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The Role of Sleep in Declarative Memory Consolidation—Direct Evidence by Intracranial EEG

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Two step theories of memory formation assume that an initial learning phase is followed by a consolidation stage. Memory consolidation has been suggested to occur predominantly during sleep. Very recent findings, however, suggest that important steps in memory consolidation occur also during waking state but may become saturated after some time awake. Sleep, in this model, specifically favors restoration of synaptic plasticity and accelerated memory consolidation while asleep and briefly afterwards. To distinguish between these different views, we recorded intracranial electroencephalograms from the hippocampus and rhinal cortex of human subjects while they retrieved information acquired either before or after a "nap" in the afternoon or on a control day without nap. Reaction times, hippocampal event-related potentials, and oscillatory gamma activity indicated a temporal gradient of hippocampal involvement in information retrieval on the control day, suggesting hippocampal–neocortical information transfer during waking state. On the day with nap, retrieval of recent items that were encoded briefly after the nap did not involve the hippocampus to a higher degree than retrieval of items encoded before the nap. These results suggest that sleep facilitates rapid processing through the hippocampus but is not necessary for information transfer into the neocortex per se.

Keywords: hippocampus, intracranial EEG, memory consolidation, plasticity, sleep

Introduction

Memory formation has been suggested to occur in 2 subsequent steps involving different brain structures: whereas an initial encoding step depends on the hippocampus, subsequent consolidation (i.e., embedding of new knowledge into the vast network of previously gained experiences) involves replay of memories and information transfer from hippocampus to neocortex (Buzsáki 1989; Hasselmo 1999; Stickgold et al. 2001; Wiltgen et al. 2004). Furthermore, these 2 steps of memory formation appear to correspond to the 2 major activity patterns in the hippocampus: an initial step of memory formation occurs during states of increased hippocampal gamma activity, when the hippocampus receives rich sensory input (Buzsáki 1989; Lisman and Idiart 1995). Off-line memory consolidation, on the other hand, occurs during states of low sensory input when hippocampal pyramidal cells elicit highly synchronized population bursts, which may serve to transfer information to the neocortex (Buzsáki 1998).

Sleep is the most obvious state when organisms receive low sensory input. Electrophysiological recordings in animals (Pavlides and Winson 1989; Wilson and McNaughton 1994; Louie and Wilson 2001) and neuroimaging and electroencephalography (EEG) studies in humans (e.g., Gais and Born 2004; Peigneux et al. 2004; Gais et al. 2006) indicate that sleep plays a major role in memory replay and consolidation (Stickgold 2005). Although memory consolidation may involve multiple steps over long time periods (Stickgold and Walker 2005), important subprocesses appear to occur already briefly after learning. A single night of sleep (Bosshardt et al. 2005) and even a short "nap" of a duration of about 1 h (Mednick et al. 2002, 2003; Takashima et al. 2006) induce significant steps of memory consolidation during a variety of tasks.

Very recent observations, however, question the specific role of sleep for the replay of memory patterns and trace transfer: O'Neill et al. (2006) observed interleaved sharp wave bursts in hippocampal brain slices even during pharmacologically induced states with a high gamma power, reminiscent of waking activity. Foster and Wilson (2006) showed in vivo that sequences of spatially specific patterns of place cell activation are replayed in reverse order during resting states immediately after encoding. Finally, Peigneux used an elegant functional magnetic resonance imaging (fMRI) study to show reactivation of spatial memories in resting state directly after their initial encoding (Peigneux et al. 2006).

Importantly, these findings do not argue against the idea that memory consolidation may occur during sleep but challenge the view that information transfer is specifically linked to sleep. There is, however, an alternative function related to memory consolidation that has been suggested to be specifically supported by sleep: the homeostatic control of synaptic plasticity (Tononi and Cirelli 2006). It has been shown that synaptic long-term potentiation (LTP) may become saturated (Moser et al. 1998) and that its decay depends on active processes (Villarreal et al. 2002). These processes are enhanced during sleep (Cirelli and Tononi 2000; Cirelli et al. 2004). Importantly, recovery of plasticity was observed not only in the hippocampus but also in the neocortex as well. This suggests that information should be processed more rapidly through the hippocampus and stored in the neocortex shortly after sleep as compared with after a prolonged waking period, when a larger amount of information has already accumulated and further plasticity requires more energy (Braun et al. 1997).

Taken together, whereas classical consolidation theory suggests that declarative information is stored in the hippocampus for a certain period and that information transfer to the neocortex occurs mainly during sleep, in particular during non-rapid eye movement (REM) sleep, novel data suggest a similar degree of consolidation during waking state. We designed an experimental paradigm to distinguish between these hypotheses. This paradigm is depicted schematically in Figure 1*A* together with the predictions of the 2 competing theories. Intracranial EEG recordings were performed on 2 subsequent days, one of them with a nap of 47.3 ± 5.8 min duration (mean ± standard error of mean) during which we

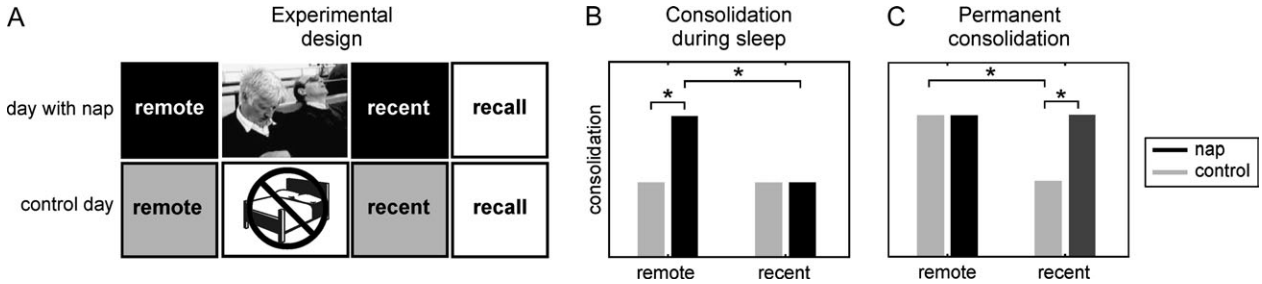

Figure 1. Experimental paradigm and predictions of 2 alternative theories of memory consolidation. (*A*) Overview of the experiment. Subjects learned 2 sets of pictures during 2 subsequent days. Whereas there was a nap between learning of remote and recent items on one of the days, subjects rested for the same duration without sleep on the control day. (*B*) "Classical" consolidation theory suggests that hippocampal–neocortical information transfer mainly occurs during sleep. It thus predicts a different degree of consolidation between remote items on the control day and the day with nap and a difference between remote and recent items on the day with nap. (*C*) The alternative consolidation theory suggests that hippocampal–neocortical information transfer occurs similarly during sleep and waking state but that sleep restores synaptic plasticity and thus facilitates learning briefly after sleep. This theory predicts a difference between the degree of consolidation on the control day and between retrieval of recent items on the 2 days.

obtained polysomnographic recordings and the other one with a rest period of similar duration. On each day, 160 pictures of landscapes and buildings were presented in 2 sessions with or without nap in between; retrieval occurred 15 min after the last learning session. Items in the first learning session are referred to as "remote" items, and items in the second learning session as "recent" items. For more details, refer to Materials and Methods. Critically, the 2 theories make competing predictions concerning behavior and brain activity during retrieval. The classical view (consolidation during sleep) predicts that consolidation occurs mainly during sleep. Therefore, only items that have been learned prior to the last sleep period should have been consolidated. In our experiment, this condition occurs only during retrieval of remote items on the day with sleep (high degree of consolidation of remote items on the day with nap in Fig. 1*B*). No sleep (and according to this theory no consolidation) occurs between encoding and retrieval of recent items on the nap day and between encoding and retrieval of either remote or recent items on the control day. Thus, there should be a difference in retrieval of remote and recent items on the day with nap and between remote items on the 2 days (small bars for all other conditions in Fig. 1*B*). If, on the other hand, consolidation occurs similarly during sleep and waking states (permanent consolidation), the critical factor for consolidation is mainly the time interval between encoding and retrieval. Therefore, remote items should have undergone consolidation on both the control day and the day with nap (Fig. 1*C*). In other words, there should be a difference in the degree of consolidation of remote and recent items even on the control day (Fig. 1*C*). This latter view is consistent with the alternative function of sleep to restore synaptic plasticity. As recent items on the day with nap are encoded briefly after sleep, they should benefit from sleep by faster consolidation than recent items on the control day, which are encoded after some time awake. This is schematically depicted in Figure 1*C* by the small degree of consolidation for recent items on the control day. Thus, the "permanent consolidation" theory further predicts a difference in consolidation of the recent items between the 2 days but not between recent and remote items on the day with nap.

Materials and Methods

Subjects

Eleven patients with pharmacoresistant temporal lobe epilepsy (5 women; mean age ± standard deviation [SD]: 36.8 ± 10.6 years) participated in the study. No seizure occurred within 24 h before the experiment. Recordings were performed from 2005 to 2006 at the Department of Epileptology, University of Bonn, Germany. All patients had bilateral mediotemporal depth electrodes that were inserted for diagnostical purposes using a computed tomography-based stereotactic insertion technique (Van Roost et al. 1998). The location of electrode contacts was ascertained by MRI in each patient and was classified as either hippocampal or rhinal or otherwise. Because our methods cannot clearly separate perirhinal and entorhinal generators, we use the term rhinal cortex without indicating an integrated rhinal processing stage. On average, patients had 2.2 ± 1.0 rhinal and 5.6 ± 1.1 hippocampal contacts (mean ± SD). The study was approved by the local medical ethics committee, and all patients gave written informed consent.

Experimental Paradigm

An overview of the experimental design is depicted in Figure 1. Each picture was presented for 1200 ms with an interval of 1800 ± 200 ms between the presentations. To monitor item processing, subjects were asked to distinguish buildings and landscapes by pressing 1 of 2 mouse buttons. The first learning session on each day was performed at 12:00 after lunch. On the day with nap, which was counterbalanced between the first and second day of the study, subjects lay after the first learning session on a bed in an electrically shielded, sound and light attenuated room for 60 min. During this period, we obtained polysomnographic recordings consisting of surface EEG, as well as measurements of horizontal and vertical eye movements, electrocardiograms, and facial electromyograms. On the day without nap, subjects had no specific instructions during this period but were asked not to sleep. A medical technical assistant controlled that subjects were awake by video monitoring and eventually by enquiry. Fifteen min after awakening from sleep, a second learning session of the same length was performed. This was followed by a 15 min rest period, during which subjects were engaged in a conversation with the experimenter to avoid rehearsal of items in short-term memory. Afterwards, subjects performed a retrieval session in which they were presented with the old as well as 80 randomly intermixed new pictures. Subjects were instructed to indicate by button press whether they had seen the picture before or not. Only trials with a correct response were taken into account for the EEG analyses. During retrieval, we recorded continuous EEG from the depth electrodes as well as from bilateral mastoid electrodes.

Recording and Analyses

Depth EEG was referenced to linked mastoids, recorded at a sampling rate of 1000 Hz, and band-pass filtered (0.01 [6 dB/octave] to 300 Hz [12 dB/octave]). EEG trials were visually inspected for artifacts (e.g., epileptiform spikes). From the contralateral (nonfocal) electrode in each patient, we analyzed data from the rhinal contact with the maximal AMTL N400 amplitude (between 200 and 600 ms) and the hippocampal contact with the maximal late positive potential (LP) (between 400 and 1500 ms). Data were analyzed using the EEGLAB package created by A. Delorme and S. Makeig (Delorme and Makeig 2004) running with

MATLAB (The Mathworks, Natick, MA). In addition to time domain analyses, we conducted frequency-based analyses to assess whether and how localized bursts of activity in specific frequency bands reflected task demands. EEG trials were filtered in the frequency range from 2 to 100 Hz (2 Hz steps) by continuous wavelet transforms. For statistical analyses, power values were averaged for nonoverlapping successive time windows of 500 ms duration from 0 to 1500 ms after stimulus onset. Afterwards, power values were normalized with respect to the prestimulus time window from –200 to 0 ms separately for each subject and each filter frequency. For the graphical depiction, power values were normalized to the prestimulus time window and then transformed into dB scale (10 * $\log_{10}$). Due to the intrinsic logarithmic frequency scaling of the wavelet decomposition, higher frequencies are not statistically independent if sampled too closely on an equidistant frequency scale. Therefore, the EEG was analyzed in broad spectral bands. *P* values in the analyses of variance (ANOVAs) were Huynh–Feldt corrected for inhomogeneities of covariance when necessary (Huynh and Feldt 1976).

Results

Study Overview and Behavioral Results

We recorded EEG from the hippocampus and the rhinal cortex of 9 epileptic patients implanted with depth electrodes for presurgical diagnostics; behavioral data were acquired from these and 2 additional patients, where no intracranial EEG was obtained for technical reasons. The intracranial EEG recordings allowed us to obtain human neural activity patterns from deep brain structures with a high sampling rate (1 kHz). The sleep statistics are depicted in the supplementary Figure 1. We first analyzed behavioral data during retrieval of items. The accuracy of memory retrieval appeared to be higher on the day with nap for both remote (53.9% vs. 51.9%) and recent items (57.1% vs. 54.2%); however, none of these effects reached significance ($P > 0.1$; 2-tailed *t*-tests; Fig. 2*A*). Our reaction time data (see supplementary Table 1 for single-subject data) were consistent with the theory that memory consolidation occurs similarly during sleep and waking state (Fig. 1*C*): ANOVAs of the behavioral data revealed a significant interaction between "category" (remote, recent, or new) and "sleep" ($P < 0.05$, 2-way ANOVA with 2 repeated measures; Fig. 2*B*). On the day without nap, recent items were retrieved more rapidly than remote items ($P < 0.05$; 2-tailed *t*-test). In contrast, there was no significant difference on the day with nap ($P > 0.1$; 2-tailed *t*-test). Furthermore, a comparison between retrieval of recent items on the 2 days revealed significantly faster retrieval at the day without nap ($P < 0.05$; 2-tailed *t*-test) while reaction times were not different during retrieval of remote items between the 2 days ($P > 0.1$; 2-tailed *t*-test). Taken together, our behavioral data show that retrieval of recent items on the control day is faster than both retrieval of remote items on the same day and retrieval of recent items on the day with nap.

A role of sleep in memory consolidation, in particular of deep sleep in declarative memory consolidation (Gais et al. 2006; Marshall et al. 2006), would predict that the total sleep time and/or the amount of deep (stage 3/4) sleep correlates with reaction times as a measure of memory consolidation. Indeed, we found that retrieval time increased with the amount of sleep and the amount of deep sleep (supplementary Fig. 2). Moreover, this correlation was observed during retrieval of both remote and recent items, supporting the idea that consolidation during sleep not only affects items learned prior to sleep but also facilitates consolidation after sleep as well.

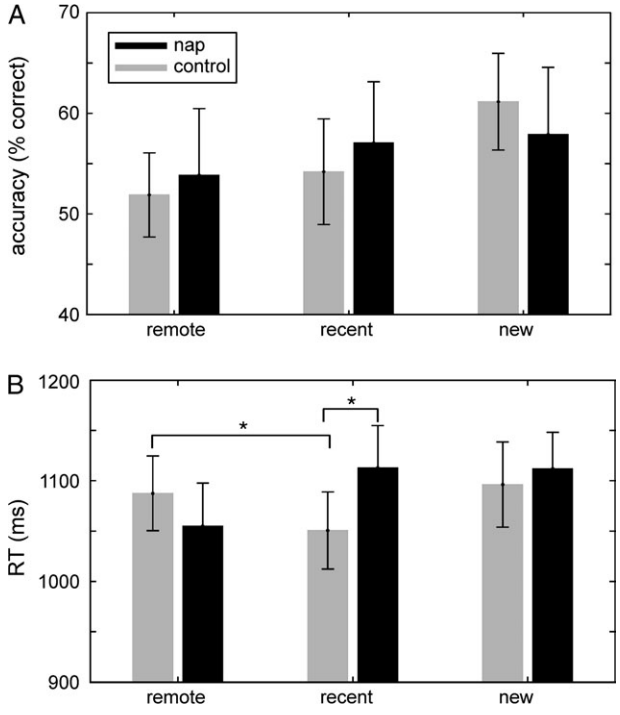

Figure 2. Behavioral data. (*A*) Our behavioral data showed no significant difference in recognition between the day with nap and the control day. (*B*) Retrieval of recent items on the control day was more rapid as compared with both recent items on the day with nap and remote items on the same day. These data are thus consistent with the view that consolidation occurs similarly during sleep and waking state.

EEG Results

Event-related potentials (ERPs) recorded during retrieval from the hippocampus and the rhinal cortex are displayed in Figure 3. Only correct trials were included in the averages. We analyzed the amplitudes of the anterior mediotemporal negative potential occurring 400 ms after stimulus presentation (AMTL N400) and the hippocampal LP by averaging the potentials in their respective peak regions (200–600 ms for the AMTL N400 and 600–900 ms for the LP). The LPs in the hippocampus were generally more pronounced when retrieval occurred after sleep ($P < 0.01$; 2-way ANOVA). Moreover, there was a significant interaction of "category" and "sleep" ($P < 0.05$; 2-way ANOVA); a direct comparison of hippocampal LPs during retrieval of recent and remote items revealed a significant difference on the day without nap ($P < 0.05$; 2-tailed *t*-test) but not on the day with nap ($P > 0.5$; 2-tailed *t*-test). Furthermore, whereas retrieval of remote items was not different between the 2 days ($P > 0.1$; 2-tailed *t*-test), there was a highly significant difference for the retrieval of recent items ($P < 0.01$; 2-tailed *t*-test).

These data are thus consistent with the behavioral findings: the hippocampal LPs during retrieval of recent items at the control day differ both from the potentials during retrieval of remote items on the same day and from retrieval of recent items on the day with nap.

The rhinal cortex is the major interface between the hippocampus and the neocortex. While the ERPs in the hippocampus were specifically altered during retrieval of recent items on the day without nap, favoring the hypothesis that memory consolidation may occur during waking state (Fig. 1*C*), we observed no differences in the AMTL N400 ($P > 0.1$; 2-way ANOVA).

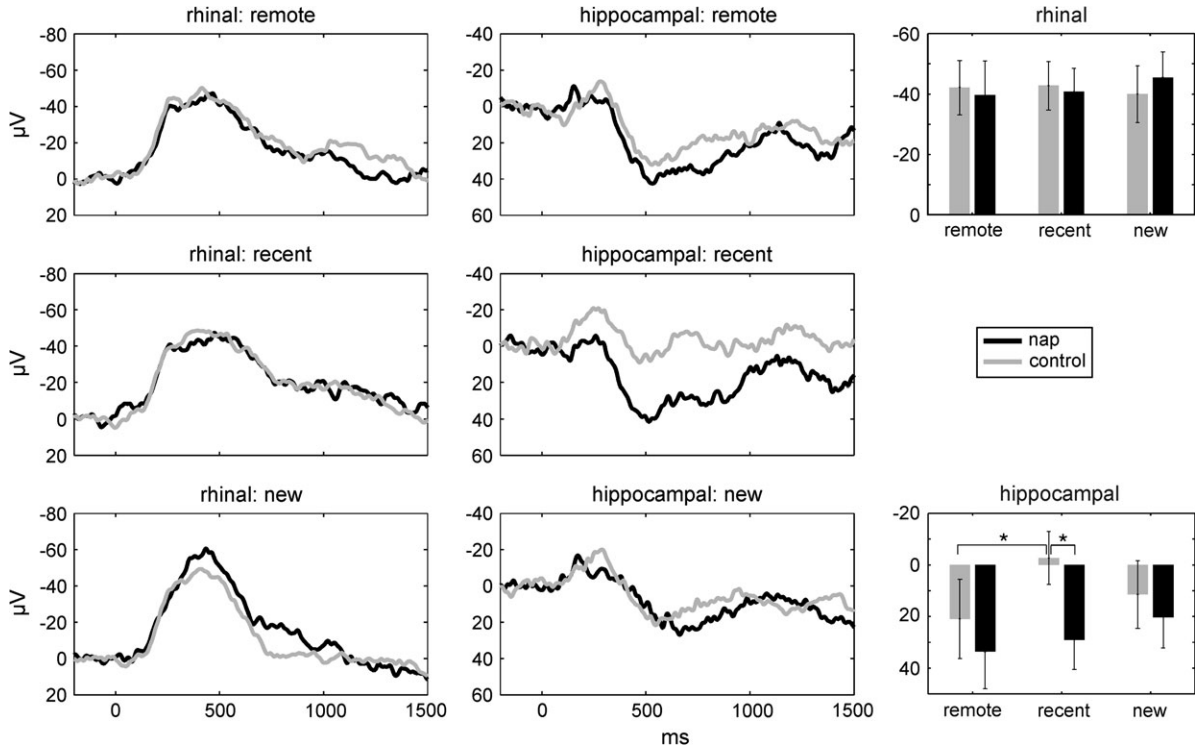

Figure 3. ERP data. Retrieval of recent items at the control day leads to a decreased hippocampal LP as compared with both remote items on the same day and recent items on the other day. There is no significant effect on the rhinal AMTL N400 potential.

ERPs are robust measures of population activity. It is hard to infer, however, whether an increased neural activity in a certain brain region corresponds to positive or negative potentials. Even more importantly, the main physiological activity of the awake hippocampus and the possible correlate of newly formed memories consists in oscillatory gamma activity, which is not reflected in unfiltered ERP data (induced, i.e., nonphase locked, gamma activity is not visible at all in averaged ERP data). We thus calculated the power in different frequency bands (theta = 3–8 Hz; alpha/beta = 8–30 Hz; $gamma_1$ = 31–60 Hz; $gamma_2$ = 61–90 Hz) for nonoverlapping time windows of 500 ms length in the hippocampus (Fig. 4). A 4-way ANOVA with "sleep" (nap or no nap), "category" (remote or recent), "window," and "band" as repeated measures revealed a trend for an interaction of "band" and "sleep" ($P = 0.063$) and a main effect of sleep in the $gamma_2$ frequency range ($P < 0.05$) but not in any other band. Based on our ERP findings, we conducted additional 2-way ANOVAs to compare whether $gamma_2$ activity during retrieval of recent and remote items differed between the day with nap and the control day. Consistent with the ERP data, we found that retrieval of recently encoded items differed significantly between the 2 days ($P < 0.05$; 2-way ANOVA with "sleep" and "window" as repeated measures) but not retrieval of remote items ($P > 0.1$; 2-way ANOVA with "sleep" and "window" as repeated measures).

A similar 4-way ANOVA of power in the rhinal cortex only yielded trivial main effects of "bands" and "window" but no effects of "sleep" or "category" and no interactions. Consistent with the rhinal ERP data, this indicates that the observed effect occurs selectively in the hippocampus.

Whereas we did not find significant effects in lower frequency bands during retrieval, recent findings from Marshall et al. (2006) as well as other groups strongly suggest that consolidation is related to oscillatory activity in lower frequency bands, in particular delta activity (slow waves). Indeed, the effects on gamma band activity reported in our study concern retrieval of consolidated as compared with unconsolidated items, not the process of consolidation per se. We thus calculated the amount of low-frequency activity in the hippocampus and rhinal cortex during the naps (power of delta band activity [1–3 Hz] multiplied with the number of non-REM sleep episodes) and computed the correlation with retrieval times (supplementary Fig. 2A, B). In the hippocampus, the amount of delta band activity during sleep was (weakly) positively correlated with retrieval times of recent items (Pearson correlation coefficient: 0.20) but less with retrieval times of remote items (Pearson correlation coefficient: 0.03). In the rhinal cortex, Pearson correlation values were 0.17 and 0.15 for the correlation between the amount of delta band activity and retrieval times of recent and remote items, respectively. Finally, we correlated total sleep time and deep sleep time with retrieval times and again observed slight positive correlations (supplementary Fig. 2C, D).

Discussion

Using intracranial EEG recordings from the hippocampus and rhinal cortex of epilepsy patients, we found a temporal gradient of hippocampal involvement in memory retrieval: whereas there was a pronounced late positive component during retrieval of items that were encoded after a time span of a few hours, this component was significantly reduced when recently encoded items were retrieved (Fig. 3). This result may at first sight be interpreted that the hippocampus is not involved in retrieval of recent items on the control day. We are not aware of

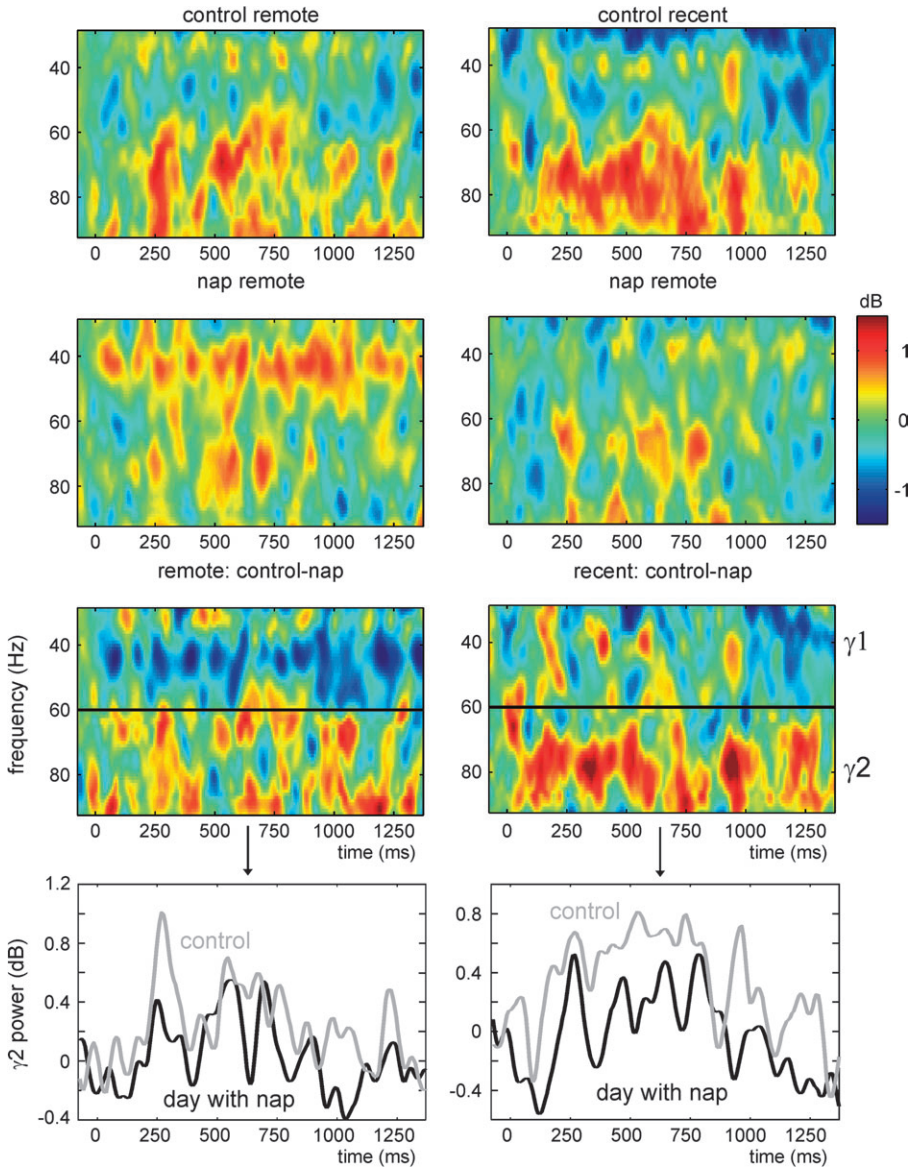

Figure 4. Power data. Power in the γ2 frequency range (60–90 Hz) in the hippocampus is specifically increased during retrieval of recent items at the control day as compared with the day with nap. There is no effect of power on retrieval of remote items. The bottom row shows averaged power in the gamma$_2$ frequency band during retrieval of items on the control day (gray) and on the day with nap (black). The color bar applies to all plots.

any previous human intracranial EEG studies investigating hippocampal ERPs during recognition memory (apart from studies using continuous recognition paradigms which simultaneously involve encoding and retrieval). Fried et al. (1997) reported increased firing of hippocampal cells during presentation of old as compared with new items regardless of subjects' response but did not analyze field potentials. However, the lack of a hippocampal involvement in recognition of recently learned material is inconsistent with a large group of findings from clinical studies of amnesic patients (e.g., Reed and Squire 1997), lesion studies in animals (e.g., Beason-Held et al. 1999; Zola et al. 2000), and experiments using fMRI (e.g., Stark and Squire 2000a). Furthermore, our time–frequency analysis revealed increased, rather than decreased, activity in the gamma frequency band during retrieval of recent items on the control day (Fig. 4). We thus suggest that the apparent lack of a hippocampal EEG response during retrieval of recently encoded items on the control day corresponds to the superposition of the late hippocampal component and a slow negative EEG shift. Indeed, slow negative potential shifts likely correspond to increased firing and/or increased synaptic activation and membrane potential depolarization of large groups of neurons (Speckmann and Elger 1999). They thus indicate an increased involvement of a region in a cognitive process (Birbaumer et al. 1990). In the hippocampus, findings from several groups indicate a direct relationship between negative field potentials and increases in cellular activity (e.g., Bragin et al. 1995; de la Prida et al. 2006; Molle et al. 2006). We therefore interpret the negative potential shift as an increased engagement of the hippocampus in retrieval of recent items on the control day. In other words, the hippocampus is mostly involved in the retrieval of recent items that have been encoded a considerable period after (night) sleep; it is significantly less involved if more time has passed during encoding and retrieval

or if encoding occurs directly after sleep. A reduced hippocampal involvement during memory retrieval likely indicates that items have been transferred to the neocortex. Taken together, these data suggest that newly acquired information is not primarily transferred from hippocampus to neocortex during sleep, as predicted by "classical" theories of memory consolidation, but during waking states as well. Directly after sleep, when no sensory input has reached the hippocampus for the period of sleep, information is rapidly processed through the hippocampus, and retrieval of this information involves less hippocampal engagement (Figs 3 and 4). These data represent the first direct electrophysiological support for the model that some steps of memory transfer from hippocampus to neocortex may not only occur during sleep but also occur during waking state (Foster and Wilson 2006; O'Neill et al. 2006; Peigneux et al. 2006).

An alternative explanation for the observed pattern of ERP responses is that the pronounced hippocampal component is actually the negative reflection of the rhinal component and that during retrieval of recent items on the control day this source moves to within the hippocampus. As a consequence, the hippocampus becomes the reversal point of the ERP and the ERP amplitude within the hippocampus is reduced. It should be noted that this explanation is consistent with an increased engagement of the hippocampus during retrieval of recent items on the control day despite a "flat" EEG trace. We argue, however, that it is less likely for the following reasons. First, the peak of the rhinal potential is earlier than the hippocampal peak, so that it is unlikely that the hippocampal peak is its negative counterpart. Second, the timing of the rhinal ERP suggests that it most likely reflects an AMTL N400 component (e.g., Halgren et al. 1980; McCarthy et al. 1995). Although this component occurs in the rhinal cortex in a variety of conditions, it has not been described so far in the hippocampus. Finally, hippocampal field potentials do not primarily reverse along the longitudinal axis of the hippocampus but between different cellular layers. For example, phase reversal occurs between stratum oriens and stratum lacunosum-moleculare for theta band activity (Bragin et al. 1995) and between pyramidal cell layer and stratum radiatum for hippocampal sharp waves (Buzsáki et al. 1983). It is unlikely that the electrodes in all patients were by chance located at the reversal point or at points with field potentials of opposite polarity because the criterion for electrode selection was the maximal peak amplitude (averaged across conditions).

In our behavioral data, we did not find a difference in accuracy between the conditions (Fig. 2*A*). Moreover, memory performance was generally rather low in our study (around 55%; Fig. 2*A*). We suggest that this is partly due to the large number of pictures and the similarity among the items. In addition, memory performance in some of the epilepsy patients was impaired as compared with normal subjects. This does not imply that the ERP data are qualitatively different from those which would be expected in healthy subjects; it has been shown that ERPs that are recorded from the contralateral site of the seizure origin in epilepsy patients are similar to ERPs recorded in healthy nonhuman primates (Paller et al. 1992). Although accuracy did not change during conditions, we observed a clear effect on reaction times: recent items on the control day are more rapidly accessible than remote items on the control day and also than recent items on the day with nap (Fig. 2*B*). This result may appear inconsistent with previous reports of improved memory recall after sleep (Gais et al. 2006). It should be noted, however, that different concepts of memory consolidation are being used in the literature: whereas some authors directly investigated memory performance (i.e., the number of correctly remembered items), others showed that consolidation specifically protects against interference (Ellenbogen et al. 2006). In a neurophysiologically inspired theory of memory consolidation, Buzsáki (2005) conceptualized consolidation as the process of embedding egocentric, or episodic, memories into an allocentric, or semantic, frame. This theory is directly linked to (and derived from) electrophysiological recordings and is thus most suited for the interpretation of the results in our study. One prediction of this theory is that consolidation increases the time required for retrieval: consolidated (semantic) information lacks the autobiographic context of a memory and thus makes it harder to remember than an episodic memory. Our result is consistent with the findings of Takashima et al. (2006) who showed that reaction times were shorter for confidently retrieved recent than for confidently retrieved remote items. Taken together, we argue that although consolidation implies that items are embedded into former experiences and thus integrated into a richer framework, this enrichment of associative context is well consistent with an impaired access to these items. Indeed, retrieval of more consolidated information containing more semantic associations has been suggested to become less vivid and slower (Nadel and Moscovitch 2001).

We found that reaction times during correct rejection of new items were similarly slow as during retrieval of remote items (Fig. 2). This might be due to the additional time needed for encoding of these new items, as observed in continuous recognition experiments for the first repetition of items (e.g., Van Strien et al. 2005). In addition, the average retrieval times of remote items on the nap day were shorter than of remote items on the control day (and almost reached the level of the recent items on the control day), even though this difference was not significant. Although we do have a clear explanation for this finding, it might be related to an increased alertness after the nap, although in this case one might expect faster reaction times to new items (as compared with the control day) as well, which was not observed.

The finding of a temporal gradient in hippocampal involvement on the control day is in apparent contrast to previous fMRI results showing that the interval between encoding and retrieval does not affect retrieval-related activity in the hippocampus (Stark and Squire 2000b; Ryan et al. 2001). Similarly, previous studies showed that retrieval-related hippocampal activity either decreased (Takashima et al. 2006) or increased (Bosshardt et al. 2005) specifically as a function of sleep. The divergence with the data presented here might be explained by the higher sensitivity of intracranial EEG to mediotemporal activity changes as compared with fMRI (Brázdil et al. 2005), suggesting that the direct measurement of hippocampal and rhinal EEG is sensitive to earlier decays of hippocampal involvement during retrieval, which already affect reaction times (Fig. 2). Importantly, we do not argue against theories suggesting that memory consolidation involves multiple steps that occur across an extended period of time, consistent with the graded retrograde memory deficit in amnesic patients (Stickgold and Walker 2005). We do argue, however, that important processes involving a measurable disengagement of the hippocampus do not strictly depend on sleep but already occur during waking state early after initial encoding. On a cellular level, these changes might correspond to early LTP, whereas further time is needed for late LTP to occur (Reymann and Frey 2007).

Interestingly, the behavioral and neurophysiological effects of prior sleep on retrieval of recent items suggest a different role for sleep in memory consolidation: instead of specifically supporting information transfer during sleep, sleep appears to restore saturated hippocampal plasticity (McNaughton et al. 1986; Moser et al. 1998; Tononi and Cirelli 2006) and allow for rapid processing through the hippocampus after sleep. It might be argued that the slower retrieval of the recent items on the day with nap as compared with the control day is due to sleep inertia (Stones 1973). However, sleep inertia has been shown to mainly decrease recognition accuracy of items that were learned briefly after sleep. In contrast, we observed a slightly higher accuracy of retrieval of recent items on the day with nap as compared with the control day (Fig. 2*A*), although this effect did not reach significance.

In our study, hippocampal involvement was measured both as a negative shift of the hippocampal LP and as an increased power of local gamma oscillations. More specifically, we found that retrieval of recent items on the control day is accompanied by both a negative shift of the hippocampal LP (as compared with retrieval during the other conditions) and an increased power of $gamma_2$ activity (Fig. 4). This result is in line with previous intracranial EEG studies of long-term memory encoding, which revealed that a negative shift of the hippocampal LP is accompanied by an increase of hippocampal gamma power (Fernández et al. 1999; Fell et al. 2001). Thus, a more negative shift in the extracellular EEG appears to be correlated with an increased activity in the gamma frequency band under a variety of conditions, maybe because of membrane potential depolarizations of large cell assemblies (Egorov et al. 2002).

Hippocampal gamma band activity is increased during exploratory behavior in animals (Bragin et al. 1995) and during waking state in humans (Fell et al. 2003) and can be induced by activation of cholinergic receptors (Fisahn et al. 1998). MTL gamma band activity during memory retrieval was analyzed by time–frequency analyses and extraction of frequency-specific power values, which reflect local synchronization of neural activity (Klimesch 1996). A direct link between hippocampal gamma activity and memory formation has been suggested by 1) increased rhinal–hippocampal synchronization and decreased power of hippocampal low-frequency (32–48 Hz) gamma oscillations during successful memory formation (Fell et al. 2001); 2) computer simulations suggesting encoding of individual items by single gamma cycles (Jensen and Lisman 2005); 3) retrieval-related reactivation of networks synchronized in the gamma frequency range (Herrmann et al. 2004); and 4) facilitated induction of hippocampal LTP following activation of cholinergic receptors in vitro (Adams et al. 2004). Gamma oscillations might thus be the most accurate measure of memory-related hippocampal activity.

Although we did not find significant effects in lower frequency bands during retrieval, the amount of delta band activity during the naps was correlated (although weakly) with the retrieval times of recent items. In contrast, there was no correlation with the retrieval times of remote items. These data support the specific role of deep sleep for the consolidation of declarative memories (e.g., Gais et al. 2006; Marshall et al. 2006). Moreover, they are consistent with recent findings that sleep does not only aid in the consolidation of memories acquired prior to sleep but also facilitates learning of new material after the sleep as well (Tononi and Cirelli 2006; Yoo et al. 2007; see Fig. 1*C*).

Taken together, our data suggest memory trace transfer from the hippocampus to the neocortex during resting state immediately following initial encoding. Our data thus argue against theories that suggest that the role of sleep in memory consolidation is to specifically facilitate information transfer during sleep. Instead, we found that sleep facilitates memory consolidation during postsleep waking state, consistent with its proposed role in synaptic downscaling and recovery of synaptic plasticity.

Supplementary Material

Supplementary material can be found at http://www.cercor.oxfordjournals.org/.

Notes

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