampus. This contribution is consistent with a central role for the anterior hippocampus in associative-relational memory processes [16, 17], raising the possibility that impairments in the ability to make strong connections between the cues and targeted memory during associative encoding in TLE+BHS patients could undermine the potential for these memories to be cue-reactivated during SWS re-exposure. Thus, during sleep, the hippocampus may function as a "gate" for the selection of targeted memories to be reactivated. Moreover, it is reasonable to assume that, among the many factors that may contribute to enhance spontaneous memory replay (e.g., salience, novelty, reward) [18], the strength of the memory association between a cue and a target memory could affect the degree by which the presentation of the cue "automatically" triggers a replay of the targeted memory during sleep.

The idea that memory reactivation is central in system consolidation during SWS has received extensive support at the mechanistic level from electrophysiological findings demonstrating rhythmic thalamocortical activity at 12–15 Hz (termed "spindles") [19], which is coupled to patterns of fast oscillations in the hippocampus (~200 Hz). This activity is associated with memory replay (termed "ripples") [5, 8, 20, 21], and such patterns of ripple-spindle events are regulated by slow waves (0.1–4 Hz), which originate in the neocortex [22]. Indeed, learning-related variations in spindles and slow-wave properties have been observed in noninvasive electroencephalographic recordings in humans [23–27]. Consistent with the mechanistic role of spindles and slow waves in memory consolidation, we explored the possibility that they could also account for the memory strengthening effects observed in our sample. Intriguingly, analysis of a number of properties of spindle activity (i.e., density, amplitude and duration) during SWS revealed consistent differences between the control subjects and TLE patients (all ANOVAs, p < 0.05) (Figures 3A and 3C–3E), where the values for these properties were consistently greater in the control subjects compared to the TLE+BHS patients (Table S5). Furthermore, we found that individual differences in spindle density during SWS showed a marginal positive correlation (p = 0.058) with the participants' memory benefit during day 2 driven by memory reactivation (measured as +R minus –R memories) (Figure 3B; Table S6). Interestingly, this correlation reached significance in the control subjects (rho = 0.70, p = 0.04) but not in the TLE patients

(including both TLE+UHS and TLE+BHS participants; rho = 0.24, p = 0.48). Finally, we observed that slow-wave density during SWS differed between groups [ANOVA, $F(2,19) = 4.85$, $p < 0.05$] (Figure 3F; Supplemental Results and Table S5), and the density of the individual's slow waves correlated positively with spindle density (rho = 0.59, $p < 0.01$). Thus, spindles and slow waves have emerged as a potential mechanistic candidate underlying the memory consolidation benefits driven by targeted memory reactivation during SWS. Although these findings may provide a valuable insight that links hippocampal structure, covert reactivation during SWS and memory consolidation, it remains to be determined how these neurophysiological indices of memory consolidation are associated with hippocampal activity networks, such as ripples, that have been observed in humans (see [28, 29]).

The association in the present study between physiological data and neural reactivation during SWS and its prediction of the degree of sleep-related memory strengthening is consistent with the idea that these findings are sleep specific. However, a potential methodological caveat of the present study is the lack of a separate wake sample of participants that could be matched with the groups included in the current investigation (i.e., healthy, TLE+UHS, and TLE+BHS). Due to the difficulty in recruiting this type of patients (given the low prevalence in the population), it was not possible to repeat this study in awake patients. Thus, some caution is required when concluding sleep specificity and the extent of the directional benefit of sleep memory reactivation compared to awake memory reactivation in our results (e.g., enhancement or prevention of deterioration [30]).

Our findings provide converging evidence of the critical role of the hippocampus in selective memory strengthening, which, via offline reactivation, underlies sleep memory consolidation in humans. These results show that the benefit of individual memory reactivation during SWS via external stimulation occurs only in patients with one preserved hippocampus, but not for patients with hippocampus that is bilaterally affected by sclerosis. Furthermore, our findings reveal that the degree of memory strengthening was correlated with the degree of hippocampal impairment, suggesting that such effects are highly sensitive to even partial lesions in this region.

The current results, which demonstrate that only a select set of memories were strengthened via re-exposure during offline SWS stages, are consistent with the view that the core mechanism by which memories are stabilized in long-term storage is through neural reactivation and that the hippocampus is critically implicated in this process. At the level of neuronal firing, compelling evidence obtained from studies in rats have shown that spatiotemporal patterns of neuronal firing present during the exploration of a novel environment and spatial tasks are replayed in the same sequential order in the hippocampus during subsequent sleep, and almost exclusively during SWS [5, 31, 32]. In humans, the active role of the hippocampus during memory re-exposure in SWS and its consequences on memory consolidation have also been reported [3]. However, the precise mechanisms by which newly encoded memory episodes are stabilized as enduring, long-term memories still remain unclear. Theoretical models have provided support for memory consolidation as a consequence of a regulated dialog between the neocortex and hippocampus. During such interactive processes, the hippocampus, either by containing time-limited memory representations (two-stage model; [30]) or by sharing such representations with the neocortex (multiple-trace theory; [33]), may act as a coordinator of disparate neocortical regions. Through the repeated orchestration of this distributed activity, the hippocampus may use feed-forward mechanisms [34, 35] to redistribute and establish new memories into long-term memories that are more resistant to interference.

Although memory reactivation during sleep has been shown to be a critical neural mechanism through which new memory episodes are transformed and gradually integrated into a long-term memory network, recent findings in animals and humans have provided evidence that other hippocampal-independent brain mechanisms might also play a role in memory consolidation [36, 37]. Thus, prior knowledge is suggested to lead to easier assimilation within an interrelated set of neocortical representations, or schema, when this new information finds multiple links within such a schema [7]. Indeed, animal studies have shown that the consolidation of new information into pre-existing schematic knowledge occurs very rapidly (i.e., within 48 hr after learning) [36]. The potential to acquire new memories and, most importantly, to make these memories durable and resistant to interference is a hallmark of patients with lesions in the medial temporal lobe. Thus, further studies are required to carefully evaluate the extent to which schema-based learning and memory reactivation could help to alleviate the deficits in memory consolidation observed in these patients.

In recent years, there has been growing interest in patients with temporal lobe epilepsy who display normal, or above normal, performance on standard delays of recall (i.e., ~30 min) but impaired performance over longer periods of retention (i.e., days or weeks), suggesting an alteration in memory consolidation mechanisms that results in an accelerated rate of forgetting [13, 14]. Our findings provide empirical evidence for the critical role of the hippocampus in the reactivation of memory events during sleep and demonstrate how an impairment in its structural integrity weakens the stabilization process of newly encoded memories, consequently accelerating the forgetfulness observed in these patients.

Experimental Procedures

Patient Information

The TLE group with unilateral sclerotic hippocampus (UHS) consisted of ten patients (seven females) with refractory TLE who were recruited after a presurgical evaluation at the University Hospital of Bellvitge (Table S1). The TLE patient group with bilateral hippocampal sclerosis (BHS) consisted of six patients (two females) with refractory TLE and recruited from a periodic clinical follow-up examination at the University Hospital of Bellvitge (Table S1). Patient diagnosis was established according to clinical electroencephalography (EEG) and magnetic resonance imaging (MRI) data. All of the patients underwent a neurological and neuropsychological examination (Table S2), continuous video-EEG monitoring, and brain MRI. Patients in the TLE+BHS group were comparable to the TLE+UHS group in terms of their explicit memory (immediate and delayed), working memory, semantic and phonetic fluency, and IQ (Supplemental Results). One TLE+UHS patient suffered from an epileptic crisis during the course of the task on day 1. This patient's data were removed from the final sample of the study. None of the remaining patients suffered a seizure during the experimental task or 24 hr before the task, and all of the patients were on habitual antiepileptic drug regimens. However, two TLE+BHS and two TLE+UHS were excluded from the final sample because they had not achieved the learning criteria on day 1. Thus, the final sample of TLE patients who participated in this study consisted of seven TLE+UHS and four TLE+BHS patients. The study was approved by the Ethical Committee of University Hospital of Bellvitge. Informed consent was obtained from all of the patients.

Behavioral Data analysis

For each participant, the memory accuracy for words during recall on day 1 and day 2 was calculated as the proportion of correct answers (i.e., hit rate).

This proportion was calculated over the tested memory pairs presented during the test. Similar calculations were performed to obtain the proportion of correctly recalled locations. A measure of behavioral memory accuracy for memories that were reactivated during SWS was extracted by calculating the proportion of correctly recalled words on day 2 that were stimulated overnight. A similar analysis was performed to obtain a measure of memory accuracy for memories that were not reactivated at night. Because the accuracy on day 1 was equalized between conditions, the difference in performance between the reactivated and nonreactivated memories on day 2 represented an index of memory benefit due to memory reactivation during SWS stage. Thus, the forgetting rate for reactivated and nonreactivated memories across individuals resulted in the range of [0–1].

A mixed repeated-measures ANOVA was performed to examine the behavioral memory differences on day 1 and day 2 within and among the three groups of participants. In addition, a mixed repeated-measures ANOVA was used to examine the forgetting rates of memories that were reactivated and not reactivated during SWS within and among the three groups of participants. Two-tailed Student's t tests were performed to determine the significant effects within (one-sample t test) and between (two-sample t test) groups. Group effects derived from three (groups; between subject) × two (experimental conditions; within subject) ANOVAs were further analyzed using a two-sample t test on the averaged within subject data. A one-tailed t test was used to analyze, at the within subject level, the presence of behavioral memory strengthening driven by memory reactivation during SWS. We used one-tailed rather than two-tailed t tests because (1) previous studies and theoretical considerations provide convincing support of memory benefit on day 2 of memories that were reactivated compared to memories that were not reactivated during SWS and because (2) a memory effect in the opposite direction would be difficult to explain, considering our current knowledge [1–3, 34]. Given the nature of the hippocampal lesion in each group of patients, a one-tailed t test was also used to test for differences in the volume of the hippocampus between groups. Importantly, p values obtained in the directional tests (i.e., one tailed) represented half of the p value obtained using two-tailed tests. The relationships between variables were determined using Spearman correlation analysis.

Neuroanatomical Data

MRI whole-brain structural scans, including T1-weighted and FLAIR acquisition protocols, were performed on the participants. An expert neuroradiologist on FLAIR imaging determined the extent of hippocampal sclerosis in each patient in the TLE+UHS group (Figure 2C) by manually determining areas of hyperintensity. Lesions were defined using the MRIcron software package and transformed into binary masks of sclerotic tissue. Volume measures for the left and right hippocampus were extracted from the structural T1 images using FreeSurfer (Supplemental Experimental Procedures and Figure S1).

Supplemental Information

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures, two figures, and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.07.006.

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